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(Dezembro de 2001 e Maio de 2002)

Sociedade Portuguesa de Protecção Contra Radiações

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**INSTITUIÇÃO DE UTILIDADE PÚBLICA**
**RUA 5 de Outubro Lote 33 1º E 2695-697 São João da Talha PORTUGAL**
Telefone: +351219552062 TELECÓPIA :+351219942077
**Email:geral@sppcr.online.pt jqbpcr@mail.telepac.pt**

**http://www.sppcr.online.pt**

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# Developmental Effects Induced by Chronic and Prolonged Exposure of Chicken Embryos to 900 MHz GSM Base Station Radiation

B. J. Youbicier-Simo

*Tecnolab Research Centre, 71100 Chalon sur Saône, France*
e-mail : youbicier-simo@wanadoo.fr

**Abstract.** Interference from base station radiation with embryonic development was investigated by chronic and prolonged exposure of developing chicken embryos. The latter were exposed under a 900 MHz GSM base station radiating microwaves at recommended safety level, i.e. 41 V/m. Total death rate was 7.7 times higher among radiation-exposed embryos (78.5 %) than among sham-exposures (10.2 %). Radiation exposure was associated with delayed hatching (3.2 days) and slightly but significantly increased ($P < 0.01$) weight of hatchlings. These findings indicate that base station radiation can induce lethal effect, as well as developmental retardation. They arouse questioning as to the adequacy of current safety guidelines.

## 1 Introduction

Scientific interested has been recently focused on non-thermal bio-effects of very low-levels of non-ionising radiation (NIR). The debate about the safety of low-energy NIR has been rekindled with the recent introduction and exponential development of mobile telephony. Information exchange between mobile phone users is bi-directional and is supported by a complex network which uses low-energy microwave radiation for the transmission of communications. The first link between the transmission network and the mobile station, i.e. the handset, is the base station [1]. Base stations are installed in cities, suburbs, rural areas and along the roads, and each one covers a limited geographic area called "cell", whose surface is inversely proportional to the density of the population [1]. Therefore there are much more base stations in towns than in rural areas and along roads. City base stations are mostly placed on/in residential or office buildings. As a result of their multiplication in the city, the general population is permanently bombarded by radiation and is immersed in an "electromagnetic smog", which adds to individual exposure resulting from the use of handsets. Such an insidious and chronic exposure of the general population to electromagnetic pollution might, in the long run, become health threatening and hence pose serious public health issues. To date, despite growing evidence of the biological effectiveness of mobile phone radiation, scientific data are rather scant as to potential health risk from exposure to base station radiation. Report from a recent epidemiological study suggested that people living in the neighbourhood of base stations (0-300 m) complained about headaches, fatigue, sleep disturbances, nausea, irritability, depression, memory loss, concentration difficulties, skin irritation, vertigo, auditory problems [2]. Similar symptoms were also reported by mobile phone users

[3, 4, 5]. Aforementioned results cast doubt as to the adequacy of safety standards currently in force in the field of mobile telephony [6]. These guidelines limit whole-body exposure level to 0.08 W/Kg (restriction level), which corresponds to reference levels of 41 V/m at 900 MHz and 58 V/m at 1800 MHz in the european GSM standard. Previous experiments performed in our laboratory [7, 8] had demonstrated that the developing chick embryo is exquisitely sensitive to electromagnetic fields including mobile phone frequencies. In the present study, the chick embryo model was applied to the investigation of the biological imp act of chronic and prolonged exposure under a 900 MHz base station radiating microwaves at the level recommended by regulatory bodies, i.e. 41 V/m. As a result, we observed worsened embryo mortality, delayed hatching and increased weight of hatchlings.

## 2 Material and methods

### 2.1 Set up for radiation exposure and incubation material

The exposure system was set up in an incubation room (3 x 3 x 2 m) equipped with a Faraday cage (1.5 x 1.5 x 1.5 m) intended to restrict the propagation of the base station radiation to the incubation area. A mini indoor base station working at 900 MHz was mounted on the ceiling of the Faraday cage. The mini indoor base station was connected to an outdoor rooftop antenna by means of an amplifier which adjusted the power output of the mini indoor base station. The rooftop antenna was installed on the roof of the laboratory, about 30 m away from the mini indoor base station.
The incubation room was heated by means of an electric resistance monitored by a thermostat (universal digital controller, Honeywell, USA). The thermostat was scheduled to maintain the temperature within the incubation room at 37.5 ± 0.5 °C. Ventilation was performed by means of a blower installed on the ceiling of the incubation room. A humidifier (HG-01 model, Hygro-Air, Italy) filled with water was connected to an hygrostat (universal digital controller, Honeywell, USA) programmed to maintain relative humidity within the incubation room at 55 ± 5 %. The egg plate was a PVC platform with numbered positions. It was placed in the Faraday cage and was centered on the vertical axis of the mini indoor base station.

### 2.2 Measurement of the level of the microwave radiation over the egg plate

The level of the microwave radiation was measured at different points over the egg plate using the EP 330 microwave probe connected to the PMM 8053 portable meter (PMM Co, Italy). Each measurement was performed over 4 min and the recorded data (r.m.s.) were used to plot the distribution map of the radiation level over the egg plate.

### 2.3 Biological material

Freshly hatched chicken eggs from the T451NI strain genetically selected by the SASSO Compagny (Sabres 40, France) were purchased from SICAMEN Hatchery (Baudrières 71, France).

### 2.4 Experimental protocol

The eggs were randomly distributed over the egg plate. Because only a single incubation room was available, sham and assay eggs were studied in independent experiments successively performed in the same incubation room. Three independent experiments were performed. The eggs were either sham-exposed (experiments 1 and 2; n = 100 eggs/group) or exposed under the base station radiating at 41 V/m (experiment 3; n = 108 eggs). All the eggs were incubated at 37.5 ± 0.5 °C, 55 ± 5 % relative humidity and permanent darkness over 21 days (duration of embryonic life in the chicken). Dead embryos were detected at 2-day intervals from 3 to 13 days of development (ED3, ED5, ED7, ED9, ED11, ED13) by candling the eggs under white light. From ED13 to ED21, embryonic mortality was not evaluated because the eggs had become so opaque that the embryos could hardly be mirrored through the shell using white light. Therefore, the embryos dead during the latter period were counted after hatching (ED21), by numbering the eggs with perforated shell or by opening those with intact shell for gross morphological examination of the embryos (embryo size, degree of withdrawal of the remains of the yolk into the body through the yolk stalk). Gross morphological examination allowed discrimination between the embryos deceased during hatching from those dead earlier. After hatching, surviving day-old chicks were weighed.

### 2.5 Statistical analysis

The data were processed by the analysis of variance (ANOVA) test using the Statview software (Abacus concenTs, Inc.).

## 3 Results

### 3.1 Mortality kinetics and total death rate (Table 1)

In the sham-exposed group, the number of dead embryos was low (3 and 4 in experiments 1 and 2 respectively) from ED3 to ED13 and slightly increased (6) from ED13 to ED21. All the embryos deceased during the second half of embryonic life (ED13 to ED21) had reached the hatching stage, as ascertained by gross morphological examination of unhatched eggs. Total death rate was 9.2 % and 10.3 % for experiments 1 and 2 respectively, and averaged mortality was 10.2 % for both experiments. When the embryos were exposed with the base station radiating at 41 V/m, not only the mortality level was higher than among sham-exposures, but it increased throughout the exposure period (24 dead embryos from ED3 to ED13 and 34 from ED13 to ED21). Twelve (35 %) of 34 embryos dead from ED13 to ED21 had reached the hatching stage. Overall mortality rate (78.5 %) was 7.7 fold higher than among sham-exposures ($P<0.01$).

### 3.2 Weight and hatching delay of hatchlings

A total of 176 (experiments 1 + 2) hatched in the sham-exposed group against 16 in the exposed group. Exposed chicks were slightly but significantly ($P<0.05$) heavier (42.7 ± 0.6 g) than sham-exposures (40.2 ± 0.1 g). Furthermore, exposed embryos hatched 3.2 days later than their sham-exposed counterparts.

## 4 Discussion

The kinetics of mortality exhibited by the sham-exposed embryos, i.e. low-level mortality during the first half of embryonic life followed by slight increment at the time of hatching, was in keeping with normal developmental pattern, which is characterized by low-level mortality and critical periods during which embryonic loss increases [9, 10]. The most critical period occurs at the time of hatching, and increased necropsy is due to stress resulting from the physical effort and energy expenditure required for hatching [9, 10]. On the other hand, necropsy occurred throughout embryonic life among exposed embryos, and embryonic defeat was observed at all the stages of development as ascertained by post-mortem morphological examination. Consistently we previously observed the same mortality profile among chicken embryos continuously exposed during 21 days either to video display unit radiation [7]or to 900 MHz GSM cell phone radiation [8]. Present findings also comply with those of another study reporting lethal and/or teratogenic effects in chicken embryos submitted for more than 20 days to 428 MHz radio-frequency radiation at a very low power density of 5.5 $cm^2$ [11]. A progressive reduction in the number of offspring, probably due to early foetal resorption, was also observed in pregnant mice continuously exposed during five gestations to radio-frequency fields emitted by an antenna park (commercial Radio FM band, UHF TV band, mobile communications towers), at the frequency range between 88.5 and 950 MHz and at power densities (168 $nW/cm^2$-1.053 $\mu$ $W/cm^2$) by far lower than recommended safety levels [12]. The authors of aforementioned studies ruled out any probability of thermal effects. On the other hand, they suggested that observed adverse effects were non-thermal cumulative end points elicited by prolonged exposure to low-dose radiation. In the present study, the gradual increase of embryonic loss observed in the embryos exposed at 41 V/m, as well as the observation of dead embryos at all the stages of development might also account for a cumulative effect resulting from chronic and protracted exposure to low-level microwave radiation.

The damaging effect of the base station radiation was associated with delayed hatching (3.2 days), as well as slightly but significantly increased weight of hatchlings. In our previous study with mobile phone handsets [8], the time of hatching was also retarded by 1-2 days. Postponement of hatching was also observed by other investigators when chicken embryos were continuously exposed for more than 20 days to 428 MHz radio-frequency radiation at a very low power density of 5.5 $cm^2$ [11]. Growth retardation was also reported for chicks exposed continuously to a 880 MHz field at intensities around 500 $\mu W/cm^2$ [13]. The adrenals of the irradiated birds were found to be smaller than those of the controls. Herein, increased weight of irradiated chicks can be explained by weight gain induced by irradiation during the retardation period of 3.2 days.

Present findings comply with the results of the epidemiological study by Santini and co-workers [2] and bring to forth the inadequacy of current safety standards, which are based on the specific absorption rate (SAR). The SAR is a measure that relates only to the rate at which energy is deposited in a given mass of biomaterial by an external microwave field. It does not -and cannot- address the question of information transfer from the irradiating field to the recipient living organism. Given that information transfer is the basis of the more subtle, athermal or non-thermal effects [14], the SAR concept is clearly not relevant to such effects [14, 15]. Thereby it is necessary to develop a new standard of electromagnetic biocompatibility basod on criteria relevant to the live state and on the biological response. In this respect, I would like to quote H.P. Schwan who, with C.Durney, was a pioneer in the field of Bioelectromagnetics: "The rationale for the specific absorption rate as a basis of health standards needs further elaboration" [15].

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