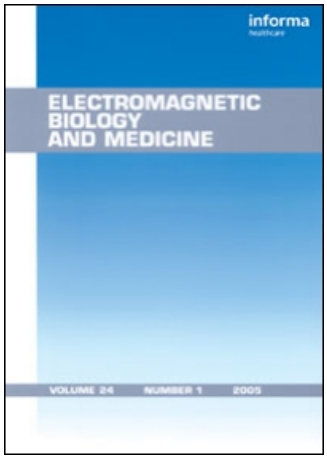

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An increasing number of studies find that pulsed Radio Frequency (RF), electromagnetic radiation of both systems of digital mobile telephony, established and commonly used in Europe during the last years, GSM 900 MHz (Global System for Mobile telecommunications) and DCS 1800 MHz (Digital Cellular System), exert intense biological action on different organisms and cells (Hardell et al., 2006; Hyland, 2000; Kundi, 2004; Panagopoulos et al., 2004, 2007). The two types of cellular telephony radiation use different carrier frequencies and give different frequency spectra, but they usually also differ in intensity, as GSM 900 MHz antennas operate at about double the power output than the corresponding DCS 1800 MHz ones. In our present experiments, we used a model biological system, the reproductive capacity of Drosophila melanogaster, to compare the biological activity between the two systems of cellular mobile telephony radiation. Both types of radiation were found to decrease significantly and non thermally the insect's reproductive capacity, but GSM 900 MHz seems to be even more bioactive than DCS 1800 MHz. The difference seems to be dependent mostly on field intensity and less on carrier frequency.

Keywords Biological effects; DCS radiation; *Drosophila*; Electromagnetic fields; GSM; Reproductive capacity; RF.

Introduction

The two systems of digital mobile telephony radiation commonly used in Europe, GSM 900 MHz and DCS 1800 MHz, differ in the carrier frequency, (900 and 1800 MHz, respectively), while both use the same pulse repetition frequency of

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217 Hz. Thereby both types of mobile telephony radiation include microwave carrier frequencies pulsed on extremely low frequencies (ELF). Additionally, both types of radiation use the TDMA (Time Division Multiple Access) code. Another difference between the two types of radiation is that GSM 900 MHz antennas of both mobile phones and base stations operate with double the output power than the corresponding DCS 1800 MHz ones (Clark, 2001; Hillebrand, 2002; Hamnerius and Uddmar, 2000; Hyland, 2000; Tisal, 1998).

Radio Frequency (RF)-microwave as well as ELF electromagnetic fields, have been reported to produce a large number of biological effects, including changes in intracellular ionic concentrations, changes in the synthesis rate of different biomolecules, changes in cell proliferation rates, changes in the reproductive capacity of animals, changes in gene expression, and even DNA damage and cell death (Aitken et al., 2005; Bawin and Adey, 1976; Bawin et al., 1975, 1978; Blackman et al., 1980, 1989; Dutta et al., 1984; Fitzsimmons et al., 1989; Goodman et al., 1983, 1995; Goodman and Henderson, 1988; Kwee and Raskmark, 1998; Lai and Singh, 1995, 1996, 1997, 2004; Liboff et al., 1984; Magras and Xenos, 1997; Nylund and Leszczynski, 2006; Ozawa et al., 1989; Panagopoulos et al., 2004; Remondini et al., 2006; Rodan et al., 1978; Schimmelpfeng and Dertinger, 1993; Velizarov et al., 1999; Xenos and Magras, 2003). Moreover, combination of RF and ELF has been reported to produce even more intense bioeffects than RF alone (Lin-Liu and Adey, 1982; Penafiel et al., 1997). In general, electromagnetic fields with changing parameters (like mobile telephony signals) are found to be more drastic than corresponding fields with constant parameters (Diem et al., 2005; Goodman et al., 1995). Recent works report DNA damage and cell death induced by GSM and DCS fields (Diem et al., 2005; Panagopoulos et al., 2007; Salford et al., 2003). At the same time, some epidemiological studies are starting to indicate a connection between the use of cellular mobile phones and certain types of cancer including acoustic neuroma (Hardell et al., 2006; Hardell and Hansson Mild, 2006; Kundi, 2004).

The reproductive capacity of *Drosophila melanogaster* has been reported by our group (Panagopoulos and Margaritis, 2002, 2003; Panagopoulos et al., 2000a, 2004, 2007) to be a very sensitive and credible indicator for the bioactivity of a stress factor like electromagnetic fields. According to our previous experiments, electromagnetic radiation from GSM 900 MHz mobile phones, "modulated" by human voice ("speaking emission" or "GSM basic"), was found to decrease the reproductive capacity of the insect *Drosophila melanogaster* by 50–60%, whereas the corresponding "non modulated" field (non speaking emission) was found to decrease the reproductive capacity by 15–20%. In both cases, exposure took place for a few minutes per day for a few days and both sexes were found to be affected (Panagopoulos et al., 2004).

Our more recent experiments have shown that the large decrease in the reproductive capacity of the female insects is due to elimination of large numbers of egg chambers during early and mid oogenesis as a result of induced death of their constituent cells. Cell death was found to be induced by both types of radiation, GSM and DCS. A first comparison showed that GSM was more bioactive than DCS (Panagopoulos et al., 2007).

Since there are discrete differences between the two types of cellular mobile telephony radiation both widely used, the question that naturally arises is which type of radiation is less harmful. Therefore, the aim of the present work was a detailed

comparison of the bioactivity between the two types of mobile telephony radiation, based on statistical experiments.

Materials and Methods

Experimental Animal

In our present work we carried out experiments with *Drosophila melanogaster* flies, Oregon R, wild-type, held in glass bottles and kept in incubator at 25°C, with 12 h periods of light and darkness and 70% relative humidity.

The reproductive capacity of this insect (oogenesis and spermatogenesis) is a model biological system, very well studied with a very good timing of its developmental processes under certain laboratory conditions (Horne-Badovinac and Bilder, 2005; King, 1970).

Following our standard protocol, the reproductive capacity was defined by the number of F_1 pupae, which under the conditions of our experiments, corresponds to the number of laid eggs (oviposition) (Panagopoulos et al., 2004).

Exposure System

We used a commercial cellular mobile phone itself as an exposure device in order to analyze effects of real exposure conditions to which a mobile phone user is subjected. We were the first, as far as we know, to use a cellular mobile phone itself as an exposure device (Panagopoulos and Margaritis, 2002, 2003; Panagopoulos et al., 2000a, 2004, 2007), instead of using simulations of digital mobile telephony signals with constant characteristics (frequency, intensity, etc.) or even test mobile phones programed to emit mobile telephony signals with constant parameters. Real GSM, DCS signals are never constant. There are continuous changes in the intensity and frequency of these signals. Electromagnetic fields with changing parameters are found to be more bioactive than fields with constant parameters (Diem et al., 2005; Goodman et al., 1995), probably because it is more difficult for living organisms to get adapted to them. Experiments with constant GSM or DCS signals can be performed, but they do not simulate actual conditions. Thereby, in order to study the bioactivity of real mobile telephony signals, we used a common cellular mobile phone itself in our experiments. Recently, other research groups have started to use cellular phones as exposure devices as well, obviously for the same reasons (Barteri et al., 2005; Diem et al., 2005; Weisbrot et al., 2003).

A dual band cellular mobile phone was used that could be connected both to either GSM 900 or DCS 1800 networks simply by changing SIM ("Subscriber Identity Module") cards on the same handset. Highest Specific Absorption Rate (SAR), given by the manufacturer for human head, is 0.89 W/Kg. Exposure procedure was the same as in previous reports of ours (Panagopoulos et al., 2004). The handset was fully charged before each set of exposures.

During our experiments, we exposed the insects to the mobile phone's GSM or DCS fields while the mobile phone was operating in speaking mode ("modulated emission"). As we have described, the intensity of the emitted radiation increases considerably when the user speaks during connection vs. when there is no speaking (Panagopoulos and Margaritis, 2002; Panagopoulos et al., 2000a, 2004). The mobile phone was held close to the experimenter's head with its antenna parallel and in

contact with the glass vials. In the most new digital cell phone handsets, the antenna is in the back and upper side of the device. This can be easily verified by measuring the emitted radiation holding the probe of the field meter in contact with different parts of the handset's surface.

Exposures and measurements of mobile phone emissions were made always at the same place where the mobile phone had full perception of both GSM and DCS signals. For power density and field measurements of the mobile phone emissions, we followed the methodology described by us before (Panagopoulos et al., 2004). Measured mean power densities in contact with the mobile phone antenna for 6 min of modulated emission were $0.407 \pm 0.061\,mW/cm^2$ for GSM 900 MHz and $0.283 \pm 0.043\,mW/cm^2$ for DCS 1800 MHz. As was expected, GSM 900 MHz intensity at the same distance from the antenna and with the same handset was higher than the corresponding DCS 1800 MHz. For better comparison between the two systems of radiation we measured the GSM power density at different distances from the antenna and found that at 1 cm distance, the GSM 900 MHz intensity was $0.286 \pm 0.050\,mW/cm^2$, almost equal to DCS 1800 MHz at zero distance. Measurements at 900 MHz and 1800 MHz were performed with a RF Radiation Survey Meter, NARDA 8718. Since both GSM and DCS signals have a pulse repetition frequency at 217 Hz, we measured the electric and magnetic field intensities in the ELF range, with a Holaday HI-3604 ELF Survey Meter. The measured values for modulated field, excluding the ambient electric and magnetic fields of 50 Hz, were $22.3 \pm 2.2\,V/m$ electric field intensity and $0.50 \pm 0.08\,mG$ magnetic field intensity for GSM at zero distance, $13.9 \pm 1.6\,V/m$, $0.40 \pm 0.07\,mG$ correspondingly for GSM at 1 cm distance and $14.2 \pm 1.7\,V/m$, $0.38 \pm 0.07\,mG$ correspondingly for DCS at zero distance. All of the above-measured values are typical for digital mobile telephony handsets and they are within the current exposure criteria (ICNIRP, 1998). All the above-measured values are averaged over ten separate measurements of each kind $\pm$ standard deviation (SD).

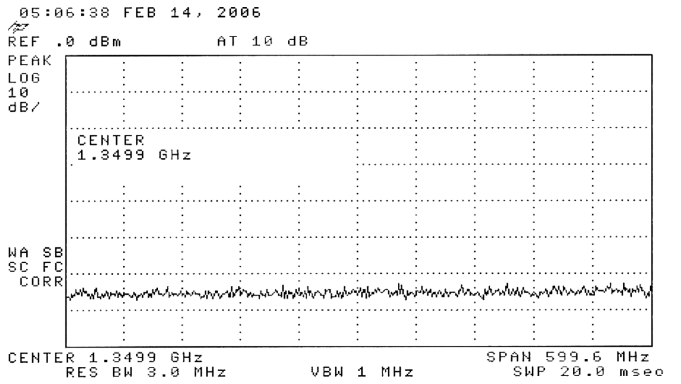
Except for the power density-field measurements of the mobile phone emissions, we obtained the spectra of both types of radiation, plus the background spectrum in our lab (Figs. 1a, 1b, 1c). Each one of the two types of radiation gave a unique frequency spectrum. While GSM 900 MHz gives a single peak around 900 MHz (Fig. 1b), DCS 1800 MHz gives a main peak around 1800 MHz and a smaller one around 900 MHz (Fig. 1c). The spectra were obtained by a Hewlett Packard 8595 E (9 kHz–6.5 GHz) spectrum analyzer (USA).

Exposure Procedure

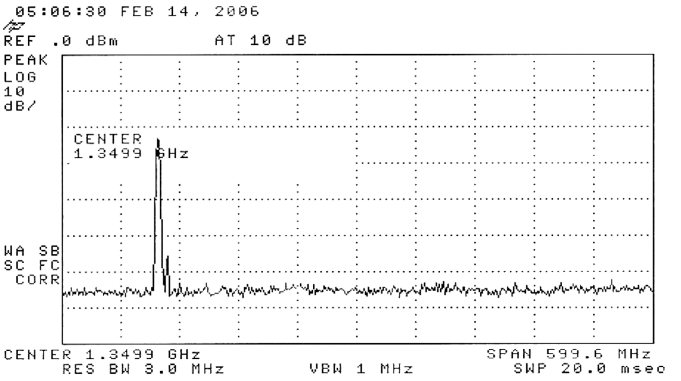
In each experiment, we collected newly eclosed adult flies from the stock and separated them into different groups following the same methodology as described before (Panagopoulos et al., 2004).

We exposed the flies within the glass vials. After each exposure, the vials were put back into the culture room. The exposures took place for a total of 5 days in each experiment starting on the first day (day of eclosion), for 6 min per day in one dose as described in detail before (Panagopoulos et al., 2004).

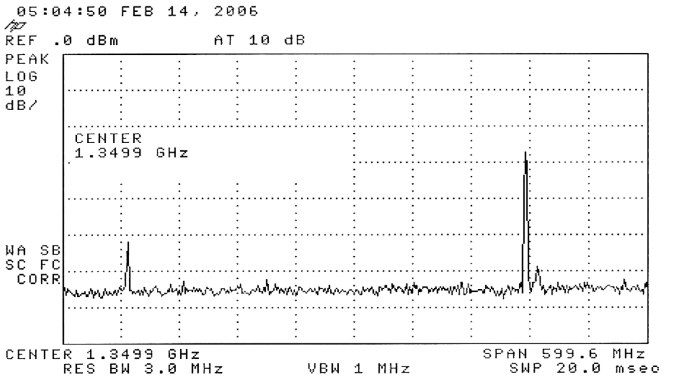
In each experiment we separated the insects into four groups: (a) the group exposed to GSM 900 MHz field with the mobile phone antenna in contact with the glass vial containing the flies (named as "900"); (b) the group exposed to GSM 900 MHz field with the antenna of the mobile phone at 1 cm distance from the



(a)



(b)



(c)

Figure 1. (a) Back ground spectrum (b) spectrum of GSM 900 MHz (c) spectrum of DCS 1800 MHz.

vial (named as "900 A"); (c) the group exposed to DCS 1800 MHz field with the mobile phone antenna in contact with the glass vial (named as "1800"); and (d) the Sham Exposed (Control) group (named as "SE"). Each one of the four groups consisted of ten female and ten male newly emerged flies. The second group (900 A)

was introduced for better comparison of possible effects between the two systems of radiation. Comparison between the first and third group represents comparison with the usual exposure conditions between GSM 900 and DCS 1800 users, while comparison between the second and third group represents comparison of possible effects between the RF frequencies of the two systems under equal radiation intensities. The sham exposed groups had identical treatment as the exposed ones, except that the mobile phone during the 6 min "exposures", was turned off.

During the first 48 h of each experiment, the males and females of each group were kept and exposed separately, while for the next 72 h during which the insect's oviposition is at its maximum, the males and females of each group (20 flies), were put together (10 pairs) and exposed in the same vial allowed to mate and lay eggs (Panagopoulos et al., 2004).

After the last 72 h (five days from the beginning of each experiment), the flies were removed from the glass vials and the vials with the developing embryos and the food within them, were maintained in the culture room for 6 additional days, without further exposure.

After the last six days, most F_1 embryos (deriving from the laid eggs) were in the stage of pupation, where they clearly can be seen macroscopically and easily counted on the walls of the glass tubes. As we have explained before (Panagopoulos et al., 2004), the number of F_1 pupae, under the conditions of our experiments corresponds to the number of laid eggs (oviposition). Hence, by counting the F_1 pupae 11–12 days after the beginning of each experiment, we get a valid estimate of each group's reproductive capacity.

Temperature during the exposures was monitored within the vials by a Hg thermometer with 0.05°C sensitivity (Panagopoulos et al., 2004). Statistical analysis was made by single factor Analysis of Variance test.

Results

We carried out ten replicate experiments. Results are listed in Table 1 and represented graphically in Fig. 2.

Table 1 shows the mean number of F_1 pupae (corresponding to the number of laid eggs) per maternal fly in the groups exposed to the GSM and DCS fields and in the corresponding sham exposed groups during the three days of the insect's maximum oviposition. This number is a direct measure of the insect's reproductive capacity.

The data show that the reproductive capacity of the exposed groups is significantly decreased compared to the sham exposed groups. The decrease is maximum in the 900 groups (48.25% compared to SE) and smaller in the 900 A and the 1800 groups (32.75 and 31.08%, respectively) (Table 1). Although the decrease was even smaller in the 1800 groups than in 900 A, differences between the 900 A and 1800 groups were found to be within the standard deviation (Table 1, Fig. 2).

The differences in the mean number of F_1 pupae per maternal fly between the groups were greater between 900 and 900 A (owing to intensity differences between the two types of radiation) and much smaller between 900 A and 1800 (owing to frequency differences between GSM and DCS) (Table 1). The statistical analysis shows that the probability that the reproductive capacity differs between groups, owing to random variations, is negligible, $P < 10^{-18}$.

We did not detect any temperature increases within the glass vials during the exposures.

Table 1
Effect of modulated GSM and DCS fields on the reproductive capacity of *Drosophila melanogaster*

Experiment no.	Groups	Mean number of F_1 pupae per maternal fly	Deviation from control (%)
1	900	7.7	−42.54
	900 A	8.9	−33.58
	1800	9.2	−31.34
	SE (Control)	13.4	
2	900	5.8	−51.26
	900 A	8.1	−31.93
	1800	7.9	−33.61
	SE (Control)	11.9	
3	900	6.8	−46.03
	900 A	7.9	−37.30
	1800	8.7	−30.95
	SE (Control)	12.6	
4	900	7.4	−47.52
	900 A	9.7	−31.21
	1800	9.9	−29.79
	SE (Control)	14.1	
5	900	6.2	−52.31
	900 A	8.5	−34.62
	1800	8.2	−36.92
	SE (Control)	13	
6	900	6.1	−43.52
	900 A	8.2	−24.07
	1800	7.8	−27.78
	SE (Control)	10.8	
7	900	6.7	−47.66
	900 A	8.3	−35.16
	1800	9	−29.69
	SE (Control)	12.8	
8	900	6	−48.72
	900 A	7.9	−32.48
	1800	8.4	−28.21
	SE (Control)	11.7	
9	900	6.7	−49.24
	900 A	8.8	−33.33
	1800	9.1	−31.06
	SE (Control)	13.2	
10	900	5.7	−53.66
	900A	8.3	−32.52
	1800	8.5	−30.89
	SE (Control)	12.3	
Average ± SD	900	6.51 ± 0.67	−48.25
	900 A	8.46 ± 0.55	−32.75
	1800	8.67 ± 0.65	−31.08
	SE (Control)	12.58 ± 0.95	

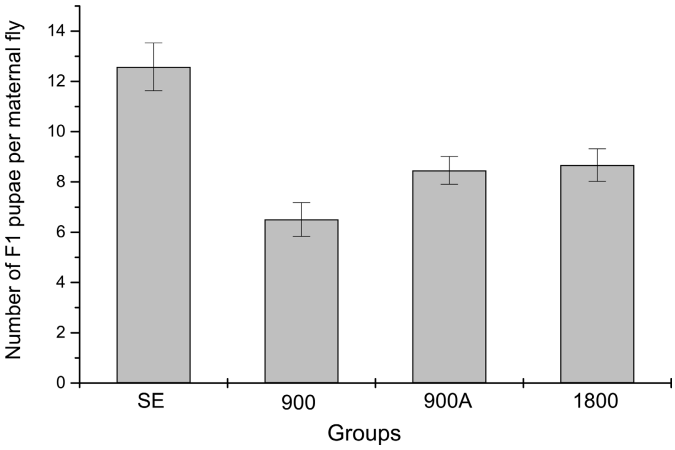


Figure 2. Reproductive capacity (mean number of F1 pupae per maternal fly) of exposed and sham exposed groups.

Discussion

Our results show that both types of mobile telephony radiation decrease considerably insect reproduction. The statistical analysis clearly shows that the exposed *Drosophila* groups differ in offspring production between them and compared to the SE groups, due to the effect of the GSM and DCS fields.

Since we did not detect any temperature increases during the exposures, the recorded effect is considered as non thermal.

Recent experiments of ours have shown that the large decrease in the reproductive capacity of the female insects, caused by the exposure to the GSM and DCS fields, is due to elimination of large numbers of egg chambers during early and mid oogenesis after death (DNA fragmentation) of their constituent cells, induced by both types of fields/radiation. Cell death was found to be induced during all developmental stages of early and mid oogenesis and in all kinds of egg chamber cells (Panagopoulos et al., 2007) phenomena that were not observed before to be produced by other stress factors like poor nutrition or chemical stress (Drummond-Barbosa and Spradling, 2001; Nezis et al., 2000), therefore suggesting that electromagnetic stress induced by cellular mobile telephony radiations is probably an even more intense type of stress than those previously examined.

As we have explained (Panagopoulos et al., 2007), similar effects on humans are certainly possible. In this case, induced cell death on a number of brain cells could explain symptoms like headaches, fatigue, sleep disturbances, etc., reported as "microwave syndrome" (Enrique et al., 2003; Hutter et al., 2006).

A biophysical explanation of the above effects can be given by the mechanism proposed by us (Panagopoulos et al., 2000b, 2002) for the action of electromagnetic fields on cells. According to this theory, ELF electric fields of the order of a few V/m are able to irregularly gate electrosensitive channels on a cell's plasma membrane and therefore disrupt cell function. Additionally, pulsed fields are shown to be more bioactive than continuous ones. Therefore, according to our proposed mechanism, the ELF components of GSM and DCS signals are able to disrupt cell function and possibly produce the above effects.

Our present experiments show that the main difference in bioactivity between GSM and DCS is related with the higher intensity of GSM under the same exposure conditions (differences between groups 900 and 900 A). Intensity differences between the two types of cellular mobile telephony radiation depend also on the ability of communication between the antennas of the mobile phone and the corresponding base station. Even if GSM usually has a higher intensity than DCS, this situation can be reversed in certain places if GSM has a much better signal perception between its antennas than DCS (Tisal, 1998). Our results count for equal signal perception conditions between the two types of radiation.

Although both types of radiation considerably affect reproduction, GSM seems to be even more bioactive than DCS, even when it is emitted at almost the same intensity (differences between groups 900 A and 1800), although the differences in this case were within the standard deviation. This might mean that lower frequency fields are more bioactive than higher frequency ones with the same rest characteristics, as is also predicted by our theory (Panagopoulos et al., 2000b, 2002), and it is also supported by other experimental evidence (Lin-Liu and Adey, 1982; Penafiel et al., 1997).

In any case, our results once more show that exposure to GSM and DCS fields has adverse effects on living organisms and should be restricted by more rigorous exposure criteria.

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