

Bioeffects of mobile telephony radiation in relation to its intensity or distance from the antenna

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Abstract

Purpose: To examine the bioactivity of GSM 900 and 1800 (Global System for Mobile Telecommunications) radiations, in relation to the distance from the antenna or to the radiation-field intensities.

Materials and methods: *Drosophila melanogaster* adult insects were exposed to the radiation of a GSM 900/1800 mobile phone antenna at different distances ranging from 0 to 100 cm, and the effect on their reproductive capacity and cell death induction in the gonads by the use of TUNEL (Terminal deoxynucleotide transferase dUTP Nick End Labeling) assay, was studied.

Results: These radiations/fields decreased the reproductive capacity by cell death induction, at all the different distances tested. The effect diminished with the distance/decreasing intensities. An increased bioactivity 'window' was revealed at distances of 20–30 cm from the mobile phone antenna, (radiation intensity around $10 \mu\text{W}/\text{cm}^2$) where the effect became highest, in relation to smaller or longer distances. The effect diminished considerably for distances longer than 40–50 cm and became not evident for distances longer than 1 m or radiation intensities smaller than $1 \mu\text{W}/\text{cm}^2$.

Conclusions: GSM bioactivity is highest for intensities down to less than $10 \mu\text{W}/\text{cm}^2$ and still evident until $1 \mu\text{W}/\text{cm}^2$ exhibiting 'window' effects.

Keywords: GSM, DCS, distances, intensity, cell death, electromagnetic fields, reproduction, bioactivity windows

Introduction

A number of biological effects from digital mobile telephony and radio frequency (RF)-microwave radiations, 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, have already been reported and documented by many research groups (Bawin et al. 1975; 1978; Bawin and Adey 1976; Lai and Singh 1995, 1996, 1997; Magras and Xenos 1997; Kwee and Raskmark 1998; Velizarov et al. 1999; Salford et al. 2003; Xenos and Magras 2003; Panagopoulos et al. 2004, 2007a, 2007b; Aitken et al. 2005; Barteri et al. 2005; Belyaev et al. 2005; 2009; Caraglia et al. 2005; Diem et al. 2005; Markova et al. 2005; Nylund and Leszczynski 2006; Remondini et al. 2006; Eberhardt et al.

2008; Garaj-Vrhovac and Orescanin 2009; Lopez-Martin et al. 2009). 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 (Kundi 2004; Hardell et al. 2006, 2007, 2009; Hardell and Hansson Mild 2006; Hardell and Carlberg 2009; Khurana et al. 2009), as well as a connection between exposure to radiation from base stations and adverse health effects reported as 'microwave syndrome' (Navarro et al. 2003; Hutter et al. 2006; Blettner et al. 2009; Kundi and Hutter 2009; Viel et al. 2009).

Most of the experiments carried out in regards to the bioactivity of mobile telephony radiation were performed either by use of commercial mobile phone devices emitting real mobile telephony signals or by test mobile phones emitting idealized mobile telephony signals with constant and controllable parameters. Until now there were no experiments

regarding the effects at different distances from mobile phone antennas corresponding to different intensities of the emitted radiation, neither experiments regarding the effects of mobile telephony base station antennas, except of statistical observations which have reported reduction of bird and insect populations around base station antennas (Balmori 2005; Everaert and Bauwens 2007).

Both systems of Digital Mobile Telephony Radiation established and commonly used in Europe, GSM 900 MHz (Global System for Mobile telecommunications), and GSM 1800 MHz, (also called DCS 1800 MHz – Digital Cellular System), except of their RF carrier signal, use a pulse repetition frequency of 217 Hz, plus other extremely low frequencies (ELF) necessary for the transmission of information (Tisal 1998; Hamnerius and Uddmar 2000; Hyland 2000; Clark 2001; Hillebrand 2002; Panagopoulos and Margaritis 2008). Thereby the signals of both systems combine RF carrier and ELF pulsing frequencies is considered to play an important role in the bioactivity of this kind of radiation (Lin-Liu and Adey 1982; Penafiel et al. 1997).

Radiation from base station antennas is almost identical to that from mobile phones of the same system (GSM 900 or 1800), except that it is about 100 times more powerful, and uses a little higher carrier frequency. GSM 900 mobile phones emit between 890 and 915 MHz (uplink operation) while base stations emit between 935 and 960 MHz (downlink operation). The corresponding GSM (or DCS) 1800 spectrums are 1710–1785 MHz (uplink operation) and 1805–1880 MHz (downlink operation) (Tisal 1998; Hamnerius and Uddmar 2000; Hyland 2000; Clark 2001; Hillebrand 2002; Panagopoulos and Margaritis 2008). Thereby, effects produced by mobile phones at certain distances, could possibly be extrapolated to represent effects from base station antennas, of the same type of radiation, at about 100 times longer distances.

The difficulty in performing experiments with base station mobile telephony antennas is due to the fact of uncontrolled conditions in the open air that do not allow the use of sham-exposed animals, (exposed to identical other conditions like temperature, humidity, light etc.). In other words, there is no way to have a sham-exposed group of experimental animals under identical environmental conditions as the exposed ones, but without being exposed to the radiation at the same time. We thought that the only way to simulate the reality of the exposure by a base station antenna is to expose the animals at different distances from a mobile phone within the laboratory.

In order to study the bioactivity of mobile telephony signals at different intensities and distances from the antenna of a mobile phone

handset, resembling effects from base station signals within residential areas, we used the same biological index as in previous experiments of ours, the reproductive capacity of the insect *Drosophila melanogaster*, defined by the number of F₁ (first filial generation) pupae derived during the three days of the insect's maximum oviposition, as this was found to be a reliable indicator for the bioactivity of electromagnetic fields (EMF) (Panagopoulos et al. 2000a, 2004, 2007a, 2007b; Panagopoulos and Margaritis 2002, 2003a).

Our previous experiments regarding a few minutes daily exposure of the same model animal to the near field of a mobile phone antenna have shown a large decrease in the reproductive capacity, affecting both sexes (Panagopoulos et al. 2004). Both systems of digital mobile telephony radiation GSM 900 MHz and GSM/DCS 1800 MHz were found to produce the same effects, but GSM 900 was found to be even more bioactive than 1800, mainly due to the higher intensity of GSM 900 antennas compared to GSM/DCS 1800 ones (Panagopoulos et al. 2007a). The decrease in the reproductive capacity was found to be due to induced cell death (DNA fragmentation) in the gonads, caused by both types of mobile telephony radiations (Panagopoulos et al. 2007b).

A widely used method for identifying cell death is TUNEL (Terminal deoxynucleotide transferase dUTP Nick End Labeling) assay. By use of this method, fluorescein dUTP is bound through the action of terminal transferase, onto fragmented genomic DNA which then becomes labelled by characteristic fluorescence. The label incorporated at the fragmented DNA is visualised by fluorescence microscopy (Gavrieli et al. 1992).

Each *Drosophila* ovary consists of 16–20 ovarioles. Each ovariole is an individual egg assembly line, with new egg chambers in the anterior moving toward the posterior as they develop, through 14 successive stages until the mature egg reaches the oviduct. The most anterior region is called the germarium. The most sensitive developmental stages during oogenesis for stress-induced cell death, are region 2 within the germarium and stages 7–8 just before the onset of vitellogenesis (Drummond-Barbosa and Spradling 2001; McCall 2004). Electromagnetic stress from mobile telephony radiations was found in our experiments to be extremely bioactive, inducing cell death to a high degree not only to the above two 'check points' (germarium and stages 7–8) but to all developmental stages of early and mid oogenesis and moreover to all types of egg chamber cells, i.e. nurse cells, follicle cells and the oocyte (OC) (Panagopoulos et al. 2007b).

In continuing our research on the biological impacts of the cellular mobile telephony radiation, the aim of the present study was to investigate the

dependence of GSM 900/1800 bioactivity on its intensity, within intensity levels that people are exposed to, from mobile phones and base station antennas as well. Finally, in the case that we would detect a decrease in the reproductive capacity at smaller intensities than in our previous experiments (Panagopoulos et al. 2004, 2007a, 2007b), our aim would be to confirm whether again the decrease is due to cell death induced by the radiation or not, by use of the TUNEL assay.

Materials and methods

Drosophila culturing

Wild-type strain Oregon R *Drosophila melanogaster* flies were cultured according to standard methods and kept in glass vials with standard food (Panagopoulos et al. 2004). Ovaries from exposed and sham-exposed flies were dissected into individual ovarioles at the sixth day after eclosion and then treated for TUNEL assay.

Exposure system

As an exposure device we used a commercial cellular mobile phone itself, in order to analyse the effects of real mobile telephony signals. As in previous experiments (Panagopoulos et al. 2007a, 2007b), we used a dual band cellular mobile phone that could be connected to either 900 or 1800 networks simply by changing SIM ('Subscriber Identity Module') cards on the same handset. The highest Specific Absorption Rate (SAR), given by the manufacturer for human head, is 0.89 W/kg. The exposure procedure was the same as in earlier experiments of ours (Panagopoulos et al. 2007b). The handset was fully charged before each set of exposures. The experimenter spoke on the mobile phone's microphone during the exposures. Thereby, the emitted 900 or 1800 radiation during the exposures was 'modulated' by the human voice, ('speaking emissions').

Exposures and measurements of mobile phone emissions were performed at the same place where the mobile phone had full perception of both 900 and 1800 signals, as described before (Panagopoulos et al. 2007a). The measured mean power densities in contact and at different distances from the mobile phone antenna for 6 min of modulated emission, for GSM 900 MHz and for DCS 1800 MHz, are shown in Table I. As explained before (Panagopoulos et al. 2007a, 2007b), the GSM 900 MHz intensity at the same distance from the antenna and with the same handset was higher than the corresponding GSM/DCS 1800 MHz. Measurements at 900 and 1800 MHz were performed with a RF Radiation Survey

Meter, NARDA 8718 (Hauppauge, NY, USA). Since both GSM 900 and 1800 signals use a pulse repetition frequency at 217 Hz plus other ELF pulses, we measured electric and magnetic field intensities in the ELF range, with a Holaday HI-3604 ELF Survey Meter (Eden Prairie, MN, USA). The measured values for the modulated ELF fields, excluding the ambient electric and magnetic fields of 50 Hz, for GSM 900 and 1800 at different distances from the antenna are also shown in Table I. All values shown in Table I are averaged over 10 separate measurements of each kind \pm standard deviation (SD). These values are typical for digital mobile telephony handsets and they are all within the established current exposure criteria (International Commission for Non-Ionising Radiation Protection [ICNIRP] 1998).

The radiation and field measurements given in Table I show that although the ELF electric and magnetic field intensities fall within the background levels for distances longer than 50 cm from both GSM 900 and 1800 mobile phone antennas, the RF components of the signals are still evident for distances up to 100 cm.

Exposure procedures

In each single experiment, we separated the collected insects into thirteen groups: The first group (named '0') was exposed to GSM 900 or 1800 field with the mobile phone antenna in contact with the glass vial containing the flies. The second (named '1'), was exposed to GSM 900 or 1800 field, at 1 cm distance from the mobile phone antenna. The third group (named '10') was exposed to GSM 900 or 1800 field at 10 cm distance from the mobile phone antenna. The fourth group (named '20') was exposed to GSM 900 or 1800 field at 20 cm distance from the mobile phone antenna, etc, the 12th group (named '100') was exposed to GSM 900 or 1800 field at 100 cm distance from the mobile phone antenna. Finally, the 13th group (named 'SE') was the sham-exposed. Each group consisted of 10 male and 10 female insects as previously (Panagopoulos et al. 2004, 2007a).

In each experiment, we collected newly eclosed adult flies from the stock early in the afternoon, and separated them into the 13 different groups following the same methodology as in previous experiments (Panagopoulos et al. 2004).

We exposed the flies within the glass vials by placing the antenna of the mobile phone outside of the vials, parallel to the vial's axis. The total duration of exposure was 6 min per day in one dose and exposures were started on the first day of each experiment (day of eclosion). In each experiment, all the 12 exposed groups were simultaneously exposed

Table I. GSM 900 and 1800 radiation and field intensities \pm SD, in the microwave and ELF regions, for different distances from a mobile phone antenna*.

| Distance from mobile phone antenna (cm) | GSM 900 radiation intensity at 900 MHz, (mW/cm ²) | GSM 900 electric field intensity at 217 Hz, (V/m) | GSM 900 magnetic field intensity at 217 Hz, (mG) | GSM 1800 radiation intensity at 1800 MHz, (mW/cm ²) | GSM 1800 electric field intensity at 217 Hz, (V/m) | GSM 1800 magnetic field intensity at 217 Hz, (mG) |
|---|---|---|--|---|--|---|
| 0 | 0.378 \pm 0.059 | 19 \pm 2.5 | 0.9 \pm 0.15 | 0.252 \pm 0.050 | 13 \pm 2.1 | 0.6 \pm 0.08 |
| 1 | 0.262 \pm 0.046 | 12 \pm 1.7 | 0.7 \pm 0.13 | 0.065 \pm 0.015 | 6 \pm 0.8 | 0.4 \pm 0.07 |
| 10 | 0.062 \pm 0.020 | 7 \pm 0.8 | 0.3 \pm 0.05 | 0.029 \pm 0.005 | 2.7 \pm 0.5 | 0.2 \pm 0.05 |
| 20 | 0.032 \pm 0.008 | 2.8 \pm 0.4 | 0.2 \pm 0.04 | 0.011 \pm 0.003 | 0.6 \pm 0.12 | 0.1 \pm 0.02 |
| 30 | 0.010 \pm 0.002 | 0.7 \pm 0.09 | 0.1 \pm 0.02 | 0.007 \pm 0.001 | 0.3 \pm 0.06 | 0.06 \pm 0.01 |
| 40 | 0.006 \pm 0.001 | 0.2 \pm 0.03 | 0.05 \pm 0.01 | 0.004 \pm 0.0007 | 0.1 \pm 0.04 | – |
| 50 | 0.004 \pm 0.0006 | 0.1 \pm 0.02 | – | 0.002 \pm 0.0003 | – | – |
| 60 | 0.002 \pm 0.0003 | – | – | 0.0016 \pm 0.0002 | – | – |
| 70 | 0.0017 \pm 0.0002 | – | – | 0.0013 \pm 0.0002 | – | – |
| 80 | 0.0012 \pm 0.0002 | – | – | 0.0011 \pm 0.0002 | – | – |
| 90 | 0.0010 \pm 0.0001 | – | – | 0.0005 \pm 0.0001 | – | – |
| 100 | 0.0004 \pm 0.0001 | – | – | 0.0002 \pm 0.0001 | – | – |

*For distances longer than 30–50 cm from the mobile phone antenna, the ELF electric and magnetic field components of both GSM 900 and 1800 radiations, fall within the background of the stray 50 Hz fields within the lab.

during the 6-min exposure sessions. The exposures took place for five days in each experiment, as previously described (Panagopoulos et al. 2004). Then there was an additional 6-min exposure in the morning of the sixth day and one hour later, female insects from each group were dissected and prepared for TUNEL assay, as described before (Panagopoulos et al. 2007b). The daily exposure duration of 6 min, was chosen for reasons we have explained before (Panagopoulos et al. 2004, 2007a) and for keeping the same exposure conditions as in our previous experiments.

After each exposure, the corresponding sham-exposure took place. The SE group was 'exposed' for 6 min at zero distance from the mobile phone antenna, following exactly the same methodology (the experimenter spoke on the mobile phone, same voice, reading the same text) but the mobile phone was turned off. Before this we had already verified that sham-exposed groups at all the 12 different locations of exposure described above, did not differ significantly between them in their reproductive capacity and additionally did not differ significantly from a Control group (named 'C') which was never taken out of the culture room during the experiments and was not exposed or sham-exposed in any way (see Appendix). Comparison between SE and C groups in relation to the reproductive capacity and ovarian cell death on the same experimental animals was discussed also in a previous work of ours (Panagopoulos et al. 2007b).

In each experiment we kept the 10 males and the 10 females of each group, in separate vials for the first 48 h, for reasons we have explained before (Panagopoulos et al. 2004). After the first 48 h of

each experiment, when both males and females of each group were sexually mature, they were put together (10 pairs) in another glass vial with fresh food. They were allowed to mate and lay eggs for the next 72 h, during which, the daily egg production of *Drosophila* is at its maximum (Panagopoulos et al. 2004).

After the last exposure in the morning of the sixth day from the beginning of each experiment, the flies were removed from the glass vials, and the ovaries of females were dissected and fixed for TUNEL assay. The vials were then maintained in the culture room for 6–8 additional days without further exposure, and then the number of F₁ pupae was counted in each group as in previous experiments (Panagopoulos et al. 2000a, 2004, 2007a). As explained in detail before (Panagopoulos et al. 2004), this number is a representative estimate of the insect's reproductive capacity.

The temperature during the exposures was monitored within the vials by a mercury thermometer with an accuracy of 0.05°C (Panagopoulos et al. 2004).

TUNEL assay

To determine the ability of GSM and DCS radiation to induce cell death during early and mid oogenesis, we used the TUNEL assay, as follows: Ovaries were dissected in Ringer's solution and separated into individual ovarioles from which we took away egg chambers of stages 11–14. In egg chambers of stages 11–14 programmed cell death takes place normally in the nurse cells and follicle cells. Thereby we kept and treated ovarioles and individual egg chambers

from germarium up to stage 10. Samples were fixed in phosphate-buffered saline (PBS) solution containing 4% formaldehyde plus 0.1% Triton X-100 (Sigma Chemical Co., Munich, Germany) for 30 min and then rinsed three times and washed twice in PBS for 5 min each. Then samples were incubated with PBS containing 20 $\mu\text{g/ml}$ proteinase K for 10 min and washed three times in PBS for 5 min each. In situ detection of fragmented genomic DNA was performed with Boehringer Mannheim kit (Boehringer Mannheim Corp., Indianapolis, IN, USA), containing fluorescein dUTP for 3 h at 37°C in the dark. Samples were then washed six times in PBS for 1 h and 30 min (total duration) in the dark and finally mounted in antifading mounting medium (90% glycerol containing 1.4-diazabicyclo (2.2.2) octane (Sigma Chemical Co.) to prevent from fading and viewed under a Nikon Eclipse TE 2000-S fluorescence microscope (Tokyo, Japan). The samples from different experimental groups were blindly observed under the fluorescence microscope (i.e., the observer did not know the origin of the sample) and the percentage of egg chambers with TUNEL positive signal was scored in each sample.

Statistical analysis

The results on reproductive capacity and cell death induction were analysed statistically by single factor Analysis of Variance test which calculates the probability (P) that differences between groups are due to random variations. The smaller this probability is, the more significantly the groups differ between them (in their reproductive capacity or in the percentages of TUNEL positive egg chambers). In addition, linear (Pearson's) and non-parametric (Kendall's) correla-

tion analysis were performed between reproductive capacity and radiation/field intensities in order to get an estimation of which parameter (RF radiation, ELF fields) might be more responsible for the effects (Weiss 1995; Maber 1999).

Results

The average mean values of reproductive capacity (mean number of F_1 pupae per maternal insect) from eight separate identical experiments with GSM 900 and GSM/DCS 1800 exposures are listed in Table II and represented graphically in Figures 1 and 2.

The data show that GSM 900 mobile telephony radiation decreases reproductive capacity at distances from 0 cm up to 90 cm from the mobile phone antenna (corresponding intensities ranging from 378 $\mu\text{W/cm}^2$ down to 1 $\mu\text{W/cm}^2$ —Table I). Table II and Figure 1 show that the effect is at a maximum at 0 cm and at 30 cm from the antenna (corresponding to radiation intensities of 378 $\mu\text{W/cm}^2$ and 10 $\mu\text{W/cm}^2$, respectively) with an overall maximum at 30 cm. For distances longer than 30 cm from the mobile phone antenna, the effect decreases rapidly and becomes very small for distances longer than 50 cm, but it is still evident for distances up to 90 cm (intensities down to 1 $\mu\text{W/cm}^2$).

The data also show that GSM/DCS 1800 mobile telephony radiation decreases reproductive capacity at distances from 0 cm up to 80 cm from the mobile phone antenna (corresponding intensities ranging from 252 $\mu\text{W/cm}^2$ down to 1.1 $\mu\text{W/cm}^2$ —Table I). Table II and Figure 2 show that the effect is maximum at 0 cm and at 20 cm from the antenna, (corresponding to radiation intensities of 252 $\mu\text{W/cm}^2$ and 11 $\mu\text{W/cm}^2$, respectively) with overall

Table II. Effect of GSM 900 and 1800 radiation-fields on the reproductive capacity at different distances from the antenna.

| Groups-Distance from mobile phone antenna, (cm) | Average mean number of F_1 pupae per maternal fly \pm SD, for GSM 900 MHz | Deviation from sham-exposed group | Average mean number of F_1 pupae per maternal fly \pm SD, for GSM 1800 MHz | Deviation from sham-exposed group |
|---|---|-----------------------------------|--|-----------------------------------|
| 0 | 7.46 \pm 0.73 | -46.14% | 9.10 \pm 0.69 | -35.09% |
| 1 | 9.35 \pm 0.62 | -32.49% | 11.35 \pm 0.63 | -19.04% |
| 10 | 11.28 \pm 0.81 | -18.56% | 11.93 \pm 0.72 | -14.91% |
| 20 | 11.55 \pm 0.79 | -16.61% | 8.33 \pm 0.7 | -40.58% |
| 30 | 7.38 \pm 0.65 | -46.71% | 12.77 \pm 0.82 | -8.92% |
| 40 | 12.81 \pm 0.97 | -7.51% | 13.52 \pm 0.86 | -3.57% |
| 50 | 13.49 \pm 0.82 | -2.60% | 13.72 \pm 0.75 | -2.14% |
| 60 | 13.62 \pm 0.83 | -1.66% | 13.81 \pm 0.92 | -1.50% |
| 70 | 13.72 \pm 0.92 | -0.94% | 13.79 \pm 0.90 | -1.64% |
| 80 | 13.68 \pm 0.80 | -1.23% | 13.85 \pm 0.81 | -1.21% |
| 90 | 13.75 \pm 0.95 | -0.72% | 14.03 \pm 1.02 | +0.07% |
| 100 | 14.01 \pm 1.01 | +1.16% | 14.05 \pm 0.99 | +0.21% |
| SE | 13.85 \pm 0.91 | | 14.02 \pm 0.98 | |

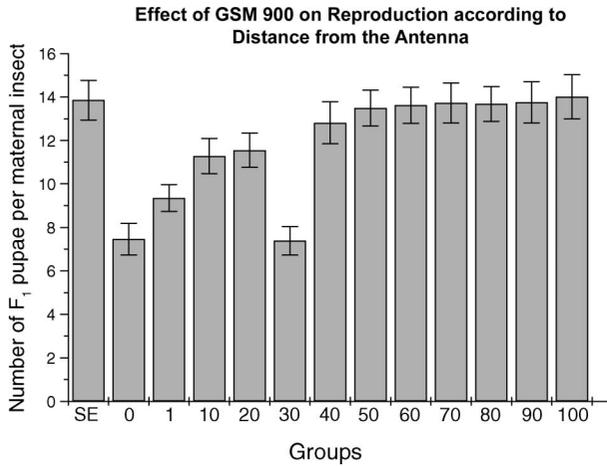


Figure 1. Reproductive capacity (mean number of F_1 pupae per maternal insect averaged over eight identical experiments) \pm SD, in relation to the distance from a GSM 900 MHz mobile phone antenna (cm). The decrease in reproductive capacity is at a maximum at zero distance and at 30 cm distance from the antenna, corresponding to RF intensities $378 \mu\text{W}/\text{cm}^2$ and $10 \mu\text{W}/\text{cm}^2$ (see Table II).

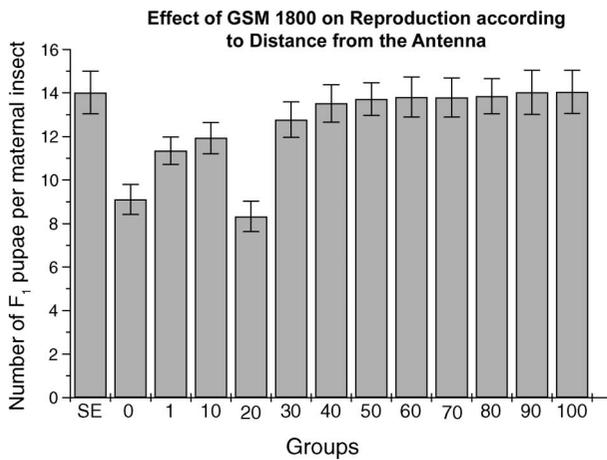


Figure 2. Reproductive capacity (mean number of F_1 pupae per maternal insect averaged over eight identical experiments) \pm SD, in relation to the distance from a GSM/DCS 1800 MHz mobile phone antenna (cm). The decrease in reproductive capacity is at a maximum at zero distance and at 20 cm distance from the antenna, corresponding to RF intensities $252 \mu\text{W}/\text{cm}^2$ and $11 \mu\text{W}/\text{cm}^2$ (see Table II).

maximum at 20 cm. For distances longer than 20 cm from the mobile phone antenna, the effect decreases rapidly and becomes very small for distances longer than 40 cm, but it is still evident for distances up to 80 cm (intensities down to $1.1 \mu\text{W}/\text{cm}^2$).

Thus, the effect of mobile telephony radiation on reproductive capacity is at a maximum at zero distance (intensities higher than $250 \mu\text{W}/\text{cm}^2$) and then becomes maximum at a distance of 30 cm or 20 cm from the antenna for GSM 900 or 1800 MHz radiation, respectively. These distances of 30 cm and 20 cm, respectively, correspond to the same RF

intensity around $10 \mu\text{W}/\text{cm}^2$ and also to the same ELF electric field intensity of about 0.6–0.7 V/m (Table I).

The statistical analysis (single factor ANOVA test) shows that the probability that the reproductive capacity differs between groups, owing to random variations, is negligible both for GSM 900 and 1800 exposures, $P < 10^{-27}$ in both cases.

There were no temperature increases within the vials during the exposures, as shown by the sensitive Hg thermometer.

In Table III, the summarised data on cell death induction in the gonads of the female insects from three separate experiments are listed. These data are represented graphically in Figures 3 and 4. The percentages of TUNEL positive egg chambers in all groups were found to be very close to the corresponding decrease in the reproductive capacity of the same groups (Table III, Figures 3 and 4), verifying the results of earlier experiments of ours (Panagopoulos et al. 2007b). The maximum percentage of TUNEL positive egg chambers of exposed animals was found in the ovaries of female insects exposed at 0 and 20 cm distance from the antenna for GSM/DCS 1800 MHz (43.39% and 55.07%) and at 0 and 30 cm distance correspondingly for GSM 900 MHz (57.72% and 57.83%), in agreement with the corresponding maximum decreases in the reproductive capacity (Table III, Figures 3 and 4).

Figure 5a, shows an ovariole from a sham-exposed (SE) female insect, containing egg chambers from germarium to stage 8, all TUNEL negative. This was the typical picture in the vast majority of ovarioles and separate egg chambers from female insects of the sham-exposed groups. In the SE groups, only few egg chambers (including germaria), (less than 8%), were TUNEL positive (Table III, Figures 3 and 4), a result that is in full agreement with the rate of spontaneously degenerated egg chambers normally observed during *Drosophila* oogenesis (Nezis et al. 2000; Baum et al. 2005; Panagopoulos et al. 2007b).

Figure 5b shows an ovariole of an exposed female insect (group 50- GSM 900), which is TUNEL positive only at the two 'check points' germarium and stage 7 and TUNEL negative at all other developmental stages. This was a typical picture of ovarioles of exposed insects from the groups 40–90 for GSM 900 and 30–80 for GSM/DCS 1800.

Figure 5c, shows an ovariole of an exposed female insect (group 20- GSM1800), with a TUNEL positive signal at all developmental stages from germarium to 8 and in all the cell types of the egg chamber (nurse cells, follicle cells and the oocyte). This was a typical picture of ovarioles of exposed insects from the groups 0–30 for GSM 900 and 0–20 for GSM/DCS 1800.

Table III. Effect of GSM 900 and 1800 radiation-fields on ovarian cell death induction at different distances from the mobile phone antenna.

| Groups- Distance from mob. phone antenna (cm) | GSM 900 | | | GSM 1800 | | |
|--|---|--|---|---|--|---|
| | Sum ratio of TUNEL-positive to total number of egg-chambers from germarium to stage 10 \pm SD | Percentage of TUNEL-positive egg-chambers (%) | Deviation from sham-exposed groups (%) | Sum ratio of TUNEL-positive to total number of egg-chambers from germarium to stage 10 \pm SD | Percentage of TUNEL-positive egg-chambers (%) | Deviation from sham- exposed groups (%) |
| 0 | 355/615 = 0.5772 \pm 0.083 | 57.72 | +50.16 | 243/560 = 0.4339 \pm 0.087 | 43.39 | +35.77 |
| 1 | 267/612 = 0.4363 \pm 0.061 | 43.63 | +36.01 | 146/483 = 0.3023 \pm 0.059 | 30.23 | +22.61 |
| 10 | 172/577 = 0.2981 \pm 0.052 | 29.81 | +22.24 | 136/532 = 0.2556 \pm 0.054 | 25.56 | +17.94 |
| 20 | 152/564 = 0.2695 \pm 0.049 | 26.95 | +19.38 | 337/612 = 0.5507 \pm 0.095 | 55.07 | +47.45 |
| 30 | 336/581 = 0.5783 \pm 0.092 | 57.83 | +50.26 | 78/452 = 0.1726 \pm 0.061 | 17.26 | +9.64 |
| 40 | 93/542 = 0.1716 \pm 0.053 | 17.16 | +9.59 | 62/577 = 0.1075 \pm 0.056 | 10.75 | +3.13 |
| 50 | 60/556 = 0.1079 \pm 0.043 | 10.79 | +3.22 | 54/511 = 0.1057 \pm 0.042 | 10.57 | +2.95 |
| 60 | 51/498 = 0.1024 \pm 0.045 | 10.24 | +2.67 | 57/580 = 0.0983 \pm 0.046 | 9.83 | +2.21 |
| 70 | 57/584 = 0.0976 \pm 0.041 | 9.76 | +2.19 | 39/427 = 0.0913 \pm 0.033 | 9.13 | +1.51 |
| 80 | 51/563 = 0.0906 \pm 0.037 | 9.06 | +1.49 | 39/485 = 0.0804 \pm 0.034 | 8.04 | +0.42 |
| 90 | 50/591 = 0.0846 \pm 0.04 | 8.46 | +0.89 | 41/534 = 0.0768 \pm 0.028 | 7.68 | +0.06 |
| 100 | 46/602 = 0.0764 \pm 0.035 | 7.64 | +0.07 | 43/557 = 0.0772 \pm 0.035 | 7.72 | +0.1 |
| SE | 47/621 = 0.0757 \pm 0.038 | 7.57 | 0 | 48/630 = 0.0762 \pm 0.034 | 7.62 | 0 |

Like in our earlier experiments (Panagopoulos et al. 2007b), although in the most egg-chambers where DNA fragmentation could be observed the TUNEL positive signal was most evident in the nurse cells, in many egg chambers of exposed animals and especially in the groups 0–30 for GSM 900 and 0–20 for GSM 1800 on which the bioactivity of the radiation was maximum, a TUNEL-positive signal was detected in all three kinds of egg chamber cells (Figure 5c).

In the SE groups, random DNA fragmentation was observed almost exclusively at the two developmental stages named check-points (germarium and stage 7–8) as also observed before (Panagopoulos et al. 2007b). Similarly, induced DNA fragmentation in the groups 40–100 for GSM 900 and 30–100 for GSM 1800 (Figure 5b), was observed mostly at the two check-points, (data not shown) and only in few cases at the other previtellogenic and vitellogenic stages, 1–6 and 9–10, correspondingly. In contrast, ovarian egg chambers of animals from the exposed groups 0–30 for GSM 900 and 0–20 for GSM 1800, were found to be TUNEL-positive to a high degree at all developmental stages from germarium to stage 10 (Figure 5c), (data not shown). In all cases (both in the SE and also in the exposed groups), the TUNEL-positive signal was observed predominantly and was most intense at the two check points, germarium and stages 7–8, as previously recorded (Panagopoulos et al. 2007b).

Statistical analysis (single factor analysis-of-variance test) shows that the probability that cell death induction differs between groups because of random variations, is $P < 10^{-10}$ both for GSM 900 MHz and 1800 MHz exposures.

The effect on the reproductive capacity, and the induced cell death in the ovaries of exposed female insects, diminishes considerably for distances longer than 40 cm from the mobile phone antenna and disappears for distances longer than 80–90 cm, corresponding to radiation intensities smaller than $1 \mu\text{W}/\text{cm}^2$ (Tables I–III, Figures 1–4). For distances longer than 50 cm where the ELF components fall within the background of the stray 50 Hz fields, the decrease in reproductive capacity as well as the increase in cell death induction, in regards to the SE groups was very small falling within the standard deviation of the SE groups (Tables II and III, Figures 1–4).

The results of Pearson's linear correlation analysis show a slightly stronger linear relationship between reproductive capacity and ELF electric field intensity (linear correlation coefficient, $r \cong -0.72$, $P < 0.01$ for GSM 900 and $r \cong -0.65$, $P < 0.03$ for GSM/DCS 1800), than between reproductive capacity and RF radiation intensity ($r \cong -0.70$, $P < 0.02$ and $r \cong -0.63$, $P < 0.03$, respectively), both for GSM 900 and 1800 exposures. Since our results show that the dependence of reproductive capacity and cell death induction on RF and ELF intensities is non-linear (Figures 1–4), we applied also Kendall's non-parametric correlation analysis for a better estimation of the non-linear correlation between the variables. This correlation analysis in contrast to the previous one, showed a slightly stronger relationship between reproductive capacity and RF radiation intensity (correlation coefficient, $r \cong -0.85$, $P < 0.001$ for GSM 900 and $r \cong -0.88$, $P < 0.001$ for GSM/DCS 1800), than between reproductive capacity and ELF electric field intensity $r \cong -0.79$, $P = 0.001$

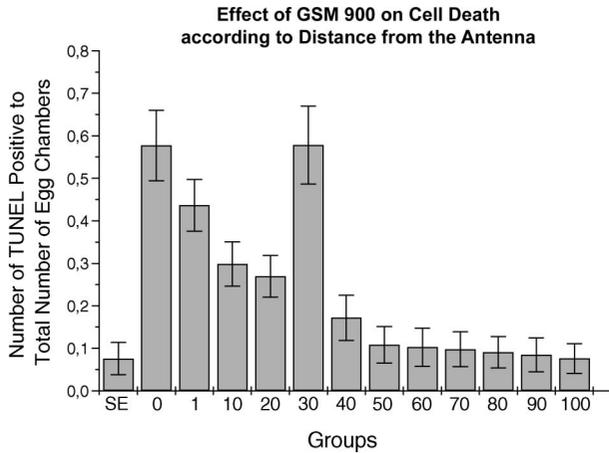


Figure 3. Mean ratio of ovarian cell death (number of TUNEL-positive to total number of egg-chambers, averaged over three identical experiments) \pm SD, in relation to the distance from a GSM 900 MHz mobile phone antenna (cm). The increase in cell death induction is at a maximum at zero distance and at 30 cm distance from the antenna, corresponding to RF intensities $378 \mu\text{W}/\text{cm}^2$ and $10 \mu\text{W}/\text{cm}^2$ (see Tables I and III).

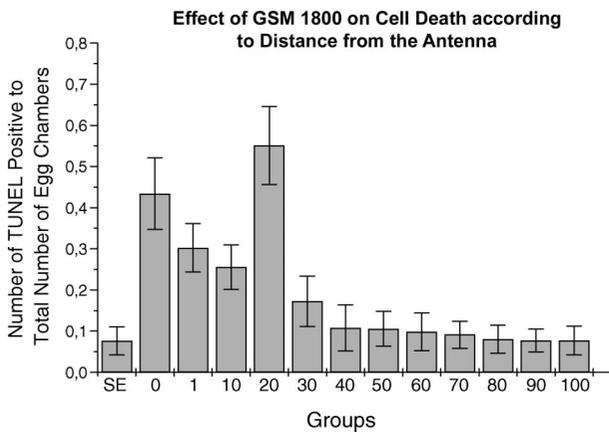


Figure 4. Mean ratio of ovarian cell death (number of TUNEL-positive to total number of egg-chambers, averaged over three identical experiments) \pm SD, in relation to the distance from a GSM/DCS 1800 MHz mobile phone antenna (cm). The increase in cell death induction is at a maximum at zero distance and at 20 cm distance from the antenna, corresponding to RF intensities $252 \mu\text{W}/\text{cm}^2$ and $11 \mu\text{W}/\text{cm}^2$ (see Tables I and III).

and $r \cong -0.78$, $P = 0.001$), both for GSM 900 and 1800 exposures. We note that the P -values (the probabilities that the corresponding r -values are due to random variation in the data points) in the case of Kendall's non-parametric correlation are smaller than the corresponding ones in Pearson's linear correlation, suggesting that non-parametric correlation analysis is perhaps more appropriate in the case of our (non-linear) results. The correlation analysis between reproductive capacity and distance from the antenna, gave the same values as between reproductive capacity and RF intensity and the correlation

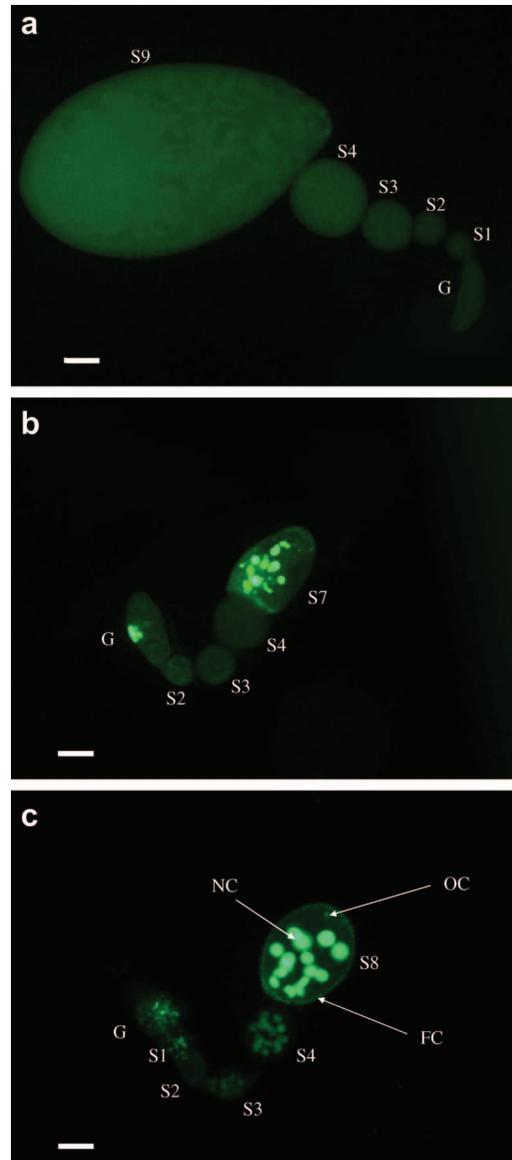


Figure 5. (a) Typical TUNEL-negative fluorescent picture of an ovariole of a sham-exposed female insect, containing egg chambers from germarium to stage 9. Bar: $10 \mu\text{m}$. (b) Ovariole of an exposed insect (group GSM 900, 50 cm) with TUNEL-positive signal only at the two check points, germarium plus stage 7 egg chamber and TUNEL-negative intermediate stages. Bar: $10 \mu\text{m}$. (c) Ovariole of exposed female insect (group GSM 1800, 20 cm) with fragmented DNA at all stages from germarium to stage 8 and in all kinds of egg chamber cells. NC, nurse cells; FC, follicle cells; OC, oocyte. Bar: $10 \mu\text{m}$.

between reproductive capacity and ELF magnetic field was found to be even weaker than with ELF electric field.

Discussion and conclusion

The effect of mobile telephony radiation on the reproductive capacity and the corresponding induced cell death in the ovaries of the exposed female insects, is very intense for distances up to 30 cm

from the mobile phone antenna, then diminishes considerably for distances longer than 40–50 cm from the mobile phone antenna where the ELF components fall within the background, but it is still evident for distances up to 100 cm (radiation intensities down to $1 \mu\text{W}/\text{cm}^2$). This fact suggests that this kind of radiation is bioactive for intensities higher than $1 \mu\text{W}/\text{cm}^2$.

The statistical analysis (single-factor Analysis of Variance) shows that the groups differ between them in reproductive capacity and cell death induction because of the GSM 900/1800 exposures at the different distances-intensities. The reason that the P value is much smaller in the case of reproductive capacity ($P < 10^{-27}$) than in cell death induction ($P < 10^{-10}$), is only that the number of experiments for cell death induction was smaller.

The fact that for distances longer than 50 cm where the ELF components fall within the background, the bioactivity of the radiation although is still evident decreases considerably and falls within the standard deviation of the SE group, might suggest that the ELF components of digital mobile telephony signals, play a crucial role in their bioactivity, alone or in conjunction with the RF carrier wave. This is in agreement with the mechanism that we have proposed for the action of EMF on living organisms, according to which, lower frequency fields are more bioactive than higher frequency ones (Panagopoulos et al. 2000b, 2002; Panagopoulos and Margaritis 2003b). According to this mechanism, ELF electric fields of the order of 10^{-3} V/m, are able to disrupt cell function by irregular gating of electrosensitive ion channels on the cell membranes. As shown in Table I, the ELF components of both GSM 900 and 1800 fields appear to possess sufficient intensity for this, for distances up to 50 cm from the antenna of a mobile phone (or about 50 m from a corresponding base station antenna).

It is interesting that the decrease in the reproductive capacity was found to be maximum not only within the near field of the mobile phone antenna (0–5.2 cm from the antenna for GSM 900 and 0–2.6 cm for GSM 1800) (Panagopoulos and Margaritis 2010), where the intensity of the radiation is maximum, but also within the far field, at 20–30 cm distance from the mobile phone antenna. Thus, in the present experiments, we have discovered the existence of increased bioactivity ‘windows’ for both GSM 900 and 1800 radiations. These ‘bioactivity windows’ appear at distances 20 or 30 cm from the GSM 1800 or 900 mobile phone antenna respectively, where the radiation intensity is in both cases close to $10 \mu\text{W}/\text{cm}^2$ and the ELF electric field intensity 0.6–0.7 V/m. At these distances, the bioeffect becomes even more intense than at zero distance from a mobile phone antenna where the RF intensity is higher than $250 \mu\text{W}/\text{cm}^2$, and the

ELF electric field intensity higher than 13 V/m (Table I). Another series of experiments is now necessary, aiming to reveal the nature of these bioactivity ‘windows’, (i.e., whether they depend on the intensity of the radiation/fields, or on any other parameter like for example the wavelength of the radiation which happens to be close to the distance where the ‘window’ appears) (Panagopoulos and Margaritis 2010).

The distance of 20–30 cm from a mobile phone antenna where the bioactivity ‘windows’ are observed, corresponds to a distance of about 20–30 m from a base station antenna (Panagopoulos and Margaritis 2008). Since mobile telephony base station antennas are usually located within residential areas, at distances 20–30 m from such antennas there are often houses and workplaces where people are exposed for up to 24 h per day. Therefore, our present findings show that mobile telephony radiation can be very bioactive at intensity levels encountered at residential and working areas around base station antennas.

We do not know which constituent of the real mobile telephony signal, (i.e., the RF carrier, the ELF pulse repetition frequencies, or the combination of both), is more responsible for the bioactivity of the signal or for the existence of the ‘windows’ found in our experiments. Real mobile telephony signals are always RF carrier signals pulsed at ELF in order to be able to transmit information. Furthermore, real mobile telephony signals are never constant in intensity or frequency. Therefore, we consider that performing experiments with idealised continuous signals corresponding to the RF carrier alone or to the ELF constituents alone would not represent reality.

Non-parametric Correlation analysis showed a slightly more increased relationship with the RF intensity than with ELF electric field intensity, while Linear Correlation analysis gave an opposite result. A possible conclusion from the Correlation analysis is that both RF and ELF parameters of the mobile telephony radiations are responsible for the effects, but since non-parametric correlation analysis might be more appropriate because of the non-linearity of our data, perhaps RF is slightly more responsible than ELF. Although the correlation analysis between reproductive capacity and distance from the antenna gave the same values as between reproductive capacity and RF intensity, distance is only indirectly related to the phenomenon. The effect of the distance depends basically on the fact that the RF and ELF intensities change with the distance. Nevertheless, other possibilities like effect of the radiation wavelength, wave interference, or effect of the differences between near and far field zone of the antenna cannot be excluded and will be investigated

and discussed in a separate series of experiments together with the nature of the observed bioactivity 'windows' (Panagopoulos and Margaritis 2010).

Although windows of increased bioactivity of RF radiations have been recorded over many years (Bawin et al. 1975, 1978; Bawin and Adey 1976; Blackman et al. 1980, 1989; Goodman et al. 1995), there is still no widely accepted explanation for their existence.

We do not know whether the bioactivity 'windows' found in our present experiments are related exclusively with the certain organism we used as experimental animal, or they would appear for other organisms too. More experiments with different experimental animals exposed at different distances from a mobile phone antenna are necessary to answer this question. Since the effect of cell death induction was observed in all three different kinds of female reproductive cells (nurse cells, follicle cells and the oocyte) and since most cellular functions are identical in both insect and mammal cells, we consider that it is possible for the above 'windows' of increased bioactivity to exist for other organisms and humans as well. The bioactivity 'windows' found in our present experiments could possibly correlate with recent results of another experimental group reporting that GSM radiation caused increased permeability of the blood-brain barrier in rat nerve cells and the strongest effect was produced by the lowest SAR values which correspond to the weakest radiation intensity (Eberhardt et al. 2008).

Our present experiments verify our earlier results (Panagopoulos et al. 2007b) that the reduction in reproductive capacity caused by digital mobile telephony radiation is due to induced cell death in the gonads. Furthermore, our present results show that induced cell death is the reason for the reduction in reproductive capacity also at longer distances from the antenna (or at lower intensities) than in our earlier experiments.

Our results show that exposure of living organisms to mobile telephony radiation is highly bioactive and able to induce cell death at intensities higher than few $\mu\text{W}/\text{cm}^2$ and this bioactivity is still evident for intensities down to $1 \mu\text{W}/\text{cm}^2$ (corresponding to distances up to 100 cm from a mobile phone, or up to about 100 m from a base station antenna). Effects were not observed at intensities lower than $1 \mu\text{W}/\text{cm}^2$ in the specific biological system that we studied. Therefore, our present results might suggest that public exposure should be restricted at intensities below this value.

As in our earlier experiments (Panagopoulos et al. 2007b), although egg chambers during early and mid oogenesis in *Drosophila* were not reported before to exhibit either stress-induced by other

stress factors than EMF, or physiological degeneration, at other stages except germarium and stages 7–8 (Drummond-Barbosa and Spradling 2001; Nezis et al. 2000, 2002; McCall 2004), mobile telephony radiation was found to induce cell death at all provitellogenic and vitellogenic stages 1–10 and the germarium. Additionally again cell death could be observed in all the cell types of the egg chamber, i.e., not only in nurse cells and follicle cells on which it was already known to be induced by other stress factors than EMF (Cavaliere et al. 1998; Foley and Cooley 1998; Drummond-Barbosa and Spradling 2001; Nezis et al. 2000, 2002; McCall 2004), but also in the oocyte (Figure 5c). A possible explanation for these phenomena as given by us before (Panagopoulos et al. 2007b) is based on the fact that the electromagnetic stress induced in the ovarian cells by the GSM 900 and 1800 fields is a new and probably more intense type of external stress, against which ovarian cells do not have adequate defence mechanisms like they do in the case of other kinds of external stresses like poor nutrition, heat or chemical stress.

The fact that electromagnetic stress induces DNA fragmentation in the oocyte (except of the nurse and follicle cells which anyway degenerate physiologically at stages 11–14) shows that the action of the electromagnetic stress is genotoxic and not just a shift of the physiological apoptotic stages in time as someone could possibly think as an alternative explanation. Besides, if it was just a shift of physiological apoptosis towards earlier stages, it would seem more likely for the organism to eliminate the defective egg chambers in the existing check points, germarium and stages 7–8, since this is the reason for the existence of the check points.

It is again important to emphasize that induced DNA fragmentation in the oocyte which undergoes meiosis during the last stages of oogenesis may result in heritable mutations upon DNA damage induction and repair, if not in cell death (Panagopoulos et al. 2007b).

Although we cannot simply extrapolate, we consider that similar effects on humans are possible for two reasons. First, insects are found to be more resistant than mammals, at least to ionising radiation (Abrahamson et al. 1973; Koval et al. 1977). Second, our results are in agreement with similar reported effects on mammals (although of course under different experimental conditions) (Lai and Singh 1995, 1996; Salford et al. 2003; Aitken et al. 2005). It is also possible that induced cell death on a number of brain cells can explain symptoms like headaches, fatigue, sleep disturbances etc., reported as 'microwave syndrome' (Navarro et al. 2003; Hutter et al. 2006).

In conclusion, we consider that our results imply the very cautious use of mobile phones at distances not shorter than 40 cm from the user's head and a reconsideration of the current exposure criteria in order to restrict public exposure from base station antennas to intensities not higher than $1 \mu\text{W}/\text{cm}^2$. According to the present study, even some of the lowest national current corresponding exposure limits might not be safe enough, like for example, the Chinese limit for public exposure ($40 \mu\text{W}/\text{cm}^2$) or the corresponding limit of Russia, Italy and Poland ($10 \mu\text{W}/\text{cm}^2$) (International EMF Project). In contrast, the recent decision of Liechtenstein to reduce its national exposure limit from $9.5 \mu\text{W}/\text{cm}^2$ (6 V/m) to $0.095 \mu\text{W}/\text{cm}^2$ (0.6 V/m) (<http://worldradio.ch/wrs/news/wrsnews/liechtenstein-to-vote-on-mobile-phone-masts.shtml?15942>) seems to be in agreement with the results of the present study, moreover including a safety factor of more than 10 times a lower limit than $1 \mu\text{W}/\text{cm}^2$.

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Appendix

Sham-exposure data

Reproductive capacity of sham-exposed at different distances from the antenna and control groups.

| SE/C groups: Distance from mobile phone antenna (cm) | Average mean number of F ₁ pupae per maternal fly ± SD |
|---|---|
| SE (0) | 13.73 ± 0.91 |
| SE (1) | 13.43 ± 1.52 |
| SE (10) | 14.07 ± 0.57 |
| SE (20) | 13.53 ± 0.80 |
| SE (30) | 14.03 ± 1.43 |
| SE (40) | 13.4 ± 1.67 |
| SE (50) | 13.13 ± 1.25 |
| SE (60) | 13.7 ± 1.01 |
| SE (70) | 14.17 ± 1.06 |
| SE (80) | 13.33 ± 1.27 |
| SE (90) | 13.67 ± 1.33 |
| SE (100) | 14.1 ± 1.28 |
| C | 14.18 ± 1.12 |

Average reproductive capacity (mean number of F₁ pupae per maternal fly) from three separate experiments ± SD for SE groups at the 12 different exposure distances from the mobile phone antenna and C groups. Single factor Analysis of Variance test showed that the reproductive capacity did not differ significantly between the 12 SE groups ($P > 0.99$), meaning that the differences between the 12 SE groups have more than 99% probability to be due to random variations.