In: Advances in Medicine and Biology. Volume 17 ISBN: 978-1-61122-790-1
Editor: Leon V. Berhardt ©2011 Nova Science Publishers, Inc.

Chapter 1

ANALYZING THE HEALTH IMPACTS OF MODERN TELECOMMUNICATIONS MICROWAVES

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ABSTRACT

While different classes of biological effects of radiation used in modern telecommunications are already confirmed by different experimenters, a lot of contradictory results are also reported. Despite uncertainties, some of the recent results reporting effects show an intriguing agreement between them, although with different biological models and under different laboratory conditions. Such results of exceptional importance and mutual similarity are those reporting DNA damage or oxidative stress induction on reproductive cells of different organisms, resulting in decreased fertility and reproduction. This distinct similarity among results of different researchers makes unlikely the possibility that these results could be wrong. This chapter analyzes and resumes our experimental findings of DNA damage on insect reproductive cells by Global System for Mobile telecommunications (GSM) radiation, compares them with similar recent results on mammalian-human infertility and discusses the possible connection between these findings and other reports regarding tumour induction, symptoms of unwellness, or declines in bird and insect populations. A possible biochemical explanation of the reported effects at the cellular level is attempted. Since microwave radiation is non-ionizing and therefore unable to break chemical bonds, indirect ways of DNA damage are discussed, through enhancement of free radical and reactive oxygen species (ROS) formation, or irregular release of hydrolytic enzymes. Such events can be initiated by alterations of intracellular ionic concentrations after irregular gating of electrosensitive channels on the cell membranes according to the Ion

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Forced-Vibration theory that we have previously proposed. This biophysical mechanism seems to be realistic, since it is able to explain all of the reported biological effects associated with exposure to electromagnetic fields (EMFs), including the so-called "windows" of increased bioactivity reported for many years but remaining unexplained so far, and recorded also in our recent experiments regarding GSM radiation exposure. The chapter also discusses an important dosimetry issue, regarding the use of Specific Absorption Rate (SAR), a quantity introduced to describe temperature increases within biological tissue (thermal effects), while the recorded biological effects in their vast majority are non-thermal. Finally the chapter attempts to propose some basic precautions and a different way of network design for mobile telephony base station antennas, in order to minimize the exposure of human population and reduce significantly the current exposure limits in order to account for the reported non thermal biological effects.

Keywords: microwaves, non-ionizing electromagnetic radiation, electromagnetic fields, mobile telephony radiation, GSM, RF, ELF, biological effects, health effects, reproduction, DNA damage, cell death, intensity windows, SAR.

INTRODUCTION

Modern Telecommunication Microwave Radiations such as GSM and 3G (3rd generation) (Curwen and Whalley 2008) is probably the main source of public microwave exposure in our time. Billions of people globally are self-exposed daily by their own mobile phones, while at the same time they are also exposed by base station antennas which are installed within residential and working areas. While exposure from mobile phones is voluntary for every user for as long daily periods as each one decides, exposure from base station antennas -although weaker- is involuntary and constant for up to 24 h a day.

A large number of biological, clinical and epidemiological studies regarding the possible health and environmental implications of microwave exposure is already published (for a review see Panagopoulos and Margaritis 2008; 2009; 2010a). While many of these studies do not report any effect, many others are indicating serious biological, clinical and health effects such as DNA damage, cell death, reproductive decreases, sleep disturbances, electro-encephalogram (EEG) alterations, and cancer induction.

Some of the studies report DNA damage or cell death or oxidative stress induction on reproductive insect and mammalian (including human) cells (Panagopoulos et al 2007a; 2010; De Iuliis et al. 2009; Agarwal et al 2009; Mailankot et al 2009; Yan et al 2007). The findings of these studies seem to explain the results of other studies that simply report insect, bird, and mammalian (including human) infertility (Panagopoulos et al 2004; 2007b; Gul et al 2009; Agarwal et al 2008; Batellier et al 2008; Wdowiak et al 2007; Magras ans Xenos 1997). Other recent reports regarding reduction of insect (especially bees) and bird populations during the last years (Stindl and Stindl 2010; Bacandritsos et al 2010; van Engelsdorp et al 2008; Everaert and Bauwens 2007; Balmori 2005), also seem to correlate with the above mentioned studies since their findings may be explained by cell death induction on reproductive cells. Other studies report DNA damage or oxidative stress induction or increase in cellular damage features in somatic mammalian and insect cells after *in vitro* or *in vivo* exposure to microwaves, (Guler et al 2010; Tomruk et al 2010; Franzellitti et al 2010; Luukkonen et al 2009; Yao et al 2008; Yadav and Sharma 2008; Sokolovic et al 2008; Lee et al 2008; Lixia et

al 2006; Zhang et al 2006; Nikolova et al 2005; Belyaev et al 2005; Diem et al. 2005). At the same time, some other studies report brain tumour induction in humans, (Hardell et al 2009; 2007; Khurana et al 2009; Johansson 2009), or symptoms of unwellness among people residing around base station antennas (Hutter et al 2006; Salama et al 2004; Navarro et al 2003).

Despite many other studies that report no effects (see Panagopoulos and Margaritis 2008; 2009; 2010a), the consistency of the above findings and their rapidly increasing number during the last years is of great importance. All the above-mentioned recent studies from different research groups and on different biological models exhibit mutually supportive results and this makes unlikely the possibility that these results could be either wrong or due to random variations. While recent experimental findings tend to show a distinct similarity between them, the need for a biophysical and biochemical explanation on the basis of a realistic mechanism of action of EMFs at the cellular level, becomes more and more demanding.

Although until today there is still no widely accepted biophysical or biochemical mechanism to explain the above findings at cellular level, many recent findings tend to support the possibility that oxidative stress and free radical action may be responsible for the recorded genotoxic effects of EMFs which may lead to health implications and cancer induction. It is possible that free radical action and/or irregular release of hydrolytic enzymes like DNases, induced by exposure to EMFs, may constitute the biochemical action leading to DNA damage. This biochemical action may be initiated by alterations in intracellular ionic concentrations after irregular gating of electro-sensitive channels on cell membranes by external EMFs. Such irregular gating of ionic channels may represent the more fundamental biophysical mechanism to initiate the biochemical one, as previously supported by us (Panagopoulos et al 2000; 2002).

EFFECTS OF MODERN TELECOMMUNICATION MICROWAVES ON A MODEL BIOLOGICAL SYSTEM

After 12 years of experimentation on the biological effects of the pulsed microwave radiation used in modern mobile telecommunications, we shall attempt a summarizing presentation of the effects of the two mobile telephony radiation systems used in Europe, GSM 900 MHz and GSM 1800 MHz (named also DCS –Digital Cellular System), on a model biological system, the reproductive capacity of the insect *Drosophila melanogaster*.

The reproductive capacity of animals depends on their ability to successfully complete subtle biological functions, as is gametogenesis (oogenesis, spermatogenesis), fertilization, and embryogenesis, in spite of any disturbing exogenous (or endogenous) factors. In the experiments that will be presented here the exogenous disturbing factor is the Radio-Frequency (RF)/microwave radiation-fields used in modern mobile telecommunications.

Gametogenesis (oogenesis, spermatogenesis) in all animals is a biological process, much more sensitive to environmental stress than other developmental - biological processes that take place at later stages of animal development. This is shown with regard to ionizing radiations as stress factors, it is in agreement with the empirical law of Bergonie-Tribondau (Coggle 1983; Hall and Giaccia 2006) and it is verified also in relation to non-ionizing

radiation by several recent experimental results, including our own, presented in the following pages.

The reproductive capacity of *Drosophila melanogaster* (especially oogenesis) is a model biological system, very well-studied, with a very good timing of its developmental processes under certain laboratory conditions (King 1970; Panagopoulos et al 2004; Horne-Badovinac and Bilder 2005).

Following a well-tested protocol of ours, the reproductive capacity is defined by the number of F_1 (first filial generation) pupae, which under the conditions of our experiments corresponds to the number of laid eggs (oviposition), since there is no statistically significant mortality of fertilized eggs, larvae or pupae derived from newly eclosed adult flies during the first days of their maximum oviposition (Panagopoulos et al. 2004).

Basic Experimental Procedure

All sets of experiments were performed with the use of commercially available cellular mobile phones as exposure devices.

The exposures were performed with the mobile phone antenna outside of the glass vials containing the flies, in contact with, or at certain distances from the glass walls. The daily exposure duration was a few minutes (depending on the kind of experiments – see below), in one dose. The exposures always started on the first day (day of eclosion) of each experiment, and lasted for a total of five or six days.

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).

In each experiment, we collected newly emerged adult flies from the stock; we anesthetized them very lightly and separated males from females. We put the collected flies in groups of ten males and ten females in standard laboratory 50-ml cylindrical glass vials (tubes), with 2.5cm diameter and 10cm height, with standard food, which formed a smooth plane surface 1cm thick at the bottom of the vials. The glass vials were closed with cotton plugs.

In each group we kept the ten males and the ten females for the first 48h of the experiment in separate glass vials. Keeping males separately from females for the first 48h of the experiment ensures that the flies are in complete sexual maturity and ready for immediate mating and laying of fertilized eggs, (Panagopoulos et al. 2004).

After the first 48h of each experiment, the males and females of each group were put together (ten pairs) in another glass vial with fresh food, allowed to mate and lay eggs for 72h. During these three days, the daily egg production of *Drosophila* is at its maximum.

After five days from the beginning of each experiment the flies were removed from the glass vials and the vials were maintained in the culture room for at least six additional days, without any further exposure to the radiation. The removed maternal flies depending on each separate experimental series, could be collected and their ovaries were dissected and treated for different biochemical assays (see below).

After the last six days, most F_1 embryos (deriving from the laid eggs) are in the stage of pupation, where they can be clearly seen with bare eyes and easily counted on the walls of the glass tubes.

We have previously shown that this number of F_1 pupae, under the above-described conditions, is a representative estimate of the insect's reproductive capacity (Panagopoulos et al 2004).

Exposures and measurements of mobile phone emissions were performed at the same place within the lab, where the mobile phone had full reception of the GSM signals.

The results were analyzed by Single Factor Analysis of Variance (ANOVA) test.

1. Comparison of Biological Activity between Non-Modulated (DTX) and Modulated (Talk Signal) GSM Radiation

In the first series of experiments, (parts 1A and 1B) we separated the insects into two groups: a) the Exposed group (E) and b) the Sham Exposed (Control) group (SE). Each one of the two groups consisted of ten female and ten male, newly emerged adult flies. The sham exposed groups had identical treatment as the exposed ones, except that the mobile phone during the "exposures" was turned off.

The total duration of exposure was 6 min per day in one dose and we started the exposures on the first day of each experiment (day of eclosion). The exposures took place for a total of 5 days.

In the first part of these experiments (1A) the insects were exposed to Non-Modulated GSM 900 MHz radiation (TDX -discontinuous transmission mode-signal) while in the second part (1B) they were exposed to Modulated GSM 900 MHz radiation (or "GSM talk signal"). In both cases, the exposures were performed with the antenna of the mobile phone in contact with the walls of the glass vials containing the insects.

The difference between the Modulated and the corresponding Non-Modulated GSM radiation is that, the intensity of the Modulated radiation is about ten times higher than the intensity of the corresponding Non-Modulated from the same handset (mobile phone) and additionally that, the Modulated radiation includes more and larger variations in its intensity within the same time interval, than the corresponding Non-Modulated one (Panagopoulos et al. 2004; Panagopoulos and Margaritis 2008).

The mean power density for 6 min of Modulated emission, with the antenna of the mobile phone outside of the glass vial in contact with the glass wall and parallel to the vial's axis, was 0.436±0.060 mW/cm^2 and the corresponding mean value for Non-Modulated (NM) emission, 0.041±0.006 mW/cm^2. The measured Extremely Low Frequency (ELF) mean values of electric field intensity of the GSM signals excluding the ambient fields of 50Hz, were 6.05±1.62 V/m for the Modulated signal, and 3.18±1.10 V/m for the Non-Modulated signal. These values are averages from eight separate measurements of each kind ± Standard Deviation (SD).

1A. Experiments with Non-Modulated GSM 900 MHz radiation ("non-speaking" emission or DTX-signal), showed that this radiation decreases insect reproduction by an average of 18.24 %, after 6 min daily exposure for 5 consecutive days (Table 1).

The exposure conditions in these experiments simulate the potential biological impact on a mobile phone user who listens through the mobile phone during a conversation, with the handset close to his/her head.

The average mean numbers of F_1 pupae from 4 identical separate experiments (corresponding to the number of laid eggs) per maternal fly in the groups E(NM) exposed to Non-Modulated (NM) GSM radiation-field, and in the corresponding sham exposed (control) groups SE(NM) during the first three days of the insect's maximum oviposition, are shown in the first two rows of Table 1.

Single factor Analysis-of-Variance (ANOVA) test, showed that the probability that differences between the groups exposed to non-modulated GSM radiation and the sham exposed groups, owing to random variations, is $P < 5\times10^{-4}$, meaning that, the decrease in the reproductive capacity is actually due to the effect of the GSM field. [A detailed description of these experiments can be found in Panagopoulos et al. 2004].

1B. Experiments with Modulated GSM 900 MHz radiation ("speaking" emission or "GSM Talk signal") exposure, showed that this radiation decreases insect reproduction by an average of 53.01 %, after 6 min daily exposure for 5 consecutive days (Table 1).

The experimenter spoke close to the mobile phone's mic during the exposures. The exposure conditions in this case simulate the potential biological impact when a user speaks on the mobile phone during a conversation, with the handset close to his/her head.

Table 1. Effect of Non-Modulated (DTX) and Modulated (Talk mode) GSM Radiation on the Reproductive Capacity of Drosophila melanogaster

Type of GSM 900 MHz Radiation	Groups	Average Mean Number of F_1 Pupae per Maternal Fly in four separate experiments ± SD	Deviation from the corresponding Sham-Exposed Groups	Probability that Differences between Exposed and corresponding Sham-Exposed Groups are due to random variations
NM or DTX-signal	E(NM)	9.97 ± 0.31	-18.24%	$P < 5\times10^{-4}$
	SE(NM)	12.2 ± 0.57		
M or Talk-signal	E(M)	5.85 ± 0.67	-53.01%	$P < 10^{-5}$
	SE(M)	12.45 ± 0.6		

The last two rows of Table 1 show the average mean number of F_1 pupae from 4 identical separate experiments (corresponding to the number of laid eggs) per maternal fly in the groups E(M), exposed to "Modulated" (M) GSM radiation- field and in the corresponding sham-exposed groups, SE(M), during the first three days of the insect's maximum oviposition.

The statistical analysis showed that the probability that mean oviposition differs between the groups exposed to modulated GSM radiation and the corresponding sham-exposed groups, owing to random variations, is very small, $P < 10^{-5}$. Thus the recorded effect is actually due to the GSM signal.

Although the intensity of the modulated signal is about ten times higher than the corresponding intensity of the non-modulated RF signal, the reproductive capacity was decreased by 53.01 % by the modulated emission, and 18.24 % by the non-modulated one. Thus the effect seems to be strongly, but non-linearly, dependent on the radiation intensity.

The results from the first set of experiments (parts 1A and 1B) are represented graphically, in Figure 1.

Temperature increases were not detected within the vials during the 6 min exposures with either DTX or "Talk signal". Therefore the described effects are considered as non-thermal.

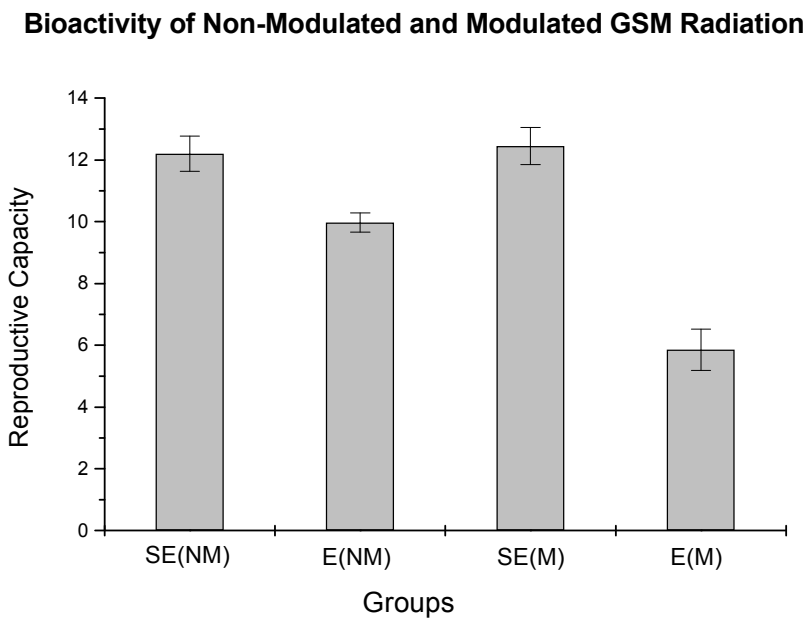

Figure 1. Reproductive Capacity (average mean number of F_1 pupae per maternal insect) ± SD of the insect groups exposed to non-modulated and modulated GSM 900 MHz radiation [E(NM), E(M)] and the corresponding sham-exposed, [SE(NM), SE(M)], groups.

2. Effect of GSM Radiation on Males and Females

In this set of experiments, we investigated the effect of GSM 900 MHz field on the reproductive capacity of each sex. The mobile phone was operating in speaking mode during the 6 min exposures, and the insects were separated into four groups (each one consisting again of 10 male and 10 female insects): In the first group (E1) both male and female insects were exposed. In the second group (E2) only the females were exposed. In the third group (E3) only the males were exposed and the fourth group (SE) was sham-exposed (control). Therefore in this set of experiments, the 6-min daily exposures took place only during the first two days of each experiment while the males and females of each group were separated and the total number of exposures in each experiment was 2 instead of 5. The exposures were again performed with the antenna of the mobile phone in contact with the glass vials containing the insects.

The average mean number of F_1 pupae per maternal fly of each group, in four separate identical experiments, are given in Table 2 and represented graphically in Figure 2.

Table 2. Effect of GSM Radiation on the Reproductive Capacity of Males and Females

Groups	Average Mean Number of F_1 Pupae per Maternal Fly ± SD	Deviation from Control
E1	7.7 ± 0.66	-42.32%
E2	8.85 ± 0.73	-33.71%
E3	11.75 ± 0.54	-11.985%
SE (Control)	13.35 ± 0.39	

The statistical analysis (single factor Analysis of Variance test) shows that the probability that the mean number of F_1 pupae differs between the four groups because of random variations is, $P < 10^{-7}$.

These results show that the GSM radiation-field decreases the reproductive capacity of both female and male insects. The reason why female insects (E2) appear to be more affected than males (E3), is probably that, by the time we started the exposures, spermatogenesis was already almost completed in male flies, while oogenesis had just started (King 1970; Panagopoulos et al. 2004). Therefore it should be expected that the GSM exposure would affect oogenesis more than spermatogenesis and the decrease in reproductive capacity would be more evident in the female than in the male insects.

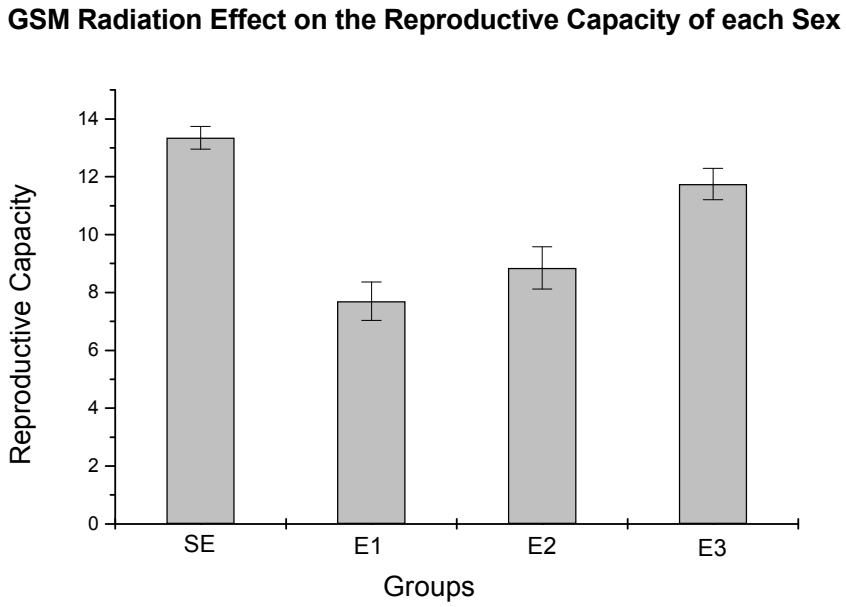

Figure 2. Effect of GSM radiation on the reproductive capacity of each sex of *Drosophila melanogaster*. Average mean number of F_1 pupae per maternal insect ± SD. SE: sham exposed groups, E1: groups in which both sexes were exposed, E2: groups in which only the females were exposed, E3: groups in which only the males were exposed.

3. Comparison of Bioactivity Between GSM 900 MHz and GSM 1800 MHz

GSM 900 MHz antennas of both handsets and base stations operate at double the power output than the corresponding GSM 1800 MHz antennas. [As mentioned before, GSM 1800 MHz radiation is also referred to as DCS]. Additionally, the two systems use different carrier frequencies (900 or 1800 MHz respectively). Therefore, a comparison of the biological activity between the two European systems of Mobile Telephony radiation is of great importance. [GSM 1900 MHz system operating in the USA, is similar to GSM 1800 MHz, except for the 100 MHz difference of their carrier frequencies].

In this and the next series of experiments, we used a dual band cellular mobile phone that could be connected to either GSM 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, was 0.89 W/Kg. The exposure procedure was the same. The handset was fully charged before each set of exposures. The experimenter spoke on the mobile phone's microphone during the exposures, thus, the GSM 900 and 1800 fields were "modulated" by the human voice, ("speaking emissions" or "GSM talk signals").

The exposures and the measurements of the mobile phone emissions were always performed at the same place within the lab, where the mobile phone had full reception of both GSM 900 and 1800 signals.

The measured mean power densities in contact with the mobile phone antenna for six min of modulated emission, were 0.407 ± 0.061 mW/cm^2 for GSM 900 MHz and 0.283 ± 0.043 mW/cm^2 for GSM 1800 MHz. As expected, GSM 900 MHz intensity at the same distance from the antenna and with the same handset was higher than the corresponding 1800 MHz. For a better comparison between the two systems of radiation we measured the GSM power density at different distances from the antenna and found that at 1cm distance, the GSM 900 MHz intensity was 0.286± 0.050 mW/cm^2, almost equal to GSM 1800 MHz at zero distance. Measured electric and magnetic field intensities in the ELF range for the modulated field, excluding the ambient electric and magnetic fields of 50Hz, were 22.3±2.2 V/m electric field intensity and 0.50±0.08 mG magnetic field intensity for GSM 900 at zero distance, 13.9±1.6 V/m, 0.40±0.07 mG correspondingly for GSM 900 at 1 cm distance and 14.2 ±1.7 V/m, 0.38±0.07 mG correspondingly for GSM 1800 at zero distance. All these values are averaged over ten separate measurements of each kind ± standard deviation (SD).

Each type of radiation gives a unique frequency spectrum. While GSM 900 MHz gives a single peak around 900 MHz, GSM 1800 MHz gives a main peak around 1800 MHz and a smaller one around 900 MHz, (Panagopoulos et al. 2007b).

In this set of experiments 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 "900"), b) the group exposed to GSM 900 MHz field with the antenna of the mobile phone at 1cm distance from the vial (named "900A"), c) the group exposed to GSM 1800 MHz field with the mobile phone antenna in contact with the glass vial (named "1800"), and d) the sham-exposed (Control) group (named "SE"). The comparison between first and third groups represents comparison of potential biological impact between GSM 900 and GSM 1800 users under the actual exposure conditions. Comparison between

the first and the second groups represents comparison of bioactivity between signals of different intensity but of the same carrier frequency, and finally, comparison between the second and third groups represents comparison of bioactivity between the RF carrier frequencies of the two systems under equal radiation intensities. Therefore the introduction of the second group (900A) contributes significantly to the better comparison of the effects between the two types of radiation.

The average mean numbers of F_1 pupae in ten replicate experiments for the different groups, are given in Table 3 and represented graphically, in Figure 3.

Table 3. Effect of GSM 900 and GSM 1800 fields on the Reproductive Capacity of Drosophila melanogaster

Groups	Average Mean Number of F_1 Pupae per Maternal Fly in ten replicate experiments ± SD	Deviation from Control
900	6.51 ± 0.67	-48.25%
900A	8.46 ± 0.55	-32.75%
1800	8.67 ± 0.65	-31.08%
SE (Control)	12.58 ± 0.95	

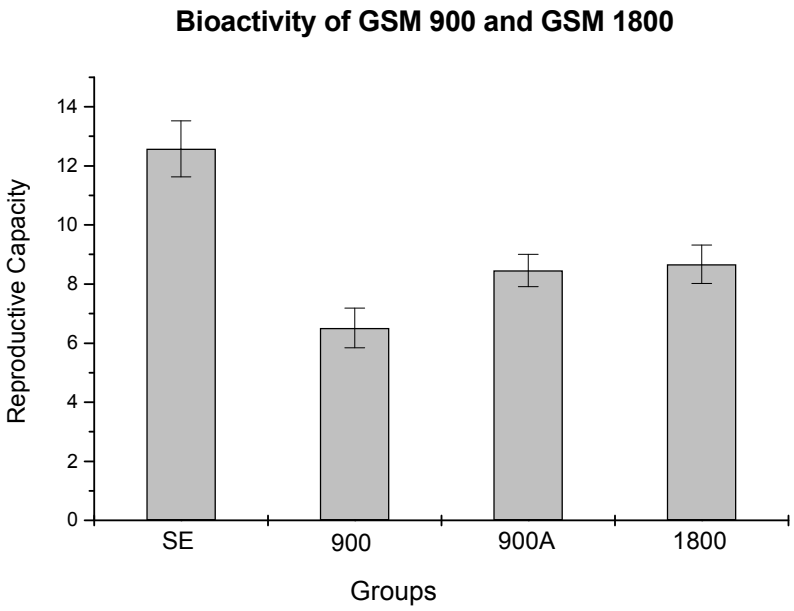

Figure 3. Reproductive Capacity (average mean number of F_1 pupae per maternal insect) ± SD of insect groups exposed to GSM 900 and GSM 1800 radiations (900, 900A, 1800) and sham-exposed (SE) groups

The results from this set of experiments show that the reproductive capacity in all the exposed groups is significantly decreased compared to the sham-exposed. The average decrease in ten replicate experiments was found to be maximum in the 900 groups (48.25%

compared to SE) and smaller in the 900A and the 1800 groups (32.75% and 31.08% respectively), (Table 3). Although the decrease was even smaller in the 1800 than in 900A groups, differences between the 900A and 1800 groups were found to be within the standard deviation, (Table 3, Figure 3).

The statistical analysis showed that the probability that the reproductive capacity differs between the four groups, owing to random variations, is negligible, $P < 10^{-18}$.

Temperature increases were again not detected within the glass vials during the exposures.

The differences in the reproductive capacity between the groups were larger between 900 and 900A (owing to intensity differences between the two types of radiation) and much smaller between 900A and 1800, (owing to the frequency difference of the carrier signal between GSM 900 and 1800), (Table 3).

This set of experiments showed that there is a difference in the bioactivity between GSM 900 MHz and GSM 1800 MHz. The GSM 900 signal is more bioactive than the corresponding GSM 1800 signal under equal other conditions and the difference is mostly due to the higher intensity of GSM 900 under the same exposure conditions, (differences between groups 900 and 900A) and less due to the different RF carrier frequencies, (differences between 900A and 1800 groups).

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 900 usually has a higher intensity than GSM 1800, this situation can be reversed in certain places where GSM 900 has a better signal reception between its antennas than GSM 1800. This is because when GSM antennas of both systems cannot easily communicate between base station and mobile phone, they emit stronger signals in order to achieve communication. Our results count for equal signal reception conditions between the two types of radiation.

A detailed description of these experiments can be found in Panagopoulos et al. 2007b.

4. GSM Bioactivity According to its Intensity (or According to the Distance from the Antenna). The Revelation of a "Window" of Increased Bioactivity

Until the recent publication of this set of experiments (Panagopoulos et al 2010; Panagopoulos and Margaritis 2008; 2009), no other experiments were reported 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, with the exception of statistical observations which had reported a reduction of bird populations around base station antennas (Everaert and Bauwens 2007; Balmori 2005).

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 slightly higher carrier frequency. GSM 900 mobile phones emit between 890 MHz and 915 MHz (uplink operation) while base stations emit between 935 MHz and 960 MHz (downlink operation). The corresponding GSM 1800 spectrums are 1710-1785 MHz (uplink operation) and 1805-1880 MHz (downlink operation). Another difference is that, although the time-averaged emitted power is significantly higher in base station antennas than in the mobile

phones, the ratio of pulse peak power versus time-averaged power is higher in the mobile phones (Hillebrand 2002; Clark 2001; Hyland 2000; Hamnerius and Uddmar 2000; Tisal 1998; Panagopoulos and Margaritis 2008). Still, the two kinds of radiation are very similar and effects produced by mobile phones at certain distances, can be extrapolated to represent effects from base station antennas, of the same type of radiation, at about 100 times longer distances. Thus, distances from mobile phone antennas can be corresponded to about 100 times longer distances from base station antennas of the same type of radiation. For example, when our distance from a mobile phone during connection is 2 m, (e.g. someone talking on the mobile phone at 2 m distance from us), then we are exposed almost equally as by a corresponding base station antenna at 200 m distance. Correspondingly, if a mobile telephony base station antenna is installed at 200 m from our place of residence, this is almost the same as when we are exposed by a mobile phone operating in talk mode 24 h a day at 2 m distance from us.

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. The only way to simulate the reality of the exposure by a base station antenna was to expose the animals at different distances from a mobile phone within the lab.

In order to study the bioactivity of mobile telephony signals at different intensities-distances from the antenna of a mobile phone handset, resembling effects of base station signals within residential areas, we used the same biological index, the reproductive capacity of the insect *Drosophila melanogaster*, defined by the number of F_1 pupae derived during the three days of the insect's maximum oviposition.

In each experiment of this set, 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 1cm 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 twelfth group (named "100") was exposed to GSM 900 or 1800 field at 100 cm distance from the mobile phone antenna. Finally, the thirteenth group (named "SE") was the sham exposed. Each group consisted of ten male and ten female insects as always.

Radiation and field measurements in contact and at different distances from the mobile phone antenna, for 6 min of modulated emission, for GSM 900 MHz and 1800 MHz in the RF and ELF ranges excluding the background electric and magnetic fields of 50 Hz, are given in Table 4. All values are averaged over ten separate measurements of each kind ± standard deviation (SD). The measurements reveal that although the ELF electric and magnetic fields, associated with the GSM signals, fall within the background of the 50 Hz electric/magnetic residential fields for distances longer than 50 cm from both GSM 900 and GSM 1800 mobile phone antennas, and for this cannot be detected, the RF components of the signals are still evident for distances up to 100 cm (Table 4).

It is important to clarify that, the fact that the ELF components of the GSM signals fall within the background levels, does not mean that they do not exist. On the contrary, they may still be bioactive even though they cannot be easily detected.

Table 4. GSM 900 and 1800 Intensities ± SD, in the Microwave and ELF regions, for different Distances from a mobile phone Antenna [(1)]

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 ±0.059	19 ±2.5	0.9 ±0.15	0.252 ±0.050	13 ±2.1	0.6 ±0.08
1	0.262 ±0.046	12 ±1.7	0.7 ±0.13	0.065 ±0.015	6 ±0.8	0. 4 ±0.07
10	0.062 ±0.020	7 ±0.8	0.3 ±0.05	0.029 ±0.005	2.7 ±0.5	0. 2 ±0.05
20	0.032 ±0.008	2.8±0.4	0.2 ±0.04	0.011 ±0.003	0.6 ±0.12	0. 1±0.02
30	0.010 ±0.002	0.7 ±0.09	0.1 ±0.02	0.007 ±0.001	0.3 ±0.06	0.06 ±0.01
40	0.006 ±0.001	0.2 ±0.03	0.05 ±0.01	0.004 ±0.0007	0.1 ±0.04	-
50	0.004 ±0.0006	0.1 ±0.02	-	0.002 ±0.0003	-	-
60	0.002 ±0.0003	-	-	0.0016 ±0.0002	-	-
70	0.0017 ±0.0002	-	-	0.0013 ±0.0002	-	-
80	0.0012 ±0.0002	-	-	0.0011 ±0.0002	-	-
90	0.0010 ±0.0001	-	-	0.0005 ±0.0001	-	-
100	0.0004 ±0.0001	-	-	0.0002 ±0.0001	-	-

[(1)] 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.

In each experiment all the 12 exposed groups were simultaneously exposed during the 6 min exposure sessions. 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. It was already verified by preliminary experiments, that SE groups at all the 12 different locations of exposure, did not differ significantly between them in their reproductive capacity.

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 1800 exposures are listed in Table 5 and represented graphically in Figures 4 and 5.

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 μW/cm^2 down to 1 μW/cm^2 – Tables 4, 5). Table 5 and Fig 4 show that the effect is at a maximum at 0 cm and at 30 cm from the antenna, (corresponding to radiation intensities of 378 μW/cm^2 and 10 μW/cm^2 respectively) with 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 μW/cm^2).

The data also show that GSM 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 μW/cm^2 down to 1.1 μW/cm^2 – Tables 4, 5). Table 5 and Fig. 5 show that the effect is maximum at 0 cm and at 20 cm from the antenna, (corresponding to radiation intensities of 252 μW/cm^2 and 11 μW/cm^2 respectively) with overall 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 μW/cm^2).

Table 5. Effect of GSM 900 and 1800 radiation-fields on 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± SD, for GSM 900 MHz	Deviation from Sham Exposed Group	Average Mean Number of F_1 Pupae per Maternal Fly± SD, for GSM 1800 MHz	Deviation from Sham Exposed Group
0	7.46 ± 0.73	-46.14 %	9.10 ± 0.69	-35.09 %
1	9.35 ± 0.62	-32.49 %	11.35 ± 0.63	-19.04 %
10	11.28 ± 0.81	-18.56 %	11.93 ± 0.72	-14.91 %
20	11.55 ± 0.79	-16.61 %	8.33 ± 0.7	-40.58 %
30	7.38 ± 0.65	-46.71 %	12.77 ± 0.82	-8.92 %
40	12.81 ± 0.97	-7.51 %	13.52 ± 0.86	-3.57 %
50	13.49 ± 0.82	-2.60 %	13.72 ± 0.75	-2.14 %
60	13.62 ± 0.83	-1.66 %	13.81 ± 0.92	-1.50 %
70	13.72 ± 0.92	-0.94 %	13.79 ± 0.90	-1.64 %
80	13.68 ± 0.80	-1.23 %	13.85 ± 0.81	-1.21 %
90	13.75± 0.95	-0.72 %	14.03 ± 1.02	+0.07 %
100	14.01 ± 1.01	+1.16 %	14.05 ± 0.99	+0.21 %
SE	13.85 ± 0.91		14.02 ± 0.98	

Thus, the effect of mobile telephony microwave radiation on reproductive capacity is at a maximum at zero distance (intensities higher than 250 μW/cm^2) and then becomes maximum at a distance of 30 cm or 20 cm from the mobile phone antenna for GSM 900 or 1800 respectively. These distances correspond to the same RF intensity of about 10 μW/cm^2 and also to the same ELF electric field intensity of about 0.6-0.7 V/m (Table 4).

Again, there were no temperature increases within the vials during the exposures at all the different distances from the mobile phone handset.

The effect diminishes considerably for distances longer than 50 cm from the mobile phone antenna, and disappears for distances longer than 80-90 cm corresponding to radiation intensities smaller than 1 μW/cm^2. For distances longer than 50 cm where the ELF components fall within the background, the decrease in reproductive capacity is within the standard deviation. This might suggest that the ELF components of digital mobile telephony signals, play a key role in their bio-activity, alone or in conjunction with the RF carrier signal.

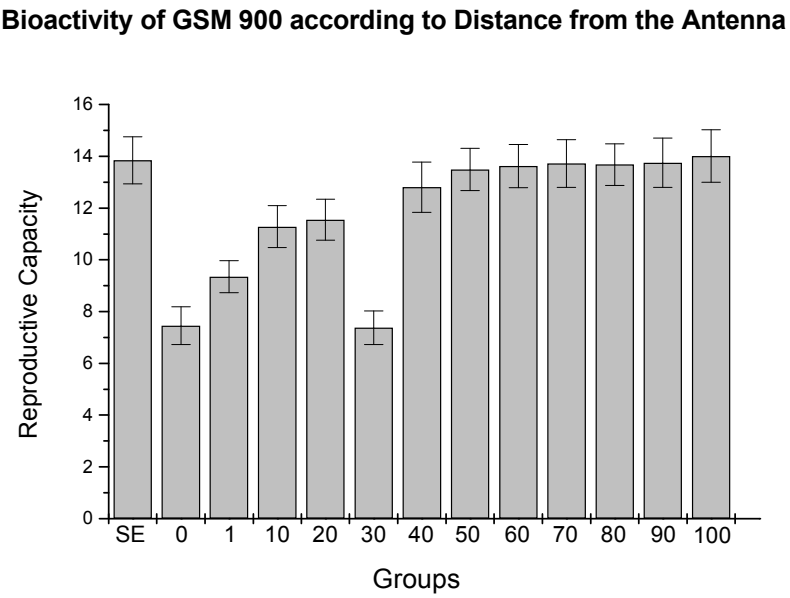

Figure 4. Reproductive Capacity (average mean number of F_1 pupae per maternal insect) ± SD in relation to the Distance from a GSM 900 MHz mobile phone antenna. The decrease in reproductive capacity is maximum at zero distance and at 30 cm distance from the antenna ("window" of increased bioactivity), corresponding to RF intensities 378μW/cm^2 and 10μW/cm^2, (Tables 4, 5).

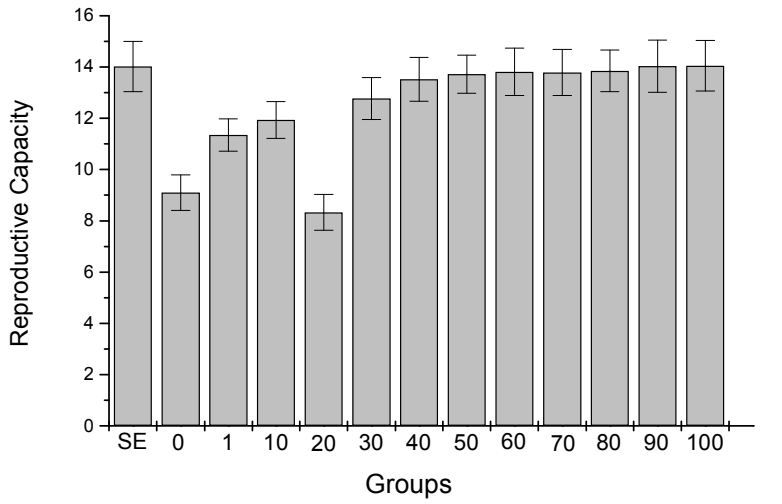

Figure 5. Reproductive Capacity (average mean number of F_1 pupae per maternal insect) ± SD in relation to the Distance from a GSM 1800 MHz mobile phone antenna. The decrease in reproductive capacity is maximum at zero distance and at 20 cm distance from the antenna ("window" of increased bioactivity), corresponding to RF intensities 252 μW/cm^2 and 11 μW/cm^2, (Tables 4, 5).

The results on reproductive capacity were analyzed statistically by single factor Analysis of Variance. In addition, linear (Pearson's) and non-parametric (Kendall's) correlation analysis were performed between reproductive capacity and radiation/field intensities in order to get an estimation of which parameter (the RF radiation, or the ELF fields) might be more responsible for the effects, (Weiss 1995; Maber 1999).

Single factor Analysis of Variance test, showed that the probability that the reproductive capacity differs between all groups, owing to random variations, is negligible both for GSM 900 and 1800 exposures, $P < 10^{-27}$ in both cases. 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 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 on RF and ELF intensities is non-linear, (Fig. 4, 5), 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 1800), than between reproductive capacity and ELF electric field intensity $r \cong -0.79$, $P = 0.001$ and $r \cong -0.78$, $P = 0.001$ respectively), 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 between reproductive capacity and ELF magnetic field was found to be even weaker than between reproductive capacity and ELF electric field.

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 2010b), where the intensity of the radiation is maximum, but also within the far field, at 20-30 cm distance from the mobile phone antenna, where the intensity is significantly decreased.

Thus, we discovered the existence of increased bioactivity "windows" for both GSM 900 and 1800 radiations. These "bioactivity windows" appear at distances 20 cm and 30 cm from the GSM 1800 and 900 mobile phone antennas respectively, where the radiation intensity is in both cases close to 10 $\mu W/cm^2$ and the ELF electric field intensity 0.6 – 0.7 V/m. At these distances, the bio-effect becomes even more intense than at zero distance from a mobile phone antenna where the RF intensity is higher than 250 $\mu W/cm^2$, and the ELF electric filed intensity higher than 13 V/m (Table 4).

The distance of 20-30 cm from the mobile phone antenna, at which the windows of increased bioactivity appear, corresponds to a distance of about 20-30 meters from a base station antenna. Since mobile telephony base station antennas are most usually located within residential areas, at distances 20-30 m from such antennas there are often houses and work places where people are exposed for up to 24 hours per day. Therefore the existence of these

"windows" may pose an increased danger for people who reside or work at such distances from mobile telephony base station antennas. 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.

From the results of these experiments, it became evident that another series of experiments was 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 – 17 cm approximately for 1800 MHz and 33 cm approximately for 900 MHz), (Panagopoulos and Margaritis 2010b).

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 GSM signals are never constant in intensity or frequency. Therefore, experiments with idealized continuous signals corresponding to the RF carrier alone or to the ELF constituents alone, do not represent real conditions.

The fact that for distances longer than 50 cm where the ELF components fall within the background, the bioactivity of the radiation, although still evident, decreasing considerably and falling 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 bio-activity, alone or in combination with the RF carrier wave. This is in agreement with the mechanism that we have proposed for the action of EMFs on living organisms, according to which, lower frequency fields are predicted to be more bioactive than higher frequency ones,. 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 4, the ELF components of both GSM 900 and 1800 fields appear to possess sufficient intensity for this, for distances at least up to 50 cm from the antenna of a mobile phone (or about 50 m from a corresponding base station antenna), where the ELF components of the GSM signals can be detected and do not fall within the background residential fields.

Non-parametric correlation analysis showed a slightly 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 could not be excluded. These possibilities are investigated and discussed in the following series of experiments together with the nature of the observed bioactivity "windows", (also in Panagopoulos and Margaritis 2010b).

The present set of experiments (a more detailed description can be found in Panagopoulos et al. 2010) showed that, the bioactivity of GSM radiation in regards to short-term exposures is evident for radiation intensities down to 1 μW/cm^2. This value of radiation intensity is encountered at about 1 m distance from a cell-phone or about 100 m distance from a corresponding base station antenna. This radiation intensity is 450 times and 900 times lower than the ICNIRP limits for 900 and 1800 MHz respectively, (ICNIRP, 1998).

It is possible for long-term exposure durations (weeks-months-years) that the effect would be evident at even longer distances/smaller intensities. For this, a safety factor of at least 10 should be introduced in the above value. By introducing a safety factor of 10, the above value becomes 0.1 μW/cm^2, which should be a reasonable limit for public exposure according to the described findings.

The bioactivity "windows" found in our 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).

Although windows of increased bioactivity of RF radiations have been recorded for many years, (Bawin et al 1975; 1978; Blackman et al, 1980; 1989), there is still no widely accepted explanation for their existence. A novel explanation for the "window" effects is given later on in this chapter.

5. The Discovered "Window" of Increased Bioactivity is an Intensity Window

The increased bioactivity "windows" of GSM 900 and 1800 MHz radiations, revealed in the previous experiments, manifesting themselves as a maximum decrease in the reproductive capacity of the insect *Drosophila melanogaster*, were examined in this series of experiments, in order to find out whether they depend on the intensity of the radiation-fields, or to any other possible factor related to the distance from the antenna.

In these experiments, one group of insects (consisting again of ten male and ten female newly eclosed adult flies) was exposed to the GSM 900 or 1800 radiation at 30 cm or 20 cm distances respectively from the antenna of a mobile phone, where the bioactivity "window" appears for each type of radiation and another group was exposed at 8 cm or 5 cm respectively, behind a metal grid, shielding both microwave radiation and the ELF electric and magnetic fields for both types of radiation in a way that radiation and field intensities were roughly equal between the two groups. Then the effect on reproductive capacity was compared between the two groups for each type of radiation.

The average mean values of reproductive capacity (number of F_1 pupae per maternal fly) ± SD from five identical experiments with each type of radiation are shown in Table 6 and represented in Figure 6.

The results show that the reproductive capacity between the two exposed groups did not differ significantly for both types of radiation, (P >0.97 in both cases, meaning that differences between the two exposed groups have more than 97% probability to be due to random variations, according to the statistical analysis). In contrast, the reproductive capacity of each exposed group was significantly decreased compared to the sham exposed group as expected, for both types of radiation, ($P<10^{-5}$ in all cases).

Table 6. Effect of GSM 900 and 1800 radiation-fields on the Reproductive Capacity of Groups Exposed at "Window" Intensity and Sham Exposed Groups

Groups	AverageMean Number of F_1 Pupae per Maternal Fly ± SD, for GSM 900 MHz, in five replicate experiments	Deviation from Sham Exposed Group	AverageMean Number of F_1 Pupae per Maternal Fly ± SD, for GSM 1800 MHz, in five replicate experiments	Deviation from Sham Exposed Group
E1	7.86 ± 0.95	-42.63 %	8.38 ± 0.93	-38.56 %
E2	7.84 ± 0.65	-42.77 %	8.36 ± 0.77	-38.71 %
SE	13.7 ± 0.70		13.64 ± 0.65	

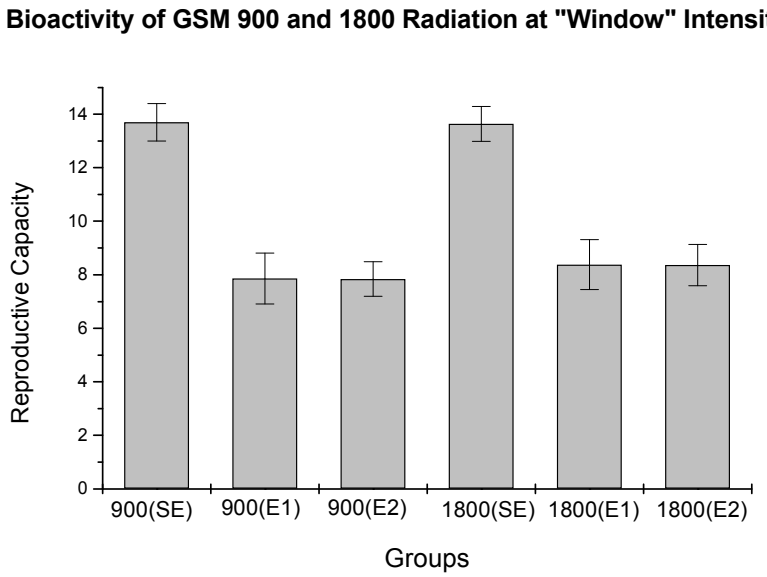

Figure 6. Reproductive Capacity (average mean number of F_1 pupae per maternal insect) ± SD of exposed and sham exposed insect groups to GSM 900 MHz and 1800 MHz radiation at "Window" Intensity (10 μW/cm^2). The decrease in reproductive capacity of the exposed groups E1 and E2 for both types of radiation is significant in relation to the sham exposed groups but there is no significant difference between them.

Therefore, since the two exposed groups do not differ significantly between them, although they were exposed at different distances from the antenna but under the same radiation-field intensities, the discovered window of increased bioactivity depends on the intensity of the radiation-field (10 μW/cm^2, 0.6-0.7 V/m) at 30 cm or 20 cm from the GSM 900 or 1800 mobile phone antenna respectively and not to any other factor that could possibly be related with the certain distances from the antenna. Thus, the increased bioactivity window of digital mobile telephony radiation found in the previous set of experiments is actually an Intensity Window around the value of 10 μW/cm^2 in regards to the RF intensity, (or around the values of 0.6-0.7 V/m and 0.10-0.12 mG in regards to the ELF electric or magnetic field intensities respectively, or to any combination of the three of them). Within this "window" the

bioactivity of mobile telephony radiation becomes even stronger than at intensities higher than 250 μW/cm^2, (or higher than 13 V/m and 0.6 mG respectively). Under normal conditions and without obstacles between the antenna and the exposed object, the intensity around 10 μW/cm^2 where the window appears is encountered at a distance of approximately 30 cm from a GSM 900 or 20 cm from a GSM 1800 mobile phone antenna, which corresponds to a distance of about 30 or 20 meters respectively from a corresponding base station antenna, since, as explained, base station antennas emit the same kind of radiation at about 100 times higher power than the corresponding mobile phones, (Panagopoulos et al 2010; Panagopoulos and Margaritis 2008; Hyland 2000).

Therefore, we have shown that the discovered window is only indirectly related to the distance from the antenna, and thus, it does not seem to be related with the wavelength (or the frequency) of the radiation. This window is directly dependent on the intensity of the radiation/field, no matter on what distance from the antenna this intensity is encountered.

A more detailed description of these experiments can be found in (Panagopoulos and Margaritis 2010b).

6. The Decrease in Reproductive Capacity is Due to DNA Damage and Cell Death Induction in the Reproductive Cells

To determine the ability of GSM 900 and 1800 MHz radiation to act as possible genotoxic factors able to induce DNA damage and/or cell death, at different intensities (or at different distances from the mobile phone antenna), we used TUNEL (Terminal deoxynucleotide transferase dUTP Nick End Labeling) assay.

This is a widely used method for identifying DNA fragmentation and cell death. By the use of this method, a fluorescent substance, 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 visualized by fluorescence microscopy, (Gavrieli et al, 1992).

The DNA fragmentation test with the use of TUNEL assay, was applied in the ovaries of the exposed and sham-exposed female insects, and especially, in the developing eggs at the stages of early and mid oogenesis, when no programmed cell death takes place, as explained below.

Each *Drosophila* ovary consists of 16 to 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). Physiological apoptosis (programmed cell death) takes place normally, in the nurse and follicle cells of developing egg chambers during the last stages (11-14) of oogenesis (choriogenesis), (Nezis et al. 2000; 2002; McCall 2004). Additionally, in cases that certain egg chambers do not develop normally, the organism itself destroys them by induction of apoptosis at either one of the two above developmental stages (germarium or stage 7-8) which are called for this reason, “check points”. This stress-induced apoptosis, is a vital process in gametogenesis and reproduction by which the organism prevents the waste of precious

nutrients. Previously known external stress factors like chemical stress, heat shock, or poor nutrition, are able to induce cell death during early and mid oogenesis, exclusively in the nurse and the follicle cells of abnormally developing egg chambers, and exclusively at the two check points, (Drummond-Barbosa and Spradling 2001; McCall 2004; Nezis et al 2000; Panagopoulos et al. 2007a). No distinction between the two check points was found before, in regards to which one is more sensitive than the other.

Electromagnetic stress from mobile telephony microwave radiations was found in earlier experiments of ours to be extremely bioactive, inducing DNA damage and cell death to a high degree during early and mid oogenesis, not only to the above "check points" (germarium and stages 7-8) but to all the developmental stages from germarium up to stage 10, and moreover to all types of egg chamber cells, i.e. nurse cells, follicle cells and the oocyte (OC), (Panagopoulos et al, 2007a).

Wild-type strain Oregon R *Drosophila melanogaster* flies were cultured according to standard methods and kept in glass vials with standard food like in the previous series of experiments, (Panagopoulos et al, 2004; 2007a; 2010).

In each single experiment of this series, we collected newly eclosed adult flies from the stock early in the afternoon, and separated them into thirteen groups exactly as in the experimental set No 4 (see above), following our standard methodology, (Panagopoulos et al, 2004). We applied TUNEL assay in the ovaries of female insects exposed at different distances and sham-exposed (for details see, Panagopoulos et al. 2007a; 2010), in order to investigate possible DNA damage at different distances from the mobile phone (or respectively for different intensities of GSM 900 and 1800 radiations).

The total duration of exposure was again, 6 min per day in one dose and the exposures were started on the first day of each experiment. All the 12 exposed groups were simultaneously exposed at the same various distances from the mobile phone as in the experiments No 4 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 their ovaries were extracted to be prepared for the TUNEL assay as follows:

TUNEL Assay

The ovaries were dissected in Ringer's solution and separated into individual ovarioles from which we excluded all the egg chambers of stages 11-14. As we have already explained, in the egg chambers of stages 11-14 programmed cell death takes place normally in the nurse cells and follicle cells. For this, 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 30min and then rinsed three times and washed twice in PBS for 5 min each. Then samples were incubated with PBS containing 20 μg/ml proteinase K for 10 minutes 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 3h at 37°C in the dark. Samples were then washed six times in PBS for 1h 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 was made by single factor Analysis of Variance test.

Table 7. Effect of GSM 900 and 1800 on ovarian DNA Fragmentation at different Distances from the mobile phone Antenna

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

In Table 7, the summarised data on cell death induction in the gonads of the female insects during early and mid oogenesis from three separate experiments are listed. These data are represented graphically in Figures 7 and 8. 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 (Tables 5, 7, Fig. 4, 5, 7, 8), as in earlier experiments of ours, (Panagopoulos et al 2007a). 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 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 (Tables 5, 7, Fig. 4, 5, 7, 8).

The effect of cell death induction in the developing eggs of the exposed female insects, just like the corresponding effect on the reproductive capacity, was very intense for distances up to 30 cm from the mobile phone antenna, then diminished considerably for distances longer than 40-50 cm from the mobile phone antenna where the ELF components decrease significantly, but it was still evident for distances up to 100 cm (radiation intensities down to 1 μW/cm^2).

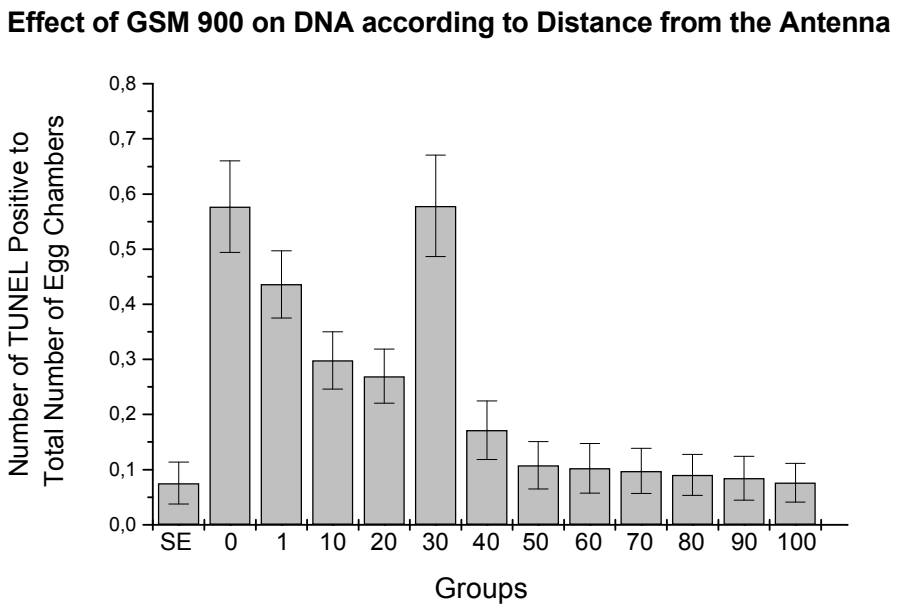

Figure 7. Mean ratio of egg-chambers with fragmented DNA (number of TUNEL- positive to total number of egg-chambers) ± SD, in relation to the Distance from a GSM 900 MHz mobile phone antenna, (cm). The increase in DNA damage and consequent cell death induction is maximum at zero distance and at 30 cm distance from the antenna (intensity "window"), corresponding to RF intensities 378 μW/cm^2 and 10 μW/cm^2, (Tables 7, 4).

Figure 9a, 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 7, Fig. 7, 8), exclusively at the two check points, 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 2007a).

Figure 9b 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 to 90 for GSM 900 and 30 to 80 for GSM 1800.

Figure 9c, shows an ovariole of an exposed female insect (group 20- GSM 1800), with a TUNEL-positive signal at all the 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 usual picture of ovarioles of exposed insects from the groups 0 to 30 for GSM 900 and 0 to 20 for GSM 1800.

Although in 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 to 30 for GSM 900 and 0 to 20 for GSM 1800 on which the impact of the radiation was maximum, a TUNEL-positive signal was detected in all three kinds of egg chamber cells, (fig. 9c).

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

Therefore, we verified that the two check points found by other experimenters (Nezis et al. 2000; 2002; Drummond-Barbosa and Spradling 2001; McCall 2004; Baum et al 2005) to be the most sensitive developmental stages in regards to other kinds of external stress, are also the most sensitive stages in regards to electromagnetic stress. Moreover, the germarium was found for the first time, to be even more sensitive than the mid-oogenesis check point (stages 7-8) in regard to the electromagnetic stress (Panagopoulos et al 2007a; 2010).

The effect of GSM radiation-field on DNA damage, and the consequent induced cell death in the ovaries of exposed female insects, diminishes considerably, just as the effect on the reproductive capacity, 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 $\mu W/cm^2$, (Tables 5, 7, Fig. 4, 5, 7, 8). For distances longer than 50 cm where the ELF components decrease significantly and fall within the background of the stray 50 Hz fields, the increase in cell death induction, just as the decrease in reproductive capacity, in regard to the SE groups was very small, falling within the standard deviation of the SE groups, (Tables 5, 7, Fig. 4, 5, 7, 8).

The 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. Therefore, the groups differ between them in cell death induction because of the GSM 900/1800 exposures at the different distances-intensities and not due to random variations. The reason that the P value is much smaller in the case of reproductive capacity ($P < 10^{-27}$ in experiments No 4) than in cell death induction

($P < 10^{-10}$), is only that the number of experiments for cell death induction (3) was smaller than the corresponding number of experiments for the effect on reproductive capacity (8).

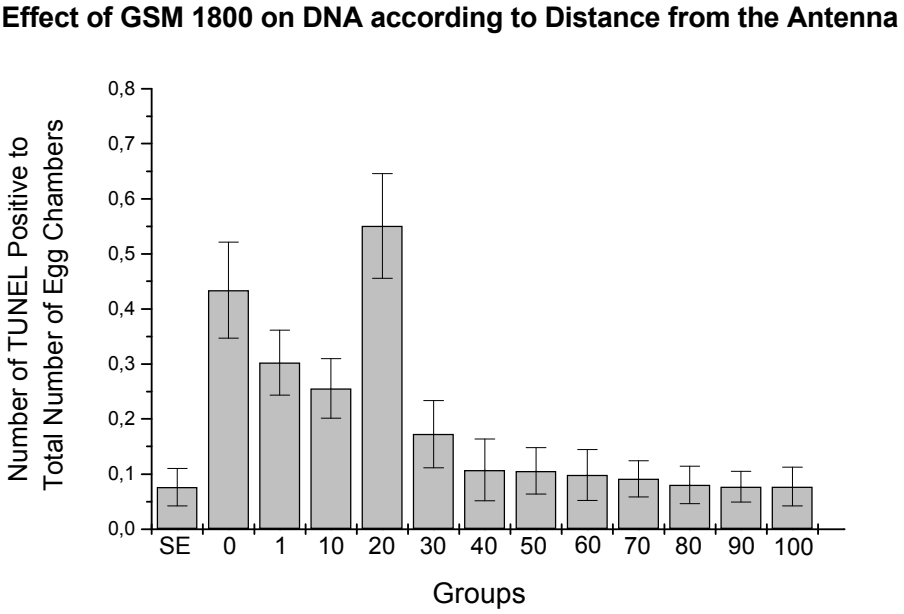

Figure 8: Mean ratio of egg-chambers with fragmented DNA (number of TUNEL- positive to total number of egg-chambers) ± SD, in relation to the Distance from a GSM 1800 MHz mobile phone antenna, (cm). The increase in DNA damage and consequent cell death induction is maximum at zero distance and at 20 cm distance from the antenna (intensity "window"), corresponding to RF intensities 252 μW/cm^2 and 11 μW/cm^2, (Tables 7, 4).

The increased bioactivity window found in our previous experiments in regard to the effect on the reproductive capacity, was also recorded in this set of experiments for the same radiation-field intensity values in regard to DNA damage. We do not know whether this intensity window is related exclusively with the certain organism that we used as experimental animal, or it 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. Nevertheless, since the effect of the GSM radiation on DNA damage 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 mammalian cells, we consider that it is possible the above intensity window to exist for other organisms and humans as well.

Our results show that exposure of living organisms to mobile telephony radiation is highly bioactive, able to induce DNA damage and cell death, at intensities higher than a few μW/cm^2 and this bioactivity is still evident for intensities down to 1μW/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 μW/cm^2 in the specific biological system that we studied, in regards to short term exposure periods.

As in earlier experiments of ours, (Panagopoulos et al 2007a), although egg chambers during early and mid oogenesis in *Drosophila* were not reported before to exhibit either stress-induced by other stress factors than EMFs, 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 EMFs, (McCall 2004; Drummond-Barbosa and Spradling 2001; Nezis et al 2000; 2002; Cavaliere et al 1998; Foley and Cooley 1998), but also in the oocyte, (fig. 9c).

Thus, electromagnetic stress from mobile telephony radiations was found in our experiments to be much more bioactive than previously known stress factors like poor nutrition, excessive heat or cytotoxic chemicals, inducing cell death to a higher degree not only to the two check points 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, 2007a).

A possible explanation for these phenomena as given by us before (Panagopoulos et al 2007a) is that, the electromagnetic stress induced in the ovarian cells by the GSM 900 and 1800 microwave 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 stress like poor nutrition, heat shock or chemical stress.

The fact that the electromagnetic stress induces DNA fragmentation also 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 these check points.

It is important to remark that DNA fragmentation in the oocyte which undergoes meiosis during the last stages of oogenesis, may result, if not in cell death, in heritable mutations transferred to the next generations after DNA damage and repair (Panagopoulos et al 2007a). Such a possibility may be even more dangerous than a reduction in the offspring.

The results of this set of experiments reveal that the large decrease in the reproductive capacity found in our previous experiments after exposure to GSM radiation, is due to elimination of large numbers of egg chambers during early and mid oogenesis, after induction of cell death on their constituent cells, caused by the mobile telephony radiations, at all the different distaces/intensities tested, up to 1m from a mobile phone antenna (or down to 1 $\mu W/cm^2$, radiation intensity).

We do not know if the induced ovarian cell death is apoptosis, i.e. caused by the organism in response to the electromagnetic stress, or necrosis, caused directly by the microwave radiation. This important issue remains under investigation.

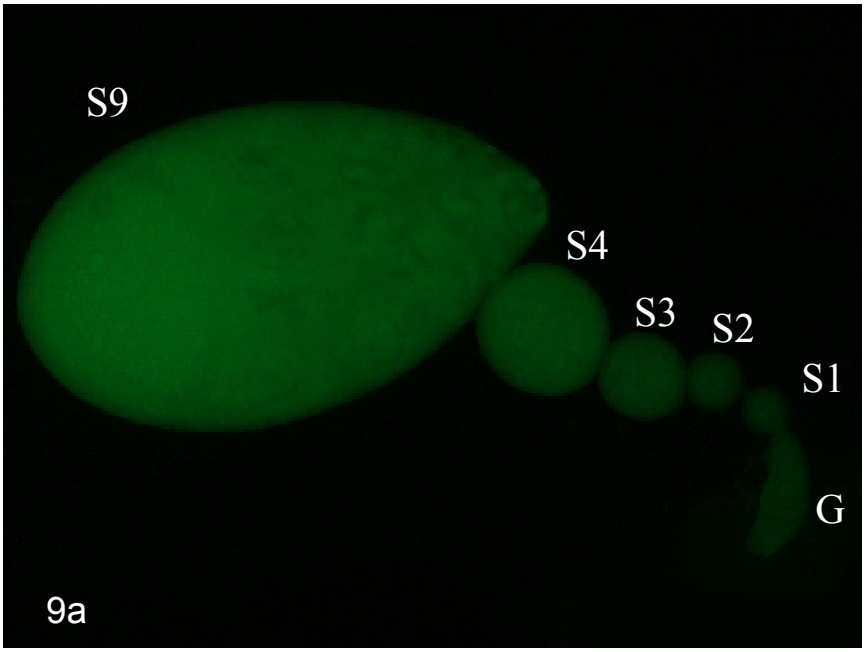

Figure 9. a) Typical TUNEL-negative fluorescent picture of an ovariole of a Sham Exposed female insect, containing egg chambers from germarium to stage 9.

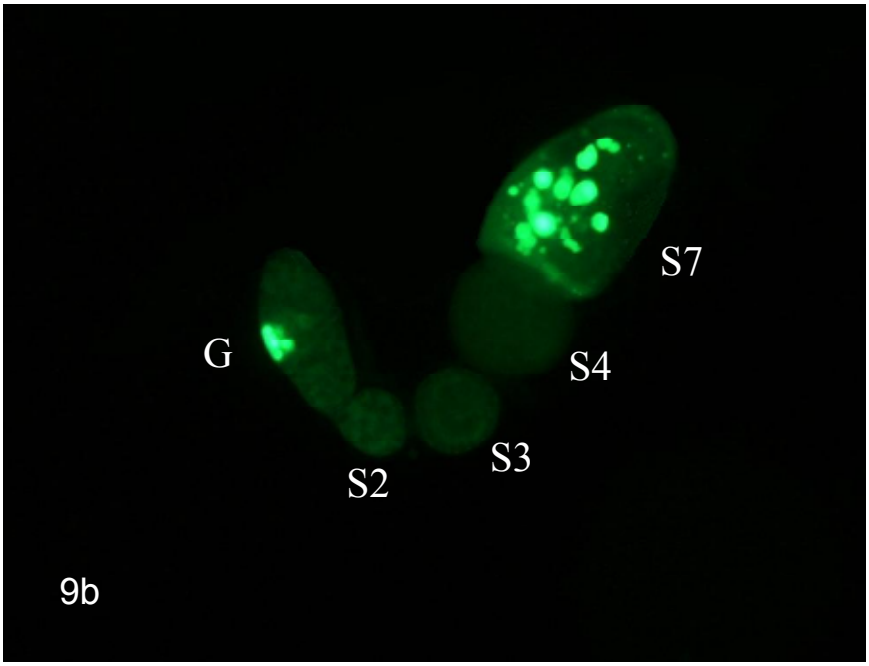

Figure 9b. Ovariole of an exposed insect (group 50-GSM 900) with TUNEL-positive signal only at the two check points, (germarium and stage 7 egg chamber) and TUNEL-negative intermediate stages.

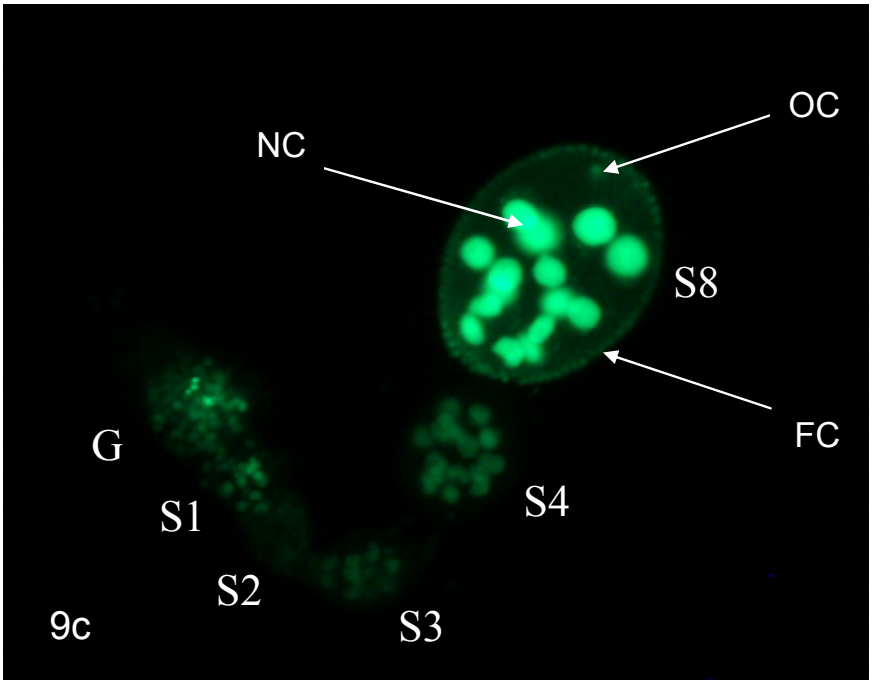

Figure 9c. Ovariole of an exposed female insect (group 20-GSM 1800) with fragmented DNA at all stages from germarium to stage 8), and in all kinds of egg chamber cells, (NC: nurse cells, FC: follicle cells, OC: oocyte).

7. The DNA Damage Induced by GSM 900 and 1800 Radiation is Accompanied by Actin Cytoskeleton Damage

In this set of experiments (a detailed description can be found in Chavdoula et al 2010), we showed that GSM radiation, induces disorganization of the actin cytoskeleton in the reproductive cells of exposed female insects during early and mid oogenesis. The disorganization of the actin cytoskeleton is another known aspect of cellular death during both apoptosis and necrosis. For this we applied rhodamine-conjugated phalloidin staining assay, as described below.

We also examined whether follicles with TUNEL-positive signal in their constituent cells had at the same time alterations in their actin cytoskeleton. For this we used double staining with rhodamine-conjugated phalloidin and TUNEL assay at the same samples following the methodology described below.

Rhodamine-Conjugated Phalloidin Staining Assay

Ovaries were dissected in Ringer's solution, fixed in PBS (Invitrogen, USA, 70013-016) containing 4% formaldehyde (Polysciences, Inc., Warrington, PA, 18814) for 20 min, and permeabilized for 35 min in PBS containing 4% formaldehyde plus 0.1% Triton X-100. The follicles were then stained for 2 h in PBS containing 1 mg/ml rhodamine-conjugated phalloidin (Invitrogen, USA, R415), washed three times (5 min each) in PBS and finally they

were mounted in 90% glycerol containing 1.4-diazabicyclo (2.2.2) octane (Sigma Chemical Co., Germany) to avoid fading (antifading mounting medium). [Rhoramine is a fluorescent substance that gets attached to the actin cytoskeleton through the binding of phalloidin].

Double Staining with Rhodamine-Conjugated Phalloidin and TUNEL

Ovaries were dissected in Ringer's solution, fixed in PBS containing 4% formaldehyde for 20 min, and permeabilized for 35 min in PBS containing 4% formaldehyde plus 0.1% Triton X-100. The follicles were then stained for 2 h in PBS containing 1 mg/ml rhodamine-conjugated phalloidin and washed three times (5 min each) in PBS. Then, they were incubated with PBS containing 20µg/ml proteinase K for 10 min. The in situ detection of fragmented genomic DNA was performed with the in situ cell death detection kit (Roche, Mannheim, Germany, 11684795910) by using fluorescein-labeled dUTP for 3 h at 37°C in the dark. Following this procedure, the follicles were washed six times in PBS over the course of 90 min in the dark and mounted in antifading mounting medium.

The simultaneous observation of the two cell death features was accomplished by double action of two different lasers on the samples and observation of the corresponding two types of fluorescence through a Nikon EZ-C1 Confocal Laser Scanning Microscope (CLSM) (Nikon Instruments, Japan).

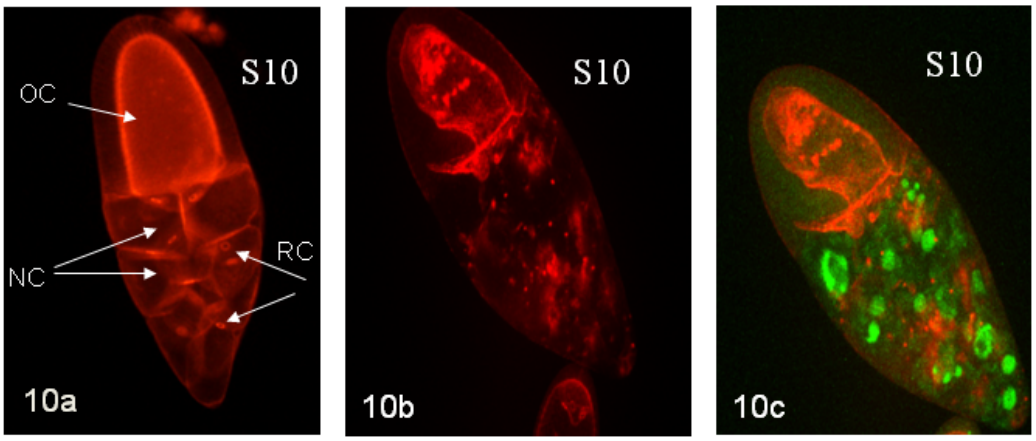

Figure 10. a) A stage 10 egg chamber from a sham-exposed insect, treated with rhodamine-conjugated phalloidin assay, with normal cytoskeleton morphology. Characteristic features of the actin cytoskeleton like the ring channels (RC) can be observed. NC: nurse cells, OC: oocyte. b) A stage 10 egg chamber of an exposed insect with disorganized actin cytoskeleton. c) The same stage 10 egg chamber as in figure 10b, treated with both TUNEL (green fluorescence) and rhodamine-conjugated phalloidin (orange fluorescence) assays, revealing that DNA fragmentation and actin cytoskeleton disorganization coexist in the damaged follicles of the exposed insects.

Figure 10a, shows a stage 10 egg chamber from a sham-exposed insect, treated with rhodamine-conjugated phalloidin assay, with normal cytoskeleton morphology. Characteristic features of the actin cytoskeleton in the nurse cells can be observed, like the ring channels (RC) which facilitate the transport of proteins and mRNAs from the nurse cells to the developing oocyte.

Figure 10b, shows a stage 10 egg chamber of an exposed insect with disorganized (damaged) actin cytoskeleton.

Figure 10c, shows the same stage 10 egg chamber as in figure 10b, treated with both TUNEL and rhodamine-conjugated phalloidin assays, revealing that DNA fragmentation and actin cytoskeleton disorganization coexist in the damaged follicles of the exposed insects.

This set of experiments demonstrated the simultaneous induction of two cell death features: DNA fragmentation and actin cytoskeleton disorganization in the egg chamber cells, during early and mid oogenesis, when no physiological apoptosis takes place. Both features of cellular death were found to coincide in the damaged follicles, (fig. 10c).

8. The Bio-Effect of the GSM Radiation Increases with Increasing Daily Exposure Duration

In this set of experiments we examined the bioactivity of different durations of a single, (continuous), daily exposure, ranging from 1 min up to 21 min, to GSM 900 and 1800 radiations. The insects were exposed to each type of radiation at an intensity of about 10 $\mu W/cm^2$, corresponding to a distance of 20 cm or 30 cm from the antenna of a GSM 1800 or a GSM 900 mobile phone handset, respectively. At these distances the bioactivity of mobile telephony radiation was shown to be maximum due to the existence of the intensity window described in the previous sets of experiments (No 4, 5).

The duration of exposure to any kind of external stimulus is an important parameter in order to know whether the biological effects related to this stimulus are cumulative or not, i.e. whether there is a difference in exposing an organism for a longer or a shorter time. It is well documented that ionizing radiations have cumulative effects on living organisms as these effects increase with the absorbed dose, i.e. the amount of energy absorbed by the unit mass of tissue (Coggle 1983; Hall and Giaccia 2006). In the case of non-ionizing radiation, and especially the RF-microwave radiation emitted by mobile telephony antennas, only few such studies were performed until the first publication of this set of experiments (Panagopoulos and Margaritis 2010a), in some cases with contradictory results.

The results of this set of experiments showed that the reproductive capacity decreases almost linearly with increasing exposure duration to both GSM 900 and 1800 radiation, suggesting that short-term exposures to these radiations have cumulative effects on living organisms.

A dual band mobile phone was used again, that could be connected to either GSM 900 or 1800 networks simply by changing the SIM ("Subscriber Identity Module") card on the same handset. The highest Specific Absorption Rate (SAR) for the human head, according to the manufacturer, was 0.795 W/Kg, while the corresponding established limit is 2 W/kg, (ICNIRP 1998). The exposure procedure was the same as in No 1 and 3-5 sets of experiments, but a recorder was used as a sound source instead of the experimenter speaking on the mobile phone during the exposures. The mobile phone was operating in speaking mode, with the same recorded voice, reading the same text during the exposures, the sham exposures and the measurements. The sound source/recorder was always at the same position in relation to the mobile phone, the insects or the probe of the field meter. The handset was fully charged before each set of exposures and measurements.

The insects within the glass vials were exposed at 20- and 30-cm distance from the mobile phone antenna to the GSM 1800 and GSM 900 signals, respectively, where the intensity of the modulated radiation (speaking emission) is roughly equal between the two types of radiation, i.e. about 10 μW/cm^2, and where the bioactivity of this radiation was previously found to reach a maximum, (Panagopoulos and Margaritis 2008; 2009; 2010b; Panagopoulos et al 2010).

In each experiment with either GSM 900 or 1800 exposures, we separated the insects into six groups: (a) the group exposed to the radiation/field for 1 min (named "E1"), (b) the group exposed for 6 min (named "E2"), (c) the group exposed for 11 min, (named "E3"), (d) the group exposed for 16 min (named "E4"), (e) the group exposed for 21 min, (named "E5") and (f) the sham-exposed group (named "SE").

Each one of the six groups in each experiment consisted of ten females and ten males, newly emerged flies, as in the previous sets of experiments. The sham-exposed groups received identical treatment to the exposed ones, except that they were not exposed to any kind of radiation, since the mobile phone was turned off during the sham exposures. The duration of the sham exposures was 21 min; it was already verified that there was no statistically important difference in the reproductive capacity between groups sham-exposed for all the different selected exposure durations from 1 min up to 21 min (data not shown).

During the exposures, the mobile phone was stabilized with its antenna parallel to the axis of the cylindrical glass vials. The insects of the different groups within their glass vials were exposed simultaneously to either GSM 900 or 1800 radiations, placed along constant intensity sectors of an arc with a 30- or 20-cm radius, respectively, at the center of which the handset was placed. This exposure arrangement was designed in order to have the different groups equally exposed during their common exposure periods, since there are constant changes in the intensity and frequency of the real mobile telephony signals. Then, during each exposure session, the different groups were taken away from the exposure bench one by one, as soon as the exposure duration of each one was completed. After each exposure, the corresponding sham exposure was performed, at the same distance from the mobile phone handset.

We carried out twelve replicate experiments, six with the GSM 900 MHz radiation and six with the GSM 1800 MHz radiation. The results are shown in Table 8 and are represented graphically in Figure 11.

The data show that the reproductive capacity of all the exposed groups is significantly decreased compared to the sham-exposed groups, for both radiation types and for all the exposure periods from 1 min to 21 min.

Moreover, the data show that the reproductive capacity decreases almost proportionally as the exposure duration increases, for both types of mobile telephony radiation.

The average decrease for the six experiments of each series compared with the sham exposed (SE) groups, was for GSM 900 MHz, 36.4% for 1 min exposure, 42.5% for 6 min, 49.2% for 11 min, 56.1% for 16 min, 63.0% for 21 min, and, correspondingly, for GSM 1800 MHz, 35.8%, 41.8%, 49.0%, 55.8% and 62.4%, (Table 8; Figure 11). The average decrease was smaller in the GSM 1800 groups than in the GSM 900 groups, for all the selected exposure durations, although differences between the GSM 900 and 1800 corresponding groups were within the standard deviations, (Table 8; Figure 11).

Table 8. Effect of different Exposure Durations of GSM 900 and 1800 radiation on the Reproductive Capacity of Drosophila melanogaster

Type of Radiation	Groups (daily Exposure Duration)	Average Mean Number of F_1 Pupae per Maternal Fly ± SD, in six identical experiments	Deviation from Sham Exposed (SE) groups
GSM 900	SE (0 min)	13.10 ± 0.95	
	E1 (1 min)	8.33 ± 0.71	-36.4 %
	E2 (6 min)	7.53 ± 0.60	-42.5 %
	E3 (11 min)	6.65 ± 0.63	-49.2 %
	E4 (16 min)	5.75 ± 0.62	-56.1 %
	E5 (21 min)	4.85 ± 0.69	-63.0 %
GSM 1800	SE (0 min)	13.05 ± 0.96	
	E1 (1 min)	8.38 ± 0.72	-35.8 %
	E2 (6 min)	7.60 ± 0.66	-41.8 %
	E3 (11 min)	6.65 ± 0.61	-49.0 %
	E4 (16 min)	5.77 ± 0.73	-55.8 %
	E5 (21 min)	4.90 ± 0.67	-62.4 %

The statistical analysis (ANOVA test) shows that for both types of radiation, the probability that the reproductive capacity differs between the six groups owing to random variations, is negligible, $P < 10^{-16}$. The corresponding probability between each exposed group and the SE was in all cases $P < 10^{-5}$ for both types of radiation. The corresponding probability between any two exposed groups that differ 5 min in exposure duration (e.g. E1-E2, E2-E3, etc), was in all cases $P < 0.07$ for GSM 900 exposures and $P < 0.08$ for GSM 1800 exposures. Finally, the corresponding probability between groups that differ 10 min in exposure duration between them (e.g. E1-E3, E2-E4 etc), was in all cases $P < 10^{-2}$.

We did not detect any temperature increases, within the glass vials during the exposures, for all the different exposure durations tested.

The statistical analysis clearly shows that the exposed *Drosophila* groups differ in offspring production between themselves and compared with the sham-exposed groups, and this difference is not due to random variations but due to the effect of the GSM fields. The reason why differences between groups differing only 5 min in daily exposure duration were not as strong ($P < 0.07$ for GSM 900 and $P < 0.08$ for GSM 1800) as between groups differing 10 min or more in exposure duration ($P < 10^{-2}$), is only that a 5-min difference in daily exposure duration was not enough to show a large difference in reproductive capacity, since all the exposures (even the shortest of 1 min daily) produced a significant effect and all the exposed groups were significantly different from the sham-exposed groups ($P < 10^{-5}$). When the difference in exposure duration is increased to 10 min or more, then the difference in reproductive capacity between exposed groups becomes highly significant.

The effect of both types of digital mobile telephony radiation on the reproductive capacity seems to increase almost linearly with increasing exposure duration from 1 to 21 min, suggesting that short-term exposures to this radiation have cumulative effects on living organisms.

We do not know whether longer short-term exposures than 21 min would result in an even higher decrease of reproductive capacity, or whether the effect would saturate after a certain exposure duration. This is left to be investigated in future experiments. Our present results represent a first indication that this radiation can have cumulative effects.

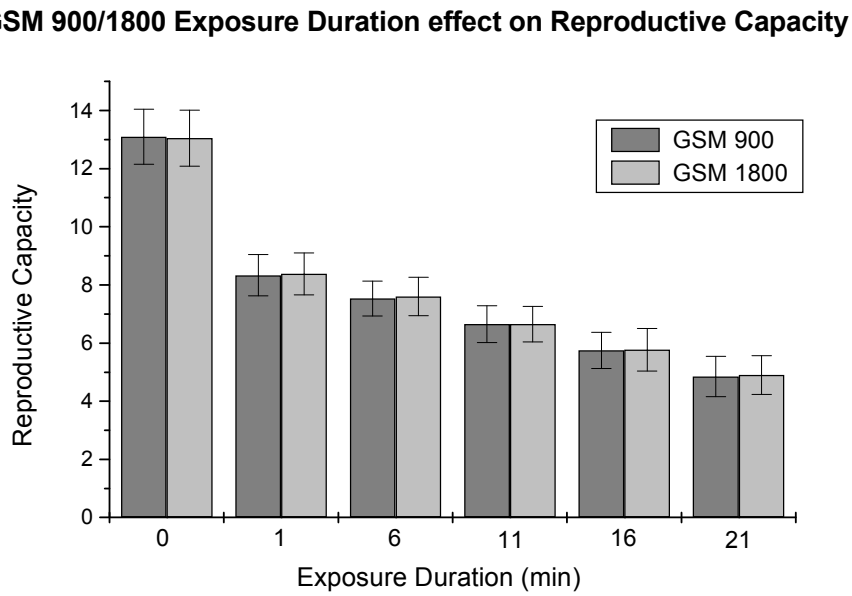

Figure 11. Reproductive Capacity (average mean number of F_1 pupae per maternal insect) ± SD of insect groups exposed to GSM 900 and GSM 1800 radiations for different daily exposure durations (1, 6, 11, 16, and 21 min) and of sham exposed groups (0 min).

It is also unknown whether long-term exposures (for weeks, months, years) of people or animals residing in areas exposed to microwave radiation by base-station antennas also have cumulative effects, but this is possible as the nature of radiation from base stations does not differ from that of mobile phones (Panagopoulos et al 2010; Hillebrand 2002; Clark 2001; Hamnerius and Uddmar 2000; Tisal 1998). Additionally, the radiation intensity used in the present experiments (~10 μW/cm^2) is usually encountered at about 20-30 m distance from mobile telephony base-station antennas where people reside or work and therefore may be exposed for up to 24 h per day.

Since we did not detect any temperature increases even during the longest exposures of 21 min, the recorded effect is considered as non-thermal. In previous experiments we exposed the same experimental animal to the near field of a mobile phone antenna (at 0 or 1 cm distances) for exposure periods up to 6 min and the recorded effects were non-thermal (Panagopoulos et al 2004; 2007a; 2007b). The effects were also non-thermal with six min exposures at different distances in the far field (Panagopoulos et al 2010). In the present experiments we exposed the insects in the far field of the mobile phone antenna, at 30 and 20 cm, respectively, for 900 and 1800 MHz, for exposure periods up to 21 min and the recorded effects were again non-thermal.

Although both types of radiation considerably affect reproduction, GSM 900 is found again to be slightly more bioactive than GSM 1800, even under equal radiation intensities and

for all the exposure durations tested, although the differences in biological activity between the two types of radiation were within the standard deviation (Table 8, Figure 11).

Our experiments with insects showed that the effect of GSM radiation is cumulative (increases with exposure duration) at least for short-term exposures. According to another study, constant exposure of mice for about 6 months to low-intensity (0.168-1.053 $\mu W/cm^2$) RF radiation from an antenna park had also cumulative effects, and resulted in progressively lower number of newborns from the first to the fifth pregnancy and finally in permanent infertility of the parent animals, (Magras and Xenos 1997).

Our results are also in agreement with other studies that have found a connection between the duration of exposure to mobile telephony radiations and increased health risks (Agarwal et al 2008; Wdowiak et al 2007; Salama et al 2004; Yadav and Sharma 2008), although contradictory findings are also reported (Zeni et al 2008; Zareen et al 2009). Moreover, our present results on insect reproduction are in agreement with certain results on human reproductive capacity (Agarwal et al 2008; Wdowiak et al 2007). In both cases, increased exposure duration to mobile telephony radiation induced increased infertility to both insects and humans.

Thus, according to the results of this set of experiments, RF-microwave radiations used in modern telecommunication systems seem to have a cumulative biological action and, for this reason, living organisms should be exposed for as short a time as possible to these radiations. Users should be informed to make cautious use of mobile phones and try to diminish the length and frequency of their phone calls.

SYNOPSIS OF EXPERIMENTAL CONCLUSIONS

The above presented experimental results have led us to the following basic conclusions:

1. GSM 900 and 1800 MHz mobile telephony radiation is found to reduce insect reproduction by up to 60%. The insects were exposed for 6 min daily during the first 5 days of their adult lives. Both males and females were found to be affected.
2. GSM 900 MHz radiation is found to be more bioactive than GSM 1800 MHz under actual conditions mainly due to the fact that, GSM 900 is emitted at double the output power than GSM 1800 (and GSM 1900). GSM 900 is also found to be slightly more bioactive than 1800 even under the same intensity.
3. The reduction of insect reproductive capacity is due to DNA damage, actin cytoskeleton damage and cell death induction in the reproductive cells (gonads).
4. The effect in regard to short-term exposures is evident for radiation intensities down to 1 $\mu W/cm^2$. This radiation intensity is found at about 1 m distance from a mobile phone or about 100 m distance from a corresponding base station antenna. This radiation intensity is 450 and 900 times lower than the current ICNIRP limits for 900 and 1800 MHz respectively (ICNIRP 1998). It is possible for long-term exposure durations, that the effect would be evident at even longer distances/smaller intensities. For this, a safety factor should be necessarily introduced in the above value. By introducing a safety factor of 10, the above value becomes 0.1 $\mu W/cm^2$,

which is the limit that reasonably results from our experiments and coincides with the limit proposed by the BioInitiative Report (2007).

5. The effect is strongest for intensities higher than 200 μW/cm^2 (0-1 cm distance from the cell phone) and within a "window" around 10 μW/cm^2 where the effect becomes even stronger. This intensity value of 10 μW/cm^2 corresponds to a distance of 20-30 cm from a mobile phone handset or to 20-30 m from a base station antenna.
6. Electromagnetic stress seems to be even more bioactive than other previously tested stress factors like poor nutrition, heat, or chemical stress, inducing DNA damage to a higher degree on insect reproductive cells.
7. The effect increases with increasing daily exposure duration in regards to short-term exposures.
8. The effect is non-thermal - there are no temperature increases during the exposures.
9. The effect at cellular level seems to be due to irregular gating of ion channels on the cell membranes caused by the EMFs, leading to disruption of cell's electrochemical balance and function. This mechanism (presented below) is non-thermal.
10. Although we cannot simply extrapolate the above results from insects to humans, similar effects on humans cannot be excluded. On the contrary, they are possible 1) because insects are in general much more resistant to radiation than mammals and 2) because the presented findings are in distinct agreement with results of other experimenters, reporting DNA damage on mammalian cells or mammalian (including human) infertility (see below).
11. Reported observations during the last years regarding reduction of bird and insect populations (especially bees) can be explained by decrease in their reproductive capacity as described in our experiments.
12. Symptoms referred to as "microwave syndrome" (headaches, sleep disturbances, fatigue etc.), among people residing around base station antennas, can possibly be explained by cellular stress induction on brain cells or even cell death induction on a number of brain cells.

AN INTRIGUING SIMILARITY OF EXPERIMENTAL FINDINGS

Our experiments published in 2007 (Panagopoulos et al. 2007a) were the first to show that GSM 900 and 1800 radiation emitted by commercially available mobile phones, induces DNA fragmentation and consequent cell death in reproductive cells after *in vivo* exposure of newly eclosed insects. Other experiments before, had indicated DNA damage (Diem et al 2005) or cell damage (Salford et al 2003; Markova et al 2005; Aitken et al 2005) induced by GSM "test" or simulated signals. Although these signals are significantly different from the real GSM signals that we used, an unquestionable similarity with our results is noticed. A similarity is also noticed with even older results reporting DNA damage in rat brain cells after exposure to microwaves from analog (1st generation) mobile phones (Lai and Singh 1995; 1996; 1997).

Recent results of other experimenters regarding mammalian (including human) infertility, (Gul et al 2009; Agarwal et al 2008; Wdowiak et al 2007; Magras and Xenos 1997) or chicken embryonic mortality (Batellier et al 2008; Grigor'ev IuG. 2003), especially those

regarding DNA damage or oxidative stress on mammalian-human reproductive cells, (De Iouliis et al 2009; Mailankot et al 2009; Yan et al 2007; Agarwal et al 2009), exhibit an even more distinct similarity with our results.

More specifically, GSM radiation from mobile phones decreased the number of follicles in the ovaries of newborn female rats exposed during their intra-uterine life, (Gul et al 2009). These results are very similar with the elimination of follicles in the ovaries of female insects after exposure to GSM radiation shown in our experiments. Other recent experiments found that GSM mobile phone radiation induced DNA damage and mitochondrial generation of reactive oxygen species (ROS) in human spermatozoa *in vitro* (De Iuliis et al 2009; Agarwal et al 2009). Induction of oxidative stress and reduction of sperm motility in rats are reported in other recent experiments after *in vivo* exposure to real mobile phone 900/1800 radiation, (Mailankot et al 2009). Sperm cell death in rats after *in vivo* exposure to mobile phone radiation, is also a very similar recent result of other experimenters (Yan et al 2007).

There is also an agreement of our results with older results of other experimenters reporting DNA damage in cell types other than reproductive, assessed by different methods than ours, after *in vivo* or *in vitro* exposure to mobile phone radiation, (Sokolovic et al 2008; Diem et al., 2005; Markova et al., 2005; Salford et al., 2003; Lai and Singh 1995; 1996).

The intriguing similarity of the above described results with ours, although in different animals and some of them in different cell types, makes unlikely the possibility that these findings can be wrong. Therefore, it seems that it is an unquestionable fact that microwaves emitted by modern mobile telecommunication antennas, may damage DNA and induce cell death, especially on reproductive cells (gonads and gametes) but not only. Additionally, DNA damage and cell death in reproductive cells may explain the recently reported population decline of bees and birds (Stindl and Stindl 2010; Bacandritsos et al 2010; van Engelsdorp et al 2008; Everaert and Bauwens 2007; Balmori 2005).

DNA damage in somatic cells may result in cancer induction. Experimental findings reporting DNA damage in somatic cells seem to be in agreement with recent reports about brain tumor induction among mobile phone users, especially among those who use mobile phones daily for more than 10 years (Khurana et al 2009; Hardell et al 2007; 2009).

MECHANISM FOR EMFS BIO-EFFECTS: THE ION FORCED-VIBRATION THEORY

The effects of EMFs at cellular level can be explained by irregular gating of electrosensitive ion channels on the cell membranes, according to the Ion Forced-Vibration Theory that we have proposed (Panagopoulos et al 2000; 2002; Panagopoulos and Margaritis 2003). This irregular gating of ion channels may lead, non-thermally, to disruption of the cell's electrochemical balance and function, as described below.

According to this theory (Panagopoulos et al 2000; 2002; Panagopoulos and Margaritis 2003), which is considered until now as the most valid one from all the proposed theories, (Creasey and Goldberg, 2001), even very weak ELF electric fields of the order of 10^{-3} V/m, are theoretically able to change the intracellular ionic concentrations and thus, disrupt cell function. Since RF-microwave radiations and especially those used in modern mobile telecommunications are always transmitted within ELF pulses, or include ELF modulating

signals of intensities thousands of times higher than 10^{-3} V/m, this theory can be applied for the explanation of their bioeffects.

The basic idea relates to the fact that any external oscillating electric or magnetic field, will induce a forced-vibration on the free ions that exist in large concentrations inside and outside of all living cells in biological tissue. When the amplitude of this forced-vibration exceeds some critical value, the electrostatic force exerted by the oscillating ions' charge on the electric sensors of the voltage-gated membrane ion channels, can irregularly gate these channels, resulting in changes to the free ions' intracellular concentrations.

These free ions play a key role in all cellular functions, and the changes in their intracellular concentrations initiate or accompany all cellular biochemical processes.

Let us consider an external oscillating electric field (or the electric component of an electromagnetic wave) of intensity E, acting on a free ion in the vicinity of a cell membrane.

The forced-vibration of each free ion due to the external oscillating field is described by the equation,

$$m_i \frac{d^2x}{dt^2} + \lambda \frac{dx}{dt} + m_i \omega_o^2 x = E_o z q_e \sin\omega t \qquad [1]$$

for the case of an external harmonically oscillating electric field : $E = E_o \sin\omega t$, with circular frequency: $\omega = 2\pi\nu$, (ν, the frequency in Hz), where: z is the ion's valence, $q_e = 1.6\times10^{-19}$ C, the elementary charge, $F_2 = -m_i\omega_o^2 x$, a restoration force proportional to the displacement distance x of the free ion, m_i the ion's mass and $\omega_o = 2\pi\nu_o$, with ν_o the ion's oscillation self-frequency if the ion was left free after its displacement x. In our case, this restoration force is found to be very small compared to the other forces and thus does not play an important role. $F_3 = -\lambda u$ is the damping force, where $u = \frac{dx}{dt}$, is the ion's velocity and λ, is the attenuation coefficient for the ion's movement, which for the cytoplasm or the extracellular medium is calculated to be $\lambda \cong 10^{-12}$ Kg/sec, while for ions moving inside channel proteins, is calculated to have a value: $\lambda \cong 6.4\times10^{-12}$ Kg/sec, (in the case of Na^+ ions, moving through open Na^+ channels) , (Panagopoulos et al 2000).

Assuming that the ions' self frequencies coincide with the frequencies of the cytosolic free ions' spontaneous oscillations observed as membrane potential spontaneous oscillations in many different types of cells with values smaller than 1 Hz and assuming that the ion's maximum vibrational velocity has a value of 0.25 m/s, as calculated for the movement of sodium ions through open sodium channels using patch-clamp conductivity data (Panagopoulos et al 2000), it comes after operations that the general solution of equation [1], is:

$$x = \frac{E_o z q_e}{\lambda\omega} \cos\omega t - \frac{E_o z q_e}{\lambda\omega} \qquad [2]$$

Since the second term of the second member of equation [2] is constant, the vibrational movement is described by the equation:

$$x = \frac{E_o z q_e}{\lambda \omega} \cos \omega t \qquad [3]$$

Equation [2] declares that, at the moment when the external field is applied and at the moment when it is interrupted, the displacement of the ion becomes twofold the amplitude of the forced-vibration.

Eq. [3] shows that the forced - vibration is in phase with the external force.The amplitude of the free ion forced-vibration is,

$$A = \frac{E_o z q_e}{\lambda \omega} \qquad [4]$$

Thus, the amplitude is proportional to the intensity and inversely proportional to the frequency of the external oscillating field.

Once this amplitude exceeds some critical value, the coherent forces that the ions exert on the voltage sensors of voltage-gated membrane channels can trigger the irregular opening or closing of these channels, disrupting in this way the cell's electrochemical balance and function, by changing the intracellular ionic concentrations.

The oscillating ions represent a periodical displacement of electric charge, able to exert forces on every fixed charge of the membrane, like the charges on the voltage sensors of voltage - gated channels.

Voltage-gated channels, are leak cation channels. The state of these channels, (open/closed), is determined by electrostatic interaction between the channels' voltage sensors, and the transmembrane voltage. They interconvert between open and closed state, when the electrostatic force, exerted by transmembrane voltage changes on the electric charges of their voltage sensors, transcends some critical value. The voltage sensors of these channels, are four symmetrically arranged, transmembrane, positively charged helical domains, each one designated S4, (Noda et al 1986; Stuhmer et al 1989).

It is known that changes of about 30 mV in the transmembrane voltage, are able to gate these electrosensitive channels by exerting the necessary electrostatic force on the fixed charges of the S4 helices (Bezanilla et al 1982; Liman et al 1991).

We have shown that a single ion's displacement ∂r, of 10^{-12} m, in the vicinity of S4, can exert an electrostatic force on each S4, equal to that exerted by a change of 30 mV, in the transmembrane voltage, (Panagopoulos et al 2000):

The intensity of the transmembrane electric field is: $E_m = \dfrac{\Delta\Psi}{s}$ [5]

where, $\Delta\Psi$ is the transmembrane potential difference and s the membrane's width.

Additionally, $E_m = \dfrac{F}{q}$ [6],

where F in this case, is the force acting on an S4 domain and q is the effective charge on each S4, which is estimated to have a value, $q \cong 1.7\ q_e$ [7], according to the available data (Liman et al 1991). From equations [5], [6], we get:

$$F = \frac{\Delta\Psi}{s}\, q \ \Rightarrow \partial\ F = \partial\,\Delta\Psi\ \frac{q}{s} \qquad [8]$$

(where $\partial\ \Delta\Psi$ is the change in the transmembrane voltage, necessary to gate the channel). For $\partial\Delta\Psi$ = 30 mV, s = 10^{-8}m and substituting q from [7], equation [8] gives: $\partial F = 8.16 \times 10^{-13}$ N.

This is the force, on the voltage sensor of a voltage-gated channel, required normally, to interconvert the channel between closed and open state.

The force acting on the effective charge of an S4 domain, via an oscillating, free *z*-valence cation, is: $F = \frac{1}{4\pi\varepsilon\varepsilon_o} \cdot \frac{q \cdot zq_e}{r^2} \Rightarrow$

$$\partial\ F = -2 \cdot \frac{1}{4\pi\varepsilon\varepsilon_o} \cdot \frac{q \cdot zq_e}{r^3}\ \partial r \Rightarrow \text{(ignoring the minus sign)},$$

$$\partial r = \frac{2\pi\varepsilon\varepsilon_o \partial F \cdot r^3}{q \cdot zq_e} \qquad [9]$$

This is the minimum displacement of a single, *z*-valence cation, in the vicinity of S4, able to generate the necessary force ∂F, to gate the channel. Where: *r*, is the distance between a free ion with charge zq_e and the effective charge q on each S4 domain, which can be conservatively taken as 1 nm, (Panagopoulos et al 2000). ε_o= 8.854×10^{-12} $N^{-1}\cdot m^{-2}\cdot C^2$, is the dielectric constant of vacuum. The relative dielectric constant ε, can have a value 80 for a water-like medium, (cytoplasm or extracellular space), or a value as low as 4, for ions moving inside channel-proteins, (Panagopoulos et al 2000; Honig et al 1986).

The concentration of free ions on both sides of mammalian cell membranes, is about 1 ion per nm^3, (Alberts et al 1994). Let us conservatively calculate ∂r for one single-valence cation, interacting with an S4 domain. If two or more single-valence cations interact, (in phase), with an S4 domain, from 1nm distance, ∂r decreases proportionally. For ions moving inside channel-proteins, we assume, that they move in single file, (Palmer 1986; Panagopoulos et al 2000).

From equation [9] and for $\partial F = 8.16 \times 10^{-13}$ N, we get:

$\partial r \cong 0.8\times10^{-10}$ m, (for ε = 80)

and: $\partial r \cong 4\times10^{-12}$ m, (for ε = 4) [10]

We can see that, a single cation's displacement of only few picometers from its initial position, is able to interconvert voltage-gated channels, between open and closed states, (for cations moving or bound within channels).

Therefore, any external field, which can induce a forced-vibration on the ions, with amplitude $A \geq 4 \times 10^{-12}$ m, is able to disrupt the cell's function. Substituting A from eq. [4], in the last condition, it comes that, a bioactive, external, oscillating electric field, of intensity amplitude E_o and circular frequency ω, which induces a forced-vibration on every single-valence ion, (z=1), must satisfy the condition:

$$\frac{E_o q_e}{\lambda \omega} \geq 4 \times 10^{-12}\,\text{m} \qquad [11]$$

Since we adopted a value for ∂r, ($\cong 4\times10^{-12}$ m), valid for cations within channels, (where $\varepsilon = 4$), we shall use the corresponding value for λ, calculated also for cations moving within channels, (Panagopoulos et al 2000), $\lambda \cong 6.4\times10^{-12}$ Kg/sec.

Thereby, the last condition becomes:

$$E_o \geq \omega \cdot 1.6 \times 10^{-4} \qquad [12] \quad \text{or}$$

$$E_o \geq \nu \times 10^{-3} \qquad [13] \quad (\nu \text{ in Hz, } E_o \text{ in V/m})$$

Moreover, in the most bioactive case of pulsed fields and for two double valence cations (i.e. Ca^{+2}) interacting simultaneously with the channel sensor, the second member of the cond. [13] is divided by 16, and the condition for irregular gating of the channel becomes, (Panagopoulos et al 2002):

$$E_o \geq \nu \times 0.625 \times 10^{-4} \qquad [14]$$

(ν in Hz, E_o in V/m). Whenever condition [14] is satisfied, the external field E can irregularly gate the ion channel.

Condition [14] declares that external ELF electric fields with intensities smaller than tenths of a mV/m should theoretically be able to disrupt cell function by irregular gating of ion channels.

According to this mechanism, lower frequency fields are the most bioactive ones and additionally pulsed fields are shown to be more bioactive than continuous, (uninterrupted), ones because of the constant term in the second member of eq. [2] which doubles the displacement of the oscillating ions at the onset and at the end of every pulse, (Panagopoulos et al., 2002).

Thereby, the ELF pulses of the mobile telephony signals are certainly within the criteria of this theory and thus, able to produce the reported biological-health effects on living organisms.

Microwave radiations are always pulsed or modulated on ELF frequencies like in mobile telephony signals in order to be able to carry and transmit information. Therefore the Ion

Forced-Vibration theory described above is applicable for the biological effects of the Radio Frequency (RF)-microwave radiations.

The Thermal Noise Problem

Free ions move anyway because of thermal activity, with kinetic energies much larger normally, than the ones acquired due to the action of an external electromagnetic field at intensities encountered in the human environment. In such a case, it has been claimed (Adair 1991) that this thermal motion masks the motion induced by the external field, making this motion unable to produce any biological effect.

But as we have explained (Panagopoulos et al 2000; 2002), thermal motion is a random motion, in every possible direction, different for every single ion, causing no displacement of the ionic "cloud" and for this it does not play any important role in the gating of channels, or in the passing of the ions through them. On the contrary, forced-vibration is a coherent motion of all the ions together in phase. The thermal motion of each ion and moreover the thermal motion of different ions, result in mutually extinguishing forces on the voltage sensor of an electrosensitive ion channel, while the coherent-parallel motion of the forced-vibration results in additive forces on the voltage sensor.

Therefore, if two or more cations interact, (in phase), with an S4 domain, from 1nm distance, ∂r in eq. [9], decreases proportionally. The concentration of free ions on both sides of mammalian cell membranes, is about 1 ion per nm^3, (Alberts et al. 1994) and for this, we have initially calculated ∂r for one cation, interacting with an S4 domain, although it is very likely that several ions interact simultaneously each moment with an S4 domain from a distance of the order of 1nm. This counts also for the ions moving already within a channel, since it is known that, although they pass through the narrowest part of the channel in single file, (Miller 2000; Palmer 1986; Panagopoulos et al. 2002), several ions fill the pore each moment as they pass sequentially, and several ion-binding sites (three in potassium channels) lie in single file through the pore, close enough that the ions electrostatically repel each other, (Miller 2000).

In the mildest case, if we consider a single ion interacting with an S4 domain, this ion moving with a drift velocity, u = 0.25 m/s, (Panagopoulos et al 2000), it needs a time interval $\delta t = \frac{\partial r}{u} \cong 1.6\times10^{-11}$ s, in order to be displaced at the necessary distance $\partial r = 4\times10^{-12}$ m. During this time interval δt, this ion will be also displaced because of thermal motion, at a total distance X_{kT}, ranging from 1.6 to 4×10^{-10} m, according to the equation: $X_{kT} = \sqrt{\frac{2kT\delta t}{\lambda}}$, for human body temperature, 37°C or T=310 °K. (X_{kT} in m, δt in s, λ in kg/s, $k = 1.381\times10^{-23}$ $J\cdot K^{-1}$ the Boltzmann's constant), (Panagopoulos et al. 2002).

The ions' mean free path in the aqueous solutions around the membrane is about 10^{-10} m, (Chianbrera et al. 1994), and it is certainly smaller within the channels, (the diameter of a potassium ion is about 2.66×10^{-10} m and the diameter of the narrowest part of a potassium channel is about 3×10^{-10} m, thereby the mean free path of a potassium ion within the channel has to be of the order of 10^{-11} m), (Panagopoulos et al. 2002; Miller 2000).

Therefore the ion within the above time interval δt, will run because of its thermal activity, several mean free paths, each one in a different direction, exerting mutually extinguishing opposing forces on the channel's sensors, while at the same time the ion's displacement because of the external field is in a certain direction, exerting on each S4 domain a force of constant direction.

In the most realistic case, if we consider several ions interacting simultaneously with an S4 domain, then the effect of the external field is multiplied by the number of ions, whereas the effect of their random thermal motions becomes even more negligible.

Thus, the claims that thermal motion masks the displacements of the free ions, caused by an external electric field, if these displacements are smaller than those caused by thermal motion (Adair 1991), are not valid according to the above analysis.

A Novel Possible Explanation of the Bioactivity "Windows"

According to the Ion Forced-Vibration theory, the action of external EMFs on cells is dependent on the irregular gating of membrane electrosensitive ion channels whenever an electric force on the channel sensors exceeds the force exerted on them by a change in the membrane potential of about 30 mV which is necessary to gate the channel normally. If in some kind of cells there is an upper limit for this value of membrane potential change, then the channel would be gated whenever the force exerted on its sensors is within this "window".

For example, the intensity window that we have recorded, in terms of the ELF electric field intensity, is around 0.6-0.7 V/m (Panagopoulos et al 2010; Panagopoulos and Margaritis 2010b). Let us assume that it ranges from 0.5 to 1 V/m. According to our theory, these limits correspond to a single-valence, single ion displacement between $\partial r_1 = 1.3\times10^{-11}$m and $\partial r_2 = 2.6\times10^{-11}$m, in the vicinity of the channel's sensors, equal to the amplitude of the induced forced-vibration in each case, according to equation [4], $\partial r = A = \frac{E_o z q_e}{\lambda\omega}$, where: E_o the amplitude of the external oscillating electric field which is equal to $E\sqrt{2}$ where E the measured (root mean square) value of electric field intensity, z the ion's valence (for example $z = 1$ for K^+ ions), q_e the unit charge (= 1.6×10^{-19} C), $\lambda \cong 6.4\times10^{-12}$ Kg/s the attenuation coefficient for the ion movement within a cation channel, $\omega = 2\pi v$ (v the frequency of the external oscillating field, in our case let us accept that, v =217 Hz the pulse repetition frequency of the GSM signals).

These displacements ∂r_1 and ∂r_2 would exert on each channel's sensor (S4 domain) corresponding forces $\partial F_1 = 2.5\times10^{-12}$ N and $\partial F_2 = 5\times10^{-12}$ N according to the equation [9], $\partial r = -\frac{2\pi\varepsilon\varepsilon_o \partial F \cdot r^3}{q \cdot z q_e}$, where $\varepsilon = 4$, the relative dielectric constant in the internal of a channel-protein, $\varepsilon_o = 8.854\times10^{-12}$ $N^{-1}\cdot m^{-2}\cdot C^2$, $r \cong 10^{-9}$ m, and $q = 1.7\, q_e$.

A force between 2.5 and 5×10^{-12} N on the channel's sensor, in turn, corresponds according to [8], $\partial F = \partial \Delta \Psi \frac{q}{s}$, to a change $\partial \Delta \Psi$ in the transmembrane voltage between 90 and 180 mV, (for $q = 1.7 q_e$ and s $\cong 10^{-8}$ m the membrane's width).

Thus we have shown that the intensity window found in our recent experiments, corresponds to a gating voltage change between 90 and 180 mV in the membrane potential.

Channel gating is usually studied on nerve cells and in this kind of cells possibly no upper limit exists, but the possibility of an upper limit (like the value of 180 mV that we found in our example), cannot be excluded for other kinds of cells which have not been studied yet in terms of their channel voltage gating. This hypothesis of ours for the explanation of the existence of bioactivity "windows" was reported recently (Panagopoulos and Margaritis 2010b) for the first time. The given numerical example is just an indication that the bioactivity windows reported for many years in bioelectromagnetic experiments but not explained so far, can possibly be explained according to the Ion Forced-Vibration theory.

BIOCHEMICAL MECHANISMS FOR DNA DAMAGE

Since microwaves are non-ionizing radiations (i.e. do not have the ability to detach electrons from molecules or break chemical bonds) it is unlikely that they can directly break DNA chains. It is possible though for the ELF pulses of the low frequency modulation signals that co-exist with the microwave carrier, to alter the intracellular ionic concentrations by irregular gating of electrosensitive cation channels on the cell membranes, according to the above described mechanism. This in turn, may initiate the following possible processes:

1. Irregular Release of Hydrolytic Enzymes

It is known that alteration of intracellular ionic concentrations, especially Ca^{+2} may initiate cell death induction through apoptosis or necrosis, (Santini et al. 2005). A common event preceding both apoptosis and necrosis, is the increase of mitochondrial calcium ion concentration released by endoplasmic reticulum, (Armstrong 2006). The mitochondrial concentration of calcium ions can be increased by irregular uptake due to direct action of the external EMF on calcium channels of the mitochondrial membrane, or indirectly due to increased calcium release in the cytoplasm by endoplasmic reticulum membrane or by plasma membrane, according to the biophysical mechanism described above. These processes may possibly lead to the release of specific hydrolytic enzymes (like DNaes) by the cytoplasmic organelles called lysosomes, (Goldsworthy 2007). The release of such enzymes may lead in turn to DNA fragmentation. Release of DNases or other hydrolytic enzymes from the lysosomes is mediated by alterations in the intracellular calcium concentrations (Santini et al. 2005; Armstrong 2006; Goldsworthy 2007). DNA fragmentation and consequent cell death induction, as it is shown in the above presented experiments of ours, is the reason for the decrease in the reproductive capacity of insects caused by mobile telephony radiations. Since an external oscillating electromagnetic field can change the intracellular ionic concentrations

by irregular gating of ion channels on cell membranes, this may lead to DNA fragmentation and cell death through the irregular release of hydrolytic enzymes.

2. Free Radical Action

Another way for indirect DNA damage by EMFs is through the action of free radicals. It is well known that ionizing radiations can detach electrons from different molecules or break molecular bonds, and form free radicals which in turn may react chemically with different biomolecules including nucleic acids, (Coggle 1983; Hall and Giaccia 2006). There is recent evidence of excessive free radical formation after RF-microwave exposures, (Phillips et al. 2009; De Iouliis et al 2009). Since the most abundant molecule in biological cells is that of water (H_2O), microwave radiation can possibly lead to the formation of water free radicals like OH•, O_2H•, H•. These molecules are extremely reactive, having a strong trend to react chemically with different biomolecules including DNA, because of an unpaired electron that they comprise, (symbolized by •). This unpaired-single electron tends to be paired and thus free radicals tend to react with other molecules in order to give, take, or contribute one electron and become stable.

The above mentioned water free radicals, except for their possible direct formation by RF-microwave exposure, can be formatted by hydrogen peroxide (H_2O_2), a product of oxidative respiration in the mitochondria, which can be converted by electromagnetic radiation (EMR) into hydroxyl free radicals via the Fenton reaction, a reaction catalyzed by iron within the cells:

$$H_2O_2 + (EMR) \xrightarrow{Fe} OH\bullet + OH\bullet \qquad [15]$$

The products of Fenton reaction (hydroxyl free radicals) are extremely reactive and able to disrupt biological macromolecules like DNA, proteins, membrane lipids, etc., (Phillips et al. 2009; Barzilai and Yamamoto 2004; Simko 2007).

It is well known that the presence of oxygen enhances the action of free radicals within the cells, by reacting with them and forming more free radicals (Coggle 1983; Hall and Giaccia 2006). These oxygen-containing free radicals are called reactive oxygen species (ROS). In aerobic cells, ROS are normally produced by mitochondrial activity, (French et al. 2001; Barzilai and Yamamoto 2004). If ROS are not properly controlled by the cell, they can damage cellular macromolecules and especially DNA. The fact that oxygen which is an essential component of life can be at the same time so dangerous, is reported as the “oxygen paradox”, (Barzilai and Yamamoto 2004). EMF exposure seems to be associated with free radical and especially ROS overproduction (Phillips et al. 2009; De Iouliis et al 2009; Simko et al 2007). In such a case, DNA damage may be expected.

Cells that are metabolically active (like the reproductive cells) or cells with a high concentration of free iron (like the brain cells) are expected to be more vulnerable to EMFs according to the above analysis. This is also supported by the experimental findings on reproduction decreases (Panagopoulos et al 2004; 2007a; 2007b; 2010; De Iouliis et al 2009; Agarwal et al 2009) and the epidemiological findings on brain cancer induction (Khurana et al 2009; Hardell et al 2009; 2007). While glial brain cells may become cancerous after DNA

damage leading to brain cancers, nerve brain cells do not divide and thus are not likely to become cancerous, (Mausset-Bonnefont et al 2004). DNA damage on nerve brain cells may then lead to cell death or malfunction which are both linked to neurodegenerative deceases such as Parkinson's and Alzheimer's.

It is also possible that already existing free radicals and ROS, produced physiologically in cells, extend their life span in the presence of external EMFs.

Finally, another possibility for the biochemical mechanism is the combination of the above two described scenarios.

The above described ways of indirect biochemical action of EMFs on cells leading to DNA damage, seem to form a realistic basis for the biochemical explanation of the recently reported experimental results regarding DNA damage and cell death, induced by EMFs. Therefore, it seems that there is a plausible complete explanation (biophysical and biochemical) for the effects of mobile telephony radiations, reported in recent studies.

DOSIMETRY OF EMFS EXPOSURES IS SAR A CREDIBLE QUANTITY ?

While some studies refer to the radiation Intensity on the surface of the exposed sample in order to describe the exposure conditions, some others refer to the Specific Absorbtion Rate (SAR- the amount of energy absorbed by the unit mass of tissue). While radiation or field intensity can be readily and objectively measured, SAR is approximately estimated, usually by complicated numerical methods simulating living tissue by inanimate objects of similar shape and mass. In this section of the present chapter, an attempt is made to discuss the necessity of using or not SAR as a dosimetric quantity.

In all the above described experiments we referred to the radiation in terms of its intensity (at the distance from the antenna where the insects were exposed), which can be readily measured objectively, rather than in terms of *SAR*, which is not measured directly and can never be accurately estimated.

Usually *SAR* values are reported in papers without any information about the way of their calculation. Let us examine this quantity:

SAR is defined as the absorbed power *P*, per unit mass of tissue (Moulder et al 1999; Panagopoulos and Margaritis 2003), (in W/Kg):

$$\mathrm{SAR} = \frac{P}{m} \qquad [16]$$

where, $m = \rho V$, is the mass of the tissue of density ρ (in Kg/m^3) and volume V.

The energy density (in J/m^3) of an electromagnetic wave is given by (Panagopoulos and Margaritis 2003):

$$W = \frac{P\delta t}{V} = \varepsilon\varepsilon_o E^2 \qquad [17]$$

where, W the energy per unit volume of tissue, transferred by the wave during a time interval δt, E the electric field component within the tissue induced by the wave (in V/m), ε_o = 8.854×10^{-12} $C^2/N\cdot m^2$ the dielectric constant of vacuum and ε, the relative dielectric constant of the tissue, (varying significantly for different tissues and different parts of a cell, for example $\varepsilon \cong 80$ for the aqueous solutions in the cytoplasm or the extracellular spaces and $\varepsilon \cong 4$ within membrane channel proteins).

By use of equation [17] and the Ohm's law: $j = \sigma E$ [18]

where j, is the induced electric current density (in A/m^2) within the tissue and σ the specific conductivity of the tissue, (in S/m), equation [16] after operations, becomes:

$$\mathrm{SAR} = \frac{\sigma \cdot \mathrm{E}^2}{\rho} \qquad [19]$$

For a homogeneous medium with specific heat c, [in J/(Kg·K)] and by use of a form of the heat transmission equation:

$$\frac{dQ}{dt} = m \cdot c \cdot \frac{\delta T}{\delta t} \qquad [20]$$

equation [16], becomes: $SAR = c \cdot \dfrac{\delta T}{\delta t}$ [21]

where: $\dfrac{dQ}{dt}$ is the wave power, transformed into heat, within the tissue of mass m, producing a temperature increase δT during the time interval δt.

SAR, is estimated by one of the following ways, (Moulder et al 1999): 1) Insertion of micro-antennas within the tissue, which detect the internal electric field. If the conductivity and the density of the tissue are known, *SAR* can be computed from eq. [19]. 2) Insertion of miniature thermal probes within the tissue. If a change δT in the temperature of the tissue is recorded, caused by the radiation/field and the tissue is supposed homogeneous with known specific heat, then *SAR* can be computed by eq. [21]. 3) Numerical modeling, like Finite Difference Time Domain, (FDTD) simulation, which simulates the spatial distribution of the radiation within a body.

Microwave energy when absorbed by matter, induces vibration on polar molecules and ions, superimposed on the thermal vibration of the same particles and therefore increasing their thermal energy. But the energy of the vibrations induced by external EMFs at environmental exposure levels, is thousands of times (about 10^4) smaller than the molecular thermal energy kT within a biological tissue, (Panagopoulos and Margaritis 2003; Panagopoulos et al. 2000; 2002). Thereby, EMFs at intensities encountered at human environment cannot cause thermal increases except if they were thousands of times more powerful, like for example the fields within a microwave oven which operates at about

1000 W in contrast to a mobile phone (~1 W) or a mobile telephony base station antenna (~100 W).

As it becomes evident from its definition, *SAR* expresses the rate at which electromagnetic energy from the external electromagnetic wave/field is converted into heat within a biological tissue (Stuchly and Stuchly 1996), therefore it assumes that EMFs bioeffects are exclusively related with thermal increases. But in our days it is well documented from many experimental studies (like the above presented experiments), that the biological effects of weak electromagnetic fields (at environmental levels) are non-thermal (Panagopoulos and Margaritis 2008; 2009), and plausible non-thermal mechanisms for the action of EMFs on cells are proposed as well, (Panagopoulos and Margaritis 2003; Panagopoulos et al. 2000; 2002). This experimental evidence is in agreement with the above argument that environmental EMFs should be thousands of times more powerful in order to be able to induce thermal increases.

Additionally, since conductivity and density vary for different tissues and moreover, conductivity varies for different field frequencies, *SAR* varies also, and therefore cannot be known accurately. Computer simulations, like FDTD method which is considered as the best way for computing *SAR*, dividing tissue volume into little homogeneous pieces (voxels) of constant conductivity and density can only be approximations. This is why earlier *SAR* estimations defining the current limits for whole body average *SAR* (ICNIRP 1998), are questioned by more recent and more accurate calculations, (Wang et al 2006). On the contrary, the characteristics of the external field, (intensity, frequency etc.), can be measured, accurately. For these reasons the “exposure criteria" are given both in power density, (or electric and magnetic field intensities) and *SAR*, (ICNIRP 1998).

The necessity or not of the use of SAR as a dosimetric quantity, is a “burning” point. Whether or not someone agrees with the above analysis, we believe that we logically support our arguments and for this the above analysis may contribute to the debate on EMFs exposure dosimetry.

PROTECTION ISSUES. A POSSIBLE WAY FOR REDUCING RADIATION LEVELS WHILE MAINTAINING THE ABILITY OF COMMUNICATION

Mobile Telephony has undoubtedly become a part of modern daily life. It is useful because people can communicate at any moment from any place and it can even save lives in difficult moments. On the other hand, the exposure to its radiations may lead to serious health implications according to the experimental findings. The intriguing similarity between some of the findings almost eliminates the possibility that these findings can be wrong, or due to randomness.

Therefore people must be seriously educated in the schools about the dangers of using mobile phones - especially the children - and make very cautious use of these devices. Similarly with other microwave emitting devices, like Internet connection wireless devices, domestic cordless phones (DECT-Digitally Enhanced Cordless Technology), local wireless networks (Wi-Fi), baby monitors, etc. Wireless telecommunication devices should not be used when similar devices can work with wire connections.

Since the effects are shown to be cumulative (Panagopoulos and Margaritis 2010a), all users should drastically reduce the length and frequency of their phone calls to the minimum. Children, being much more vulnerable to radiations, should not use mobile phones, except for emergency situations. Mobile telephones must not be carried on the bodies unless they are turned off. This is because at the "standby" mode, every few minutes they emit a periodic signal lasting a few seconds, to maintain connection with the nearest base station antenna. These periodic signals are as powerful as the usual "talk signals" during a conversation. The users must make use of the mobile phone's loudspeaker and keep the handset at least 40 cm away from their heads and other most sensitive organs of their bodies like the heart or the reproductive organs, during conversations. All other ways of protection (like wire-connected ear-phones – "hands free"), are less effective, or maybe even more dangerous (like the "blue tooth" devices) than the mobile phone itself, due to the existence of the intensity window described before. The mobile phone must not be held close to the head except for emergency situations.

The electronic circuits of the mobile telephony antennas of both mobile phones and base stations should be redesigned by the manufacturers in order to make the receiver circuits operate at max power while keeping the emitter's power to the minimum.

With regard to the involuntary exposure from base station antennas, a different network design should be attempted by the mobile phone industry. Instead of installing antennas everywhere within residential and working areas, they should perhaps try the following: Install powerful antennas on mountains and hills around the towns and a minimum number of low-power antennas at certain places within the towns at the largest possible distances from inhabitants, (in the middle of wide streets, on the roofs of the highest buildings with appropriate electromagnetic shielding on the roofs, within parks on appropriate towers etc). These low-power base station antennas within the towns should be just a little more powerful than a mobile phone, (5 W maximum output power instead of 100 W of the usual base station antennas). If there is sea or lake or even river adjacent to the town, then base station antennas could be installed on towers upon floating platforms as well.

Installing powerful base station antennas on the satellites could also be useful and complementary to the above ways.

Perhaps the engineers of the mobile telephony industry will argue that these proposals would not work, but what we propose here is reasonable and worth trying. Perhaps the signal will not be available everywhere by the ways we propose, but we think that safety is more important than signal availability everywhere.

GENERAL CONCLUSION

In the present study we showed that microwave radiation used in modern mobile telecommunications can damage DNA and induce cell death or heritable mutations which may in turn result in reproductive decreases, degenerative deceases, or cancer. We analyzed the biophysical and biochemical mechanism underlying this biological impact, and discussed dosimetry and protection issues.

All healthy organisms have defense mechanisms in order to repair biological damage. But defense mechanisms are weaker in children and old individuals, and become also weaker

during sicknesses or during stress conditions. Although even the most serious biological effects may not necessarily lead to health effects in an exposed individual, all health effects are initiated by corresponding biological ones. Thereby, biological effects - especially the most serious ones as is DNA damage or cell death induction - may potentially lead to health effects.

Ways for the safe use of microwave technology must be further developed in order to minimize the exposure levels. A reasonable proposed exposure limit for the general population in terms of the radiation intensity, resulting from our experiments, is 0.1 $\mu W/cm^2$.

The mission of technology is putatively to improve the living conditions of the human race. Technological evolution is accomplished by use of the natural powers, such as electromagnetism. But the use of these powers and the improvement of the living conditions must always be carried out without violating the natural environment, the powers of which we are actually using, and without undermining human health.

Knowing the dangers of each new technological achievement and finding ways to use technology safely, might be even more important than technology itself.

"This work is a tremendous leap forward in terms of consolidating the science around wireless communication technology. Dr Panagopoulos' presentation is well thought out and presented in a very clear and thorough manner. I hope others move forward from this wonderfully thought-provoking paper. Dr Panagopoulos clearly is emerging as a thought-leader in this field".

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