Chapter 1
Mobile Telephony EMFs Effects on Insect Ovarian Cells. The Necessity for Real Exposures Bioactivity Assessment. The Key Role of Polarization, and the "Ion Forced-Oscillation Mechanism"

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Abstract Exposure of *Drosophila melanogaster* young adult insects to Electromagnetic Fields (EMFs)/Radiation (EMR) emitted by an active GSM (Global System for Mobile telecommunications) mobile phone handset during a usual "talk" operation for a few minutes daily for 2–5 days, revealed an impressive decrease (up to 57%) in reproductive capacity (fecundity) (Panagopoulos et al. 2004). That effect directed us to focus our next studies on the effects of this type of EMF/EMR on the DNA and proteins of the insect's reproductive cells (gametes). More specifically, we focused on the effects on the female ovarian cells. We used the TUNEL (Terminal deoxynucleotide transferase dUTP Nick End Labeling) assay, to detect fragmented DNA in the ovarian cells. Moreover, we used the Rhodamine-conjugated Phalloidin staining assay, to detect possible damage in the actin cytoskeleton of the ovarian cells. We found a high degree of DNA fragmentation in the nuclei of ovarian cells of the exposed insects (up to +55% compared to the sham-exposed insects) (Panagopoulos et al. 2007a). The DNA fragmentation was highly dependent on the intensity of radiation (distance from the handset) and was found to be maximum for intensities higher than 250 μW/cm^2 (in close proximity with the handset) and within a "window" around 10 μW/cm^2 (at 20–30 cm distance from the handset) (Panagopoulos et al. 2010). The DNA fragmentation in the nuclei of the exposed ovarian cells was found to be accompanied by actin cytoskeleton damage (Chavdoula et al. 2010). These effects caused a destruction of a significant percentage of egg chambers in the ovaries of the

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exposed females (Panagopoulos 2012a). New data (Panagopoulos et al. 2015a, b) suggest that the continuous and unpredictable variability of the mobile telephony signals, in combination with the fact that they are totally polarized (just like every type of man-made EMF), and the inclusion of Extremely Low Frequencies (ELF) - due to pulsing and modulation of the microwave carrier - in all modern mobile telecommunication microwave signals, constitute the main reasons for their intense bioactivity. A significant opposition is found between the results of experimental studies employing real exposures of biological samples from commercially available mobile phones, and the results of studies employing simulated exposures from generators or "test" phones as suggested by health authorities (Health Protection Agency 2012; IARC 2013). While experimental studies employing simulated EMF-emissions present a strong inconsistency among their results with nearly 50% of them reporting no effects, studies employing real-life emissions demonstrate an almost 100% consistency in showing adverse effects (Panagopoulos et al. 2015a). Finally, in the present chapter we show why polarized (man-made) EMFs are significantly more bioactive than natural (unpolarized) ones, and we describe the "Ion Forced-Oscillation Mechanism" for the action of polarized EMFs on biological systems.

Keywords Electromagnetic fields • Microwave radiation • Mobile phones • Real exposures • Biological effects • DNA damage • Protein damage • Reproduction • Ovarian cells • Insects • *Drosophila melanogaster* • Polarization • Mechanism of action

1.1 Introduction

Microwaves are called the electromagnetic waves produced by electronic oscillators of human technology, with frequencies higher than those which can be reflected by the ionosphere (thus higher than ~ 200 MHz) and up to the low limit of infrared (~ 300 GHz). Microwaves occupy the higher frequency part of a wider category called Radio-Frequency (RF) electromagnetic waves and start from frequencies around 10 kHz. In other words, microwaves are not reflected by the ionosphere unlike the electromagnetic waves of lower frequencies, and thus the receiving and the emitting microwave antennas need to have optical contact between them. The continuous demand for increasing the volume of transmitted information by microwave antennas leads to the continuous increase in the microwave frequencies, and the consequent approximation closer to the low limit of infrared (Lioliousis 1979).

In addition to the artificially produced microwaves which constitute the vast majority in our modern environment, there are natural microwaves of broad spectrum (10 MHz – 10 GHz) and cosmic origin which reach the earth's surface at very low intensities (~10^{-17} mW/cm^2/MHz) (Presman 1977). These are called cosmic microwaves. An important difference between man-made and natural (cosmic) microwaves is that the first are totally polarized like every form of man-made electromagnetic radiation (EMR)/field (EMF), while the second are unpolarized

just like every form of natural EMR/EMF. This difference is of decisive importance when it comes to the issue of the mechanism of how microwaves (and EMFs in general) interact with living matter, as we shall explain later on.

One of the main applications of man-made microwaves is in modern telecommunications, such as digital mobile telephony, domestic cordless phones (DECT-Digitally Enhanced Cordless Technology), or internet connection wireless devices and local wireless networks (Wi-Fi), or satellite communications. Radars employ microwaves as well.

With the tremendous development of telecommunications during the past few decades, the levels of RF/microwave radiation are exponentially increased especially in modern urban environments, and there is consequent increasing concern among the scientists and the public about the possible adverse health effects of these radiation types.

It is very important to emphasize that all (wireless) telecommunication devices emitting microwaves, emit extremely low frequencies (ELF) as well since this is absolutely necessary for pulsing and modulating the RF carrier signal in order to be able to transmit increasing amount of information (Tisal 1998; Hyland 2000; Hillebrand 2002; Tuor et al. 2005; Curwen and Whalley 2008). In other words, exposure to EMFs from mobile or cordless phones, Wi-Fi and other wireless communication devices, is simultaneous exposure to several frequencies both RF and ELF. It is suspected that the ELF emissions included in all telecommunication signals are those more responsible for the biological/health effects, and not the carrier RF signal itself (Panagopoulos et al. 2015b).

Hundreds of biological, clinical, and statistical/epidemiological studies investigating the potential health effects of microwave radiation are carried out so far. A first look shows contradicting results (Verschaeve 2009; Verschaeve et al. 2010; Bourthoumieu et al. 2010; Vijayalaxmi 2012; Health Protection Agency 2012; Ingole and Ghosh 2012; Ros-Llor et al. 2012; Karaca et al. 2012; Vignera et al. 2012; Waldmann et al. 2013; Cucurachi et al. 2013; IARC 2013; Balmori 2014; Paul et al. 2015; Lerchl et al. 2015; Roggeveen et al. 2015; Morgan et al. 2015; Singh et al. 2016; Manna and Ghosh 2016; Shahin-Jafari et al. 2016). But a closer look shows that there is a significant difference between studies employing simulated mobile phone signals from generators or "test" phones programmed to emit constant signals of fixed frequency, waveform and output power, and studies employing real signals from commercially available mobile phones or other microwave devices. While experimental studies employing simulated EMF-emissions present a strong inconsistency among their results with nearly 50% of them reporting no effects (Health Protection Agency 2012; Vijayalaxmi 2012; IARC 2013), studies employing real mobile phone exposures demonstrate an almost 100% consistency in showing adverse effects (Panagopoulos et al. 2015a). This consistency is in addition supported by studies showing association with brain tumors, symptoms of un-wellness, and declines in animal populations. These statistical/epidemiological studies concern exposures to real emissions as well, mostly from mobile phone handsets and base station antennas (Navarro et al. 2003; Salama and Abou El Naga 2004; Kundi 2004; Hutter et al. 2006; Balmori

2005, 2010; Balmori and Hallberg 2007; Everaert and Bauwens 2007; Khurana et al. 2009; Blettner et al. 2009; Kundi and Hutter 2009; Viel et al. 2009; Hardell et al. 2007, 2013; Bhattacharya and Roy 2014; Singh et al. 2016).

Determination of realistic exposures from mobile phones and other wireless telecommunication devices is one of the most important issues in scientific studies examining the biological/health effects of microwaves, since it is key to defining public health protection. The situation becomes confusing by the divergent results reported in the literature which can very well be due to unrealistic exposures, which in turn leads to wrong conclusions and ineffective and misdirected regulations (Panagopoulos et al. 2015a).

While the International Agency for Research on Cancer (IARC) has classified both ELF and RF EMFs as possibly carcinogenic to humans (IARC 2002, 2013), in its 2013 report IARC criticized and excluded from consideration experimental studies that used commercially available mobile phone handsets in exposing biological samples, as having "unreliable dosimetry" without further scientific explanation (IARC 2013). Similarly the Health Protection Agency criticized this exposure methodology reporting that the exposure is "highly variable" with "lack of control" due to network reasons (number of subscribers each moment) and movement of the animals within the vials/boxes in case of freely moving animals, but recognizes that restriction of the animals during the exposures will result in additional stress. Their critique recommended that exposures should be performed by devices or handsets set to produce emissions at fixed frequency and output power by use of engineering or hardware controls (Health Protection Agency 2012). In both reports the critique was based on the fact that real mobile phone emissions always include large variations in their intensity, frequency, etc., especially in the near-field of the antenna.

But billions of mobile phone users are daily exposed for increasing periods to real emissions from their handsets in the near-field of the antenna in contact with their ears/bodies, not to any simulated emissions with fixed parameters. Is it then scientifically correct to study the effects of a "highly variable" field by using fields with fixed parameters? In our opinion, it is definitely not. Especially since the varying nature of the field seems to be an important reason for its increased biological activity.

A plausible biophysical mechanism explaining how man-made EMFs can alter cell function by irregular gating of electrosensitive ion channels on the cell membranes is published (Panagopoulos et al. 2000a, 2002, 2015b) and verified by numerical test while previously suggested other mechanisms failed to pass the same test for realistic conditions within living cells (Halgamuge and Abbetrathne 2011). This mechanism known as "Ion Forced-Oscillation Mechanism" is based on the property of polarization of all man-made EMFs. Any externally applied polarized EMF will cause a parallel and in phase forced-oscillation of all charged particles - such as the mobile/free ions existing in large concentrations in all living cells - which can then exert constructive (additive) forces on any other charge such as the voltage-sensors of electrosensitive ion channels. These additive forces can be much more effective in gating (opening or closing) this type of ion channels than

the chaotic forces in every possible direction exerted by the same ions due to their random thermal motion, or due to any random individual oscillations caused by any unpolarized (natural) EMF.

In the present chapter we review a series of experiments with real mobile phone exposures that show important effects on DNA in insect ovarian cells. Then we examine the opposition between the results of studies employing real telecommunication microwave emissions and the results of studies employing exposures by simulated microwave emissions, as we think this is a point of utmost importance. Finally, we analyze the differences in bioactivity between polarized and unpolarized EMFs emphasizing on mobile telephony EMFs, and we provide an explanation of the reported biological effects on a biophysical basis according to the "Ion Forced-Oscillation Mechanism" for man-made (polarized) EMFs.

1.2 Effect of GSM Mobile Phone EMFs on Insect Ovarian Cells

1.2.1 Exposure Device and EMFs Measurements

We were the first to use a commercially available mobile phone handset to expose biological samples to microwave radiation (Panagopoulos et al. 2000b, 2004; Panagopoulos and Margaritis 2002). The reason was (and still is) obvious: We wanted to test the effects of the real EMFs which expose daily billions of humans globally.

In each study, in spite of the fact that we used a commercially available and therefore approved for the market mobile phone handset, we measured the intensity of radiation in the RF/microwave region during a normal phone-conversation. Moreover, we measured the electric and magnetic field intensities in the ELF region, since it is known that GSM emissions except for their carrier microwave frequency around 900, 1800 (Europe), or 1900 (North America) MHz, employ ELF pulsing fields at 217 Hz, and other even lower ELFs such as 8.34 Hz (Tisal 1998; Hyland 2000). The measurements and the experiments were always carried out with the battery of the handset fully charged, and at the same bench within the lab with full signal reception, and same positions of all items around (Panagopoulos et al. 2004, 2007a, b, 2010).

From the first sets of measurements we found out that when the mobile phone user talked during a phone conversation ("talk signal" or "voice-modulated signal" or "GSM basic") the intensities of the emissions increased about tenfold than when the user listened and there was no sound in the room ("listening mode" or "non-modulated signal" or discontinuous transmission mode-DTX). The biological effect of the voice-modulated exposures on the fecundity of young adult Drosophila insects was similarly much more intense than the corresponding effect of the DTX exposures with the same handset. While the DTX 900 MHz GSM exposures

Table 1.1 GSM 900 and 1800 radiation and field intensities ± SD, in the microwave and ELF bands averaged over 6 min of voice-modulated emission, for different distances from a mobile phone antenna[a]

Distance from mobile phone Antenna (cm)	**GSM 900** Radiation Intensity at 900 MHz, (mW/cm^2)	GSM 900 ELF Electric Field Intensity, (V/m)	GSM 900 ELF Magnetic Field Intensity, (mG)	**GSM 1800** Radiation Intensity at 1800 MHz, (mW/cm^2)	GSM 1800 ELF Electric Field Intensity, (V/m)	GSM 1800 ELF Magnetic Field Intensity, (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	–	–

[a]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

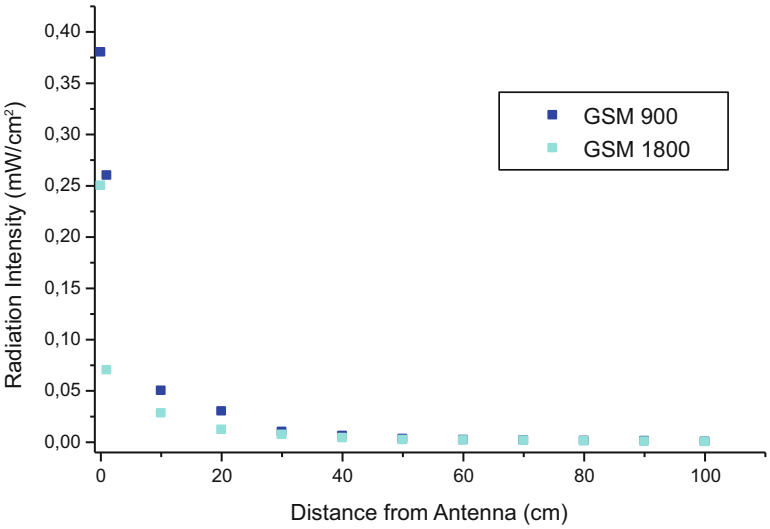

Fig. 1.1 Mobile phone radiation intensity values for GSM 900 and GSM 1800 MHz, according to the distance from the mobile phone antenna

of six min daily for 5 days reduced fecundity by approximately 18% compared to the unexposed insects, the voice-modulated ("talk") corresponding exposures reduced fecundity by approximately 53% respectively (Panagopoulos et al. 2004).

Representative intensity measurements of voice-modulated emissions averaged over 6 min, both in the microwave and ELF bands, and at different distances from the antenna of a mobile phone operating at 900 MHz and (the same handset with a different SIM - "subscriber identity module" card) at 1800 MHz, are shown in Table 1.1 (Panagopoulos et al. 2010). All mesured intensities are within the exposure criteria established by health authorities (ICNIRP 1998). The average radiation intensity levels of the microwave carrier (900 and 1800 MHz) according to the distance from the mobile phone antenna are graphically represented in Fig. 1.1. The standard deviations (SD) of the different intensity values given in Table 1.1 are not shown in Fig. 1.1. The ELF electric and magnetic field values also are given in Table 1.1 but not represented in the diagram as they are more or less proportional to the radiation intensity values.

1.2.2 Experimental Animals

Our experimental animals were *Drosophila melanogaster* flies, strain Oregon R wild-type, held in glass bottles with their food and kept in an incubator at 25 °C, with 12-h periods of light and darkness, and 70–75% relative humidity slightly varying from one experimental series to another but kept constant during each specific

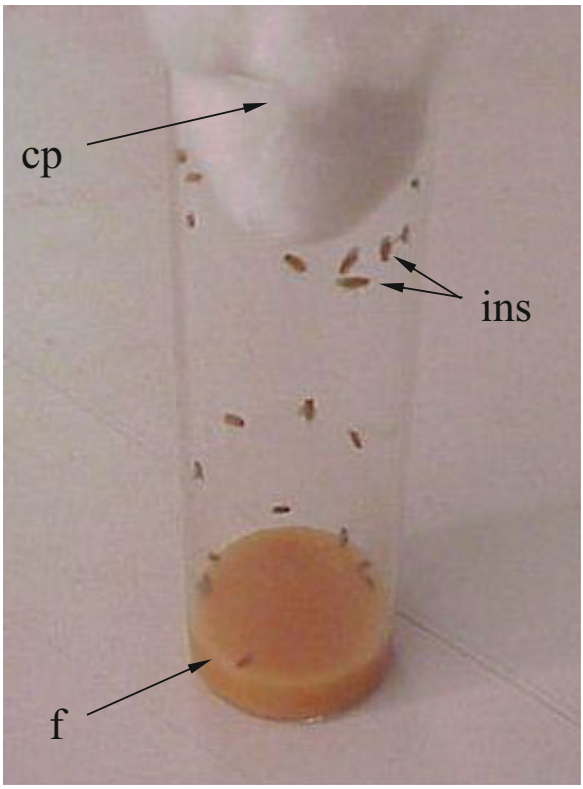

Fig. 1.2 A typical glass vial used in our experiments, containing a group of 20 insects (ins) and food (f), closed with cotton plug (cp)

experimental series. In each individual experiment we collected newly emerged adult flies which we separated into different groups of usually ten males and ten females (except when otherwise mentioned), and put within identical 50-ml cylindrical glass vials with 2.5 cm diameter and 10 cm height, with equal amounts of food of identical quality prepared at the same time, as previously described (Panagopoulos et al. 2004). Figure 1.2 shows one group of flies within a 50-ml glass vial with food.

The oogenesis of this insect is a model biological system, with a very good timing of developmental processes under controlled conditions (King 1970; Panagopoulos 2012b).

The reproductive capacity was assessed by the number of first filial generation (F_1) pupae derived from eggs laid during the first 3 days of the insect's maximum fecundity (oviposition). This number - under the conditions of our experiments - equals the number of fertilized laid eggs, 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, 2007a, b, 2010, 2013a; Panagopoulos 2012b).

1.2.3 Exposure Procedure

In each experiment, the collected newly emerged flies from the stock were anesthetised very lightly with diethyl ether and separated males from females. We put the collected flies in groups of usually ten males and ten females (twenty flies in each group) in the 50-ml vials with food, closed with cotton plugs.

In each experiment, the males and the females of each group were kept and exposed/sham-exposed in separate vials during the first 48 h (two separate vials for each exposed/sham-exposed group). Newly emerged adult *Drosophila* flies are not sexually mature immediately after eclosion. Male flies become sexually mature about 12 h after eclosion and females about 45 h after eclosion (King 1970; Panagopoulos 2012b). Keeping males separately from females for the first 48 h of each experiment ensures that when the two sexes are put together they are in complete sexual maturity and ready for immediate mating and laying of fertilized eggs. This in turn ensures that all laid eggs during the next 72 h are fertilized, and this minimizes variability in oviposition counts.

After the first 48 h, the males and females of each group (20 flies) were lightly anesthetized again and put together in a single vial with fresh food (one vial for each group) to continue being daily exposed/sham-exposed and allowed to mate and lay eggs for the next 72 h, during which the insect's oviposition is at its maximum (King 1970; Bos and Boerema 1981; Panagopoulos et al. 2004; Panagopoulos 2012b).

The exposure to the GSM fields started from the first day of the experiments, 1 h after the insects were fully awaken from the first anesthesia and - in most experiments - lasted for 6 min daily in a single exposure. In experiments testing the effect of exposure duration the daily exposures ranged from 1 to 21 min between the different groups (Panagopoulos and Margaritis 2010a). The exposures took place for the first 2–6 days of the insects' adult lives, depending on the experimental protocol. In experiments comparing the effect of the EMF on the reproductive capacity between the two sexes the exposures took place only for the first 2 days (while the two sexes in all groups were separated) (Panagopoulos et al. 2004). In experiments testing the effect on reproductive capacity of both sexes the EMF exposures took place for the first 5 days of the insects' adult lives (Panagopoulos et al. 2004, 2007b; Panagopoulos and Margaritis 2010a, b). In experiments testing the effect on DNA and proteins, there was an additional exposure in the sixth day before dissection and treatment of the ovaries (Panagopoulos et al. 2007a, 2010; Chavdoula et al. 2010).

In the different sets of experiments we separated the insects into: a) the Exposed group(s) and b) the Sham-Exposed group(s). The sham-exposed groups had identical treatment with the exposed groups, except that the mobile phone handset was turned off during the sham-exposures. We performed several replicate experiments for each different experimental protocol in the different studies.

The number of groups depended on the specific experimental protocol. For example, in the first experiments testing the effect of GSM radiation on

reproduction (fecundity) we separated the insects into two groups (one exposed and one sham-exposed) (Panagopoulos et al. 2000b, 2004; Panagopoulos and Margaritis 2002). In the set of experiments testing the dependence of fecundity/DNA damage on the intensity of the radiation/fields, we exposed simultaneously 12 groups at 0, 1, 10, 20,. . ., 100 cm distance from the mobile phone, and thus we had 12 Exposed groups plus one sham-exposed (13 groups in each replicate experiment) (Panagopoulos et al. 2010). In the set of experiments testing the effect of exposure duration on reproductive capacity (Panagopoulos and Margaritis 2010a), we separated the insects into six groups: (a) a group exposed to the GSM EMFs for 1 min, (b) a group exposed for 6 min, (c) a group exposed for 11 min, (d) a group exposed for 16 min, (e) a group exposed for 21 min, and (f) the sham-exposed group (SE). [Details for each study can be found in the original publications].

The temperature in the laboratory during the experimental procedures was maintained at 24 ± 0.5 °C. No detectable temperature increase took place within the vials with the insects or within the mass of the food due to the exposures at all distances between the handset and the vials, and with all tested exposure durations. That was checked during preliminary exposures in each study by the use of a Hg-thermometer with 0.05 °C sensitivity.

1.2.4 Reproductive Capacity Assessment

When the male and female flies of each group had been together in the same vial for 3 days (72 h), that is after 5 days from the beginning of each experiment, the flies were removed from the glass vials. These vials containing the developing embryos were then kept in the culture room for at least six additional days without any further EMF exposure/sham-exposure.

After the six additional days that the vials without the parental flies were kept in the culture room without exposure, most F_1 embryos (deriving from the fertilized laid eggs) were at the stage of pupation, where they could be clearly seen macroscopically and easily counted on the walls of the glass vials. [At the last larvae stages the larvae go out of the food, crawl up on the walls of the glass vials and get immobilized there to become pupae (Panagopoulos 2012b)].

By counting the F_1 pupae 11 days after the beginning of each experiment (2 days separated males from females, plus 3 days mating and egg laying, plus 6 days for the F_1 embryos to reach the pupation stage), we had a representative estimate of each group's reproductive capacity. In this way, instead of counting eggs under a stereo microscope on the surface and within the food which is subject to large errors, we simply counted the number of pupae with bare eyes on the walls of the glass tubes with no error at all. That was also an important innovation in assessing the fecundity of this insect, in addition to keeping the males and females of each group in separate vials for the first 48 h of each experiment (Panagopoulos et al. 2004). All procedures and counts were performed blindly (the experimenter did not know the identity of the groups).

The blinded scoring was repeated for the next 2 days in order to ensure that no larvae had remained within the food and all F_1 embryos had reached the pupation stage and were counted. In this way, no error can occur in the scoring of F_1 pupae.

Comparing the numbers of F_1 pupae between exposed and sham-exposed groups, we get a credible estimate of the effect of EMF exposure on reproductive capacity.

1.2.5 Assessment of DNA Fragmentation in the Ovarian Cells

Oogenesis in *Drosophila* starts during the last stages of pupation. At eclosion, the ovaries of female flies contain already eggs at the first preyolk stages. The eggs develop through 14 distinct stages until they are ready to be fertilized and laid. This process for every egg lasts about 48 h at 25 °C (King 1970; Panagopoulos 2012b).

In order to investigate the ability of the EMFs to damage DNA during early and mid oogenesis (when programmed cell death does not occur) we applied the TUNEL (Terminal deoxynucleotide transferase dUTP Nick End Labeling) assay which is a marker for DNA fragmentation. In this assay fluorescein dUTP (a fluorescent substance) binds through the action of terminal transferase (an enzyme that catalyzes the specific biochemical reaction), onto broken chains (phosphodiester bonds) of genomic DNA which then become labelled by this fluorescent substance. The label incorporated at the damaged sites of DNA is visualized by fluorescence microscopy, as it emits characteristic visible radiation when it is irradiated by ultraviolet ("TUNEL-positive signal").

In the studies testing the effect of EMFs on ovarian DNA and proteins there was an additional exposure in the morning of the sixth day of each individual experiment as already reported. After 1 h from this additional exposure, the parental flies were removed from the glass vials, and they were anesthetized and sacrificed. The ovaries from all exposed and sham-exposed female flies were dissected in Ringer's solution and either were collected intact to measure the ovarian size (Panagopoulos 2012a), or separated into individual ovarioles and egg chambers from which the egg chambers of stages 11–14 were excluded (Panagopoulos et al. 2007a, 2010, 2013a; Chavdoula et al. 2010; Panagopoulos 2016). In egg chambers of stages 11–14 programmed cell death takes place physiologically in the nurse and follicle cells (McCall 2004; Nezis et al. 2000, 2002). Thereby we kept and treated ovarioles and individual egg chambers from germarium up to stage 10. A few representative samples were selected from both ovaries of all the exposed and sham-exposed females in each experiment. The selected samples of ovarioles and egg chambers were then fixed for the TUNEL assay or the rhodamine-conjugated phalloidin staining assay, to search for DNA damage or actin cytoskeleton damage (Panagopoulos et al. 2007a, 2010, 2013a; Chavdoula et al. 2010; Panagopoulos 2012a, 2016).

The application of the TUNEL assay is described in detail in Panagopoulos et al. (2007a). The samples were viewed under a Nikon EZ-C1 fluorescence microscope (Nikon Instruments, Japan). Samples from different 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.

1.2.6 Assessment of Actin Cytoskeleton Damage in the Ovarian Cells

In this set of experiments - a detailed description can be found in Chavdoula et al. (2010) - we examined whether the GSM exposure 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 a known aspect of cellular death during both apoptosis and necrosis. For this we applied the rhodamine-conjugated phalloidin staining assay. Rhoramine is a fluorescent substance that gets attached to the actin cytoskeleton through the binding of phalloidin. When this is done, the morphology of the actin cytoskeleton is observed by fluorescence microscopy.

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. The simultaneous observation of DNA fragmentation and actin cytoskeleton damage 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 (Nikon Instruments, Japan) (Chavdoula et al. 2010).

1.2.7 Results

1.2.7.1 GSM EMFs Dramatically Decrease Reproduction

The results of the experiments investigating the effect on reproduction showed that the 6 min daily exposure to GSM EMFs in “talk” mode at close proximity (near-field) to the mobile phone antenna during the first 5 days of the insects’ adult lives, induced an impressive average decrease by 53.01% in their reproductive capacity (fecundity). The corresponding average decrease in DTX mode was 18.24%. The GSM field was found to decrease the reproductive capacity in both male and female insects (Panagopoulos et al. 2004).

1.2.7.2 The Decrease in Reproduction Is Due to Elimination of Egg Chambers After DNA Fragmentation and Consequent Cell Death of Their Constituent Cells

The dramatic decrease in reproductive capacity was found to be due to destruction of significant numbers of egg chambers after severe DNA damage in the reproductive cells (gametes). This was found both for GSM 900 and GSM 1800 radiation types. The effect with GSM 900 was more intense than the corresponding effect with GSM 1800 mainly due to its higher intensity, and in a much lesser degree due to its lower carrier frequency (Panagopoulos et al. 2007a, b).

Figure 1.3 shows an ovariole of a sham-exposed female insect with no DNA fragmentation at all developmental stages. This TUNEL-negative picture was representative of the ovarioles of all sham-exposed insects. Figure 1.4 shows an ovariole of an exposed insect with DNA fragmentation (TUNEL-positive signal) only at the two most sensitive developmental stages/checkpoints (germarium and stage 7–8), and no DNA fragmentation (TUNEL-negtive signal) at all other developmental stages. Figure 1.5 shows ovarioles of exposed insects with DNA fragmentation (TUNEL-positive signal) at all developmental stages from germarium up to stage 8. Figure 1.6 shows a stage 10 egg chamber with DNA fragmentation (TUNEL-positive signal) in the nurse and follicle cells.

What was novel and impressive with these findings was that the GSM EMFs induced DNA fragmentation, (1) at all developmental stages of early and mid oogenesis while previously examined stress factors such as starvation or chemicals were found to induce DNA damage only at the two most sensitive developmental stages/checkpoints (germarium and stages 7–8). (2) The DNA fragmentation was observed in all three types of egg chamber cells (nurse cells, follicle cells, and the oocyte), while the above previously examined stress factors induced DNA damage only in the nurse and follicle cells, not in the oocyte (Nezis et al. 2000, 2002; Drummond-Barbosa and Spradling 2001; Panagopoulos et al. 2007a, 2010).

1.2.7.3 The DNA Damage in the Gametes and the Consequent Decrease in Reproduction Is Primarily Dependent on the Intensity of the GSM Fields

The effects on reproduction and on DNA damage decreased non-linearly with increasing distance from the mobile phone handset, or with decreasing EMFs intensities. The effects were maximum for radiation intensities higher than 250 $\mu W/cm^2$ (close proximity with the mobile phone antenna) and within a "window" around 10 $\mu W/cm^2$ (20–30 cm from the handset) (Panagopoulos et al. 2010). More specific experiments showed that the discovered "window" of maximum bioactivity was not dependent on any specific position but on the specific of EMF-intensity values (Panagopoulos and Margaritis 2010b).

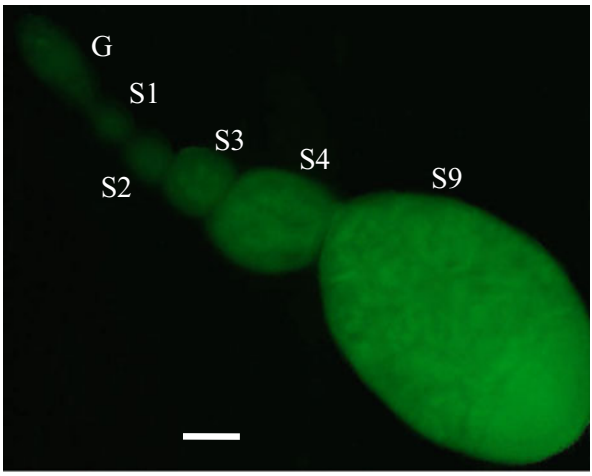

Fig. 1.3 Typical TUNEL-negative fluorescent picture of an ovariole of a sham-exposed female insect, containing egg chambers from germarium to stage 9. Bar: 10 μm

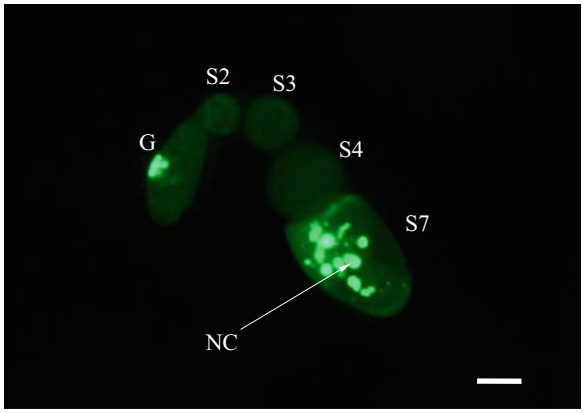

Fig. 1.4 Ovariole of an exposed female insect with TUNEL-positive signal in the nurse cells (NC) only at the two check points, germarium (G) and stage 7 egg chamber, and TUNEL-negative intermediate stages. Bar: 10 μm

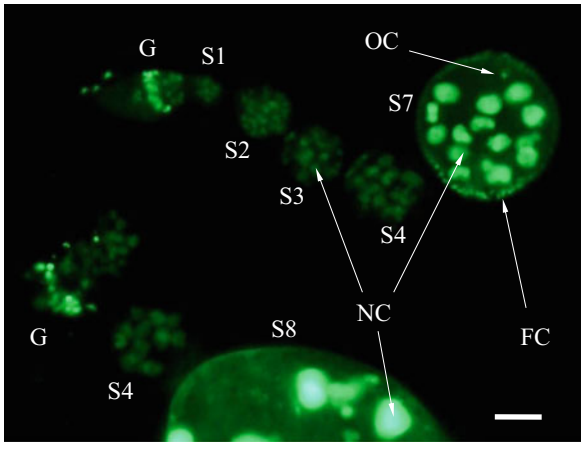

Fig. 1.5 Ovarioles of an exposed female insect with fragmented DNA at all developmental stages from germarium (G) to stage 8, and in all kinds of egg chamber cells, (NC: nurse cells, FC: follicle cells, OC: oocyte). Bar: 10 μm

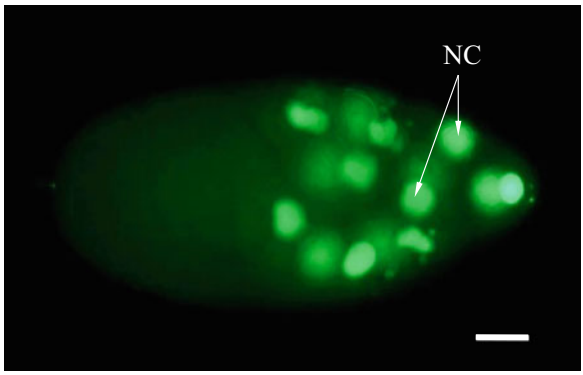

Fig. 1.6 A stage 10 egg chamber of an exposed female insect with fragmented DNA in the nurse cells, (NC). Bar: 10 μm

Figures 1.7 and 1.8, show the percentage of egg chambers with fragmented DNA and the reproductive capacity, in accordance to the distance of the vials from the mobile phone handset for GSM 900 and 1800 respectively. The effects manifested for intensities down to 1 $\mu W/cm^2$ corresponding to approximately 1 m distance from the handset (Panagopoulos et al. 2010).

The carrier frequency of the GSM radiation (900 or 1800 MHz) was found to have only a minimal effect on ovarian DNA fragmentation and the reproductive capacity, with 900 MHz being slightly more bioactive than 1800 MHz under the same EMFs intensities (Panagopoulos et al. 2007a, b, 2010). For example with radiation intensity approximately 285 $\mu W/cm^2$, GSM 900 decreased fecundity by 32.75% in relation to the sham-exposed group while GSM 1800 decreased fecundity by 31.08% (Panagopoulos et al. 2007b). [The "Ion Forced-Oscillation Mechanism" described below shows that the bioactivity is inversely proportional to the frequency of the EMF (Eq. 1.24)].

1.2.7.4 The Effect on Reproduction Increased with Increasing Daily Exposure Durations

In this set of experiments the insects were exposed at the far field of the antenna where previous experiments (Panagopoulos et al. 2010) showed that we have the largest effect, that is at 30 cm distance from the handset for GSM 900 or at 20 cm distance for GSM 1800 where the intensity of both radiation types was ~10 $\mu W/cm^2$. The groups were exposed simultaneously, placed along constant intensity sectors of an arc with a 30- or 20-cm radius - for GSM 900 or GSM 1800 respectively - at the center of which the handset was placed. 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. (Panagopoulos and Margaritis 2010a).

The experiments showed that even 1 min of daily exposure during the first 5 days of the insects' adult lives is capable to induce a significant decrease in reproduction on the order of 35% in relation to the sham-exposed insects. Then, as the duration of

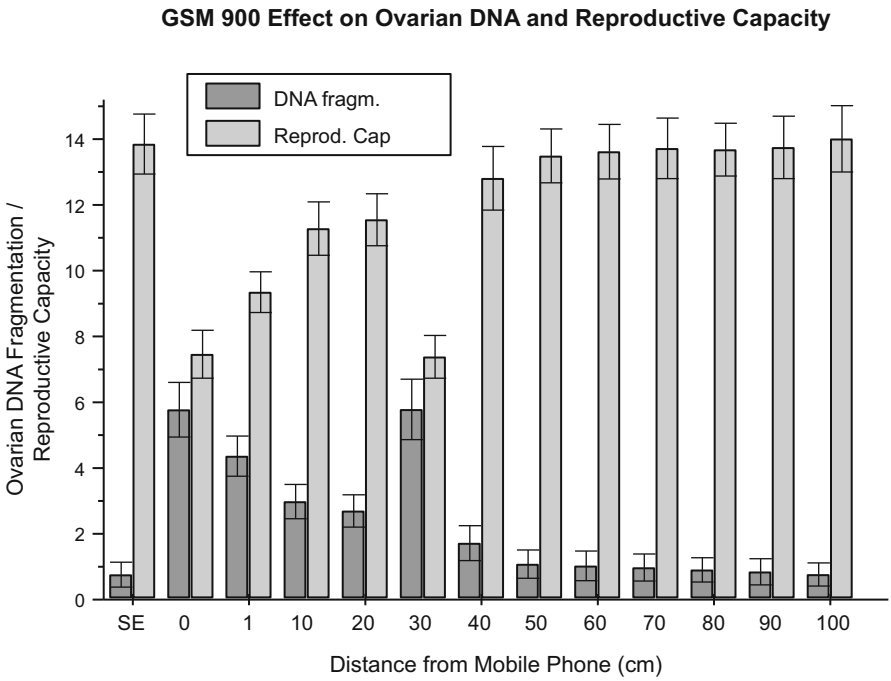

Fig. 1.7 [Mean ratio of ovarian DNA fragmentation (number of TUNEL- positive to total number of egg-chambers) ± SD]×10, and Mean number of F_1 pupae per maternal insect ± SD, versus Distance from mobile phone antenna (cm), for GSM 900

the single daily GSM exposure gradually increased from 1 to 21 min, fecundity was decreasing almost linearly. The effect was shown both with GSM 900 or GSM 1800 fields, but again, GSM 900 was found to be slightly more bioactive than GSM 1800 (Panagopoulos and Margaritis 2010a).

Figure 1.9 shows the decrease in reproduction in relation to the exposure duration for GSM 900, and 1800.

1.2.7.5 The DNA Damage in the Exposed Ovarian Cells Was Found to be Accompanied by Actin Cytoskeleton Damage

The same cells that suffered DNA fragmentation after exposure to the GSM fields, also suffered actin cytoskeleton disorganization as it was shown by double staining of the ovarian samples with both TUNEL and rhodamine-conjugated phalloidin, and observation by two different lasers under a Confocal Laser Scanning Microscope. The percentages of egg chambers with actin cytoskeleton damage in both exposed and sham-exposed samples were very close to corresponding percentages of DNA damage. The analysis by double-staining with TUNEL and rhodamine conjugated phalloidin revealed that DNA fragmentation and actin-cytoskeleton

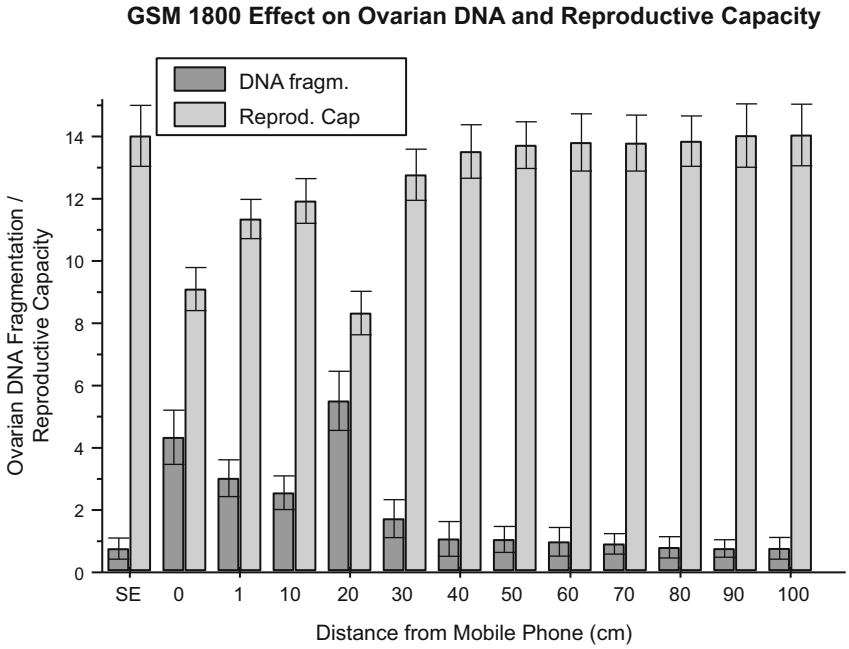

Fig. 1.8 [Mean ratio of ovarian DNA fragmentation (number of TUNEL- positive to total number of egg-chambers) ± SD]×10, and Mean number of F_1 pupae per maternal insect ± SD, versus Distance from mobile phone antenna (cm) for GSM 1800

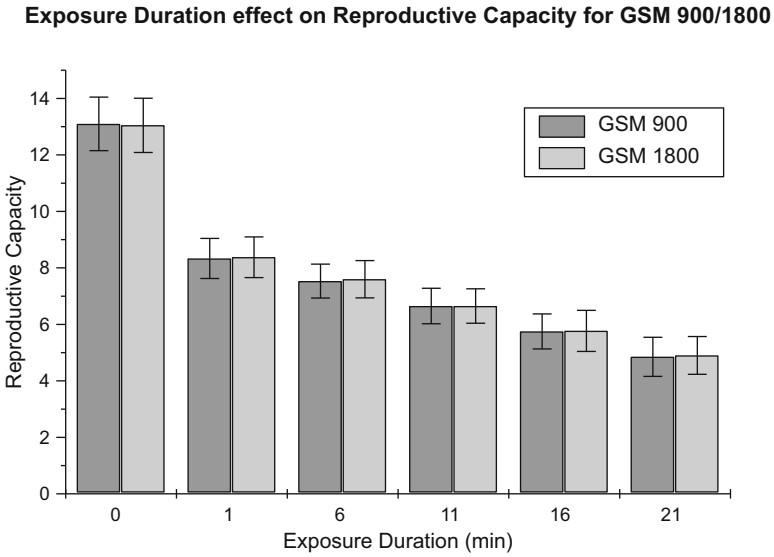

Fig. 1.9 Reproductive capacity ± SD of groups exposed to GSM 900 or 1800 fields for different daily exposure durations (1, 6, 11, 16, and 21 min) and of sham-exposed groups (0 min)

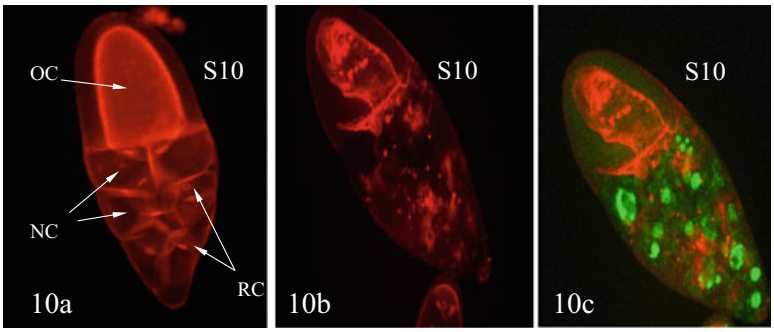

Fig. 1.10 (**a**) A stage 10 egg chamber from a sham exposed insect, treated with rhodamine-conjugated phalloidin assay, with normal cytoskeleton morphology. Characteristic features of the actin cytoskeleton like the ring channels (RC) can be observed. *NC* nurse cells, *OC* oocyte. (**b**) A stage 10 egg chamber of an exposed insect with disorganized actin cytoskeleton. (**c**) The same stage 10 egg chamber as in (**b**), 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

disorganization coincide in the affected egg chambers. Since the actin cytoskeleton damage is a known sign of cell death, this result shows that the affected cells most usually do not survive, and are led to apoptosis (Chavdoula et al. 2010).

Figure 1.10a shows the actin cytoskeleton of a stage 10 egg chamber of a sham-exposed insect with normal morphology. Specific features such as the ring channels can be clearly seen. Figure 1.10b shows a stage 10 egg chamber of an exposed insect with damaged actin cytoskeleton. Figure 1.10c shows that the actin cytoskeleton damage accompanies the DNA damage in the same cells (Chavdoula et al. 2010).

1.2.7.6 The Ovarian Development in the Exposed Females Was Significantly Decreased

Due to the DNA and actin cytoskeleton damage induced in the egg chamber cells by the GSM EMFs and the consequent cell death in the affected cells, a large percentage of the developing egg chambers in the ovaries of exposed females were eliminated. As a result, the ovarian size of the exposed females was significantly decreased compared to the ovarian size of the unexposed insects. This we showed with just eclosed virgin female adult insects which we exposed for 6 min 3 h after eclosion, and subsequently every 10 h. Then the ovaries of exposed and sham-exposed insects were dissected and photographed under the same magnification at different developmental stages during the first 2 days (48 h) of their adult lives which is a little more than the time needed for the complete development of the first egg chambers in the ovaries under certain controlled laboratory conditions (~ 45 h). The average ovarian size was compared between exposed and sham-exposed insects according to the photographs. Figure 1.11a, b show ovaries of

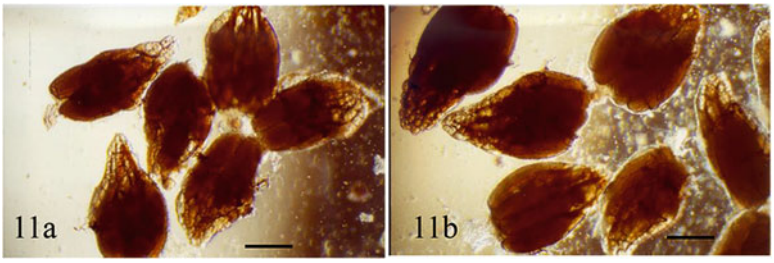

Fig. 1.11 Ovaries of exposed (**a**) and sham-exposed (**b**) female insects 45 h after eclosion. Ovaries of exposed insects are significantly smaller than those of sham-exposed, due to elimination of egg chambers after cell death of their constituent cells induced by the GSM field. Bars: 50 μm

exposed and sham-exposed insects respectively, 45 h after eclosion. The average "descriptive ovarian size" (DOS) of the exposed ovaries was decreased by 29.75% compared to the sham-exposed (Panagopoulos 2012a).

1.2.7.7 Microwave GSM Field Was Found to be Considerably More Bioactive than ELF Magnetic Field or Pulsed Electric Field. The Differential Bioactivity Between Different Types of EMFs Revealed a Differential Sensitivity Between Different Types of Cells, and Between the Two Checkpoints of Oogenesis

The exposure to the GSM field induced more DNA damage on the same biological system than exposure to 50 Hz alternating magnetic field with intensities 1–21 G, or 8 kHz electric field pulsed on 44.4 Hz with intensities 100–400 kV/m, even though the exposures to these fields were of significantly longer daily exposure durations, and of intensities exceeding the environmentally accounted ones (Panagopoulos et al. 2007a, 2013a; Panagopoulos 2016). While the GSM 6-min exposures for a few days induced DNA damage in the ovarian cells up to +55% compared to the sham-exposed cells, the 50 Hz alternating magnetic field induced DNA damage up to +7.52% with the strongest intensity (21 G), and the pulsed electric field up to +3.87% with the strongest intensity (400 kV/m).

This differential bioactivity of the different types of EMFs, revealed that there is a differential sensitivity between the three types of egg chamber cells (NC-nurse cells, FC-follicle cells, OC-oocyte). It was found that cellular sensitivity to EMFs decreases (or resistivity increases) from the NC to the FC, and from the FC to the OC. In other words it was found that the OC (the single cell in each egg chamber that will give the offspring after fertilization) is the most resistant cell type, and the NC the most vulnerable from the three cell types to EMF-exposure (Panagopoulos 2007a, 2013a; Panagopoulos 2016).

Moreover, it was found that the two checkpoints of oogenesis (germarium, and stage 7–8) present different sensitivities in regards to exposures to different types of

EMFs. With the GSM exposure which was found to be the most bioactive/stressful one, germarium was found to be more sensitive than stage 7–8 (the percentage of TUNEL-positive germaria was higher than the percentage of TUNEL-positive egg chambers of stages 7–8) (Panagopoulos et al. 2007a, 2010). On the contrary, with the 50 Hz magnetic field and with the 8 kHz pulsed electric field there was more DNA damage at the stage 7–8 than at the germaria (Panagopoulos et al. 2013a; Panagopoulos 2016).

1.3 The Key Role of Field-Variability in the Bioactivity of EMFs

1.3.1 Adaptation to EMFs

Living organisms have been constantly exposed throughout evolution to terrestrial static electric and magnetic fields of average intensities ~130 V/m and ~0.5 G respectively. While no adverse health effects are connected with normal exposure to these natural ambient fields, variations in their intensities on the order of 20% during "magnetic storms" or "geomagnetic pulsations" due to changes in solar activity with an average periodicity of about 11 years are connected with increased rates of animal/human health incidents, including nervous and psychic diseases, hypertensive crises, heart attacks, cerebral accidents, and mortality (Presman 1977; Dubrov 1978).

It is clear that living organisms perceive EMFs as environmental stressors (Presman 1977; Goodman et al. 1995; Weisbrot et al. 2003; Panagopoulos 2013). But since man-made EMFs constitute a very new stressor for living organisms within the billions of years of biological evolution, the cells have not developed defensive mechanisms, e.g. special genes to be activated for protection against electromagnetic stress of man-made EMFs. This is probably the reason why in response to man-made EMFs, cells are found to activate heat-shock genes and produce heat-shock proteins very rapidly (within minutes) and at a much higher rate than for heat itself (Weisbrot et al. 2003). It seems to be for the same reason that mobile phone radiation is found to induce DNA damage and cell death in insect reproductive cells at a higher degree than other types of external stressors examined before like food deprivation or chemicals, (Nezis et al. 2000, 2002; Drummond-Barbosa and Spradling 2001; Panagopoulos et al. 2007a). Thus it appears that cells are much more sensitive to man-made EMFs than to other types of stress previously experienced by living organisms in the course of evolution such as heat, cold, starvation, or chemicals.

One reason for the increased biological activity of man-made EMFs can be that cells/organisms adapt more easily to any external stressor - and to EMFs - when this stressor is not of significantly varying type, in other words when its parameters are kept constant or vary only slightly. Since living organisms do not have defense

mechanisms against variations on the order of 20% of natural EMFs as reported, it is realistic to expect that they do not have innate defenses against unnatural (man-made) EMFs, which are mostly not static but varying (alternating, pulsed, modulated fields, including simultaneously several different frequencies, etc.) and totally polarized in contrast to natural EMFs.

Indeed, RF signals pulsed or modulated by ELF are found in numerous studies since the mid-seventies to be more bioactive than continuous RF signals of identical other parameters (intensity, frequency, duration, waveform, etc) (Bawin et al. 1975, 1978; Bawin and Adey 1976; Blackman et al. 1980; Lin-Liu and Adey 1982; Somosy et al. 1991; Veyret et al. 1991; Bolshakov and Alekseev 1992; Thuroczy et al. 1994; Penafiel et al. 1997; Höytö et al. 2008; Franzellitti et al. 2010; Campisi et al. 2010). Moreover, intermittent exposure to mobile phone radiation with short intermittence durations (which makes the field even more variable) is repeatedly found to be more bioactive than continuous exposure both with simulated and real fields (Diem et al. 2005; Chavdoula et al. 2010). This experimental evidence further supports the argument that the more complicated and variable the field/stressor is, the more difficult it is for a living organism to adapt to it.

1.3.2 Increased Variability of Mobile Telephony EMFs

All types of digital mobile telephony radiation, except for their RF carrier signal, employ ELF fields necessary for the modulation and for increasing the capacity of transmitted information by pulsing the signal. The combination of the RF carrier and the ELF pulsing and modulation frequencies has been found to be more bioactive than the RF carrier alone (Lin-Liu and Adey 1982; Veyret et al. 1991; Penafiel et al. 1997). Moreover, according to the "Ion Forced-Oscillation Mechanism" (Panagopoulos et al. 2000a, 2002, 2015b), (a) the ELF frequencies included in any pulsed or modulated RF signal, are those more responsible for the biological effects, (b) changes in field intensity play a major role, and (c) the pulsing of the signal makes it twice more bioactive.

A constant carrier RF wave modulated by a constant ELF field can certainly be simulated but this is not the case in real mobile telephony signals, in which both the carrier and the modulation are constantly and unpredictably varying in intensity, frequency, and waveform during a phone-conversation (Tisal 1998; Hillebrand 2002; Curwen and Whalley 2008; Holma and Toskala 2004; Panagopoulos 2011).

The intensity of radiation varies significantly each moment during a usual phone-conversation depending on signal reception, number of subscribers sharing the frequency band each moment, air conductivity, location within the wireless infrastructure, presence of objects and metallic surfaces, "speaking" versus "non-speaking" mode, etc. These variations are much larger than 20% of the average signal intensity (as opposed to the periodical variations in the terrestrial fields known to cause health effects). Moreover the phase of the carrier signal varies continuously during a phone-conversation, and the RF frequency constantly

changes between different available frequency channels, especially in third generation (3G) radiation. The wave shape is also constantly changing depending on how the changing information transmitted each moment modulates the carrier wave. Thus, the parameters of this radiation change constantly and unpredictably each moment and large, sudden, unpredictable variations in the emitted EMFs/radiation take place constantly during a usual phone-conversation. The more the amount of carried information is increased (by adding text, speech, pictures, music, video, internet, etc) in more recent phone generations (G)/types (2G, 3G, 4G, etc), the more complicated and unpredictably varying the cell phone signals become (Tisal 1998; Hillebrand 2002; Curwen and Whalley 2008; Holma and Toskala 2004; Panagopoulos 2011).

Thus, real digital mobile phone - and other wireless communication devices - emissions change constantly and unpredictably. As a consequence, living organisms cannot adapt to such a highly varying type of stress. Moreover, due to the unpredictably varying type of the real emissions, it is impossible to simulate them by EMFs of fixed parameters.

1.3.3 Real Exposure Studies as Opposed to Studies with Simulated Exposures

A significant number of studies which employed commercially available mobile phones for exposure to a wide variety of animals (including humans)/biological samples have already been published. These include human volunteers *in vivo* (Ferreri et al. 2006; Vecchio et al. 2007, 2010, 2012; Yadav and Sharma 2008; D'Costa et al. 2003; Cam and Seyhan 2012; Luo et al. 2013; Mandala et al. 2014; Movvahedi et al. 2014), human sperm *in vitro* (Agarwal et al. 2009; Gorpinchenko et al. 2014), mice or rats or guinea-pigs or rabbits *in vivo* (Irmak et al. 2002; Dasdag et al. 2003; Ilhan et al. 2004; Ferreira et al. 2006; Elhag et al. 2007; Yan et al. 2007; Meral et al. 2007, 2014; Balci et al. 2007; Mailankot et al. 2009; Gul et al. 2009; Imge et al. 2010; Aldad et al. 2013; Al-Damegh 2012; Koca et al. 2013; Meo and Rubeaan 2013; Motawi et al. 2014), *Drosophila* (Weisbrot et al. 2003; Panagopoulos et al. 2004, 2007a, b, 2010; Panagopoulos and Margaritis 2010a, b; Panagopoulos 2012a; Margaritis et al. 2014; Chavdoula et al. 2010), bees (Sharma and Kumar 2010; Kumar et al. 2011; Favre 2011), ants (Cammaerts and Johansson 2014), chicken eggs (Batelier et al. 2008; Ingole and Ghosh 2012), quails (Tsybulin et al. 2013), mouse cells *in vitro* (Liu et al. 2013), human breast cancer cells *in vitro* (Cig and Naziroglu 2015), protozoa (Cammaerts et al. 2011), and even purified proteins *in vitro* (Barteri et al. 2005). An impressive percentage (96%) of these studies (48 out of 50 studies with real-life exposures) have recorded significant adverse biological or clinical effects, ranging from loss of orientation, kinetic changes, behavioral, or electroencephalographic (EEG) changes, to decrease in male and female reproductive capacity, reproductive declines, molecular changes,

changes in enzymatic activity, DNA damage and cell death, protein damage, and histopathological changes in the brain. From the remaining two studies, one reported no effect (Dasdag et al. 2003) and one reported an increase in short-term memory of children (Movvahedi et al. 2014) which we did not count as an adverse effect although it may be.

On the contrary, about 50% of the studies performed with simulated signals have reported no effects (IARC 2013; Health Protection Agency 2012; Verschaeve et al. 2010; Bourthoumieu et al. 2010; Waldmann et al. 2013; Shahin-Jafari et al. 2016), even though several recent review studies suggest an overall predominance of studies showing effects regardless of real or simulated exposures (Verschaeve 2009; Vignera et al. 2012; Cucurachi et al. 2013; Balmori 2014; Manna and Ghosh 2016). A meta-analysis of 88 studies published during 1990–2011 investigating genetic damage in human cells from RF radiation, 87 of which did not employ real telecommunication EMFs, reported no overall association with genotoxicity (Vijayalaxmi 2012).

Although we may have missed a few more studies with real mobile phone exposures, it becomes evident that there is a strong conflict between the overall results of studies performed with real mobile phone exposures and the overall results of studies with simulated exposures from generators and "test" phones. Moreover, while within the group of studies with simulated exposures there is also a conflict between studies that find effects and studies that do not, the group of studies with real exposures demonstrates an impressive consistency in showing effects almost at 100%. This impressive consistency is corroborated by increasing epidemiological evidence - especially during the last years - for an association between (real-life) mobile phone use and brain tumors (Kundi 2004; Khurana et al. 2009; Hardell et al. 2007, 2013), by statistical studies reporting symptoms of unwellness among people residing around mobile telephony base station antennas or among mobile phone users (Navarro et al. 2003; Salama and Abou El Naga 2004; Hutter et al. 2006; Blettner et al. 2009; Kundi and Hutter 2009; Viel et al. 2009; Singh et al. 2016), as well as by open field studies reporting declines in amphibian and bird populations around mobile telephony base station antennas (Balmori 2005, 2010; Balmori and Hallberg 2007; Everaert and Bauwens 2007; Bhattacharya and Roy 2014).

Although in most studies with real mobile phone exposures the biological samples were exposed in close proximity (within the near-field up to approximately 5 cm) with the mobile phone handset, in several studies the samples/animals were exposed at greater distances in the far-field up to 1 m (Ilhan et al. 2004; Ferreira et al. 2006; Yan et al. 2007; Balci et al. 2007; Batellier et al. 2008; Panagopoulos et al. 2010; Panagopoulos and Margaritis 2010a, b; Vecchio et al. 2010) where the intensity variations are much smaller and the dosimetry absolutely "reliable" as is generally accepted for far-field antenna measurements (Slater 1991). In one of these studies it was found that at 20–30 cm distance from the mobile phone the biological effect (DNA damage) was even more intense than at zero distance (Panagopoulos et al. 2010). [A mobile phone antenna's near-field extends to a distance of 5.2 or 2.6 cm, for 900 or 1800 MHz respectively (most commonly employed carrier

frequencies in 2G mobile telephony radiation), according to the relation $r = \lambda/2\pi$, (r the distance of near-field far limit from the antenna when the length of the antenna is smaller than the wavelength λ of the emitted radiation (WHO 1993)].

In studies with real mobile phone exposures investigating the dependence of observed effects on dose (radiation intensity and/or exposure duration) the effects were found to be dose-dependent (Panagopoulos et al. 2004, 2007a, b, 2010; Panagopoulos and Margaritis 2010a, b; Batellier et al. 2008; Tsybulin et al. 2013; Aldad et al. 2013). The dependence on dose was in most cases non-linear, although in two studies the dependence of certain effects on exposure duration was approximating linearity (Panagopoulos and Margaritis 2010a; Aldad et al. 2013).

Certainly the results of experiments with real-life (variable) mobile phone EMFs cannot be identically reproducible, since between successive exposures at any specific location, the exact characteristics of the emitted signal are always different. But the average field values over a few minutes (or more) period, are close to each other, and thus the results of different replicate experiments with real emissions as the independent variable, although not identical quantitatively, are qualitatively similar. Statistical significance in the results can be increased by increasing the number of experimental replications while keeping rigorous control of all other parameters (such as animal/sample conditions, temperature, humidity, light, stray EMFs within the lab, etc). Then, as the number of replications increases, the variability of the field becomes less significant (Maber 1999). Moreover, in order to have a measure of the emitted EMFs variability, RF and ELF measurements of average intensity ± SD of the emitted real EMFs should be included in the studies, in addition to the Specific Absorption Rate (*SAR*) information supplied by the manufacturer (referring to a simulated human head (Gandhi et al. 2012)). With increasing number of measurements the SD decreases enough for the dosimetry to be judged as reliable (Maber 1999; Panagopoulos et al. 2015a).

1.4 The Key Role of Polarization in the Bioactivity of EMFs

We shall show now why man-made EMFs are actually very different and much more bioactive than natural EMFs, due to the fact that the first are always totally polarized, a property that only partially exists in natural EMFs.

1.4.1 Man-Made EMR Is More Bioactive Than Natural Non-ionizing EMR

Many studies during the past few decades have indicated a variety of adverse biological effects to be triggered by exposure to man-made EMFs, especially RF, and ELF. The recorded biological effects - many of which already described in the

previous pages - range from alterations in the synthesis rates and intracellular concentrations of different biomolecules, to DNA and protein damage which may result in cell death, reproductive declines, or even cancer (Goodman et al. 1995; Phillips et al. 2009; Blackman 2009; Johansson 2009; Khurana et al. 2009; Panagopoulos 2011; Panagopoulos et al. 2013a). Under the weight of this evidence the International Agency for Research on Cancer (IARC) has classified both ELF magnetic fields and RF EMFs as possibly carcinogenic to humans (IARC 2002, 2013). The intensities of radiation and durations of exposure in the majority these studies were significantly smaller than those of corresponding exposures from natural EMFs in the terrestrial environment. Moreover, the field intensities applied in the studies were several orders of magnitude smaller than the physiological fields in cell membranes, or fields generated by nerve and muscle excitations (Alberts et al. 1994; Stryer 1996).

Solar EMR intensity incident upon a human body ranges normally between 8 and 24 mW/cm^2 (depending on season, atmospheric conditions, geographical location, etc) while corresponding intensity from a digital mobile phone handset upon a human head (even in contact with the ear) during "talk" emission is normally less than 0.2 mW/cm^2 (Roller and Goldman 1968; Parsons 1993; Panagopoulos et al. 2010). Similarly, terrestrial electric and magnetic fields, or infrared radiation from every human body at normal temperature, have significantly larger incident intensities and exposure durations on any human than most artificial EMF sources (Presman 1977; Dubrov 1978; Gulyaev et al. 1995). Why is then the first beneficial while the latter seem to be detrimental?

Below we shall explain theoretically that the increased adverse biological action of man-made EMFs is due to the fact that they are polarized in contrast to the natural ones. First we must provide some definitions and equations on polarization, field intensity, wave intensity, and superposition/interference of EMFs/EMR which will be necessary in order to explain our reasoning.

1.4.2 Man-Made EMR Is Polarized, While Natural EMR Is Not

A field/wave is called linearly polarized when it oscillates on a certain plane which is called the "polarization plane". A combination of linearly polarized fields/waves can give circularly or elliptically polarized fields/waves. Circularly and elliptically polarized 50–60 Hz electric and magnetic fields produced by 3-phase electric power transmission lines are accused for association with cancer (IARC 2002; Panagopoulos et al. 2013a).

Natural EMR/EMFs (cosmic microwaves, infrared, visible light, ultraviolet, gamma rays) and several forms of artificially triggered electromagnetic emissions (such as from light bulbs with thermal filaments, gas discharge lamps, x-rays, lasers, etc.) are not polarized. They are produced by large numbers of molecular,

atomic, or nuclear transitions of random orientation and random phase difference between them (except for the lasers which are coherent). These are de-excitations of molecules, atoms, or atomic nuclei (Beiser 1987). Each photon they consist of oscillates on a distinct random plane, and therefore it has a different polarization. Moreover the different photons are not produced simultaneously but they have random phase differences among them.

In contrast, man-made electromagnetic waves are produced by electromagnetic oscillation circuits ("Thomson" circuits), forcing free electrons to oscillate back and forth along a metal wire (electric circuit). Thus, they are not produced by excitations/de-excitations of molecules, atoms, or nuclei, and because the electronic oscillations take place macroscopically in specific directions/orientations they are polarized (most usually linearly polarized). The plane of polarization is determined by the geometry of the circuit. [Lasers are coherent light emissions, not necessarily polarized, and condensed within a narrow beam with high intensity, but they may also be polarized].

Oscillating polarized EMFs/EMR (in contrast to unpolarized) have the ability to induce coherent forced-oscillations on charged/polar molecules within a medium. In case that the medium is biological tissue, the result is that all charged molecules will be forced to oscillate in phase with the field and on planes parallel to its polarization (Panagopoulos et al. 2000a, 2002, 2015b). Several oscillating electromagnetic fields of the same polarization - such as the fields from different antennas vertically oriented - may also produce constructive interference effects and thus, amplify at certain locations the local field intensity, and the amplitude of oscillation of any charged particle within the medium (and within living tissue) (Sangeetha et al. 2014). At such locations, living tissue becomes more susceptible to the initiation of biological effects.

Only coherent polarized fields/waves of the same polarization and frequency are able to produce standing interference effects (fringes of maximum and minimum intensity) (Arago and Fresnel 1819). When the polarization is fixed (e.g. vertically oriented antennas) but there are differences in coherence and/or frequency between the sources, the interference effects are not standing at fixed locations, but change with time creating transient peaks at changing locations.

Natural light from two or more different sources does not produce interference effects, except under the specific conditions of the Young experiment, where the light from a single source passes through two identical slits which - in turn - become two identical-coherent secondary sources (Pohl 1960; Alonso and Finn 1967).

Unpolarized electromagnetic radiation can become polarized when it passes through anisotropic media, as are certain crystals. In fluids (gases and liquids) the molecules are randomly oriented, and macroscopically are considered isotropic inducing no polarization in the electromagnetic waves transmitted through them. Unpolarized natural light can become partly polarized to a small average degree after diffraction on atmospheric molecules, or reflection on water, mirrors, metallic surfaces, etc. (Alonso and Finn 1967). Thus, living organisms exposed to natural radiation since the beginning of life on Earth, although have been exposed to partially polarized light at a small average degree under certain circumstances

(Chen and Rao 1968; Cronin et al. 2006), have never been exposed to totally polarized radiation as is EMR/EMFs of modern human technology.

1.4.3 Field Intensity and Wave Intensity of Electromagnetic Waves

A plane harmonic electromagnetic wave in the vacuum or the air has electric and magnetic field intensity components, given by the equations:

$$E = E_o \sin(k_w r - \omega t) \tag{1.1}$$

$$B = B_o \sin(k_w r - \omega t) \tag{1.2}$$

E_o, B_o are the amplitudes of electric and magnetic field intensities respectively, r is the distance from the source, t is the time, $\omega = 2\pi\nu = k_w \cdot c$ is the circular frequency of the wave (ν the frequency), k_w ($=2\pi/\lambda$) is the wave number (λ the wavelength), and c the velocity of the wave.

The velocity of the electromagnetic wave (and of any wave), is:

$$c = \lambda \cdot \nu \tag{1.3}$$

The wave intensity $\vec{J}$ (also called "Poynting vector"), is:

$$\vec{J} = \vec{c}\,\varepsilon_o E^2 = c^2\,\varepsilon_o\,\vec{E} \times \vec{B} \tag{1.4}$$

And the average value of its amplitude:

$$J_{ave} = \frac{1}{2} c \varepsilon_o E_o^2 \tag{1.5}$$

Thus, the wave intensity depends upon the square of the electric field intensity.

1.4.4 Superposition of Unpolarized EMR/EMFs

Consider two incoherent, unpolarized electromagnetic rays with electric components E_1, E_2, reaching a certain point P in space at a certain moment t in time. Each ray consists of innumerous elementary plane harmonic waves (e.g. photons) of random but discrete polarization. Let us pick for simplicity two elementary plane harmonic waves, one from each ray. The two vectors $\vec{E}_1$, $\vec{E}_2$ due to the different polarizations oscillate on different planes. Since the two rays are not polarized, the polarizations of their constituent plane harmonic elementary waves vary randomly

at point P each moment. The total angle ϕ between the two vectors each moment at point P is determined by the different polarizations, plus the different phases, and varies randomly in time.

The resultant electric field $\vec{E}$ (electric component of the resultant electromagnetic wave) each moment at point P, is given by the equation:

$$E = \sqrt{{E_1}^2 + {E_2}^2 + 2E_1E_2\cos\phi} \tag{1.6}$$

E varies with time due to the temporal variations of E_1, E_2, $\cos\phi$. But the average value of $\cos\phi$ is zero: $\frac{1}{2\pi}\int_0^{2\pi}\cos\phi\, d\phi = 0$, and the averages of E^2, ${E_1}^2$, and ${E_2}^2$ are ${E_o}^2/2$, ${E_{o1}}^2/2$ and ${E_{o2}}^2/2$ respectively (E_o, E_{o1}, E_{o2} the amplitudes of E, E_1, E_2).The average resultant electric field is then:

$$E_{ave} = \sqrt{\frac{1}{2}\left(E_{o1}^2 + E_{o2}^2\right)} \text{ or } E_o^2 = E_{o1}^2 + E_{o2}^2 \quad (= \text{constant})$$

and (according to Eq. 1.5):

$$J_{ave} = J_{1,ave} + J_{2,ave}\,(= \text{constant}) \tag{1.7}$$

Even when the two component waves have the same frequency and phase, due to the randomly changing polarizations, the result is still the same.

Thus, the total time average wave intensity due to the superposition of two (or more) rays consisting of elementary plane harmonic waves of random polarizations (natural EMR/EMFs) is the sum of the two individual average intensities, and it is constant at every point and - macroscopically - there is no local variation in the resultant intensity, i.e. no interference effects.

1.4.5 Wave Intensity Versus Field Intensity of Unpolarized EMR

Although the sum average wave intensity due to superposition of natural unpolarized rays is the sum of individual average intensities each one depending on the square amplitude of individual electric field (Eq. 1.7), the sum electric field intensity from infinite number of individual elementary waves constituting each ray (as e.g. with natural light), is zero:

$$\lim_{n\to\infty} \sum_{i=1}^{n} \vec{E}_i = \quad \vec{E}_1 + \vec{E}_2 + \vec{E}_3 + \ldots + \vec{E}_n = 0 \tag{1.8}$$

Let us explain this in more detail: Consider many photons of natural unpolarized light superposed on each other at a particular point in space. Let us assume for simplicity that these photons have equal amplitudes and are of the same frequency but have different polarizations meaning that their electric vectors have all possible orientations forming angles between each two of them from 0^o to 360^o. Since all possible orientations have equal probabilities, the superposition of a large number of such equal vectors applied on the same point in space will be the sum of vectors applied on the centre of a sphere with their ends equally distributed around the surface of the sphere. The sum of an infinite number of such vectors (all applied on the same point – centre of the sphere – and with their ends evenly distributed at all points of the sphere surface) tends to be zero.

In other words, at any given location, any moment, the sum electric field of a large number of incident photons of random polarization tends to be null, since the individual vectors are in all possible directions diminishing each other when superimposed (destructive interference of electric vectors). Similarly for the sum magnetic field: $\lim_{n\to\infty} \sum_{i=1}^{n} \vec{B}_i = 0$

Thus, the result of superposition of a large number of incident natural waves is increased wave intensity, but negligible electric and magnetic fields approaching zero with infinite number of individual waves/photons. Since the electric forces on charged particles depend directly not on the wave intensity $\vec{J}$, but on the electric and magnetic field intensities ($\vec{E}$, $\vec{B}$), unpolarized EMFs/EMR cannot induce any net forced-oscillations on any charged particles (e.g. biological molecules). They may only induce heat, i.e. random oscillations in all possible directions due to momentary non-zero field intensities, but this does not result to any net electric or magnetic field, or to any net forced-oscillation of charged molecules. This conclusion is very important for our whole reasoning.

1.4.6 Constructive and Destructive Interference of Polarized Waves/Fields

When two or more waves/fields of the same polarization and frequency are in addition coherent, in other words, when their phase difference at the location of superposition is:

$$\varphi = 2n\pi, (\text{with}\, n = 1, 2, 3, \ldots), \tag{1.9}$$

the result is constructive interference, meaning that the resultant wave has an amplitude (intensity) equal to the sum of amplitudes of the single waves that interfere at the particular location.

When two waves of same polarization have opposite phases at another location, in other words, when their phase difference is:

$$\varphi = (2n+1)\pi, \tag{1.10}$$

then the result of their superposition is destructive interference, i.e. a wave of the same polarization but with diminished intensity.

The electrical components of two such waves (plane harmonic waves of the same polarization and frequency) reaching a certain location after having run different distances r_1, and r_2 from their two coherent sources, are given by the equations:

$$E_1 = E_{o1} \sin(k_w r_1 - \omega t) \tag{1.11}$$

$$E_2 = E_{o2} \sin(k_w r_2 - \omega t) \tag{1.12}$$

Again, the amplitude E_o of the resultant electric field $\vec{E}$ (electric component of the resultant electromagnetic wave), is:

$$E_o = \sqrt{{E_{o1}}^2 + {E_{o2}}^2 + 2E_{o1}E_{o2}\cos\varphi} \tag{1.13}$$

where $\varphi = \frac{2\pi}{\lambda}(r_1 - r_2)$ depending in this case only upon the difference in the distances run by the two waves, and not upon polarization.

At any location where: $\varphi = 2n\pi$, Eq. 1.13 gives:

$$E_o = \sqrt{E_{o1}^2 + E_{o2}^2 + 2E_{o1}E_{o2}} \quad (= |E_{o1} + E_{o2}|) \tag{1.14}$$

At these locations we have constructive interference.

At any location where: $\varphi = (2n+1)\pi$, Eq. 1.13 gives:

$$E_o = \sqrt{E_{o1}^2 + E_{o2}^2 - 2E_{o1}E_{o2}} \quad (= |E_{o1} - E_{o2}|) \tag{1.15}$$

At these locations we have destructive interference.

The intensity of the resultant wave at any location is:

$$\vec{J} = \vec{J}_1 + \vec{J}_2 \tag{1.16}$$

The amplitude of the resultant wave intensity will be, correspondingly:

$$J_o = c\varepsilon_o(E_{o1} + E_{o2})^2 \tag{1.17}$$

(at the locations of constructive interference), and

$$J_o = c\varepsilon_o(E_{o1} - E_{o2})^2 \tag{1.18}$$

(at the locations of destructive interference).

Thus, at the locations of constructive interference, the electric field vectors of the two waves/fields are parallel and in the same direction, and both the resultant field and the resultant wave intensity are maximum (Eqs. 1.14 and 1.17).

For two identical sources $(E_{o1} = E_{o2}) : E_o = 2E_{o1}$ and $J_o = 4c\varepsilon_o E_{o1}^2 = 4J_{o1}$

$$\text{For } N \text{ identical sources} : E_o = NE_{o1} \tag{1.19}$$

$$\text{and } J_o = N^2 J_{o1} \tag{1.20}$$

This is why series of parallel RF/microwave antennas are often used to produce high-intensity beams in certain directions (Alonso and Finn 1967).

At the locations of destructive interference the electric field vectors of the two waves are anti-parallel, and thus, both the resultant field and the resultant wave intensity are minimum (Eqs. 1.15 and 1.18). For identical sources ($E_{oI} = E_{o2}$): $E = 0, J = 0$.

Thus, for N number of polarized coherent electromagnetic sources of the same polarization, frequency, and different intensities, with electric components E_1, E_2,..., E_N, it comes that at the locations of constructive interference, the resultant electric field is the sum electric field from all the individual sources (e.g. antennas):

$$E = E_1 + E_2 + E_3 + \ldots + E_N \tag{1.21}$$

The bigger the number of coherent superimposed waves/fields (from the same or different sources), the higher and narrower the peaks (Alonso and Finn 1967). That situation can create very sharp peaks of wave and field intensities at certain locations, not easily detectable by field meters, where any living organism may be exposed to peak electric and magnetic field intensities.

Thus, the difference between superposition of unpolarized and polarized electromagnetic waves/fields, is that while in the first case we have increased average wave intensity but zeroed net fields at any location, in the second case we have increased both wave intensity and fields at certain locations where constructive interference occurs. This difference is of crucial importance for understanding the differences in biological activity between natural and man-made EMFs/non-ionizing EMR.

1.5 Theoretical Explanation of the Effects: "Ion Forced-Oscillation Mechanism" for Polarized EMFs. Why ELF and Pulsed EMFs Are More Bioactive

A review of the whole EMF-bioeffects literature reveals that the most bioactive EMFs are the lower frequency ones, especially the ELF fields (Goodman et al. 1995). Moreover it is shown that pulsed EMFs are more bioactive than continuous fields of same rest characteristics (Goodman et al. 1995; Veyret et al. 1991; Penafiel et al. 1997). The pulse repetition frequency is always a low frequency, most usually ELF. We argue that the reason of the intense bioactivity of modern low-intensity microwave fields is most likely the ELF pulsing and modulation frequencies that they include and not the RF carrier wave itself. Below we shall explain this.

All critical biomolecules are either electrically charged or polar (Alberts et al. 1994). While natural unpolarised EMF/EMR at any intensity cannot induce any specific/coherent oscillation on these molecules, polarized man-made EMFs/EMR will induce a coherent and parallel forced-oscillation on every charged/polar molecule within biological tissue. This is fundamental to our understanding of the biological phenomena. This oscillation will be most evident on the free (mobile) ions which carry a net electric charge and exist in large concentrations in all types of cells or extracellular tissue determining practically all cellular/biological functions (Alberts et al. 1994). Although all molecules oscillate randomly with much higher velocities due to thermal motion, this has no biological effect other than increase in tissue temperature. But a coherent polarized oscillation of even millions of times smaller energy than average thermal molecular energy (Panagopoulos et al. 2013b) can initiate biological effects.

A forced-oscillation of mobile ions, induced by an external polarized EMF, can result in irregular gating of electrosensitive ion channels on the cell membranes. That was described in detail in Panagopoulos et al. (2000a, 2002). According to this theory - the plausibility of which in actual biological conditions was verified by numerical test (Halgamuge and Abeyrathne 2011) - the forced-oscillation of ions in the vicinity of the voltage-sensors of voltage-gated ion channels can exert forces on these sensors equal to or greater than the forces known to physiologically gate these channels. Irregular gating of these channels can potentially disrupt any cell's electrochemical balance and function (Alberts et al. 1994), leading to a variety of biological/health effects including the most detrimental ones, such as DNA damage, cell death, or cancer (Pall 2013, 2015).

Most cation channels (Ca^{+2}, K^{+}, Na^{+}, etc) on the membranes of all animal cells, are voltage-gated, or as they are usually called, "electrosensitive" (Alberts et al. 1994). They interconvert between open and closed state, when the electrostatic force on the electric charges of their voltage sensors due to transmembrane voltage changes, transcends some critical value. The voltage sensors of these channels are four symmetrically arranged, transmembrane, positively charged helical domains, each one designated S4. Changes in the transmembrane potential on the order of 30 mV, are normally required to gate electrosensitive channels (Noda et al. 1986;

Liman et al. 1991). Several ions may interact simultaneously each moment with an S4 domain from a distance on the order of 1 nm, since - except for the single ion that may be passing through the channel pore when the channel is opened - a few more ions are bound close to the pore of the channel at specific ion-binding sites (e.g. three in potassium channels) (Miller 2000).

Consider e.g. four potassium ions at distances on the order of 1 nm from the channel-sensors (S4), and an externally applied oscillating EMF/EMR. The electric (and the magnetic) force on each ion due to any unpolarized field is zero (Eq. 1.8). On the contrary, the force due to a polarized field with an electrical component E, is $F = Ezq_e$. It is shown that for a sinusoidal alternating field $E = E_o \, sin\omega \, t$, the movement equation of a free ion of mass m_i, is (Panagopoulos et al. 2000a, 2002):

$$m_i \frac{d^2 r}{dt^2} + \lambda \frac{dr}{dt} + m_i \, \omega_o^2 \, r = E_o \, z \, q_e \sin \omega \, t \tag{1.22}$$

r is the ion displacement due to the forced-oscillation, z is the ion's valence ($z = 1$ for potassium ions), $q_e = 1.6 \times 10^{-19}$C the elementary charge, λ the damping coefficient for the ion's displacement (calculated to have a value within a channel $\lambda \cong 6.4 \times 10^{-12}$Kg/s), $\omega_o = 2\pi\nu_o$ (ν_o the ion's oscillation self-frequency taken equal to the ion's recorded spontaneous intracellular oscillation frequency on the order of 0.1 Hz), $\omega = 2\pi\nu$ (ν the frequency of the field/radiation), and E_o the amplitude of the applied electric field.

The general solution of Eq. 1.22, is:

$$r = \frac{E_o z q_e}{\lambda \omega} \cos \omega t + \frac{E_o z q_e}{\lambda \omega} \tag{1.23}$$

The term $\frac{E_o z q_e}{\lambda \omega}$ in the solution, represents a constant displacement, but has no effect on the oscillating term $\frac{E_o z q_e}{\lambda \omega} \, cos\omega \, t$. This constant displacement doubles the amplitude $\frac{E_o z q_e}{\lambda \omega}$ of the forced-oscillation at the moment when the field is applied or interrupted, or during its first and last periods, and the ion's displacement will be twice the amplitude of the forced-oscillation. For pulsed fields (such as most fields of modern digital telecommunications) this will be taking place constantly with every repeated pulse. Thus, pulsed fields are - theoretically - twice more drastic than continuous/non-interrupted fields of the same other parameters, in agreement with several experimental data (Goodman et al. 1995; Veyret et al. 1991; Penafiel et al. 1997).

The amplitude of the forced-oscillation (ignoring the constant term in Eq. 1.23), is:

$$A = \frac{E_o z q_e}{\lambda \omega} \tag{1.24}$$

The force acting on the effective charge q of an S4 domain, via an oscillating single-valence free cation, is: $F = \frac{1}{4\pi\varepsilon\varepsilon_o} \cdot \frac{q \cdot q_e}{r^2}$, ($r$ is the distance of the free ion from the effective charge of S4). Each oscillating cation displaced by dr, induces a force on each S4 sensor:

$$dF = -\frac{q \cdot q_e}{2\pi\varepsilon\varepsilon_o r^3} dr \tag{1.25}$$

While in the case of a non-polarized applied field $\sum d\vec{r} = 0$, and $\sum d\vec{F} = 0$, in the case of a polarized applied field, the sum force on the channel sensor from all four cations, is: $4dF = -2\frac{q \cdot q_e}{\pi\varepsilon\varepsilon_o r^3} dr$.

This is an even more crucial difference between polarized and unpolarized EMFs in regard to biological activity than the ability of constructive interference.

The effective charge of each S4 domain is found to be: $q = 1.7\ q_e$ (Liman et al. 1991). The minimum force on this charge required normally to gate the channel – equal to the force generated by a change of 30 mV in the membrane potential (Liman et al. 1991) – is calculated (Panagopoulos et al. 2000a) to be: $dF = 8.16 \times 10^{-13}$ N.

The displacement of one single-valence cation within the channel, necessary to exert this minimum force is calculated from Eq. 1.25 to be:

$$dr = 4 \times 10^{-12}\text{m}$$

For 4 cations oscillating in phase and on parallel planes due to an external polarized field/radiation, the minimum displacement is decreased to: $dr = 10^{-12}$ m.

Therefore, any external polarized oscillating EMF able to force free ions to oscillate with amplitude $\frac{E_o z q_e}{\lambda\omega} \geq 10^{-12}$m, is able to irregularly gate cation channels on cell membranes. For $z = 1$ (potassium ions), and substituting the values for q_e, λ on the last condition, we get:

$$E_o \geq 0.25\,\nu \times 10^{-3} \tag{1.26}$$

(ν in Hz, E_o in V/m)

For double-valence cations ($z = 2$) (e.g. Ca^{+2}) the condition becomes,

$$E_o \geq \nu \times 10^{-4} \tag{1.27}$$

(ν in Hz, E_o in V/m)

For pulsed fields the second part of Condition 27 is divided by 2, and becomes:

$$E_o \geq 0.5\,\nu \times 10^{-4} \tag{1.28}$$

(ν in Hz, E_o in V/m)

For digital mobile telephony fields/radiation emitting ELF pulses with a pulse repetition frequency $\nu = 217$ Hz (among other ELF frequencies they transmit) (Tisal 1998), Condition 28 becomes:

$$E_o \geq 0.01\,\mathrm{V/m} \tag{1.29}$$

For the pulse repetition frequency of $\nu = 8.34$ Hz (also included in mobile telephony signals) (Tisal 1998; Hyland 2000; Tuor et al. 2005), Condition 28 becomes:

$$E_o \geq 0.0004\,\mathrm{V/m} \tag{1.30}$$

Thus, ELF electric fields emitted by mobile phones and base stations stronger than 0.0004 V/m are also potentially able to disrupt the function of any living cell. This ELF intensity value is emitted by regular cell phones at distances up to a few meters and base stations at distances up to a few hundred meters (Tuor et al. 2005; Panagopoulos 2011). For N number of mobile telephony antennas vertically oriented, the last value is divided by N (according to Eq. 1.19) at locations of constructive interference.

We do not distinguish between externally applied EMFs and internally induced ones within living tissue, especially in the case of ELF for the following reasons: (1) Living tissue is not metal to shield from electric fields and certainly is not ferromagnetic metal (Fe, Co, Ni) to shield from magnetic fields. Moreover, it is known that especially ELF fields cannot be easily shielded even by Faraday cages and in order to significantly minimize them it is recommended to totally enclose them in closed metal boxes (Panagopoulos 2011). Thus, ELF electric fields penetrate living tissue with certain degree of attenuation, and magnetic fields penetrate with zero attenuation. (2) Even in case that the ELF fields are significantly attenuated in the inner tissues of a living body, the eyes, the brain, the skin cells, or the myriads of nerve fiber terminals that end up on the outer epidermis, are directly exposed to the field intensities measured externally on the surface of the living tissue.

It has been shown that tissue preparations (such as bovine fibroblasts or chicken tendons) respond to externally applied pulsed or sinusoidal ELF electric fields (by changes in DNA or protein synthesis rates, proliferation rates, alignment with respect to the field direction, etc), at very low thresholds ~10^{-3} V/m (Goodman et al. 1995; McLeod et al. 1987; Cleary et al. 1988; Lee et al. 1993). These thresholds are very close to those predicted above by the described mechanism.

We note that the external field does not gate the channel by forces exerted directly on the channel sensors. It would take a field on the order of the transmembrane field (10^6–10^7 V/m) for that. It is the mediation of the oscillating ions in close proximity to the S4 channel sensors that allows such weak fields to be able to exert the necessary forces to gate the channel.

Except for direct electric field exposure by an external field, there can be an electric field within tissues induced by an externally applied oscillating magnetic one,

which - as explained - penetrates living tissue with zero attenuation. Tuor et al. (2005) measured ELF magnetic fields from mobile phones on the order of 1 G ($= 10^{-4}$ T) at 217 Hz. This can induce electric fields on the order of ~0.1 V/m within the human body, as can be shown by application of Maxwell's law of electromagnetic induction:

$$\oint_l \vec{E}_{ind} \cdot d\vec{l} = -\frac{d}{dt} \int_S \vec{B} \cdot \vec{u}_N dS \tag{1.31}$$

($\vec{B}$, $\vec{E}_{ind}$, the magnetic and the induced electric field intensities respectively, $d\vec{l}$ an incremental length along a closed path l of induced electric field circulation. $\vec{u}_N$ is the unit vector vertical to the surface S).

Assuming $\vec{E}_{ind}$ parallel to and independent of l, $\vec{B}$ vertical to and independent of S, and l a circular path of radius α including the surface S, Eq. 1.31 becomes:

$E_{ind} \oint_l dl = -\frac{dB}{dt} \int_S dS$

which gives:

$$E_{ind} = 0.5\alpha \frac{dB}{dt} \tag{1.32}$$

(E_{ind} in V/m, B in T, α in m.).

By replacing in the last equation $\alpha = 0.20$ m (a reasonably large radius for a circumference within an adult human body), and $\frac{dB}{dt} = 1\,\mathrm{T/s}$, [according to Tuor et al. (2005)], we get $E_{ind} \sim 0.1$ V/m. This is the induced electric field intensity within a human body by the 217 Hz pulses of mobile telephony, and it is about ten times larger than the minimum estimated value able to initiate biological effects at this frequency according to Condition 29.

Thus, we have shown that - according to the presented "Ion Forced-Oscillation Mechanism" - the EMFs emitted by mobile telephony antennas possess enough energy to disrupt any living cell's electrochemical balance and function, and consequently induce adverse biological/health effects.

1.6 Discussion

Our experiments showed that GSM mobile telephony EMFs induced DNA damage and actin cytoskeleton damage in a high degree on female insect ovarian cells. This in combination with a corresponding effect on the male gametes (sperm cells) is what causes the impressive decrease in reproduction recorded since our first experiments (Panagopoulos et al. 2000b, 2004). The affected cells are most usually led to apoptosis, as we showed that the induced DNA damage is accompanied by actin cytoskeleton disorganization which is a known aspect of cellular death. But in cases that this will not occur, cancer induction (in the case of somatic cells), or inherited mutations transferred to the next generations (in the case of the oocyte or

the sperm cells), may take place. Therefore our studies have shown that this type of microwave radiation is highly bioactive and dangerous for health, and a cautious use of mobile phones should necessarily be adopted, as well as a strict governmental policy regarding the positioning of the base station antennas. Moreover, our experimental results explain biologically the increased cancer rates reported by recent epidemiological studies among long-term mobile phone users (Khurana et al. 2009; Hardell et al. 2007, 2013).

The effects found in our experiments (DNA damage, actin cytoskeleton damage, reproductive decrease, decrease in ovarian development) are also found by other investigators in a variety of other animal (including human) cells. For example the decrease in ovarian development was also found for female rats exposed to GSM radiation (Gul et al. 2009). The large decrease in reproduction was also found for bees (Sharma and Kumar 2010), and this explains the disappearing of bees reported during the past ~15 years especially in Europe and North America (Hamzelou 2007; Cucurachi et al. 2013). Corresponding effects were found for birds (Balmori 2005), amphibia (Balmori 2010), and decrease in human male fertility (Agarwal et al. 2008, 2009). This unique similarity of reproductive/fertility declines in different organisms found by different research groups is explained by the observed cell death induction in reproductive cells due to DNA damage found in our experiments (Panagopoulos et al. 2007b, 2010; Panagopoulos 2012a), and reported also for human and rat sperm cells (De Iuliis et al. 2009; Yan et al. 2007; Mailankot et al. 2009). It is evident that such a similarity of effects cannot be due to randomness.

Moreover, this similarity of effects in different organisms is not surprising, since cells are essentially the same in all animals (including humans). Both insect and mammalian (including human) cells (as well as cells from all other animals) have identically constructed membranes and membrane channels, identical intracellular organelles (nuclei, mitochondria, ribosomes, endoplasmic reticulum, Golgi apparatus, etc), identical free/mobile ions initiating practically every cell function, identically constructed biomolecules such as DNA, RNA, proteins, etc. (Alberts et al. 1994; Stryer 1996).

In the present chapter we have shown that polarized EMFs/EMR, such as every type of man-made EMF, have the ability to create interference effects and amplify their field intensities at specific locations where constructive interference occurs, and that this phenomenon cannot occur with natural EMFs/EMR which are not polarized. Locations at equal distances from identical sources (antennas), in other words locations along the midperpendicular to the distance d between the two sources, are locations of constructive interference and increased field and wave intensities. As the number of sources (e.g. antennas) increases, the amplification of the resultant field intensities (E, B) at certain locations increases as well (Eq. 1.19), and for a large number of sources, field intensities may become very sharp. This explains the detected "hot spots" from mobile telephony base stations in urban environments (Sangeetha et al. 2014). The result of field superposition at those locations are standing waves (i.e. they do not change with time) when the two or more sources of the same polarization are in addition coherent (i.e. same frequency, same phase difference). Within biological tissue, at those locations of constructive

interference we can have increased biological activity. The most usual case is when the multiple incident fields/waves are of the same polarization but not coherent (i.e. different frequencies and/or varying phase differences), as e.g. the waves from all different radio, television, and mobile telephony antennas vertically oriented. Then, the resultant fields/waves are not standing but timely varying, creating momentary constructive interference at unpredictably different locations each moment. This fact may represent an extraordinary ability of man-made/polarized EMFs to trigger biological effects.

By use of the Ion Forced-Oscillation Mechanism (Panagopoulos et al. 2000a, 2002, 2015b) we showed that the resultant force exerted on the S4 sensors of electrosensitive ion channels on cell membranes by several ions forced to oscillate on parallel planes and in phase by an applied polarized EMF (and even more by constructively superimposed fields from several polarized EMF-sources), is able to irregularly gate these channels. The result can then be the disruption of the cell's electrochemical balance, leading to a variety of biological/health effects (Pall 2013, 2015). This is in contrast to the null force exerted by any number of ions oscillating on non-parallel random planes and with different phases from each other due to any number of non-polarized applied EMFs, and in contrast to the null force exerted by the random thermal movement of the same ions (Panagopoulos et al. 2002, 2013b).

Thus, we showed that polarized EMFs/EMR can have increased biological activity, due to: (1) Ability to produce constructive interference effects and amplify their intensities at many locations. (2) Ability to force all charged/polar molecules and especially free ions within and around all living cells to oscillate on parallel planes and in phase with the applied polarized field. These features render man-made EMFs/EMR considerably more bioactive than natural non-ionizing EMFs/EMR. This explains the increasing number of biological effects discovered during the past few decades to be induced by man-made EMFs, in contrast to the absence of effects attributed to natural EMFs at normal intensities in the terrestrial environment which have always been present throughout evolution, although human exposure to the latter ones is normally of significantly higher intensities/energy and longer durations.

This is the reason why polarized microwave radiation of maximum power 1–2 W emitted by a mobile phone can damage DNA and cause adverse health effects (Philips et al. 2009; Blackman 2009; Khurana et al. 2009; Panagopoulos 2011), while non-polarized infrared, visible, and ultraviolet radiation from a 100 W light bulb, or ~400 W infrared and visible EMR from a human body (Gulyaev et al. 1995), cannot. Similarly with solar EMR the intensity of which incident upon a human body (~ 8–24 mW/cm^2) is hundreds of times higher than radiation intensity incident from e.g. a mobile phone on a user's head/body during a usual phone-conversation with the handset in touch with the head (less than 0.2 mW/cm^2), or incident intensities from other RF, ELF sources of human technology (Roller and Goldman 1968; Parsons 1993; Panagopoulos et al. 2010). The total daily duration of human exposure to the sunlight is also much longer than the total daily duration of mobile phone exposure during conversations. Yet, there are no adverse biological effects due to normal/non-excessive exposure to sunlight. On the contrary, it is

beneficial and vital/necessary for human/animal health, in contrast to mobile phone radiation. Similarly, there are no adverse biological effects due to exposure (mainly in the infrared and visible bands) from one human body to another (with an incident intensity ~ 20 mW/cm^2) (Gulyaev et al. 1995).

Although all animals on Earth have adapted throughout evolution to exposures to EMFs from the sun and the earth, these fields are non-polarized (even though natural light may become partially polarized in a small average degree due to atmospheric scattering or reflections). Moreover, terrestrial electric and magnetic fields are mainly static, emitting very weak non-polarized ELF radiation due to slight variations in their intensities. However, larger variations on the order of 20% of their normal intensities due to solar activity with a periodicity of about 11 years result in increase of human/animal health incidents (Dubrov 1978). Therefore, living organisms on Earth are adapted to natural (non-polarized or even partially polarized) EMFs since the beginning of life, but not to variations in their normal intensities on the order of 20%, and thus we would not expect them to adapt to unnatural (man-made) and totally polarized EMFs/EMR.

The role of polarization in the ability of EMFs/non-ionizing EMR to induce biological effects, is - up to today - largely underestimated in the EMF-bioeffects literature. Exposure to polarized EMFs may even be beneficial in certain cases of applied static electric or magnetic fields of specified orientation and intensities that enhance the action of endogenous physiological fields within living cells/organisms e.g. during development, wound healing, bone fracture healing etc. (Lee et al. 1993; Panagopoulos 2013).

We should emphasize that the increased bioactivity of man-made EMFs does not necessarily result in observable biological/health effects, since there are adaptive mechanisms operating at cellular-tissue-organism levels in response to ever occurring changes. However, these mechanisms may not always be totally effective, especially when the organism is under additional stress or increased metabolic needs (e.g. sickness, childhood/development, old age, etc.). Then exposure to polarized (man-made) and highly variable EMFs may considerably increase the probability for the initiation of adverse health effects.

In the present chapter we also underscored the need for real-life exposure assessment in experimental studies investigating the biological/health effects of EMFs. We showed that the percentages of positive results differ significantly between studies employing real mobile phone exposures and studies employing simulated exposures, regardless of biological samples or other procedure details. The basic difference between real and simulated mobile telephony EMFs is the inherent significant variability of the first which we believe is the reason for their increased bioactivity.

Any variability in the field and correspondingly in the dosimetry, does not change the fact that people are actually exposed daily for increasing periods to this “highly variable” field in contact with their heads/bodies and at different distances. For this reason it is unrealistic for experimental studies to use simulated mobile phone signals with fixed parameters to expose biological samples. Using non-realistic simulations, especially when real conditions are easily accessible to be

studied in the lab with well-controlled other parameters, is in our opinion a serious scientific flaw. Simulated signals with fixed parameters bear little - if any - resemblance to what mobile phone users actually experience, even when they employ combinations of simulated signals (Kuster and Schoenborn 2000; Ndoumbè Mbonjo Mbonjo et al. 2004; Czyz et al. 2004). To investigate the biological/health effects from a widely accessible device exposing daily billions of humans we should not try to simulate its emissions, but simply use the device itself. Especially, we should not try to simulate its real varying emissions with totally unrealistic invariant ones. This is a serious scientific flaw that may lead to totally devious results with enormous adverse consequences for public health. In order for the biological/clinical studies testing the bioactivity of mobile telephony radiation to account for real conditions, exposures should be performed by real EMFs as these are emitted by commercially available mobile phones. The same holds for experiments with other types of EMFs employed in modern telecommunication systems such as DECT phones, Wi-Fi, etc. Simulated emissions may be used complementarily to real-life ones, in order to study e.g. the effects of separate parameters of the real EMFs, but in no way should simulated emissions substitute the real ones.

In Panagopoulos et al. (2015a) we analyzed the need for real-life exposures assessment in experimental studies in detail. One of the co-authors of the study, Dr. Olle Johansson wrote about the study:

> *Any paper moving mankind closer to the final mechanistic understanding of the association between electromagnetic fields and health effects is very important and valuable. I count this paper as one of the very best and most important in my career. To work together with Dr. Panagopoulos and Dr. Carlo is a genuine blessing. [Professor Dr. Olle Johansson, Department of Neuroscience, Karolinska Institute, Stockholm, Sweden]*

In the present chapter we also provided a detailed theoretical explanation for the increased bioactivity of man-made EMFs (emphasizing on mobile telephony EMFs) - based on the irregular gating of membrane ion channels in all living cells - due to their totally polarized nature. One of the main arguments of those who support that low-intensity EMFs of human technology do not justify any biological/health effects has always been the lack of a plausible biophysical mechanism to theoretically explain the reported effects (Adair 1991). We did show that the Ion Forced-Oscillation Mechanism is indeed a plausible biophysical mechanism that explains the reported biological effects, and therefore, "lack of mechanism" is not the case anymore. Dr. Martin Pall wrote about this issue:

> *The argument that has been made by the advocates of the current safety standards is that the low intensity, non-thermal EMFs produce only very weak forces on charged groups, weaker than those due to thermal motions at body temperature. They argue, therefore that any effect would be no more than effects produced all the time spontaneously in the body. That physics argument has been disproven by Panagopoulos and his colleagues when they published two biophysical studies in 2000 and 2002. The problem with that can be seen when one looks at the voltage-gated calcium channels (VGCCs). The VGCCs have a specific number of charged amino acid residues each of which has a role in the opening and closing of the channels. Each of these are pushed by weak forces, acting in the same direction when a change in the electrical potential across the plasma membrane opens the channel. In much the same way, weak forces ACTING IN THE SAME DIRECTION*

produced by the EMFs should be able to open the channel as well. Whereas thermal motions act randomly in three dimensions and will therefore only extremely rarely be all acting in the same direction, the forces produced by these fields like the forces produced by changes in plasma membrane electrical potential, do act coordinately in the same direction and can, therefore open these channels. This was the insight that led Panagopoulos to formulate his "Ion Forced-Oscillation" theory and it is, in my view, a brilliant insight! The whole basis of the heating/thermal/SAR paradigm of action of these fields is entirely based on the claim that "there is no biophysically viable mechanism for the action of these weak non-thermal or micro-thermal fields and that claim was shown by Panagopoulos to be wrong and the empirical evidence shows that Panagopoulos is right. In addition to that there are literally thousands of studies that falsify the heating/thermal/SAR paradigm. This is THE best example I have seen of a clearly strongly supported paradigm shift within the last 50 years." [Dr. Martin L. Pall, Professor Emeritus, Washington State University, Portland, USA]

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