

# Comparing chromosome damage induced by mobile telephony radiation and a high caffeine dose: Effect of combination and exposure duration

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**Abstract.** I recently reported induction of chromatid-type aberrations in human peripheral blood lymphocytes after a single 15 min exposure to Universal Mobile Telecommunications System (UMTS) Mobile Telephony (MT) Electromagnetic Field (EMF) from a mobile phone. Lymphocytes from six healthy subjects were stimulated for mitosis, and exposed during the G<sub>2</sub>/M phase at 1 cm distance from the handset during an active phone call in “talk” mode. The same type of cells from the same subjects treated with a high caffeine dose (~ 290 times above the permissible single dose for an adult human) exhibited the same type of aberrations in a little smaller but comparable degree. The combination of this caffeine dose and the 15 min MT EMF exposure increased dramatically the number of aberrations in all subjects. The combined effect increased almost linearly with increasing duration of exposure to the MT EMF. Thus, MT EMF exposure ~ 136 times below the official limit (ICNIRP 2020) exerts a genotoxic action even greater than that of a caffeine dose ~ 290 times above the corresponding limit. Therefore, with a reasonable approximation, the limit for MT EMFs should be lowered by at least ~ 4×10<sup>4</sup> times (136×290) for short-term exposures, and ~ 4×10<sup>6</sup> times for long-term exposures.

**Key words:** Electromagnetic fields — Mobile phone radiation — Caffeine — Human lymphocytes — Chromatid aberrations — DNA damage

## Introduction

The scientific community and the general public are increasingly concerned about the adverse effects of man-made Electromagnetic Fields (EMFs) especially those employed in modern microwave telecommunications including Mobile Telephony (MT). All microwave telecommunications EMFs always combine Radio-Frequency (RF)/microwave carrier waves (on the order of GHz) with Extremely Low Frequency (ELF) (up to 3000 Hz) pulsing and modulation in order to

increase the amount and speed of transmitted information (speech, text, images, video, Internet, etc.) (Panagopoulos 2011, 2017, 2019a, 2019b). Both RF and ELF EMFs have been classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans (group 2B) under the weight of the accumulating scientific evidence (IARC 2002, 2013; Baan et al. 2011). Based on additional scientific evidence after the 2011 IARC classification for RF EMFs, several studies have suggested that RF (including MT) EMFs should be re-evaluated and classified as probably carcinogenic (group 2A) or carcinogenic (group 1) to humans (Hardell et al. 2013; Carlberg and Hardell 2017; Hardell 2017, 2019; Miller et al. 2018; Panagopoulos 2019b; Hardell and Carlberg 2020; Hardell and Nyberg 2020). Moreover it is shown that the majority of studies performed with simulated/invariable microwave EMFs emitted by generators (following IARC's guidelines) grossly underestimate the biological activity of

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real-life highly variable exposures by commercially available devices (Panagopoulos et al. 2015a; Panagopoulos 2017, 2019a, 2019b; Kostoff et al. 2020).

Universal Mobile Telecommunications System (UMTS) or third generation (3G) MT EMFs/radiation emitted by commercially available mobile phone handsets and base antennas is still the most usual type of modern MT radiation exposing daily billions of mobile phone users and non-users throughout the world. While 2G Global System for Mobile telecommunications (GSM) is still in use and 4G – a combination of 3G MT and a different microwave radiation with carrier frequency up to 2.6 GHz for broadband Internet access – is the newest widely in use type, telecommunication industry has already begun the installation of the 5G MT system. This involves an even higher carrier frequency (up to 100 GHz) in order to be able to transmit higher amounts of data *per second*, and a much denser network of base antennas of possibly increased power in order to compensate the energy scattering loss due to the higher carrier frequency (Sauter 2011; Singh et al. 2017; Hardell and Carlberg 2020; Hardell and Nyberg 2020; Kostoff et al. 2020). A part of the scientific community has expressed strong objections to 5G installation with concerns of highly increased health risk (McClelland and Jaboin 2018; Miller et al. 2018, 2019; Pall 2018; Panagopoulos 2019a, 2019b; Hardell and Carlberg 2020; Hardell and Nyberg 2020; Kostoff et al. 2020).

Numerous studies have already reported genotoxic effects of RF/microwave and especially MT EMFs on a variety of organisms and cell/tissue types (Panagopoulos 2019b). A recent study of the USA National Toxicology Program (NTP) found that rats exposed for 2 years, 9 h *per day*, to a simulated near-field of a mobile phone antenna emitting 2G or 3G MT EMFs developed brain and heart cancer. Moreover the study found significantly increased DNA damage (strand breaks) in the brains of exposed animals (NTP 2018; Melnick 2019; Smith-Roe et al. 2020), confirming the fact that DNA damage is intimately related with epigenetic cancer. In a similar Italian life-span study, rats were exposed to a simulated GSM 1800 far-field, and were also found to get heart schwannomas and brain glial tumors in agreement with the NTP study (Falcioni et al. 2018). A study that compared the bioactivity between 2G and 3G MT EMFs/radiation emitted by an active mobile phone, found both types of MT EMFs inducing DNA damage and histological changes on the developing liver of chick embryos, with 3G (UMTS) being even more genotoxic/bioactive than 2G (GSM) (D’Silva et al. 2017).

Human peripheral blood lymphocytes – naturally arrested in  $G_0$  phase and usually stimulated for mitosis (M) – are a well-known model for the assessment of genotoxicity of environmental agents such as ionizing radiation, chemicals, smoking, pharmaceuticals, EMFs, etc. (IAEA 2011). Before my previous experimental report (Panagopoulos 2019a) several other studies had already been conducted to

search the effects of MT EMFs on human peripheral blood lymphocytes *in vitro* (Zeni et al. 2003, 2012; Baohong et al. 2005, 2007; Belyaev et al. 2005, 2009; Markova et al. 2005; Stronati et al. 2006; Manti et al. 2008; Schwarz et al. 2008; El-Abd and Eltoweissy 2012). The majority of these studies have found genotoxic effects induced by the MT EMFs alone or in combination with other genotoxic agents (see Panagopoulos 2019a). One of these studies found DNA strand breaks and chromosomal aberrations induced by UMTS-like MT EMF at degrees increasing with longer exposure duration. The effects were attributed to oxidative stress induced by the EMF exposure (El-Abd and Eltoweissy 2012). Stronati et al. (2006) did not find any DNA or chromosomal damage induced by a GSM-like exposure during the  $G_0$  phase. All of these studies employed simulated/invariant MT EMFs produced by generators or test phones which as already mentioned are significantly less bioactive than real-life MT EMFs (Panagopoulos et al. 2015a; Panagopoulos 2017, 2019b; Kostoff et al. 2020). Two laboratory studies were found that employed real-life MT EMF exposure of human lymphocytes by commercially available mobile phones: In the older one (Ji et al. 2004), volunteers were exposed *in vivo* by talking on their GSM (2G) mobile phones for 4 h. After the exposure, DNA damage in their blood samples was significantly increased compared to their blood samples before the exposure. In the newer study (Danese et al. 2017) blood samples were exposed *in vitro* for 30 min to a GSM (2G) real signal emitted by an activated mobile phone and no significant effect on DNA double strand breaks was reported. While the authors of this study used a 3G mobile phone for the exposures, they did not test the 3G signal but the much older (and less bioactive) 2G signal. The authors do not report whether they exposed during “talk” mode (modulated/speaking emission) or during “listening” or other mode. No EMF-measurements were reported, and they applied an assay (the foci method) which does not detect other types of DNA damage apart from double strand breaks, without showing any foci pictures from exposed and control samples. Most importantly, the blood samples were exposed during their resting  $G_0$  phase (like in Stronati et al. 2006) while it is known that proliferating cells are much more vulnerable than resting cells of the same kind with most sensitive phases of the cell division cycle being M, and  $G_2$  (Nias 1998; Terzoudi et al. 2011). Certainly, *in vivo* studies inevitably employ exposures during  $G_0$  since lymphocytes are normally in this phase. In such a case the duration of exposure has to be longer, like in Ji et al. (2004).

Two studies examined peripheral blood lymphocytes from people residing in the vicinity of MT base stations and thus exposed *in vivo* to real-life MT EMFs/radiation emitted by the base antennas. Both studies Gulati et al. (2016) and Zothansiana et al. (2017) found significantly increased genetic damage compared to control groups residing more

than 800 or 300 m, respectively, away from the antennas/cell towers.

A most sensitive assay to record sensitivity of human peripheral blood lymphocytes to environmental stressors is the G<sub>2</sub> assay. This allows observation at metaphase of unrepaired DNA damage induced during G<sub>2</sub> or early M-phase and converted into chromatid-type aberrations in cells activated for mitosis (Terzoudi and Pantelias 2006; Pantelias and Terzoudi 2010, 2011; Terzoudi et al. 2011; Panagopoulos 2019a).

The reason for the increased sensitivity of the G<sub>2</sub> phase of the mitotic cycle is related with the existence of a checkpoint. Checkpoints in biological systems exist at the most sensitive stages of metabolic procedures and are cellular damage detectors and repair activators. This checkpoint during the G<sub>2</sub> phase identifies cellular damage and either activates repair mechanisms, or drives the cell to apoptosis when the damage is not reparable, in order to prevent proliferation of cells with genetic damage (Mendelsohn et al. 1981; Pantelias and Terzoudi 2011). Caffeine in high doses is considered to be a blocker/abrogator of the G<sub>2</sub> checkpoint. Moreover, it is well-known that addition of caffeine in cell cultures results in DNA damage and chromatid aberrations (Kuhlmann et al. 1968; Pincheira and Lopez-Saez 1991; Hatzi et al. 2015). Caffeine was previously classified by IARC as possibly carcinogenic to humans (IARC 1991), but was recently re-evaluated as non-carcinogenic (IARC 2016).

I recently reported that a 15 min single exposure of human peripheral blood lymphocytes to UMTS EMF emitted by a commercially available mobile (“smart”) phone during an active phone call in “talk” mode at 1 cm distance from the blood samples induced chromatid breaks and gaps at highly significant percentages (up to +275%) compared to the sham-exposed (control) samples in all six healthy subjects tested. The lymphocytes were stimulated to enter the mitotic cycle. Those exposed during the G<sub>2</sub> or early M phase were arrested at metaphase by colcemid treatment and observed by light microscopy (Panagopoulos 2019a).

The purpose of the present study was to compare the genotoxic activity of UMTS (3G) MT EMFs/radiation on human cells described in my previous report (Panagopoulos 2019a), with that of a high caffeine dose, and test the genotoxicity of the combination of the two stressors. No other study has up to today compared the genotoxicity, or investigated the combined effect of caffeine and MT EMF exposure, and therefore the present study is novel.

## Materials and Methods

### *Blood culture and separation into groups*

After obtaining consent, blood samples were collected from six healthy non-smoker adult donors (one sample from one donor

in each experiment) in heparinized glass tubes, for analysis of chromosomal sensitivity to mobile phone exposure. The donors/subjects were both males and females, 28–42 years old, with “moderate” mobile phone use (no more than ~ 30 min total daily conversation on their mobile phones), and no reported history of major illnesses or any regular medication. Apart from this, no specific differences between the subjects were searched, since each subject had its own control sample. Whole blood samples were cultured in RPMI 1640 medium (Biochrom AG, Germany) containing 10% fetal bovine serum (FBS), 1% L-glutamine (2 mM), and 1% antibiotics (penicillin: 100 U/ml; streptomycin: 100 µg/ml). Phytohaemagglutinin (PHA) 2% of the final medium volume (dissolved in water at a concentration of 0.24 mg/ml) was added to stimulate the lymphocytes (normally arrested in the G<sub>0</sub> phase) to enter the mitotic cycle (Panagopoulos 2019a).

For each subject, a single culture was prepared in a 200 ml flask (which was later divided into individual samples/groups) to ensure identical culture conditions and treatment for all individual samples/groups in each experiment. The culture was incubated for 72 h, at 37°C in a humidified incubator with an atmospheric content of 5% CO<sub>2</sub> and 95% air.

After 72 h of incubation the single blood culture was subdivided into individual samples/groups in identical 30 ml rectangular plastic flasks. [Each individual group contained: 0.5 ml blood, 5 ml culture medium, 100 µl PHA]. One sample would be exposed to the UMTS EMF for 15 min, and another one would be sham-exposed as previously described (Panagopoulos 2019a). Two additional samples/groups were treated with caffeine for all six individuals. One of them would be exposed for 15 min to the UMTS EMF, and the other sham-exposed. In three (out of the six) individuals (subjects No. 4, 5, 6) two more additional samples/groups were treated with caffeine, one exposed for 5 min and the other for 25 min to the UMTS EMF, in order to study the effect of exposure duration (5, 15, 25 min) to the UMTS EMF combined with the caffeine dose. Therefore the number of individual samples/groups concerning the present experiments were either 4 (one sham-exposed/control, one UMTS 15 min-exposed, one caffeine-treated alone and sham-exposed to the EMF, and one treated with caffeine and UMTS EMF for 15 min), or 6 (same groups as described plus the two additional caffeine-treated groups one exposed 5 min and the other 25 min to the UMTS EMF). Right after the separation of the initial culture into individual groups/samples and the addition of caffeine in the specific samples as described above, the blood samples were either exposed to the UMTS MT EMF in another room of the laboratory (called “exposure room”), or sham-exposed (simply transferred for 15 min to the exposure room). Preliminary experiments had shown that there was no notable difference between groups sham-exposed for 15 min and groups sham-

exposed for 5 or 25 min. For this reason all sham-exposures took place for 15 min.

#### *EMF exposure system and Caffeine dose evaluation*

Exposures were performed by a UMTS (3G) commercially available “smart” mobile phone handset in order to test the effects of real-life exposures experienced daily by billions of MT users around the world. For description of the parameters of the UMTS EMF (modulation, pulsing, etc.) see Panagopoulos (2019a). SAR value of the handset for the human head according to the manufacturer is 0.66 W/kg. The Internet connection (data), Wi-Fi, and Bluetooth antennas of the “smart” phone were disabled like in Panagopoulos (2019a).

The RF radiation intensity, emitted by the handset during the exposures was measured at 1 cm distance from the handset by a Cornet ED85EXpluss RF meter (Cornet Microsystems Inc., USA), and a Spectran HF-4040V3 spectrum analyzer (Aaronia AG, Germany), both with a near-field antenna. The ELF electric and magnetic field intensities (ELF-E and ELF-B) emitted by the handset were measured at 1 cm distance by a Spectran NF-1010E (Aaronia AG, Germany) spectrum analyzer. Representative average peak power density (from five representative peak instant measurements excluding background) in the RF band  $\pm$  Standard Deviation (SD) was  $92 \pm 27 \mu\text{W}/\text{cm}^2$ . Averaged power density over six min (in compliance with the International Commission on Non-Ionizing Radiation Protection-ICNIRP limits) was  $29 \pm 14 \mu\text{W}/\text{cm}^2$ , which is  $\sim 34$  times below the corresponding ICNIRP (1998) general public limit of  $1000 \mu\text{W}/\text{cm}^2$  for the frequency of 2–6 GHz, and  $\sim 136$  times below the latest ICNIRP (2020) corresponding limit which is raised (!) to  $4000 \mu\text{W}/\text{cm}^2$ . The carrier frequency was variable  $\sim 1920$ – $1960$  MHz during the exposures. Representative average ELF-E and ELF-B (from five representative instant measurements excluding background)  $\pm$  SD at 100 Hz was  $12 \pm 4.2$  V/m, and  $0.9 \pm 0.4$  mG, respectively. Corresponding average ELF-E and ELF-B (from five instant measurements excluding background)  $\pm$  SD at 1500 Hz was  $8 \pm 4.6$  V/m, and  $0.06 \pm 0.02$  mG, respectively. All measurements were carried out separately from the exposures in order to have the measuring devices at the same location with the samples during the exposures.

In each individual sample/group treated with caffeine, 200  $\mu\text{l}$  caffeine solution was added (containing 0.2 g caffeine per 10 ml PBS). In other words, each individual caffeine-treated sample with total volume 5.8 ml (5 ml culture medium, 0.5 ml blood, 100  $\mu\text{l}$  PHA, and 200  $\mu\text{l}$  caffeine solution) contained 4 mg caffeine. Thus the final caffeine concentration was  $\sim 3.4$  mM. This caffeine dose is considered to abrogate the G<sub>2</sub> checkpoint (Pantelias and Terzoudi 2011; Hatzi et al. 2015). Thus, to 0.5 ml blood of each sample (8.6% of the

sample volume) correspond  $\sim 0.34$  mg caffeine (8.6% of the caffeine in the sample), and to  $\sim 5$  l whole blood contained in a 70 kg adult human body correspond  $\sim 3.4$  g caffeine. In the case of drinking coffee, caffeine does not go directly into the blood since first passes through the peptic system and distributed to all tissues. It is estimated that from an initial amount A g of caffeine administered by coffee drinking, about  $\sim A/17$  is dissolved into the blood (Cook et al. 1996; Higdon and Frei 2006). Therefore, the amount of caffeine administered directly in each caffeine-treated blood sample corresponds to approximately  $3.4 \text{ g} \times 17 = 57.8$  g caffeine taken by coffee by an adult 70 kg individual. The permitted single caffeine dose for a 70 kg adult is 0.2 g, which is  $\sim 290$  times smaller than the 57.8 g which correspond to the dose used in the experiments (EFSA 2015). Thus, the caffeine dose used in the experiments was  $\sim 290$  times higher than the permitted single dose for an adult individual of 70 kg body weight. This is an important result for the comparison between caffeine and MT EMF exposures.

#### *EMF exposure procedure*

Two blood samples from each subject (one with caffeine and one without) were simultaneously exposed within the 30 ml flasks by the UMTS (3G) mobile phone handset during an active phone-call (“talk” signal) for 15 min at 1 cm distance from the proximal flask wall. This took place in the exposure room so that the controls (in the culture room) would not be exposed. Two additional blood samples of individuals No. 4, 5, 6 treated with caffeine were simultaneously with the others exposed for 5 and 25 min to the UMTS EMF in order to study the effect of exposure duration to the UMTS EMF in combination with the caffeine dose (each sample that its exposure was completed was taken back to the culture room while the other samples continued to be exposed). After all the exposed samples were back in the culture room, the corresponding control (sham-exposed) samples were also transferred in the exposure room for 15 min at the same location as the exposed samples, without being exposed to the MT EMF. This was done because the background ELF-E and ELF-B and the light conditions in the two rooms were different.

The temperature in the two rooms was the same during the procedures/exposures and was kept at  $22 \pm 1^\circ\text{C}$ . In both rooms the RF background was below  $0.01 \mu\text{W}/\text{cm}^2$ . In the exposure room the ELF-E background was  $\sim 2$  V/m, and the ELF-B background  $\sim 0.3$  mG (0.03  $\mu\text{T}$ ). In the culture room the corresponding ELF background fields were higher (E  $\sim 10$  V/m, B  $\sim 1$  mG) due to electrical devices, such as the culture chamber and the hood, existing in all biological laboratories. Temperature increases within the blood samples during the longest 25 min exposures did not exceed  $0.2^\circ\text{C}$  as measured within an identical culture



and flask by a HANNA CheckTemp 1 calibrated electronic thermometer (USA).

#### Metaphase arrest, fixation and observation

After exposures/sham-exposures were completed (~ 30–60 min after the beginning of the exposure procedure) and all the exposed and sham-exposed samples were returned back to the culture room, all individual groups/samples were treated with colcemid (50  $\mu$ l added to each sample) for 60 min, to arrest dividing cells at metaphase. Colcemid prohibits dividing cells to proceed from metaphase to anaphase by prohibiting the formation of attractus. Keeping the cells in metaphase makes their condensed chromosomes clearly observable by light microscopy for possible aberrations. The duration of colcemid treatment (60 min) right after the termination of exposure/sham-exposure plus the exposure/sham-exposure time (1.5–2 h in total) determines in which phases of the cell-division cycle the arrested in metaphase lymphocytes were exposed. In this case, the 1.5–2 h period determines that the cells collected for observation were at the mid-late G<sub>2</sub> or early M (prophase) stages during the exposure/sham-exposure. It also determines the duration of caffeine-treatment of the specific samples.

Cells were then collected by centrifugation, treated for 10 min with hypotonic KCl solution 75 mM (Sigma-Aldrich, USA), fixed in methanol: glacial acetic acid (3:1 v/v), and stained for 10 min with 5% Giemsa solution (Merck, Germany) to be observed by light microscopy. Light microscopy was coupled with an image analysis system (Ikaros MetaSystems, Germany) to facilitate scoring.

Chromosomal damage was evaluated by the number of chromatid gaps (achromatic lesions) and chromatid breaks (terminal deletions) in cells at metaphase. For each of the 4 or

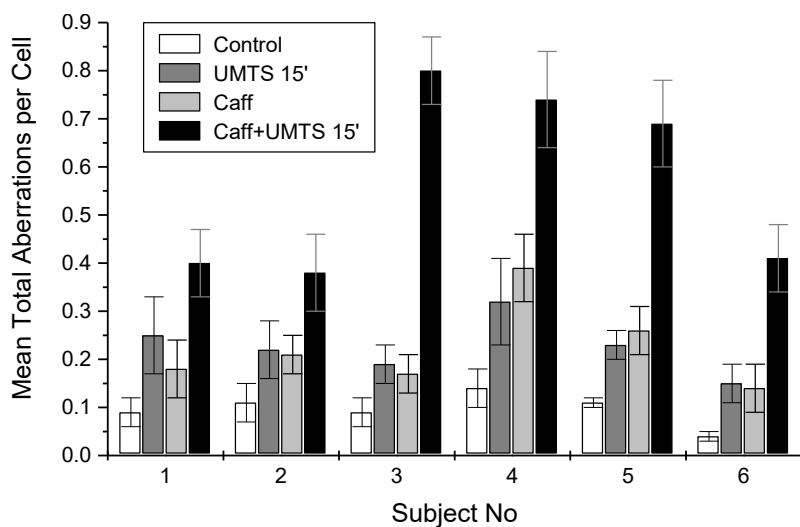
6 samples of each subject (described above) 400 metaphases identically processed from 4 different slides (100 cells from each slide), were blindly scored for gaps and breaks. Mean values of total number of aberrations (gaps and breaks) *per cell* and SD in all samples were calculated for each individual. Gaps were scored only when extended across the full chromatid width. An aberration was considered as “break” when the gap width was equal to or greater than the chromatid width.

#### Statistical analysis

Results were statistically analysed by application of the Student's *t*-test for unequal variances (Microsoft Excel program) between exposed and control groups for each individual. The *p*-values < 0.05 for the probability that differences between groups are due to random variations were accepted as statistically significant.

#### Results

Results from experiments with the six healthy subjects (No. 1–6) with 1600 metaphases scored from each one (400 exposed to UMTS alone, 400 to caffeine alone, 400 to combination of UMTS plus caffeine, and 400 from control/sham-exposed blood samples) are listed in Table 1 and represented graphically in Figure 1. A single 15-min exposure by the UMTS mobile phone during a phone call in “talk” mode at 1 cm distance, increased the total number of chromosomal aberrations (chromatid gaps and breaks) by 100–275% in regards to the sham-exposed/control samples, while caffeine alone (and sham-exposure to the EMF) increased the same number by 89–250%. In four out of the six subjects the number



**Figure 1.** Mean total number of aberrations (gaps and breaks) *per cell* in 400 cells (peripheral blood lymphocytes) of each group: Control, UMTS 15' (exposed to UMTS for 15 min), Caff (caffeine-treated), and Caff+UMTS 15' (exposed to combination of caffeine and UMTS for 15 min), for each one of the six subjects (No. 1–6). Data are mean  $\pm$  SD.

**Table 1.** Chromatid-type aberrations in human lymphocytes induced by caffeine or/and UMTS MT EMF 15 min exposure

Subject No. (age, sex)	Groups (Samples)	Gaps in 400 cells	Breaks in 400 cells	Total Aberr. in 400 cells	Mean Total Aberr. <i>per cell</i> ± SD	Deviation from Control (%)	<i>p</i> -value	Deviation from Caff (%)	<i>p</i> -value
1 (42, Male)	Control	30	5	35	0.09 ± 0.03				
	UMTS	84	17	101	0.25 ± 0.08	+178	< 0.02	+39	> 0.05
	Caff	50	23	73	0.18 ± 0.06	+100	< 0.04		
	Caff+UMTS	110	51	161	0.40 ± 0.07	+344	< 0.01	+122	< 0.01
2 (33, Female)	Control	37	7	44	0.11 ± 0.04				
	UMTS	70	19	89	0.22 ± 0.06	+100	< 0.03	+5	> 0.05
	Caff	63	22	85	0.21 ± 0.04	+91	< 0.02		
	Caff+UMTS	106	46	152	0.38 ± 0.08	+245	< 0.01	+81	< 0.03
3 (28, Male)	Control	28	9	37	0.09 ± 0.03				
	UMTS	63	15	78	0.19 ± 0.04	+111	< 0.02	+12	> 0.05
	Caff	50	18	68	0.17 ± 0.04	+89	< 0.03		
	Caff+UMTS	160	162	322	0.80 ± 0.07	+789	< 0.01	+371	< 0.01
4 (40, Male)	Control	43	15	58	0.14 ± 0.04				
	UMTS	102	26	128	0.32 ± 0.09	+129	< 0.03	-18	> 0.05
	Caff	97	61	158	0.39 ± 0.07	+179	< 0.01		
	Caff+UMTS	190	106	296	0.74 ± 0.10	+429	< 0.01	+90	< 0.01
5 (35, Female)	Control	42	2	44	0.11 ± 0.01				
	UMTS	82	12	94	0.23 ± 0.03	+109	< 0.01	-12	> 0.05
	Caff	70	34	104	0.26 ± 0.05	+136	< 0.02		
	Caff+UMTS	217	59	276	0.69 ± 0.09	+527	< 0.01	+165	< 0.01
6 (30, Male)	Control	15	2	17	0.04 ± 0.01				
	UMTS	56	5	61	0.15 ± 0.04	+275	< 0.01	+7	> 0.05
	Caff	47	8	55	0.14 ± 0.05	+250	< 0.03		
	Caff+UMTS	124	39	163	0.41 ± 0.07	+925	< 0.01	+193	< 0.01

Aberr.: aberrations; Caff: caffeine.

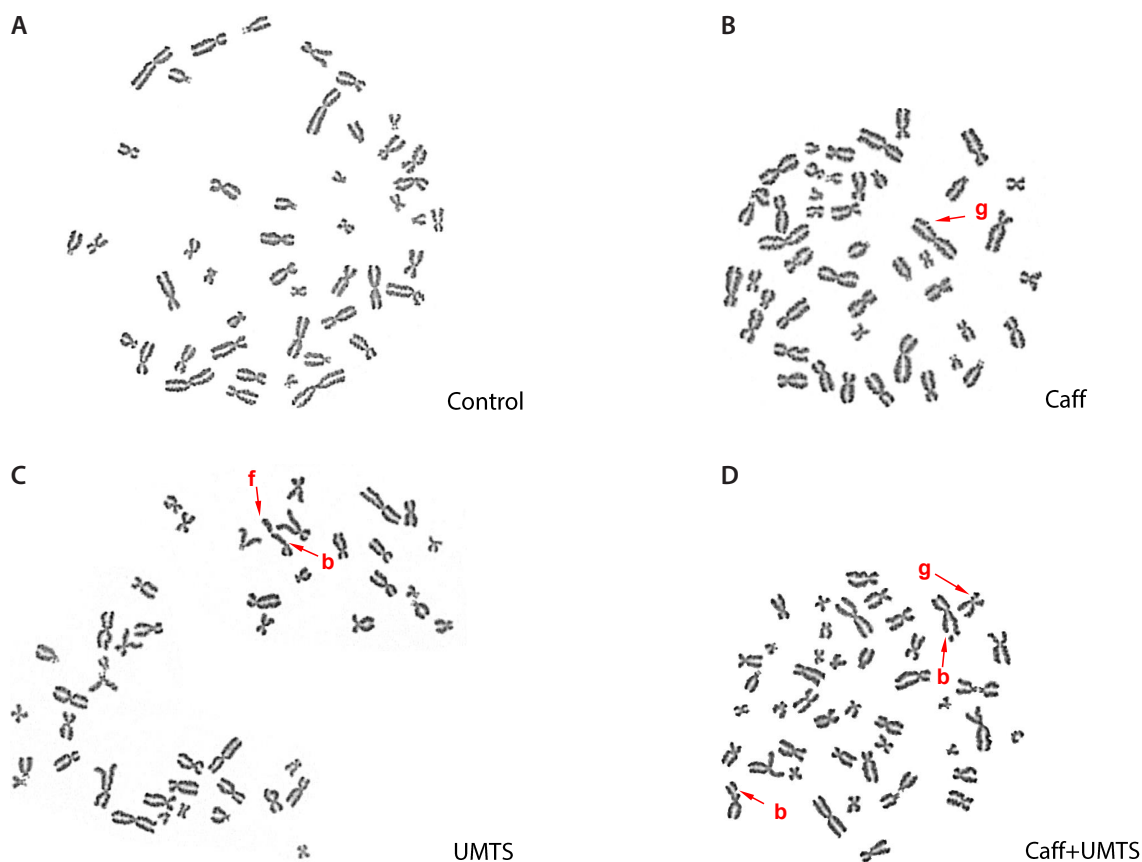
of aberrations induced by UMTS exposure was higher than the number of aberrations induced by caffeine. The combination of caffeine and the 15 min UMTS exposure increased dramatically the corresponding number of induced aberrations by 245–925% compared to the controls (Table 1, Fig. 1). In all subjects, all UMTS-exposed samples (with or without caffeine) and caffeine treated samples differed significantly from the corresponding control/sham-exposed samples ( $p < 0.04$ ) (Table 1). In contrast, in all subjects the UMTS alone-treated samples did not differ significantly from the caffeine alone-treated samples ( $p > 0.05$ ) (Table 1). Thus, while the effect of each stressor was very intense on the human lymphocytes, the effects of the UMTS 15 min exposure alone and the high caffeine dose alone were comparable between them.

In Figure 2A a metaphase of a control blood sample is shown from subject No. 5 (female). This is a representative picture of a metaphase from a control sample/group with all 46 chromosomes intact. Figure 2B shows a metaphase of a blood sample of the same subject, exposed to caffeine only (and sham-exposed to the UMTS EMF), with one chromatid achromatic lesion – gap (g). Figure 2C shows a metaphase of a blood sample of the same subject exposed to UMTS MT EMF (15 min) with one chromatid terminal deletion – break

(b) with displaced fragment (f). Figure 2D shows a metaphase of a blood sample of the same subject (No. 5), exposed to the combination of caffeine and 15 min UMTS MT EMF with two chromatid breaks (b), and one chromatid gap (g).

Each subject exhibited a different sensitivity to each stressor (caffeine or MT EMF exposure). The differential sensitivity was also recorded in the control blood samples due to genetic and environmental factors. The mean number of total aberrations *per cell* between the six different healthy individuals varied in the control samples from 0.04 to 0.14, in the UMTS-exposed samples from 0.15 to 0.32, in caffeine alone-treated samples from 0.14 to 0.39, and in the samples exposed to the combination of UMTS plus caffeine from 0.38 to 0.80. In some cases, subjects with fewer aberrations in their control samples exhibited higher sensitivity to the MT EMF exposure, while this was not observed with caffeine (Table 1).

The MT EMF exposure or the high caffeine dose alone, induced mainly gaps, but also breaks in smaller percentages. The number of gaps induced by the UMTS exposure was 4–7 times greater than the corresponding number of induced breaks in all subjects, while the number of gaps induced by caffeine-alone was 2–6 times greater than the correspond-



**Figure 2.** A. Metaphase of Control blood sample from subject No. 5 (female). All 46 chromosomes are intact. B. Metaphase of caffeine-treated blood sample from the same subject with 1 achromatic lesion – gap (g). C. Metaphase of exposed blood sample to UMTS MT EMF for 15 min, from the same subject with 1 terminal deletion – break (b) with displaced fragment (f). D. Metaphase of a blood sample from the same subject, exposed to combination of caffeine and UMTS MT EMF for 15 min, with 2 terminal deletions – breaks (b), and 1 achromatic lesion – gap (g).

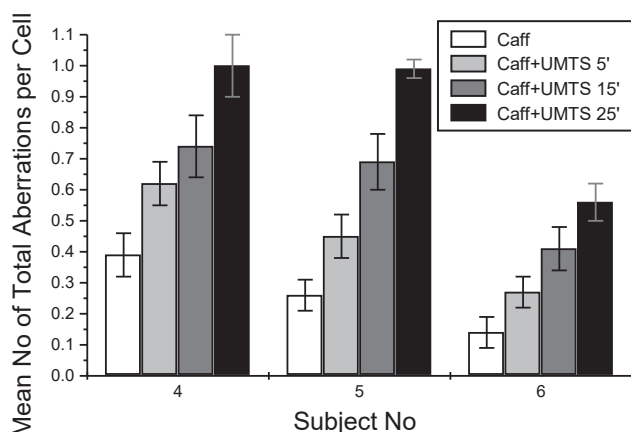
ing number of induced breaks in all subjects. Both gaps and breaks and the total number of aberrations induced either by UMTS alone or by caffeine alone, were significantly increased in regards to the sham-exposed/control sample of each subject (Table 1, Fig. 1).

The combined effect of caffeine with the three different exposure durations to UMTS MT EMF (5, 15, and 25 min) on the chromosomes of 3 subjects (No. 4–6) in terms of induced chromatid gaps and breaks, is shown in Table 2 and represented graphically in Figure 3. The number of induced chromosomal aberrations (chromatid gaps and breaks) *per* cell increased almost linearly with increasing exposure duration to the MT EMF, compared to caffeine treatment alone, showing that the UMTS EMF exposure effect on human lymphocytes is dose-dependent. Figure 4A shows a metaphase of a control/sham-exposed blood sample from subject No. 4 (male) with all 46 chromosomes intact. This was a representative picture for most metaphases from control samples. Figure 4B shows a metaphase of a caffeine alone-treated blood sample from the

same subject with 1 achromatic lesion – gap (g). Figure 4C shows a metaphase from the same subject exposed to combination of caffeine and UMTS MT EMF 5 min, with 1 terminal deletion – break (b). Figure 4D shows a metaphase from the same subject exposed to combination of caffeine and UMTS MT EMF 15 min, with one terminal deletion – break (b) with displaced fragment (f), and one achromatic lesion – gap (g). Figure 4E shows a metaphase from the same subject exposed to combination of caffeine and UMTS MT EMF 25 min, with two terminal deletions – breaks (b), the one with a displaced fragment (f), and one achromatic lesion – gap (g).

## Discussion

It is shown that while a single 15 min exposure of human blood samples to an active 3G (UMTS) mobile phone in “talk” mode at 1 cm distance from the handset increased chromatid-type aberrations from 100% up to 275%, the cor-



**Figure 3.** Mean total number of aberrations (gaps and breaks) per cell in 400 cells (peripheral blood lymphocytes) of each group (caffeine treated only, and exposed to combination of caffeine and UMTS for 5, 15, or 25 min), for subjects No. 4–6. Data are mean  $\pm$  SD

responding increase induced by the high caffeine dose ranged from 89% up to 250% (Table 1). Thus, a single MT EMF exposure  $\sim$  136 times lower than the newest 2020 ICNIRP exposure limit (or  $\sim$  34 times lower than the corresponding 1998 ICNIRP limit), induced chromosomal aberrations in a little higher degree than a caffeine dose  $\sim$  290 times higher than the permissible single caffeine dose for an adult human.

This comparison shows that the exposure limits set for microwave EMFs by ICNIRP (1998; 2020) are enormously less stringent ( $\sim$   $34 \times 290$  times or  $\sim$   $136 \times 290$  times, respectively) than those for caffeine. Assuming linearity between caffeine dose and MT radiation intensity ( $\mu\text{W}/\text{cm}^2$ ) regarding their abilities to induce chromatid aberrations and assuming the caffeine single dose limit to be correct (since the

effects of caffeine on human organism are fast and evident in contrast to EMF-effects), the ICNIRP (2020) microwave EMF exposure limits averaged over 6 min (short-term) exposures should be lowered by  $\sim$  40000 ( $= 4 \times 10^4$ ) times (or the corresponding 1998 ICNIRP limits by  $\sim$   $10^4$  times). By lowering the 2020 ICNIRP limit of  $4000 \mu\text{W}/\text{cm}^2$  (for 2 GHz averaged for 6 min exposure) by  $4 \times 10^4$  times (or the 1998 ICNIRP limit by  $10^4$  times) the limit would become  $0.1 \mu\text{W}/\text{cm}^2$  for short-term exposures, and by lowering this at least by 100 for long-term exposures it would become  $0.001 \mu\text{W}/\text{cm}^2$ . These limits for short and long exposures would be compatible with the caffeine consumption limit, and realistic according to the EMF-bioeffects literature data (Panagopoulos et al. 2010; Panagopoulos 2011, 2017).

Moreover, the present study showed that when MT EMF exposure is combined with a high caffeine dose, the number of induced chromatid aberrations is dramatically increased compared to the effect of each stressor alone (Table 1, Fig. 1). Even though the caffeine dose used in the experiments was  $\sim$  290 times higher than the permissible single dose for an adult individual, this result possibly implies that heavy coffee consumption combined with mobile phone use may significantly increase health risks and should be tested in future experiments. In addition, it shows that the combination of two (or more) separate stressors (called co-stress condition) may have much greater biological effect than the sum of the individual effects of each stressor alone. Since EMF exposure at different bands of the spectrum (RF, ELF, etc.) constitutes a new reality in daily life for everyone, its combination with a variety of other existing stressors (such as development, aging, sickness, infections, ionizing radiation, chemicals, pharmaceuticals, smoking, psychological stress, etc.) should be examined by future studies.

Finally, the present study showed that the effects of MT EMF exposure on humans are dose-dependent and increase

**Table 2.** Aberrations induced by caffeine and its combination with 3 different exposure durations to UMTS MT EMF 5, 15, 25 min exposure

Subject No. (age, sex)	Groups (Samples)	Gaps in 400 cells	Breaks in 400 cells	Total Aberr. in 400 cells	Mean Total Aberr. per cell $\pm$ SD	Deviation from Caff (%)	<i>p</i> -value
4 (40, Male)	Caff	97	61	158	$0.39 \pm 0.07$		
	Caff+UMTS 5 min	153	97	250	$0.62 \pm 0.07$	+59	< 0.01
	Caff+UMTS 15 min	190	106	296	$0.74 \pm 0.10$	+90	< 0.01
	Caff+UMTS 25 min	256	143	399	$1.00 \pm 0.10$	+156	< 0.01
5 (35, Female)	Caff	70	34	104	$0.26 \pm 0.05$		
	Caff+UMTS 5 min	125	55	180	$0.45 \pm 0.07$	+73	< 0.01
	Caff+UMTS 15 min	217	59	276	$0.69 \pm 0.09$	+165	< 0.01
	Caff+UMTS 25 min	291	105	396	$0.99 \pm 0.03$	+281	< 0.01
6 (30, Male)	Caff	47	8	55	$0.14 \pm 0.05$		
	Caff+UMTS 5 min	78	30	108	$0.27 \pm 0.05$	+93	< 0.01
	Caff+UMTS 15 min	124	39	163	$0.41 \pm 0.07$	+193	< 0.01
	Caff+UMTS 25 min	164	60	224	$0.56 \pm 0.06$	+300	< 0.01

Aberr.: aberrations; Caff: caffeine.

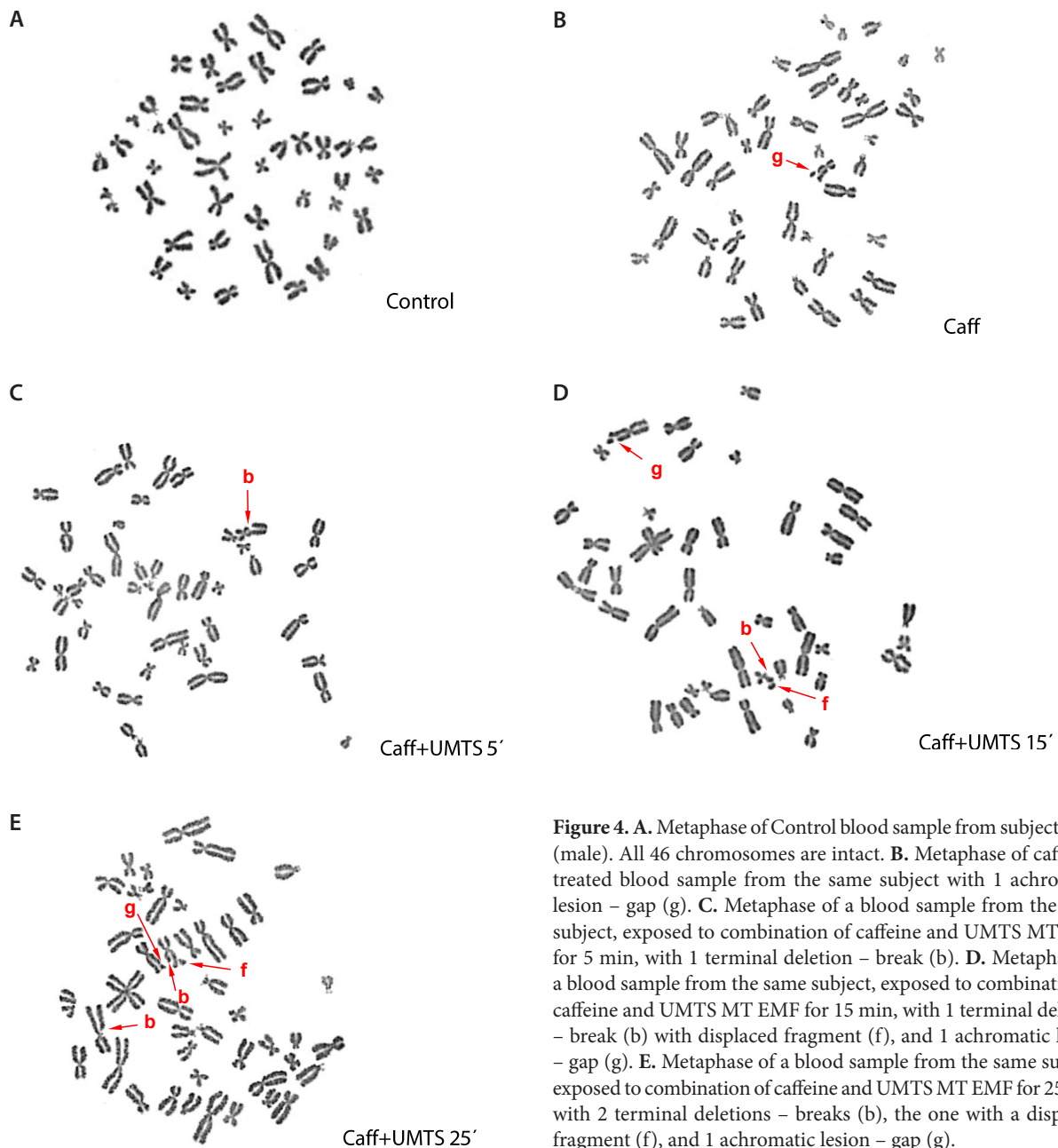


almost linearly with exposure duration. This is in agreement with El-Abd and Eltoweissy (2012), as well as with previous results of my group regarding 2G MT EMF exposures on fruit fly reproduction and DNA damage on reproductive cells (Panagopoulos and Margaritis 2010; Panagopoulos 2011, 2017).

The present study employed a high caffeine dose (~ 290 times higher than the permissible dose for an adult human). One reason was that this dose is frequently used in lymphocyte experiments to abrogate the G<sub>2</sub> checkpoint and study the effect of other stressors such as ionizing radiation

on unprotected cells (Pantelias and Terzoudi 2011; Hatzi et al. 2015). Another reason was that preliminary experiments showed that this high caffeine dose induces a comparable effect with that of a few min UMTS exposure. Certainly this is not a representative dose for coffee consumption as shown in the study (see section *Caffeine dose evaluation*). Future experiments should be conducted combining MT EMF exposure with different caffeine doses ( $\leq 300$  times smaller) representative of coffee consumption.

In previous studies of my group, 2G (GSM) mobile phone radiation exposure on fruit flies induced extensive DNA



**Figure 4.** A. Metaphase of Control blood sample from subject No. 4 (male). All 46 chromosomes are intact. B. Metaphase of caffeine-treated blood sample from the same subject with 1 achromatic lesion – gap (g). C. Metaphase of a blood sample from the same subject, exposed to combination of caffeine and UMTS MT EMF for 5 min, with 1 terminal deletion – break (b). D. Metaphase of a blood sample from the same subject, exposed to combination of caffeine and UMTS MT EMF for 15 min, with 1 terminal deletion – break (b) with displaced fragment (f), and 1 achromatic lesion – gap (g). E. Metaphase of a blood sample from the same subject, exposed to combination of caffeine and UMTS MT EMF for 25 min, with 2 terminal deletions – breaks (b), the one with a displaced fragment (f), and 1 achromatic lesion – gap (g).

damage in the gametes leading to cell death and reproductive decline (Panagopoulos et al. 2007, 2010; Chavdoula et al. 2010; Panagopoulos 2012). Since DNA damage is converted into chromosomal damage during the early M phase of the cell division cycle (Terzoudi and Pantelias 2006; Pantelias and Terzoudi 2010; Terzoudi et al. 2011; Tian et al. 2018), the recorded chromosomal damage induced by the UMTS (3G) MT EMF and/or caffeine is apparently due to DNA damage caused by these stressors.

The DNA damage recorded in our previous studies was induced only by a few min daily exposures for a few days for radiation intensities down to  $1 \mu\text{W}/\text{cm}^2$  (Panagopoulos et al. 2010). Setting a short-term exposure limit based on this result, that would reasonably be  $0.1 \mu\text{W}/\text{cm}^2$ , which is the same with the corresponding limit deduced from the results of the present study.

The recorded effects on human peripheral blood lymphocytes are therefore in complete agreement with previous results of my group (Panagopoulos et al. 2007; 2010; Chavdoula et al. 2010; Panagopoulos 2012, 2017, 2019b) showing once more that digital MT microwave EMFs are very genotoxic/bioactive, able to induce DNA damage and consequent chromosome damage in the human cells as well as in other animals. This should be anticipated since cells are essentially the same in all animals, and all biological/health effects are initiated at the cellular level (Panagopoulos 2019b). It is important to note that the present study based on *in vitro* exposure of human blood cells implies the same limits for short-term exposures ( $0.1 \mu\text{W}/\text{cm}^2$ ) and long-term exposures ( $0.001 \mu\text{W}/\text{cm}^2$ ) to MT EMFs as the previous studies of my group based on *in vivo* animal exposures.

The main type of aberrations induced by either MT EMF exposure or caffeine or the combination of the two stressors were chromatid gaps (achromatic lesions). While chromatid breaks are more intense damages and easier to be recognized (Conger 1967), both gaps and breaks are damages of the same nature and gaps are actually incomplete breaks (Brecher 1977). Ignoring the gaps and counting only the breaks, may be another reason why certain previous studies, e.g. Stronati et al. (2006), did not find aberrations in human blood lymphocytes, in addition to exposing during more resistant cell conditions (e.g. during the  $G_0$  phase instead of during the cell division cycle and especially its most sensitive phases M,  $G_2$ ), and to employing simulated MT signals.

The recorded chromatid-type aberrations induced by the MT EMF exposure is a non-thermal effect since it was not accompanied by any significant temperature increase of the exposed blood samples. The  $0.2^\circ\text{C}$  highest temperature increase during the 25 min exposures is well tolerated by the blood cells as previously explained (Panagopoulos 2019a). The upcoming 5G technology with significantly higher carrier frequencies up to 100 GHz, much denser antenna networks, and more intense and collimated radiation beams,

is expected to induce significant thermal effects in addition to the non-thermal ones which may not be tolerated by the human/animal body (Singh et al. 2017; Neufeld and Kuster 2018; Thielens et al. 2018, 2020; Panagopoulos 2019a; Hardell and Carlberg 2020). This may represent a great danger for public health which the health authorities should carefully investigate before allowing 5G installation.

It is shown that real-life MT EMFs emitted by commercially available mobile phone devices or base antennas/cell towers are far more bioactive than simulated corresponding signals with invariable parameters emitted by generators (Panagopoulos et al. 2015a; Panagopoulos 2017, 2019b; Kostoff et al. 2020). This is probably one reason why in some of the previous studies no effects of simulated MT EMFs on human lymphocytes were reported (Zeni et al. 2003, 2012; Stronati et al. 2006; Schwarz et al. 2008), while in the present study in which a real UMTS exposure was employed, a very intense effect was found (up to 275% increase in chromatid aberrations in regards to the control samples). From the five previous studies with human lymphocytes exposed to real-life MT EMFs (Ji et al. 2004; Gulati et al. 2016; Danese et al. 2017; Zothansiana et al. 2017; Panagopoulos 2019a), four found effects (Ji et al. 2004; Gulati et al. 2016; Zothansiana et al. 2017; Panagopoulos 2019a) in agreement with the majority of lymphocyte studies, while one (Danese et al. 2017) did not. This is the only study found employing real-life MT exposure that reported no effect on human lymphocytes, and one of the very few on any biological model (Panagopoulos 2017, 2019b). In this study, in addition to other issues discussed in the introduction, they exposed the cells during their resting  $G_0$  phase, alike Stronati et al. (2006), instead of exposing them during the cell division cycle, and especially the most sensitive phases M,  $G_2$  (Nias 1998; Terzoudi et al. 2011).

The study that found real-life UMTS (3G) exposure to be even more genotoxic than real-life GSM (2G) (D'Silva et al. 2017) is in line with the fact that newer types of MT EMFs (3G, 4G, etc) transmit increasingly higher amount/density of variable information (speech, text, images, video, Internet) making the signal increasingly complicated, unpredictably varying each moment, and increasingly more bioactive due to the inability of living organisms to adapt. Thus, the effects of the upcoming 5G MT EMF are expected to be even more intense than those of 2G, 3G, 4G. This should be seriously considered by the responsible public health authorities.

Since the health effects of all microwave telecommunication EMFs (including MT, Wi-Fi, domestic cordless phones, Bluetooth wireless connections etc.) are of utmost importance in our days, studies should be conducted to test the most sensitive biological conditions with real-life exposures, and in combination with other environmental stressors, otherwise the results may be misleading in terms of public health protection. Exposures by any type of simulated signals and within any type of exposure chambers used to produce

“uniform” exposures such as “reverberation chambers” or “TEM chambers” (Ardoino et al. 2005; Wu et al. 2009) do not represent real-life exposure conditions and may produce misleading outcomes towards “no effect” findings (Panagopoulos 2017, 2019b; Pall 2018).

The disruption of cell electrochemical balance by man-made (polarized) EMFs through irregular gating of voltage-gated ion channels on cell membranes is described by the “ion forced-oscillation mechanism” (Panagopoulos et al. 2000, 2002, 2015b, 2020). This may lead to DNA damage by intracellular release of free radicals or hydrolytic enzymes like DNases (Barzilai and Yamamoto 2004; Phillips et al. 2009; Panagopoulos 2011; Pall 2013; Yakymenko et al. 2016). This is in line with the attribution of the DNA and chromosome damage to oxidative stress by El Abd and Eltoweissy (2012). What is referred to by Pall (2018) as voltage-gated calcium channel mechanism (“VGCC mechanism”) is the application of the above mechanism specifically on the calcium voltage-gated ion channels. The same mechanism refers to all cation voltage-gated channels, and calcium – although more extensively studied – should not be considered more important than other cations.

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