Cytogenetic Effects of Exposure to 2.3 GHz Radiofrequency Radiation on Human Lymphocytes *In Vitro*

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Abstract. *Background: No previous in vitro studies have tested radio frequency radiation for at least one full cell cycle in culture. The aim was to test if exposure used in mobile phones and wireless network technologies would induce DNA damage in cultured human lymphocytes with and without a known clastogen. Materials and Methods: Lymphocytes from six donors were exposed to 2.3 GHz, 10 W/m^2 continuous waves, or 2.3 GHz, 10 W/m^2 pulsed waves (200 Hz pulse frequency, 50% duty cycle). Mitomycin C was added to half of the cultures. DNA synthesis and repair were inhibited in one experiment. Results: No statistically significant differences were observed between control and exposed cultures. A weak trend for more chromosomal damage with the interaction of pulsed fields with mitomycin C compared to a constant field was observed. Conclusion: Exposure during the whole cell cycle in inhibited cultures did not resulted in significant differences in chromosomal aberrations as compared to controls.*

In recent years there have been considerable advances in the development of devices emitting microwaves and radiofrequency electromagnetic radiation (RFR) such as mobile phones. Sienkiewicz and Kowalczuk (1) in a review of 26 previous reviews found that most of the reports reached a similar conclusion, namely that exposure to low level RFR may cause a variety of subtle biological effects. However, the possibility of exposure causing adverse health effects remained unproven. Gøtrik *et al*. (2), in a common approach for the Nordic competent authorities, agreed that there is no scientific evidence for any adverse health effects from mobile telecommunication systems, but concluded that a knowledge gap existed that justified more research in this field. Moulder *et al*. (3) in a recent review concluded that the current evidence for a causal association between cancer and exposure to RFR is weak and unconvincing. When epidemiological evidence is weak, partly due to short follow-up periods, laboratory studies are essential and *in vitro* studies are a good supplement if the results are replicable and consistent across different methods of testing.

Several recent *in vitro* studies on genotoxic effects of RFR have been published. A few of them reported that exposure of cultured cells to mobile phone RFR caused DNA damage in some assays (4, 5), while other *in vitro* studies reported no effect on DNA (6-10). Based on a meta-analysis of 63 publications from 1990 to 2005, Vijayalaxmi and Prihoda (11) concluded that the overall genotoxicity indices were similar in the RFR-exposed and control groups when *in vitro* investigations were conducted under the recommended safety guidelines. A direct genotoxic action is generally thought to be unlikely because of the low energy of microwave photons (12), but several studies have suggested that RFR exposure may enhance the genotoxic effects of other cancer-inducing substances (3, 13, 14).

What kind of exposure is of interest? The third generation of mobile phones, UMTS (Universal Mobile Telecommunication) was introduced in Europe in 2002, using frequencies around 2.0-2.2 GHz. Blue-tooth equipment (2.4 GHz) is used

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in hands-free equipment for mobile phones and many workplaces have installed equipment for wireless local area network (LAN) communication in rooms and corridors operating in the frequency range 2-2.4 GHz, employing modulated fields. Thus, an exposure for 2.0-2.4 GHz electromagnetic fields might be of particular interest. Since these frequencies have only been used extensively since 2002, the short latency period reduces the chances of an effect being seen in cancer studies. Chromosomal aberrations in lymphocytes have proven to be associated with cancer risk in humans (15-17) and are considered informative indicators of exposure; moreover they can be used in a more immediate screening for adverse effects.

The aim of the present pilot study was therefore to investigate the clastogenetic effects of 2.3 GHz radiation, both pulsed and continuous exposures, alone or in combination with the known clastogen mitomycin C (MMC) on cultured human lymphocytes. In order to enhance the likelihood of discovering a possible effect, we chose to use lymphocytes from several donors, to expose the cells during the whole *in vitro* period and in one experimental setup to inhibit DNA synthesis and repair in the G_2-phase of the cell cycle.

The level of exposure we have chosen corresponds roughly to the safety limit recommended by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) (13). Safety limits are based on induced currents in the human body, and the difference in geometry and material properties makes it difficult to compare exposures in an *in vitro* experiment to the human body. This problem can be investigated by detailed finite difference method calculations. Since our aim is not to discuss safety guidelines, but rather to perform a pilot study to identify potentially interesting effects that could be followed up more closely in further study, we have not carried out such detailed theoretical calculations. However, we have given enough information about the experimental design so that others can perform finite difference calculations.

Materials and Methods

Lymphocyte donors. After informed consent, four non-smoking healthy individuals, two females (25 and 44 years old) and two males (40 and 44 years old), and two smoking females (41 and 55 years old) were chosen as blood donors. The hospital authorities approved the study, otherwise the research was exempted from review since the blood samples were only used for *in vitro* studies and the results were registered in an anonymous database. All donors were genotyped for polymorphisms in the glutathione-*S*-transferase (*GST*) genes as polymorphisms in these detoxifying enzymes are common in the Caucasian population (18) and are therefore important to control for (19). Individuals with *GSTM1* null and *GSTP1* heterozygote or wild-type alleles were chosen as donors. Polymorphisms of the *GSTM1* (null), *GSTT1* (null) and *GSTP1* ($Ile^{105}Val$) genes were analyzed simultaneously by multiplex PCR as described by Nedelcheva Kristensen *et al*. (20) with minor modifications.

Cytogenetic analysis. Phytohemagglutinin-stimulated whole blood in RPMI medium (1640; BioWhittaker Cambrex, Verviers, Belgium) was cultured using methods as described elsewhere (21). A cell density of 1×10^6 cells/ml of medium was ensured. Before the experiments were initiated, cultures with different concentrations of mitomycin C (MMC) (Sigma-Aldrich, Oslo, Norway) were performed and harvested at different time intervals. After 48 hours of incubation, very few cells were in mitosis. For the experiments Colcemid (Sigma-Aldrich, Oslo, Norway) (0.3 μg /ml) was added three hours before harvesting at 53 hours. Previous experiences in handling blood from adults have suggested a similar delay in first cell division in our assay system. The cells were harvested and the slides stained with Giemsa according to conventional methods. In addition, chromosomal aberrations were scored in cells where DNA synthesis and repair were inhibited *in vitro* with hydroxyurea (Sigma-Aldrich) and caffeine (Sigma-Aldrich) both added at a concentration of 7.5×10^{-2} M three hours before harvesting together with Colcemid (0.3 μg /ml) (22, 23). For the uninhibited cultures, 200 cells per test were scored blind on coded slides by the same three microscopists. For the inhibited cultures, 50 cells per culture were scored due to the increased aberration frequencies in this assay. The same microscopist scored both the exposed and control cultures from the same blood donor. The scoring criteria used were as described by Brøgger *et al*. (24) and Savage (25) and harmonized among scorers. The effect parameters in the present study were number of cells with aberrations (CA), cells with aberrations and gaps (CAG), chromosome type aberrations (CSA), chromosomal breaks (CAB), chromatid type aberrations (CTA), chromatid breaks (CTB), and chromosome and chromatid gaps (CSG and CTG).

Study design. Positive and negative controls were included in each experiment. Eight cultures of 10 ml of medium were set up for each donor for the uninhibited cultures. Half of the cultures were exposed for 53 hours to RFR either as continuous or pulsed-field exposure. As a positive control and to test if the combination of a known clastogen and RFR exposure would be more detrimental to the cells, MMC (100 ng/ml) was added to half of the cultures with and without RFR exposure for the last 23 hours *in vitro*. Each experimental setup included cultures from two blood donors (either two females or two males). For each donor, two cultures with and two without MMC were exposed, and two cultures with and two without MMC were controls using 10 ml of medium per culture flask (EasYFlasks™ Nunclon™ flask; Nunc A/S, Roskilde, Denmark). For the setups where inhibition of DNA synthesis and repair were applied, the two cultures for each treatment regime were replaced with four cultures of 3 ml of medium in culture tubes (TubeNunclon™; Nunc A/S) otherwise the setups were identical. For the uninhibited cultures blood, samples from two males and two non-smoking females were exposed to continuous RFR. For the exposure to pulsed RFR, blood from two smoking females were included in the study. The inhibited cultures from all six donors were exposed to pulsed RFR only.

Exposure. The experiments were performed in a large anechoic chamber (5×4×4 m) with temperature stabilized at 37˚C (details below). A diagram with a detailed description of the instrumental setup is shown in Figure 1 and the system is illustrated in Figure 2. Both exposed and control samples were kept in the same room, but well separated. The exposure of the control samples was approximately 40 dB lower than that of the exposed samples. The RFR exposure was

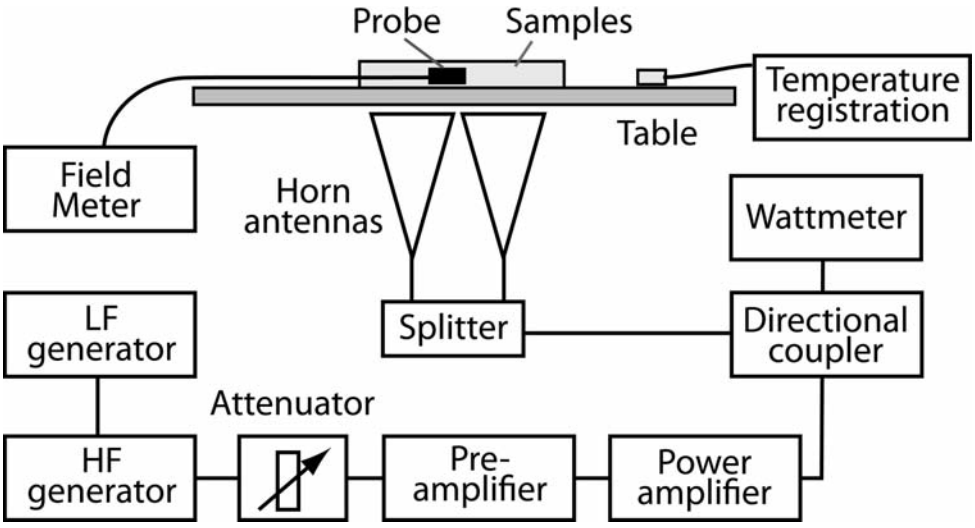

Figure 1. *Block diagram for the experimental setup for the continuous-wave experiment. For the pulsed-wave, the setup was similar. The temperature regulation setup is not included in the drawing. The incident power desity was measured with a Wandel & Goltermann EMR300 instrument with a 9C probe. The probe was used to map the exposure level across the full area where the samples were placed. The probe was removed during the exposure of the cells. The control samples are not included in this drawing. LF, Low frequency; HF, high frequency.*

2.3 GHz, 10 W/m^2 incident continuous wave, or 2.3 GHz, 10 W/m^2 incident pulsed-wave (200 Hz pulse frequency, 50% duty cycle). Time averaged values are given. The cells were exposed to the RFR through a rectangular horn antenna (Narda 615, Odd Tvedt & Co, Bergen, Norway). The opening of the antenna was approximately 6×8 cm and was directed upwards, placed approximately 1.2 m beneath the cultures. The exposed area (ca. 30 cm diameter) was constructed to cover only the number of exposed cultures. The culture flasks were placed horizontally on 5-6 mm thick cardboard. The samples were oriented so that the magnetic field was parallel to the long side of the flask or tube, with the electric field horizontally and perpendicular to this direction. The exposure level was determined with a Wandel & Goltermann EMR 300 instrument with a Type 9C probe (Odd Tvedt, Bergen, Norway). For the continuous wave, the electric field was adjusted to 61 V/m rms just outside the cell growth medium containing the cells. This corresponds to 10 W/m^2 for a far field situation, which we expect is valid for our geometry. The intensity varied by 1.5 dB across the area where the samples were placed, with the highest intensity in the middle. To compensate for the fact that exposure is somewhat unevenly distributed within the flask and to some degree depends on the position of the flasks within the exposed area, the cultures were stirred and replaced randomly in the exposed area three times during the culture period. The cells were placed in EasY Flasks Nunclone™ culture flasks with 25 cm^2 culture area (4 cm wide), or flat-bottomed Nunc Tubes Nunclon™ culture tubes with 5.5 cm^2 culture area (1.0 cm wide). The level of the medium was 4 mm in the flat-bottomed flasks and ranged from 2 to 9 mm within the tubes. The flasks or tubes were positioned relatively close to each other as indicated in Figure 2.

The anechoic chamber was temperature stabilized to 37.0±0.3˚C and the temperature of the samples was determined by the all-room regulation only. The temperature was measured and recorded continuously with two thermocouples interfaced to an INTAB data logger AAC-2 (Intab Interface-teknik AB, Sternkulle, Sweden). The absolute precision of the temperature registration was better than 0.5˚C, and the resolution in the recording was 0.1˚C. Each probe was placed in an additional culture flask with comparable amount of water as the samples, and placed near the exposed and control area. The temperature recordings showed 37.0±0.3˚C throughout the culture period. The standard deviation given represents the fluctuation of the temperature only. Separate control measurements revealed that the temperature in the exposed samples was 0.5˚C higher than in the control samples. This difference was not compensated for in this study. The heating elements and fans circulating the room air generated a background magnetic field (50 Hz) of 0.03 μT at the position of the cells. This magnetic field was determined with a Wandel & Goltermann EMR 300 instrument with an EFA-3 probe. The calculated depth of penetration (skin depth) in the vertical direction for the frequency used in this study was approximately 1 cm in the culture medium.

Statistics. For statistical comparisons of the mean values, the Mann-Whitney non-parametric test was performed. The Kruskal-Wallis test was performed to test for possible differences between the control cultures for each individual. A probability level of 0.05, two-tailed, was used to indicate statistical significance. Anova was used to analyze differences between exposed and control cultures for all conventional cultures with gender, MMC, pulsed and continuous field exposure in the model. SPSS 12.0 for windows (SPSS, Chicago, USA) was used for the statistical analyses.

Results

The results are presented as mean values per 100 cells, with standard deviation for the two or four parallel cultures for CA, CSA, CTA and CTG for each experimental setup. The results for the uninhibited cultures exposed to continuous-

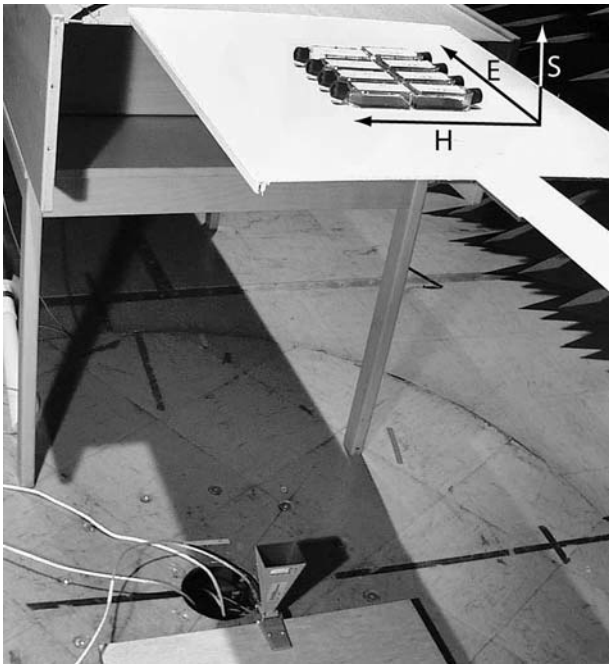

Figure 2. *The culture flasks or tubes were placed relatively closely to each other. This figure, in combination with the text, provides sufficient information if one wants to investigate the field pattern inside the samples further, using finite difference methods or similar numerical techniques. The diameter of the outer circle is slightly more than 30 cm. LF, Low frequency; HF, high frequency.*

wave radiation are presented in Table I, with males and non-smoking females separate and summarized. In Table II, the results for cultures exposed to pulsed-wave radiation are presented. In this experiment, two smoking female blood donors were included. There was a significant difference for all types of aberrations between cultures with and without MMC as expected ($p<0.05$ for CSA, $p=0.001$ for CA, CTA, CTG). No statistically significant differences were found between control and RFR-exposed cultures regardless of MMC and RFR treatment for either gender nor for combined results using the Mann-Whitney test. No difference between control and RFR-exposed cultures were observed for the remaining effect parameters either (data not shown).

The effect of inhibition of DNA synthesis and repair in the G_2-phase of the cell cycle was studied for cultures exposed to pulsed RFR with and without MMC for all six blood donors. The results are presented in Table III. Compared to the uninhibited cultures (Table II), a significant increase was observed for all types of aberrations, except for CSA in cultures without MMC, despite the fact that only 50 cells per culture were scored in the inhibited cultures. No statistical significant differences were observed for any of the chromosomal parameters for controls compared to RFR-exposed cultures, in spite of the increase in number of aberrations.

A comparison of the effect of continuous contra-pulsed RFR exposure was possible for the uninhibited cultures for the non-smoking blood donors. The mean frequencies combined for all donors are presented in Table IV for the exposed cultures, for all the chromosomal parameters scored. A consistent trend for a slightly non-significant increase in mean frequencies for all chromosomal parameters for pulsed-RFR exposure combined with MMC treatment was found. To include the control cultures in the statistical evaluation of continuous contra-pulsed-RFR exposure, the frequency difference between the exposed and control culture was calculated for each individual for each experimental setup separately because non-significant variations were observed for control cultures from the same individual in the different experimental settings using the Kruskal-Wallis test (data not shown). The mean frequency difference for each experimental setting was then used as the effect parameter in an Anova analysis including gender, MMC, pulsed- and continuous-field exposure in the model. None of the included parameters had a significant impact on the effect parameters. A weak trend towards more damage from pulsed-rather than constant-field exposure was observed, as well as a trend towards more damage from the interaction of pulsed fields and MMC. This was observed for all the chromosomal parameters (Table IV).

Discussion

The primary focus of this *in vitro* pilot study was to evaluate if exposure to continuous or pulsed 2.3 GHz electromagnetic radiation of the type used in different mobile phone and wireless network technology would induce chromosomal damage in human lymphocytes.

Inhibition of DNA synthesis and repair in the G_2-phase of the cell cycle was carried out in one experimental setup in order to test if an increase in aberrations would be a more sensitive method for detecting possible differences between control and exposed cultures. The focus was on whether RFR could cause chromosomal damage either alone or in combination with the known clastogenic agent MMC when RFR was applied during a full cell cycle in culture. We used lymphocytes from both smoking and non-smoking individuals in order to see if smoking habits would influence the sensitivity of our test system.

Test cultures were performed before the experiments were initiated. Since few mitoses were observed in the early cultures, most of the cells collected in mitosis from 50 to 53 hours were assumed to be in the first cell division. Even if a few cells should be in the second cell division at 53 hours, the cells were continuously exposed and aberrations could also be expected to appear during the second cell division. Exact cell proliferation measurements were not performed.

Table I. *Aberrations recorded for uninhibited cultures exposed to 2.3 GHz continuous-wave (CW) radiation. Mean frequencies per 100 cells (standard deviation in brackets) are given for two parallel cultures for two male (M) and two female (F) blood donors.*

Variable	N/Gender	No. of cells	CA	CSA	CTA	CTG
Without mitomycin C						
Control	4 M+F	1600	1.13 (0.95)	1.31 (2.59)	0.56 (0.69)	3.56 (1.01)
CW-RFR	4 M+F	1600	1.00 (0.76)	0.44 (0.42)	0.69 (0.70)	3.69 (1.91)
Control	2 M	800	1.25 (0.96)	2.00 (3.67)	0.63 (0.95)	3.25 (0.65)
CW-RFR	2 M	800	1.25 (0.87)	0.75 (2.29)	0.75 (0.87)	2.75 (1.85)
Control	2 F	800	1.00 (1.08)	0.63 (0.95)	0.50 (0.41)	3.88 (1.31)
CW-RFR	2 F	800	0.75 (0.65)	0.13 (0.25)	0.63 (0.63)	4.63 (1.65)
With mitomycin C						
Control	4 M+F	1600	8.50 (2.79)	2.00 (1.00)	10.00 (3.72)	9.31 (2.14)
CW-RFR	4 M+F	1600	7.75 (4.40)	2.50 (4.36)	10.06 (5.29)	8.94 (2.56)
Control	2 M	800	8.00 (2.38)	2.13 (1.31)	8.88 (4.27)	9.25 (1.55)
CW-RFR	2 M	800	6.38 (2.32)	1.50 (1.22)	8.38 (3.20)	8.88 (1.38)
Control	2 F	800	9.00 (3.44)	1.88 (0.75)	11.13 (3.28)	9.38 (2.87)
CW-RFR	2 F	800	9.13 (5.89)	3.50 (6.34)	11.75 (6.90)	9.00 (3.98)

CA, Number of cells with aberrations; CSA, chromosome type aberrations; CTA, chromatid type aberrations, CTG chromatid gaps.

Table II. *Aberrations recorded for uninhibited cultures exposed to 2.3 GHz pulse-wave (PW) radiation. Mean frequencies per 100 cells (standard deviation in brackets) are given for two parallel cultures for two male (M), two non-smoking female (F) and two smoking female (FS) blood donors.*

Variable	N/Gender	No. of cells	CA	CSA	CTA	CTG
Without mitomycin C						
Control	6 M+F+FS	2400	1.41 (0.95)	1.16 (1.27)	0.63 (0.94)	4.53 (1.86)
PW-RFR	6 M+F+FS	2400	1.34 (0.98)	1.63 (3.34)	0.93 (1.11)	3.98 (1.31)
Control	2 M	800	1.13 (0.75)	1.00 (0.71)	0.38 (0.75)	4.00 (0.71)
PW-RFR	2 M	800	1.25 (0.29)	5.00 (5.81)	0.50 (0.41)	3.13 (1.44)
Control	2 F	800	1.50 (1.58)	1.13 (1.03)	0.88 (1.03)	4.38 (1.03)
PW-RFR	2 F	800	1.35 (1.12)	0.75 (0.96)	0.73 (0.52)	3.80 (0.91)
Control	2 FS	800	1.50 (0.76)	1.25 (1.67)	0.63 (1.06)	4.88 (2.53)
PW-RFR	2 FS	800	1.38 (1.19)	0.38 (0.52)	1.25 (1.49)	4.50 (1.31)
With mitomycin C						
Control	6 M+F+F S	2400	11.22 (3.77)	1.94 (1.11)	14.47 (5.72)	11.81 (3.58)
PW-RFR	6 M+F+F S	2400	11.81 (3.55)	3.03 (1.62)	14.34 (4.34)	13.75 (5.76)
Control	2 M	800	8.63 (4.13)	1.38 (0.48)	10.38 (6.63)	8.00 (2.12)
PW-RFR	2 M	800	9.00 (1.41)	2.50 (1.58)	11.38 (1.31)	9.00 (1.08)
Control	2 F	800	13.25 (2.33)	2.88 (0.63)	17.25 (3.12)	12.50 (2.89)
PW-RFR	2 F	800	14.50 (4.81)	3.13 (1.49)	16.75 (5.43)	13.00 (2.86)
Control	2 FS	800	11.50 (3.82)	1.75 (1.28)	15.13 (5.72)	13.38 (3.25)
PW-RFR	2 FS	800	11.86 (2.75)	3.25 (1.83)	14.63 (4.31)	16.50 (6.72)

CA, Number of cells with aberrations; CSA, chromosome type aberrations; CTA, chromatid type aberrations; CTG, chromatid gaps.

Blood samples were collected twice with a month's interval for the uninhibited cultures and with a time lap of more than a year before the inhibited cultures were initiated. Repeated analysis of peripheral blood lymphocytes from the same individual demonstrated good reproducibility of the assay for the control cultures, although a non-significant variation was observed, stressing the importance of including both positive and negative controls in each experiment. Higher frequencies in the control culture corresponded to higher frequencies in the RFR culture. Glutathione-*S*-transferases (GSTs) encode a family of detoxifying phase II enzymes catalyzing the conjugation of glutathione to electrophilic compounds. The *GSTM1, GSTT1* and *GSTP1* genes are known to be polymorphic, and some *in vitro* experiments with human lymphocytes include the influence of the genotype of the donors on the background level of DNA damage (19, 26, 27). Therefore, to secure equal ability to detoxify possible chemical exposures, all blood donors

Table III. *Aberrations recorded for inhibited cultures exposed to 2.3 GHz pulsed-wave radiation. Mean frequencies per 100 cells (standard deviation in brackets) are given for four parallel cultures for two male (M), two non-smoking female (F) and two smoking female (FS) blood donors.*

Variable	N/Gender	No. of cells	CA	CSA	CTA	CTG
Without mitomycin C						
Control	2 M	400	5.25 (2.12)	2.00 (1.51)	5.00 (2.83)	21.50 (8.73)
PW-RFR	2 M	400	4.75 (2.60)	1.50 (1.41)	3.50 (2.56)	20.00 (8.75)
Control	2 F	220	5.86 (3.50)	1.00 (2.00)	5.36 (2.78)	20.57 (9.23)
PW-RFR	2 F	200	6.00 (5.16)	2.00 (2.83)	4.50 (3.00)	22.50 (8.23)
Control	2 FS	400	11.50 (5.53)	3.00 (3.85)	12.00 (4.41)	25.75 (10.22)
PW-RFR	2 FS	300	7.00 (5.18)	1.67 (1.51)	6.33 (6.25)	21.33 (14.51)
With mitomycin C						
Control	2 M	350	39.1 (14.6)	28.3 (24.7)	64.3 (41.3)	95.7 (20.2)
PW-RFR	2 M	400	35.3 (10.4)	23.0 (15.4)	77.3 (30.5)	114.8 (36.1)
Control	2 F	200	45.5 (10.8)	47.0 (21.0)	133.0 (56.7)	156.0 (32.7)
PW-RFR	2 F	200	34.0 (10.8)	34.5 (17.8)	69.0 (23.5)	102.0 (24.7)
Control	2 FS	400	47.3 (9.3)	32.5 (16.1)	107.5 (44.8)	147.0 (41.8)
PW-RFR	2 FS	400	50.3 (9.3)	36.8 (16.6)	115.8 (29.3)	144.3 (35.6)

CA, Number of cells with aberrations; CSA, chromosome type aberrations; CTA, chromatid type aberrations; CTG, chromatid gaps.

were of *GSTM1* null genotype, *GSTP1* heterozygote or wild type, and only one donor was *GSTT1* null genotype.

The exposed samples experienced a temperature approximately 0.5˚C higher than that for the control samples. We believe that this should not have a significant effect on the results, since in a similar experimental set up with 16.5 GHz exposure we tested cultures at both 37˚C and 38˚C. The temperature difference had very little effect on the chromosomal aberration frequencies Hansteen *et al*. (28). In the present experiment where RFR was applied during all phases of the cell cycle no significant differences were observed between control and RFR-exposed cultures for any of the chromosomal parameters for each blood donor, regardless of gender (results not shown), or for all results combined. This is consistent with previous reports for different RFR wavelength exposure for different cytogenetic assays (6-8, 11, 14, 28-31) even for intermittent exposures at different stages of the cell cycle (32). Other studies reported that exposure to RFR signals under extended conditions were capable of inducing DNA damage in human lymphocytes *in vitro* (5, 33), and Tice *et al*. (5) and Zotti-Martelli *et al*. (4) reported individual differences between blood donors.

In the present study, no cytogenetic effect of 2.3 GHz radiation combined with MMC treatment was documented. RFR exposure with or without treatment with MMC gave no increase in chromosomal damage, neither in lymphocytes from non-smoking nor from smoking individuals. This is consistent with some earlier studies where no synergistic effects were observed for a range of genotoxicity endpoints of a combined exposure of cells to RFR and cytotoxic drugs (34-37). Maes *et al*. (38) reported a weak synergistic effect of RFR with co-exposure to MMC on cytogenetic endpoints, but this was not reproduced in a later study (39). Zhang *et al*. (40) also reported a synergistic increase in both micronuclei and DNA damage in human lymphocytes upon co-exposure to MMC.

Lai and Sing (41) and d'Ambrosio *et al*. (42) suggested a different response to continuous- and pulsed-wave RFR exposure. We did not find statistically significant results in our experiment, but the Anova analysis showed a weak trend towards more damage with the pulsed- than the continuous-

Table IV. *Aberrations compared for 2.3 GHz continuous and pulsed field exposure to lymphocytes from non-smoking males and females combined. Mean frequencies per 100 cells (standard deviation in brackets) are shown*.*

	Without mitomycin C		With mitomycin C	
Variable	Continuous	Pulsed	Continuous	Pulsed
CAG	4.19 (2.14)	3.89 (1.33)	12.50 (2.74)	13.94 (3.46)
CA	1.00 (0.76)	1.30 (0.81)	7.75 (4.40)	11.75 (4.41)
CTG	3.69 (1.91)	3.46 (1.17)	8.94 (2.76)	11.00 (2.93)
CSG	0.81 (0.84)	0.61 (0.45)	5.19 (1.31)	6.31 (1.65)
CTB	0.68 (0.70)	0.61 (0.59)	5.31 (3.26)	7.56 (3.50)
CTA	0.69 (0.70)	0.61 (0.45)	10.06 (5.29)	14.06 (4.65)
CSB	0.31 (0.37)	2.44 (3.73)	2.06 (3.67)	2.31 (1.39)
CSA	0.44 (0.42)	2.88 (4.48)	2.50 (4.36)	2.81 (1.46)

CAG, Number of cells with gaps; CA, number of cells with aberrations; CTG, chromatid gaps; CSG, chromosome gaps; CTB, Chromatid breaks; CTA, all chromatid type aberrations; CSB, chromosome breaks; CSA, all chromosome type aberrations. Number of cultures=8. *The results for the control cultures are not shown, but they are included in the Anova analysis (see text).

field exposure and a higher damage rate from the interaction between pulsed fields and MMC. As seen by the mean frequencies listed in Table IV, the increase is not dramatic and the implication on health effects is uncertain (3).

In conclusion, no statistically significant differences were observed between control and the 2.3 GHz radiation-exposed cultures, with or without addition of MMC, for any of the chromosomal parameters for any of the blood donors in the uninhibited assay, or in the cultures where DNA synthesis and repair were inhibited. The increase in chromosomal damage due to unrepaired aberrations was not a more sensitive method for detecting possible differences between control and exposed cultures in this setting. An Anova analysis was performed with gender, MMC, pulsed- and continuous-field exposure in the model, and the difference between the control and exposed aberration frequencies as effect parameter. None of the included parameters had a significant impact on the effect parameter. A weak trend was observed towards more chromosomal damage from pulsed- than constant-field exposure, especially from the interaction of pulsed fields and MMC.

References

1 Sienkiewicz ZJ and Kowalczuk CI: A summary of Recent Reports on Mobile Phones and Health (2000-2004). http://www.hpa.org.uk/radiation/publications/w_series_reports/2005/nrpb_w65.htm: 2005.

2 Gøtrik JK, Laaksonen J, Magnusson SM, Harbitz O and Holm L-E: Mobile Telephony and Health. A common approach for the Nordic competent authorities. Nordic Mobil Press: 1-2, 2004.

3 Moulder JE, Foster KR, Erdreich LS and McNamee JP: Mobile phones, mobile phone base stations and cancer: a review. Int J Radiat Biol *81*: 189-203, 2005.

4 Zotti-Martelli L, Peccatori M, Maggini V, Ballardin M and Barale R: Individual responsiveness to induction of micronuclei in human lymphocytes after exposure *in vitro* to 1800-MHz microwave radiation. Mutat Res *582*: 42-52, 2005.

5 Tice RR, Hook GG, Donner M, McRee DI and Guy AW: Genotoxicity of radiofrequency signals. I. Investigation of DNA damage and micronuclei induction in cultured human blood cells. Bioelectromagnetics *23*: 113-126, 2002.

6 Zeni O, Romano M, Perrotta A, Lioi MB, Barbieri R, d'Ambrosio G, Massa R and Scarfi MR: Evaluation of genotoxic effects in human peripheral blood leukocytes following an acute *in vitro* exposure to 900 MHz radiofrequency fields. Bioelectromagnetics *26*: 258-265, 2005.

7 Sakuma N, Komatsubara Y, Takeda H, Hirose H, Sekijima M, Nojima T and Miyakoshi J: DNA strand breaks are not induced in human cells exposed to 2.1425 GHz band CW and W-CDMA modulated radiofrequency fields allocated to mobile radio base stations. Bioelectromagnetics *27*: 51-57, 2006.

8 Chemeris NK, Gapeyev AB, Sirota NP, Gudkova OY, Tankanag AV, Konovalov IV, Buzoverya ME, Suvorov VG and Logunov VA: Lack of direct DNA damage in human blood leukocytes and lymphocytes after *in vitro* exposure to high power microwave pulses. Bioelectromagnetics *27*: 197-203, 2006.

9 Komatsubara Y, Hirose H, Sakurai T, Koyama S, Suzuki Y, Taki M and Miyakoshi J: Effect of high-frequency electromagnetic fields with a wide range of SARs on chromosomal aberrations in murine m5S cells. Mutat Res *587*: 114-119, 2005.

10 Figueiredo ABS, Alves RN and Ramalho AT: Cytogenetic analysis of the effects of 2.5 amd 10.5 GHz microwaves on human lymphocytes. Genet Mol Biol *27*: 1-10, 2004.

11 Vijayalaxmi, Prihoda TJ: Genetic damage in mammalian somatic cells exposed to radiofrequency radiation: A meta-analysis of data from 63 publications (1990-2005). Radiat Res *169*: 561-574, 2008.

12 Maes A, Verschaeve L, Arroyo A, De Wagter C and Vercruyssen L: *In vitro* cytogenetic effects of 2450 MHz waves on human peripheral blood lymphocytes. Bioelectromagnetics *14*: 495-501, 1993.

13 ICNIRP (International Commission on Non-Ionizing Radiation Protection): Guidelines for limiting exposure to time-varying electric, magnetic, and electromagnetic fields (up to 300 GHz). Health Phys *74*: 494-522, 1998.

14 Vijayalaxmi, Obe G: Controversial cytogenetic observations in mammalian somatic cells exposed to radiofrequency radiation. Radiat Res *162*: 481-496, 2004.

15 Hagmar L, Brøgger A, Hansteen IL, Heim S, Høgstedt B, Knudsen L, Lambert B, Linnainmaa K, Mitelman F, Nordenson I, Reuterwall C, Salomaa S, Skerfving S and Sorsa M: Cancer risk in humans predicted by increased levels of chromosomal aberrations in lymphocytes: Nordic Study Group on the Health Risk of Chromosome Damage. Cancer Res *54*: 2919-2922, 1994.

16 Bonassi S, Hagmar L, Strømberg U, Montagud AH, Tinnerberg H, Forni A, Heikkila P, Wanders S, Wilhardt P, Hansteen IL, Knudsen LE and Norppa H: Chromosomal aberrations in lymphocytes predict human cancer independently of exposure to carcinogens. European Study Group on Cytogenetic Biomarkers and Health. Cancer Res *60*: 1619-1625, 2000.

17 Bonassi S, Norppa H, Ceppi M, Strømberg U, Vermeulen R, Znaor A, Cebulska-Wasilewska A, Fabianova E, Fucic A, Gundy S, Hansteen I-L, Knudsen LE, Lazutka J, Rossner P, Sram RJ and PB: Chromosomal aberration frequency in lymphocytes predicts the risk of cancer: results from a pooled cohort study of 22,358 subjects in 11 countries. Carcinogenesis *29*: 1178-1183, 2008.

18 Garte S, Gaspari L, Alexandrie AK, Ambrosone C, Autrup H, Autrup JL, Baranova H, Bathum L, Benhamou S, Boffetta P, Bouchardy C, Breskvar K, Brockmoller J, Cascorbi I, Clapper ML, Coutelle C, Daly A, Dell'Omo M, Dolzan V, Dresler CM, Fryer A, Haugen A, Hein DW, Hildesheim A, Hirvonen A, Hsieh LL, Ingelman-Sundberg M, Kalina I, Kang D, Kihara M, Kiyohara C, Kremers P, Lazarus P, Le Marchand L, Lechner MC, van Lieshout EM, London S, Manni JJ, Maugard CM, Morita S, Nazar-Stewart V, Noda K, Oda Y, Parl FF, Pastorelli R, Persson I, Peters WH, Rannug A, Rebbeck T, Risch A, Roelandt L, Romkes M, Ryberg D, Salagovic J, Schoket B, Seidegard J, Shields PG, Sim E, Sinnet D, Strange RC, Stucker I, Sugimura H, To-Figueras J, Vineis P, Yu MC and Taioli E: Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol Biomarkers Prev *10*: 1239-1248, 2001.

19 Bernardini S, Hirvonen A, Jarventaus H and Norppa H: Trans-stilbene oxide-induced sister chromatid exchange in cultured human lymphocytes: influence of *GSTM1* and *GSTT1* genotypes. Mutagenesis *16*: 277-281, 2001.

20 Nedelcheva Kristensen V, Andersen TI, Erikstein B, Geitvik G, Skovlund E, Nesland JM and Borresen-Dale AL: Single-tube multiplex polymerase chain reaction genotype analysis of *GSTM1, GSTT1* and *GSTP1*: relation of genotypes to TP53 tumor status and clinicopathological variables in breast cancer patients. Pharmacogenetics *8*: 441-447, 1998.

21 Skyberg K, Hansteen IL, Jelmert O and Rønneberg A: A cytogenetic and haematological investigation of oil-exposed workers in a Norwegian cable manufacturing company. Br J Ind Med *46*: 791-798, 1989.

22 Jelmert O, Hansteen IL and Langard S: Enhanced cytogenetic detection of previous *in vivo* exposure to mutagens in human lymphocytes after treatment with inhibitors of DNA synthesis and DNA repair *in vitro*. Mutat Res *271*: 289-298, 1992.

23 Skyberg K, Hansteen IL and Vistnes AI: Chromosomal aberrations in lymphocytes of employees in transformer and generator production exposed to electromagnetic fields and mineral oil. Bioelectromagnetics *22*: 150-160, 2001.

24 Brøgger A, Norum R, Hansteen IL, Clausen KO, Skardal K, Mitelman F, Kolnig AM, Strombeck B, Nordenson I, Andersson G, Jakobsson K, Makipaakkanen J, Norppa H, Jarventaus H and Sorsa M: Comparison between five Nordic laboratories on scoring of human lymphocyte chromosome aberrations. Hereditas *100*: 209-218, 1984.

25 Savage JR: Classification and relationships of induced chromosomal structual changes. J Med Genet *13*: 103-122, 1976.

26 Kocabas NA, Karahalil B, Karakaya AE and Sardas S: Influence of *GSTM1* genotype on comet assay and chromosome aberrations after induction by bleomycin in cultured human lymphocytes. Mutat Res *469*: 199-205, 2000.

27 Schlade-Bartusiak K, Sasiadek M and Kozlowska J: The influence of *GSTM1* and *GSTT1* genotypes on the induction of sister chromatid exchanges and chromosome aberrations by 1,2:3,4-diepoxybutane. Mutat Res *465*: 69-75, 2000.

28 Hansteen IL, Lågeide L, Clausen KO, Haugan V, Svendsen M, Eriksen JG, Skiaker R, Hauger E, Vistnes AI and Kure EH: Cytogenetic effects of 18.0 and 16.5 GHz microwave radiation on human lymphocytes *in vitro*. Anticancer Res *29*: 2885-2892, 2009.

29 Verschaeve L and Maes A: Genetic, carcinogenic and teratogenic effects of radiofrequency fields. Mutat Res *410*: 141-165, 1998.

30 Zeni O, Gallerano GP, Perrotta A, Romano M, Sannino A, Sarti M, D'Arienzo M, Doria A, Giovenale E, Lai A, Messina G and Scarfi MR: Cytogenetic observations in human peripheral blood leukocytes following *in vitro* exposure to THz radiation: a pilot study. Health Phys *92*: 349-357, 2007.

31 Vijayalaxmi. Cytogenetic studies in human blood lymphocytes exposed *in vitro* to 2.45 GHz or 8.2 GHz radiofrequency radiation. Radiat Res *166*: 532-538, 2006.

32 Zeni O, Schiavoni A, Perrotta A, Forigo D, Deplano M and Scarfi MR: Evaluation of genotoxic effects in human leukocytes after *in vitro* exposure to 1950 MHz UMTS radiofrequency field. Bioelectromagnetics *29*: 177-184, 2008.

33 Diem E, Schwarz C, Adlkofer F, Jahn O and Rudiger H: Non-thermal DNA breakage by mobile-phone radiation (1800 MHz) in human fibroblasts and in transformed GFSH-R17 rat granulosa cells *in vitro*. Mutat Res *583*: 178-183, 2005.

34 Ciaravino V, Meltz ML and Erwin DN: Absence of a synergistic effect between moderate-power radiofrequency electromagnetic radiation and adriamycin on cell-cycle progression and sister chromatid exchange. Bioelectromagnetics *12*: 289-298, 1991.

35 Meltz ML, Eagan P and Erwin DN: Absence of mutagenic interaction between microwaves and mitomycin C in mammalian cells. Environ Mol Mutagen *13*: 294-303, 1989.

36 Meltz ML, Eagan P and Erwin DN: Proflavin and microwave radiation: absence of a mutagenic interaction. Bioelectromagnetics *11*: 149-157, 1990.

37 Kerbacher JJ, Meltz ML and Erwin DN: Influence of radiofrequency radiation on chromosome aberrations in CHO cells and its interaction with DNA-damaging agents. Radiat Res *123*: 311-319, 1990.

38 Maes A, Collier M, Van Gorp U, Vandoninck S and Verschaeve L: Cytogenetic effects of 935.2-MHz (GSM) microwaves alone and in combination with mitomycin C. Mutat Res *393*: 151-156, 1997.

39 Maes A, Collier M and Verschaeve L: Cytogenetic effects of 900 MHz (GSM) microwaves on human lymphocytes. Bioelectromagnetics *22*: 91-96, 2001.

40 Zhang MB, He JL, Jin LF and Lu DQ: Study of low-intensity 2450-MHz microwave exposure enhancing the genotoxic effects of mitomycin C using micronucleus test and comet assay *in vitro*. Biomed Environ Sci *15*: 283-290, 2002.

41 Lai H and Singh NP: Acute low-intensity microwave exposure increases DNA single-strand breaks in rat brain cells. Bioelectromagnetics *16*: 207-210, 1995.

42 d'Ambrosio G, Massa R, Scarfi MR and Zeni O: Cytogenetic damage in human lymphocytes following GMSK phase modulated microwave exposure. Bioelectromagnetics *23*: 7-13, 2002.

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