Cytogenetic Effects of 18.0 and 16.5 GHz Microwave Radiation on Human Lymphocytes *In Vitro*

INGER-LISE HANSTEEN[1], LARS LÅGEIDE[1,2*], KJELL OSKAR CLAUSEN[1], VERA HAUGAN[1], MARIT SVENDSEN[1], JAN GUNNAR ERIKSEN[3], ROLF SKIAKER[3], EGIL HAUGER[3], ARNT INGE VISTNES[4] and ELIN H. KURE[1,2,5,6]

[1]*Department of Laboratory Medicine, Section of Medical Genetics, Telemark Hospital, Skien;*
[2]*Telemark University College, Department of Environmental and Health Studies, Bø in Telemark;*
[3]*Telenor Research and Development, Kjeller;*
[4]*Department of Physics, University of Oslo;*
[5]*Laboratory of Molecular Oncology, Department of Research and Development, The Cancer Center, and*
[6]*Section of Molecular Pathology, Department of Pathology, Oslo University Hospital, Ulleval, Oslo, Norway*

Abstract. *Background: There are few cell studies on the direct genotoxic effects of microwave radiation. In this study, cytogenetic effects of microwave radiation alone or in combination with mitomycin C (MMC) were investigated. Materials and Methods: Lymphocytes from two smoking and four non-smoking donors were exposed for 53 hours in vitro to 1.0 W/m^2 continuous-wave radiation at 18.0 GHz or 10 W/m^2 pulsed-wave at 16.5 GHz, alone or in combination with MMC. DNA synthesis and repair were inhibited in vitro in some cultures. Results: No synergistic effect was observed in cells exposed to combinations of microwave radiation and in vitro exposure to MMC, or to cells pre-exposed in vivo to tobacco smoke. For the 16.5 GHz pulsed exposure, a non-significant trend consisting of an increase in aberration frequencies with microwave radiation was shown for the DNA synthesis and repair inhibited cultures both with and without MMC. Conclusion: Neither 18.0 GHz continuous-wave nor 16.5 GHz pulsed-wave exposure to human lymphocytes in vitro induced statistically significant increases in chromosomal aberration frequencies. 16.5 GHz pulsed-wave exposure requires further documentation before a true negative conclusion can be drawn.*

**Present address:* StatoilHydro ASA, EPN ONS HSE, Stavanger, Norway.

Correspondence to: Elin H. Kure, Laboratory of Molecular Oncology, Department of Research and Development, The Cancer Center, Oslo University Hospital, Ulleval, 0407 Oslo, Norway. Tel: +47 23027823, Fax: +47 23027822, e-mail: elin.kure@uus.no

Key Words: Chromosomal aberrations, radio frequency, microwave radiation, pulsed-wave, continuous-wave, promotion.

The widespread presence of radiofrequency radiation (RFR) has for many years caused concern regarding its potential health effects and extensive research has been conducted to elucidate the problem. Most studies have concentrated on exposure to low levels of RFR (1). Available experimental evidence shows that high-frequency RFR may cause an increase in tissue temperature and that adverse biological effects can be caused by elevated temperature in tissue exceeding 1.0˚C (2, 3). Reports on direct genotoxic effects of super high-frequency RFR controlling for temperature as assessed from incidence of cytogenetic damage in mammalian cells are few (4). The tentative conclusion is that *in vitro* RFR exposure under non-thermal conditions appears not to induce cytogenetic damage. Mason *et al*. (5) studied the effect of 94 GHz RFR exposure in an animal model of skin carcinogenesis also testing the notion that RFR energy might serve as a promoter or co-promoter. They did not show any effect of RFR exposure in this system. Mobile radios, mobile phones and microwave ovens operate at a radiating frequency of 300 MHz to 3 GHz, radio communication links operate at 3 to 30 GHz (6), while a Local Multipoint Distribution System (LMDS) may operate at around 40 GHz (7). As few studies have been performed with exposures to ultra high RFR *in vivo* or *in vitro*, the aim of the present pilot study was to investigate clastogenetic effects on cultured human lymphocytes exposed to 18.0 GHz continuous-wave and 16.5 GHz pulsed-field alone or in combination with the known clastogen mitomycin C (MMC) to test for a possible synergistic effect with microwave radiation. In this study, microwave radiation exposure was applied during at least one full cell cycle in culture to test if the cells would be more sensitive to cytogenetic damage when grown under continuous exposure. Most exposures in previous *in vitro* studies were applied before the cells were cultured (8-10).

Chromosomal aberrations in lymphocytes have proven to be associated with cancer risk in humans (11-13) and are considered informative indicators of exposure. We therefore decided to use the same test system for this *in vitro* experiment using microwave radiation.

Materials and Methods

Lymphocyte donors. In this model system, blood from two non-smoking males (40 and 44 years old), and two non-smoking (25 and 44 years) and two smoking females (41 and 55 years) was used. Informed consent was collected. The hospital authorities approved the study, the research was otherwise exempted from review because the blood was 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 (14) and therefore important to control for (15). The polymorphisms were analyzed as described by Nedelcheva Kristensen *et al*. (16). Individuals with *GSTM1* null and *GSTP1* heterozygote or wild-type genotypes were chosen as donors

Cytogenetic analysis. Phytohemagglutinin-stimulated lymphocyte cultures from heparinized whole blood were used according to methods described elsewhere (17) using RPMI medium (1640; BioWhittaker Cambrex, Verviers, Belgium). Before the experiments were initiated, cultures with different concentrations of 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) (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 slides were stained with Giemsa according to conventional methods. All aberrations were scored blind on coded slides by three microscopists. For the series with 18.0 GHz exposure 200 cells per culture were scored; for the series with 16.6 GHz exposure, 100 cells per culture were scored while 50 cells per culture were scored for the inhibited cultures. The same microscopist scored both the exposed and control cultures from the same series. The scoring criteria used were as described by Brøgger *et al*. (18) and Savage (19) and harmonized among the scorers. The aberrations were grouped as number of cells with aberrations (CA), cells with aberrations and gaps (CAG), chromosome type aberrations (CSA), chromosome breaks (CSB), chromatid type aberrations (CTA), chromatid breaks (CTB), and chromosome and chromatid gaps (CSG and CTG).

Study design. Positive and negative control cultures were included in each experimental series. Half of the cultures were exposed to microwave radiation during the 53 hours in culture. MMC (100 ng/ml) was added after 30 hours to half of the cultures with and without microwave radiation. Each experimental series included cultures from two blood donors (either two females or two males). For the 18.0 GHz exposure experiments, eight cultures in all were exposed two with and two without MMC, and two with and two without MMC were controls using 10 ml of medium per EasYFlasks™ Nunclon™ flask (Nunc A/S, Roskilde, Denmark) (25 cm^2 culture area). For the 16.5 GHz exposure experiments, 16 cultures were set up in TubeNunclon™ (Nunc A/S) (5.5 cm^2 culture area) with 3 ml of medium. The two cultures for each treatment regime for the 18 GHz exposure were replaced with four cultures of 3 ml of medium in culture tubes, otherwise the series were identical. For the experiments with 16.5 GHz microwave radiation, chromosomal aberrations were also 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 together with Colcemid™ three hours before harvesting (20, 21).

Exposure. The experiment was performed in a large anechoic chamber (5×4×4 m) which was temperature stabilized at 37˚C (details below). The instrumental setup is detailed in Figure 1 and illustrated in Figure 2. The exposed and the control samples were initially kept in the same room, but well separated with microwave radiation at the control samples approximately 40 dB below that of the exposed samples.

One scenario of microwave exposure was at 18.0 GHz with 1 W/m^2 incident continuous wave. We also wanted to use pulsed exposure. Initially, it proved difficult to construct an 18.0 GHz pulsed-field exposure set-up with the instruments available, and 16.5 GHz was chosen. The pulsed exposure was therefore at 16.5 GHz, 10 W/m^2 incident pulsed-wave (1 kHz pulse frequency, 50% duty cycle). Time-averaged values are given. The cells were exposed to microwave radiation through two rectangular horn antennas placed underneath the samples. The antennas were separated to ensure negligible overlap between the two exposure areas. The antennas were directed upwards and placed approximately 1.2 m below the cultures. The exposed area (slightly more than 30 cm in diameter) was constructed to cover only the number of exposed cultures. The culture flasks were placed horizontally on a 5 mm-thick cardboard. The samples were oriented so that the magnetic field was along the long side of the flask or tubes, 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 19 V/m rms just outside the growth medium containing the cells. This corresponds to 1.0 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 culturing period. The cells were placed in EasY Flasks Nunclon™ culture flasks with 25 cm^2 culture area (6 cm wide), or flat-bottomed TubeNunclon™ culture tubes with 5.5 cm^2 culture area (1.6 cm wide). We used 10 ml cell suspension for the former and 3 ml for the latter. The thickness/height of the medium was 4 mm in the flat-bottomed flasks and ranged from 2 to 9 mm in the tubes. The flasks/tubes were positioned relatively close to each other as indicated in Figure 2.

The level of exposure chosen corresponds roughly to the safety limit recommended by ICNIRP (2). However, safety limits are based on induced currents in a human body, and the difference in geometry and material properties makes it difficult to compare exposures in an *in vitro* experiment with one in the human body. This problem can be investigated by detailed finite difference method calculations. Since our aim is not to discuss safety

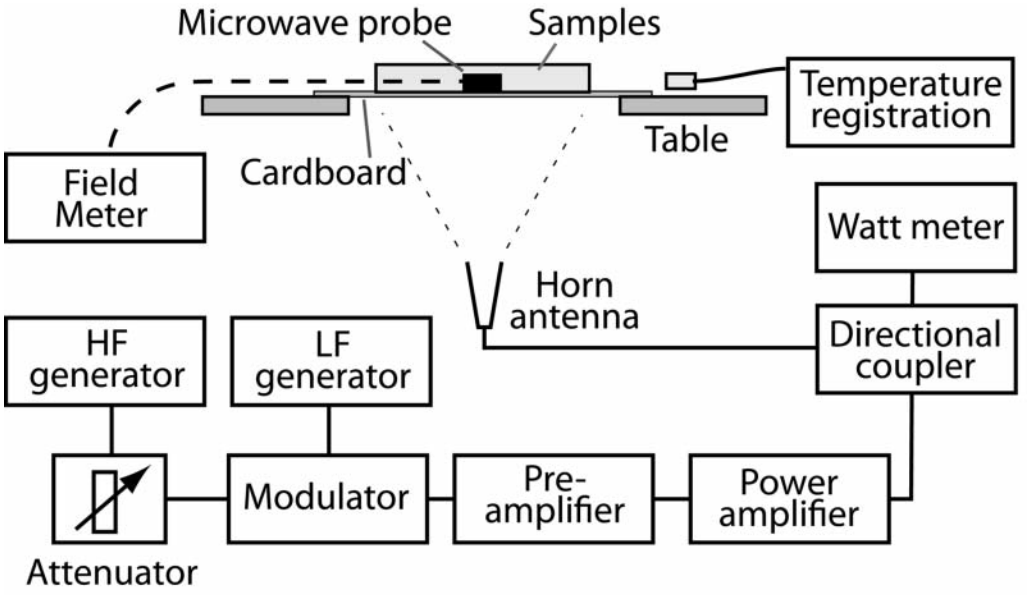

Figure 1. *Block diagram for the experimental setup for the continuous wave experiment. Two horn antennas fed through a splitter. They were positioned far enough from each other to form two separate exposure areas. For the pulsed wave the setup was similar. The temperature regulation setup is not included in the drawing. The incident power density was measured with a Wandel & Goltermann EMR300 instrument with a type 9C probe. The probe was used to map the exposure level across the full area where the samples were placed. It was removed during exposure. The control samples are not included in this drawing.*

guidelines, but rather to perform a pilot study to see if we could find interesting effects that could be followed up more closely in further studier, we have not carried out such detailed theoretical calculations. We have given enough information about the experimental setup so that it is possible for others to complete the finite difference calculations.

The anechoic chamber was initially temperature stabilized to 37.0±0.4˚C, and the temperature of the samples was determined by regulation of room temperature only. The temperature was measured and recorded continuously with two thermocouples interfaced to an INTAB data logger AAC-2 (Intab Interface-teknik AB, Sternkullen, Sweden). The temperature regulation led to a temperature oscillation in the samples of approximately ±0.25˚C. The absolute precision of the temperature registration was lower 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 the control areas. The temperature recordings in the control samples as well as in the continuous wave exposed samples were 37.0±0.3˚C throughout the culture period (standard deviation given represents the fluctuation of the temperature only). However, sporadic measurements in the 16.5 GHz pulsed exposed samples revealed that the mean temperature there was 38.0±0.4˚C. The temperature in the anechoic chamber was then reduced to 36.0±0.3˚C and the control cultures were moved to an incubator at 37.0±0.2˚C for the remaining series of experiments.

Experiments with blood from the female smokers were then repeated with a chamber temperature at 36.0±0.3˚C. Exposed cultures from the male donors were set up only at 36.0±0.3˚C. Results from both temperature series are presented, but kept

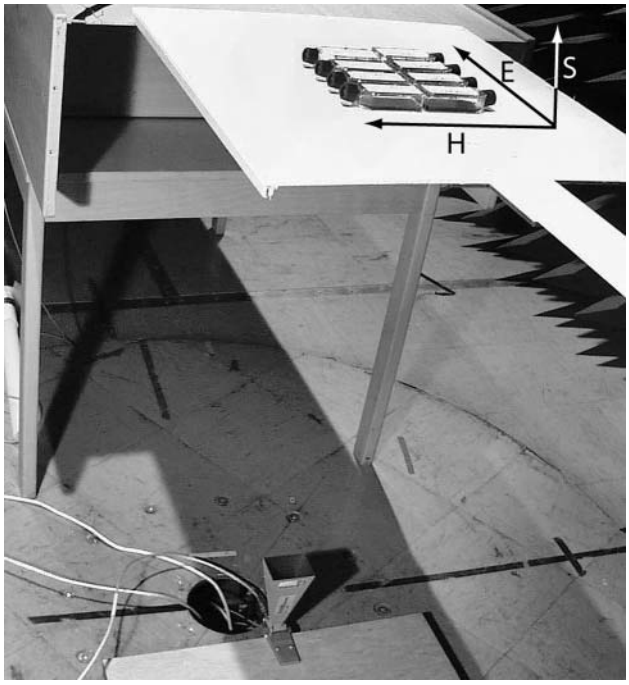

Figure 2. *The culture flasks/tubes were placed relative close to each other. This figure, in combination with the text, provides sufficient information if one wants to investigate the field pattern inside the samples, using finite difference methods or a similar numerical techniques. The diameter of the outer circle is slightly more than 30 cm.*

Table I. *Aberrations recorded for cultures exposed to 18.0 GHz, 1.0 W/m^2 continuous microwave radiation. Mean frequencies per 100 cells (standard deviation in brackets) are given for non-smoking male (M) and female (F) blood donors.*

Variable	N/ Gender	CA	CSA	CTA
Without mitomycin C				
Control	2 F	**0.88** (0.25)*	0.75 (0.65)	0.25 (0.50)
RFR	2 F	0.38 (0.25)	0.25 (0.50)	0.25 (0.29)
Control	2 M	1.25 (1.85)	1.25 (2.50)	0.75 (1.19)
RFR	2 M	1.00 (1.08)	0.63 (0.63)	0.50 (0.71)
With mitomycin C				
Control	2 F	9.13 (2.56)	1.50 (1.08)	10.63 (3.54)
RFR	2 F	11.50 (3.29)	2.13 (1.31)	13.38 (4.33)
Control	2 M	7.88 (1.89)	1.13 (1.03)	9.88 (2.25)
RFR	2 M	7.38 (2.50)	2.00 (0.71)	7.75 (2.73)

CA, Number of cells with aberrations; CSA, chromosome type aberrations; CTA, chromatid type aberrations. *p<0.05, Mann-Whitney non-parametric test. Number of cells=800.

separate, to show that an increase of 1.0˚C in culture temperature gave little variation in the aberration frequencies.

The heating elements and fans circulating the air in the room generated a background magnetic field (50 Hz) of 0.04 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 3 mm in the culture medium.

Statistical analysis. For statistical comparisons of the mean values of the cytogenetic parameters, 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. SPSS 12.0 for windows (SPSS, Chicago, USA) was used for the statistical analyses.

Results

The results for the blood cultures exposed to 18.0 GHz continuous-wave microwave radiation are presented in Table I for the non-smoking males and females separately for CA, CSA and CTA. The other cytogenetic parameters studied (CAG, CSB, CTB, CSG and CTG) showed similar results and are not presented in any of the series. No statistically significant differences between control and exposed cultures were observed for any of the experimental series except for a statistically significant protective effect of exposure to cultures from the female donors for CA.

Exposure to pulsed 16.5 GHz microwave radiation resulted in an increase in temperature to a mean of 38.0˚C for cultures from both female smokers and non-smokers. Experiments adjusting the temperature to 37.0˚C were performed with blood from the two smoking females and for the two male donors. Results from both temperature series are reported. For the uninhibited cultures with and without MMC (Table II), there was no statistical significant increase in the mean frequencies of CA, CSA or CTA in exposed cultures as compared to controls for any of the blood donors, except for two or three of the twelve aberration frequencies reported for cultures grown at 38.0˚C from smoking female donors. The control cultures for female smokers were kept at 37.0˚C for both temperature series. A non-significant variation in the chromosomal aberration frequencies was observed between the control cultures in the two experiments with a statistical significant difference only for CA for the cultures with MMC (mean frequency per 100 cell: 14.50 *vs*. 7.00).

The significant difference in CSA levels between control and exposed cultures grown at 38.0˚C without MMC should be interpreted with caution. The control value of 0.00 in this series was the only occasion where no aberration was observed.

For the cultures where DNA synthesis and repair were inhibited, an increase in all aberration frequencies was found as expected. The increase in culture temperature did not seem to have an effect on the chromosomal aberration frequencies (Table III). No statistically significant differences between control and exposed cultures were found for any of the cultures with and without MMC, except for a significant increase in CA in exposed cultures without MMC from male and in CSA in cultures at 37.0˚C from smoking female blood donors. However, a non-significant increased trend in aberration frequencies with exposure to microwave radiation could be seen for cultures both with and without MMC. MMC was used as a positive control in all experiments. There was a significant increase in all chromosomal aberration frequencies for the MMC positive cultures compared to the cultures without MMC (Tables I, II and III) except for three CSA frequencies.

Discussion

Very few mitoses were observed before and at 48 hour in culture. Fifty-three hours in culture was judged to give enough mitoses for scoring an appropriate number. As so few mitoses were observed in the early cultures, most of the cells collected in mitosis from 50 to 53 hours were predicted to be in the first cell division. Even if a few cells should be in the second cell division, the cells were continuously exposed and aberrations could also be expected to occur during the second cell division. Exact cell proliferation measurements were not performed and cell proliferation studies on human lymphocytes exposed to microwave radiation have to our knowledge not been published. However, studies on low electromagnetic field exposure have indicated both no effect (22-24), and an increase in cell proliferation (25).

Table II. *Aberrations recorded for the cultures exposed to 16.5 GHz, 10 W/m^2 pulsed microwave radiation. Mean frequencies per 100 cells (standard deviation in brackets) are given for non-smoking male (M) and female (F) and smoking female (FS) blood donors. Mean culture temperature is indicated.*

Variable	N/Gender	Temp.	CA	CSA	CTA
Without mitomycin C					
Control	2 F	37˚C	0.88 (0.83)	0.50 (0.76)	0.63 (0.74)
RFR	2 F	38˚C	1.38 (1.30)	0.75 (0.89)	0.88 (0.99)
Control	2 FS	37˚C	0.38 (0.74)	0.00 (0.00)	0.38 (0.74)
RFR	2 FS	38˚C	**2.00** (1.77)*	**1.50** (1.77)(*)	1.13 (1.81)
Control	2 FS	37˚C	0.75 (1.04)	0.63 (1.06)	0.38 (0.74)
RFR	2 FS	37˚C	1.25 (1.39)	2.13 (1.96)	0.38 (0.74)
Control	2 M	37˚C	1.00 (1.31)	0.63 (1.06)	0.38 (0.52)
RFR	2 M	37˚C	1.13 (0.64)	1.13 (1.36)	0.63 (0.92)
With mitomycin C					
Control	2 F$^+$	37˚C	9.00 (3.95)	2.00 (2.61)	11.50 (3.08)
RFR	2 F	38˚C	10.38 (3.25)	2.88 (1.73)	11.50 (5.76)
Control	2 FS	37˚C	14.50 (3.16)	3.00 (2.07)	16.63 (3.96)
RFR	2 FS	38˚C	18.00 (5.29)	**5.63** (3.02)*	17.88 (7.20)
Control	2 FS	37˚C	7.00 (1.93)	1.75 (1.16)	7.00 (1.77)
RFR	2 FS	37˚C	7.63 (2.67)	1.63 (1.69)	7.63 (2.83)
Control	2 M	37˚C	4.88 (1.55)	1.13 (0.83)	5.13 (2.64)
RFR	2 M	37˚C	5.00 (3.59)	0.38 (0.52)	6.38 (6.12)

*$p\leq0.05$, Mann-Whitney non-parametric test. Number of cells=800, except $^+$600.

Table III. *Aberrations recorded for inhibited cultures exposed to 16.5 GHz, 10 W/m^2 pulsed microwave radiation. Mean frequencies per 100 cells (standard deviation in brackets) for cultures from non-smoking male (M) and female (F) and smoking female (FS) blood donors. Mean culture temperature is indicated.*

Variable	N/Gender	Temp.	No. of cells	CA	CSA	CTA
Without mitomycin C						
Control	2 F	37˚C	450	3.6 (1.8)	2.0 (1.2)	3.2 (3.9)
RF R	2 F	38˚C	480	5.3 (5.8)	2.6 (2.4)	5.1 (5.8)
Control	2 FS	37˚C	400	9.8 (5.2)	3.3 (3.0)	8.3 (5.5)
RF R	2 FS	38˚C	400	9.3 (3.7)	4.8 (4.1)	9.0 (2.8)
Control	2 FS	37˚C	350	8.9 (5.6)	1.4 (1.5)	9.1 (6.6)
RFR	2 FS	37˚C	400	11.0 (3.7)	**4.3 (2.9)***	12.0 (5.5)
Control	2 M	37˚C	340	4.9 (2.5)	3.6 (1.6)	4.0 (2.8)
RFR	2 M	37˚C	300	**11.0 (2.1)***	4.3 (4.6)	11.3 (6.8)
With mitomycin C						
Control	2 F	37˚C	600	27.0 (6.7)	20.7 (13.5)	47.8 (14.7)
RFR	2 F	38˚C	792	31.4 (8.4)	39.3 (27.7)	58.9 (25.7)
Control	2 FS	37˚C	400	49.0 (8.5)	31.3 (12.8)	87.5 (21.2)
RFR	2 FS	38˚C	400	52.0 (4.7)	32.5 (15.0)	89.5 (19.4)
Control	2 FS	37˚C	400	43.0 (6.1)	24.5 (7.1)	61.5 (14.5)
RFR	2 FS	37˚C	400	51.3 (15.3)	32.5 (22.0)	83.5 (32.1)
Control	2 M	37˚C	350	55.7 (9.1)	16.3 (16.9)	60.3 (20.1)
RFR	2 M	37˚C	300	58.3 (13.2)	22.0 (9.0)	81.3 (25.7)

*$p<0.05$, Mann-Whitney non-parametric test.

Regarding exposure, the distribution of the induced microwave radiation in *in vitro* experiments are dependent upon many physical factors such as frequency, polarization, the size and form of the culture flask, the amount of medium and the height of the medium within the flask, in addition to the position of the flasks in the exposed area. The variation in the exposure of the cells within the culture would be less in tubes compared to flasks if the electric field was oriented across the length of the tube (26, 27). This orientation of the field was applied in the present experiments when using culture flasks in

the 18.0 GHz or culture tubes in the 16.5 GHz pulsed-field exposure experiments. We found, however, a temperature increase in the 16.5 GHz pulsed cultures compared to the continuous exposure to 18.0 GHz, and the temperature in the anechoic chamber was reduced to 36.0±0.3˚C to keep the culture temperature at approximately 37.0˚C to control for a possible thermal effect. The penetration of the radiation for frequencies above 10 GHz is low (2, 5), but will reach the monolayer cells with irradiation from beneath. The positions of the flasks/tubes in the exposed area were changed randomly to ensure as even exposure as possible.

Tobacco smoke was chosen as an *in vivo* exposure to carcinogens in addition to exposing the lymphocytes *in vitro* for MMC in order to test if microwave radiation increases susceptibility to chromosomal damage. Most published reports do not support the hypothesis that RFR radiation results in direct genotoxic effects such as chromosomal aberrations, micronuclei, DNA strand breaks or mutations (28-30). Many studies have tested the possibility of RFR having a promotion effect with differing results (9, 31-35). A wide variation in RFR exposure parameters such as frequency, modulation, polarization and pulsed contra continuous emission, the length of exposure, the difference in test material and whether the cells are exposed before culturing or at short intervals (2–24 hours) during the initial culturing could potentially influence the results. Most studies have tried to mimic possible human exposure situations. One phase of the cell's life cycle could, however, potentially be more susceptible to damage than another phase. The present study addressed this question by applying exposure during all 53 hours in culture (the whole experiment). No increase in aberration frequencies could be documented with this exposure regime either for 18.0 GHz continuous or 16.5 GHz pulse-wave microwave radiation. Only one study on the effect of 94 GHz RFR in an animal model of skin carcinogenesis is reported (5). They concluded that exposure to 94 GHz RFR applied once or twice a week for 12 weeks did not promote or co-promote the effect of DMBA-induced papilloma development in their model.

Most reports concern effects of RFR exposure up to 2.4 GHz. Kerbacher *et al*. (32) reported no effect on chromosome aberrations from increases in temperature during 2.45 GHz pulsed-wave exposure, while Takashima *et al*. (22) reported a drastic increase in temperature using intermittent RFR field exposure to 2.45 GHz with specific absorption rate (SAR) of 50 W/kg for 2 hours causing cell proliferation disorder. They suggested that the adverse effect was caused by an increase in temperature and not by the RFR field itself. Continuous-wave exposure to human cells *in vitro* to 2.14 GHz (36) or 2.3 GHz (Hansteen *et al*. in preparation) did not induce an increase in chromosomal aberrations compared to controls, while pulsed-wave microwave radiation exposure may be slightly more detrimental to the cells.

Taking the above arguments into consideration some conclusions may be drawn from the present study. Firstly, exposure throughout the whole experiment to 18.0 GHz continuous-wave microwave radiation (1.0 W/m^2) did not lead to a noticeable temperature increase or increase in aberration frequencies, while 16.5 GHz pulsed exposure (1 kHz pulse frequency, 50% duty cycle, 10 W/m^2 average mean value) increased the mean temperature by approximately 1.0˚C. Of the total of 24 aberration frequencies scored in the conventional and inhibited cultures grown at 38.0˚C with and without MMC (Tables II and III), only three frequencies (Table II) showed a statistically significant increase with microwave radiation exposure compared to controls, indicating that the temperature increase of 1.0˚C was of minor importance.

Secondly, for the conventional cultures, exposure to 18.0 GHz continuous-wave or 16.5 GHz pulsed-wave microwave radiation did not affect the chromosomal aberration frequencies in the lymphocytes from the smoking or non-smoking donors, and thus failed to show an effect of microwave radiation on cells exposed to tobacco smoke *in vivo*. Additional exposure to MMC *in vitro* served as a positive control, but no further increase in chromosomal aberrations was observed in combination with 18.0 GHz continuous or 16.5 GHz microwave radiation pulsed exposure controlling for temperature. Thus the MMC exposure combined with microwave radiation did not seem to increase the cells' susceptibility to chromosomal damage.

Thirdly, inhibition of DNA synthesis and repair increased the frequencies of all the different chromosomal parameters, the hypothesis being that an increase in the number of aberrations would give more robust measurements and be a more sensitive method for picking up differences. A non-significant trend for increasing aberration frequencies with microwave radiation was shown for the cultures with and without MMC, reaching statistical significance only for CSA for smoking females and for CA for the males in cultures without MMC grown at 37.0˚C.

Finally, with so many parameters tested, there will be a few significant differences due to chance only. Furthermore, unpredictable biological effects *in vivo* or *in vitro* are difficult to fully control for, as shown by the variation in frequencies in control cultures for the same individual.

In summary, neither 18.0 GHz continuous-wave nor 16.5 GHz pulsed-wave exposure of human lymphocytes during 53 hours *in vitro*, with or without MMC, induced a convincing statistically significant increase in chromosomal aberration frequencies measured as CA, CSA, CTA, CSB, CTB, CSG or CTG. However, the non-significant trend with increased frequencies of chromosomal damage in the 16.5 GHz pulsed-wave exposed DNA synthesis and repair inhibited cultures requires further documentation before a true negative conclusion can be drawn.

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 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.

3 Repacholi MH: Health risks from the use of mobile phones. Toxicol Lett *120*: 323-331, 2001.

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

5 Mason PA, Walters TJ, DiGiovanni J, Beason CW, Jauchem JR, Dick Jr EJ, Mahajan K, Dusch SJ, Shields BA, Merritt JH, Murphy MR and Ryan KL: Lack of effect of 94 GHz radio frequency radiation exposure in an animal model of skin carcinogenesis. Carcinogenesis *22*: 1701-1708, 2001.

6 European Committee for Electrotechnical Standardization, Brussels: Human exposure to electromagnetic fields. High Frequency (10 kHz to 300 GHz). ENV *50166-2*: 1-44, 1995.

7 EBU. Digital Video Broadcasting (DVB); LMDS Base Statio and User Terminal Implementation Guidelines for ETSI EN 301 199. ETSI Technical Report 101 205 v1.1.2: 1-46, http://www.etsi.org, 2001.

8 Vijayalaxmi, Leal BZ, Meltz ML, Pickard WF, Bisht KS, Roti Roti JL, Straube WL and Moros EG: Cytogenetic studies in human blood lymphocytes exposed *in vitro* to radiofrequency radiation at a cellular telephone frequency (835.62 MHz, FDMA). Radiat Res *155*: 113-121, 2001.

9 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.

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

11 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.

12 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.

13 Bonassi S, Norppa H, Ceppi M, Stromberg U, Vermeulen R, Znaor A, Cebulska-Wasilewska A, Fabianova E, Fucic A, Gundy S, Hansteen I-L, Knudsen LE, Lazutka J, Rossner P and Sram RJ, P. B: 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.

14 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.

15 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.

16 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.

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

18 Brøgger A, Norum R, Hansteen IL, Clausen KO, Skårdal 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.

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

20 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.

21 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.

22 Takashima Y, Hirose H, Koyama S, Suzuki Y, Taki M and Miyakoshi J: Effects of continuous and intermittent exposure to RF fields with a wide range of SARs on cell growth, survival, and cell cycle distribution. Bioelectromagnetics *27*: 392-400, 2006.

23 Aldinucci C, Garcia JB, Palmi M, Sgaragli G, Benocci A, Meini A, Pessina F, Rossi C, Bonechi C and Pessina GP: The effect of exposure to high flux density static and pulsed magnetic fields on lymphocyte function. Bioelectromagnetics *24*: 373-379, 2003.

24 Pessina GP and Aldinucci C: Short cycles of both static and pulsed electromagnetic fields have no effect on the induction of cytokines by peripheral blood mononuclear cells. Bioelectromagnetics *18*: 548-554, 1997.

25 Pessina GP and Aldinucci C: Pulsed electromagnetic fields enhance the induction of cytokines by peripheral blood mononuclear cells challenged with phytohemagglutinin. Bioelectromagnetics *19*: 445-451, 1998.

26 Kuster N and Schonborn F: Recommended minimal requirements and development guidelines for exposure setups of bio-experiments addressing the health risk concern of wireless communications. Bioelectromagnetics *21*: 508-514, 2000.
27 Guy AW, Chou CK and McDougall JA: A quarter century of *in vitro* research: A new look at exposure methods. Bioelectromagnetics *20*: 21-39, 1999.
28 Brusick D, Albertini R, McRee D, Peterson D, Williams G, Hanawalt P and Preston J: Genotoxicity of radiofrequency radiation. Environ Mol Mutagen 32: 1-16, 1998.
29 IEGMP. Mobile Phones and Health. Chilton; NRPB http://www.iegmp.org.uk/IEGMPtxt.htm:2000.
30 Verschaeve L and Maes A: Genetic, carcinogenic and teratogenic effects of radiofrequency fields. Mutat Res *410*: 141-165, 1998.
31 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.
32 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.
33 Maes A, Collier M, Slaets L and Verschaeve L: 954 MHz microwaves enhance the mutagenic properties of mitomycin C. Environ Mol Mutagen *28*: 496-501, 1996.
34 Maes A, Van Gorp U and Verschaeve L: Cytogenetic investigation of subjects professionally exposed to radiofrequency radiation. Mutagenesis *21*: 139-142, 2006.
35 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.
36 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.

Received March 20, 2009
Revised May 22, 2009
Accepted May 27, 2009