1800 MHz Radiofrequency Electromagnetic Fields Cause Energy-Dependent Genotoxic Effects in Human Promyelocytic HL-60 Cells

Abstract.
Different SAR levels have been examined with respect to the effect on micronuclei induction and comet formation in HL-60 cells. Comparing RF-EMF exposure (1800 MHz, continuous wave, 24 h) at SAR levels ranging from 0.2 W/kg to 3.0 W/kg with sham-exposure and incubator controls under blinded conditions indicates that both effects, micronuclei induction and comet formation, appear to be energy-dependent within a window of more than 1.0 W/kg and below 3.0 W/kg. The maximal effect was observed at a SAR of 1.3 W/kg. Experiments on the influence of the exposure duration showed that short exposure periods for MN induction (6h) caused no and for comet formation (2h, 6h) caused less pronounced effects as compared to longer exposure periods of 24h. While micronucleus frequencies were further increased after exposure for 72 h, comet formation after 72 h of exposure was less expressed as compared to 24 h exposure.

First experiments on the influence of RF-signals showed that at a “maximum effect” SAR level of 1.3 W/kg all RF-signals tested, i.e. continuous wave, continuous wave 5 min on/ 10 min off, continuous wave 217 Hz pulse, and GSM Talk exhibited similar effects on MN induction and on comet formation. So far no hints for alteration of HL-60 genomic integrity due to apoptosis, necrosis or cytotoxicity has been detected. Additionally, RF-EMF exposure (1800 MHz, 1.3 W/kg, 24 h) had no effect on the cellular doubling time and the activity of the enzyme thymidine kinase of HL-60 cells, indicating that RF-field exposure does not influence cellular growth rates.

1. Introduction
With regard to RF-EMF used in mobile phone communication, the potential influence of the exposure on genotoxic, mutagenic or carcinogenic processes in the last years has become an important topic of interest for the general public, health politics and also industry. Results of several epidemiological studies as well as animal studies cast suspicion on RF-signals to promote cancer in exposed subjects. At present, numerous in vitro studies have been carried out, investigating the genotoxic and carcinogenic potential of RF-exposure. These studies used different test methods for genotoxic and epigenetic activity (Wiedemann et al. 2002). Most of the studies reported on negative results. Putatively non-thermal, immediate and reversible responses in exposed subjects have been described by several authors (Roschke and Mann 1997, Borbely et al. 1999, Preece et al. 1999). Nevertheless, these effects, because of their unspecific nature have been regarded as indications of potential biological responses to an electrical excitation, rather than harmful effects with the ability induce permanent damages in individuals. In most recent years, a number of investigations, using advanced methods, provided some evidence that GSM signals cause cellular responses such as alterations in gene expression, changes in protein phosphorylation or induction of micronuclei (Leszinsky et al. 2002, Tice et al. 2002, Mashevich et al. 2003).

2. Material and Methods
Cell culture. Human leukemia HL-60 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and maintained at 37°C in a humidified atmosphere of 95% air and 5% CO₂ in RPMI 1640 supplemented with 10% fetal bovine serum, penicillin G (50 IU/ml) and streptomycin (50 µg/ml). The cells were transferred to new medium every 3-4 days and not longer used than 15 passages. Cells were seeded in petri dishes at a density of approximately 0.75 x 10⁶ cells
per dish before exposure. In addition as incubator controls cells were incubated under these conditions in a separate incubator. RF-EMF- and sham-exposure were performed in a Heraeus incubator in an atmosphere of 95% air and 5% CO\textsubscript{2} with temperature kept constant at 37 ± 0.1 °C using a ITIS exposure setup (see below). In addition, pH of the cell medium was regularly controlled at the end of the exposure period (pH = 7.11 ± 0.06 for sham-exposed cells and pH = 7.12 ± 0.07 for RF-field exposed cells; 1800 MHz, continuous wave, SAR 1.3 W/kg, 24 h, N= 10).

**Exposure setup.** Cells were exposed or were sham-exposed in waveguides connected with a RF-generator system provided by ITIS (Prof. Kuster, Zurich, Switzerland). The system enables RF-EMF exposure of cells under defined conditions with respect to field strengths, polarization, modulation and temperature and is operated within the GSM DCS mobile frequency band. Each waveguide is equipped with a fan for rapid environmental atmospheric exchange. In order to ensure stable exposure independent of the loading and drifts, monopole antennas are integrated to monitor and control the incident field. The system is adapted to the exposure of cell suspensions in Petri dishes with a nonuniformity of SAR of less than 30% and an efficiency of better than 20 W/kg per W input power. Field strengths, temperatures and fan currents as well as all commands are continuously logged to encrypted files which are evaluated after the experiments in order to ensure studies under 'blind' conditions (exposure and sham conditions are blindly assigned to the two waveguides by the computer-controlled signal unit). The signal unit allows the application of the exposure signal continuous wave (CW).

**Cytokinesis-block micronucleus assay.** The micronuclei (MN) assay was carried out as described by Natarajan and Darroudi (1991) according to the guidelines developed by Fenech (2000) and Garriott et al. (2002). In order to evaluate the frequency of MN in binucleated human HL-60 cells (BNC), cytochalasin B (final concentration 3.0 µg/ml) was added to the growth medium after exposure and washing. Cytochalasin B prevents the cells from completing cytokinesis, resulting in the formation of multinucleated cells (Fenech and Morley 1985). As a positive control cells were exposed to 6 MeV γ-irradiation (0.5 Gy; exposure time: 5.2 s). Experiments were repeated at least three times independently. The results reported are the mean values ± standard deviation (SD) of the number of MN per 1000 BNC.

**Alkaline single cell gel electrophoresis assay (Comet assay).** The alkaline SCGE assay was carried out as described by Singh et al. 1988 according to the guidelines developed by Tice et al. 2000; Fairbairn et al. 1995 and Klaude et al. 1996. To determine DNA migration of exposed, sham-exposed or incubator control cultures, 100 cells were scored microscopically for comet formation on 2 slides for each experimental point. As a positive control hydrogen peroxide at a final concentration of 100 µmol/L (1 h) was used. Experiments were repeated at least three times independently. The results reported are the mean values ± standard deviation (SD) of the Olive Tail Moment.

### 3. Results

**Different SAR levels** have been examined with respect to the effect on micronuclei induction and comet formation in HL-60 cells (exposure conditions: 1800 MHz, continuous wave, 24 h). SAR levels ranging from 0.2 W/kg to 3.0 W/kg were compared with sham-exposure and incubator controls under blinded conditions. The results indicate that both effects, micronuclei induction and comet formation, appear to be energy-dependent within a window of more than 1.0 W/kg and below 3.0 W/kg. The maximal effect was observed at a SAR of 1.3 W/kg (figure 1+2).

**Exposure duration.** Experiments on the influence of the exposure duration showed that short exposure periods for MN induction (6h) caused no and for comet formation (2h, 6h) caused less pronounced effects as compared to longer exposure periods of 24h (figure 3+4). While micronucleus frequencies were further increased after exposure for 72 h, comet formation after 72 h of exposure was less expressed as compared to 24 h exposure (figure 3+4).

First experiments on the influence of RF-signals showed that at a SAR level of 1.3 W/kg all RF-signals tested, i.e. continuous wave, continuous wave 5 min on/ 10 min off, continuous wave 217 Hz pulse, and GSM Talk exhibited similar effects on MN induction and on comet formation.
Figure 1: Micronucleus frequencies in binucleated HL-60 cells after exposure to RF-field (1800 MHz, continuous wave) for 24 h ranging from SAR 0.2 to 3.0 W/kg, compared to control and sham-exposure (N=3).

Figure 2: Comet formation in HL-60 cells after exposure to RF-field (1800 MHz, continuous wave) for 24 h ranging from SAR 0.2 to 3.0 W/kg expressed as Olive Tail Moment, compared to control and sham exposure (N=3).

Figure 3: Micronucleus frequencies in binucleated HL-60 cells after exposure to RF-fields (1800 MHz, continuous wave, SAR 1.3 W/kg) for 6, 24 and 72 h, compared to control and sham-exposure (N=3). Positive control: 6 MeV γ-irradiation; 0.5 Gy, 5.2 s (N=3).

Figure 4: Comet formation in HL-60 cells after exposure to RF-fields (1800 MHz, continuous wave, SAR 1.3 W/kg) for 2, 6, 24 and 72 h, expressed as Olive Tail Moment, compared to control and sham-exposure (N=3).

Figure 5: Effect of RF-field exposure (1800 MHz, continuous wave, SAR = 0.2, 1.0, 1.3, 1.6, 2.0 and 3.0 W/kg, 24 h) on HL-60 cell growth with respect to growth velocity compared to control and sham-exposure, determined by the cellular doubling time. Each bar represents the mean ± SD of results obtained in at least three independent experiments, except for control (N=6) and SAR 1.3 W/kg (N=6).
**Cellular doubling time.** So far no hints for alteration of HL-60 genomic integrity due to apoptosis, necrosis or cytotoxicity has been detected. Additionally, RF-EMF exposure (1800 MHz, 1.3 W/kg, 24 h) had no effect on the cellular doubling time of HL-60 cells (figure 5) and the activity of the enzyme thymidine kinase, indicating that RF-field exposure does not influence HL-60 cellular growth rates.

4. Conclusions
Comparing RF-EMF exposure (1800 MHz, continuous wave, 24 h) of HL-60 cells at SAR levels ranging from 0.2 W/kg to 3.0 W/kg indicates that comet formation as well as micronuclei induction appear to be energy-dependent. The maximal effect was observed at a SAR of 1.3 W/kg. At higher SAR levels from 2.5 to 3.0 W/kg micronucleus frequencies and comet formation declined as compared to the effect noted at a SAR of 1.3 W/kg.

RF-EMF exposure (1800 MHz, 1.3 W/kg, 24 h) had no effect on the cellular doubling time and the activity of the enzyme thymidine kinase of HL-60 cells, indicating that RF-field exposure does not influence cellular growth rates.

Within a narrow energy window, RF-fields under the conditions used are clearly genotoxic in HL-60 cells without affecting cell proliferation or cell progression.

5. REFERENCES
Singh NP, McCoy MT, Tice RR, Schneider EL (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 175:184-191

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