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Induction of DNA strand breaks by intermittent exposure to extremely-low-frequency electromagnetic fields in human diploid fibroblasts

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Abstract

Results of epidemiological research show low association of electromagnetic field (EMF) with increased risk of cancerous diseases and missing dose–effect relations. An important component in assessing potential cancer risk is knowledge concerning any genotoxic effects of extremely-low-frequency-EMF (ELF-EMF).

Human diploid fibroblasts were exposed to continuous or intermittent ELF-EMF (50 Hz, sinusoidal, 24 h, 1000 μT). For evaluation of genotoxic effects in form of DNA single- (SSB) and double-strand breaks (DSB), the alkaline and the neutral comet assay were used.

In contrast to continuous ELF-EMF exposure, the application of intermittent fields reproducibly resulted in a significant increase of DNA strand break levels, mainly DSBs, as compared to non-exposed controls. The conditions of intermittence showed an impact on the induction of DNA strand breaks, producing the highest levels at 5 min field-on/10 min field-off. We also found individual differences in response to ELF-EMF as well as an evident exposure–response relationship between magnetic flux density and DNA migration in the comet assay.

Our data strongly indicate a genotoxic potential of intermittent EMF. This points to the need of further studies in vivo and consideration about environmental threshold values for ELF exposure.

Keywords: ELF-EMF; Comet assay; Intermittent exposure; 50 Hz

1. Introduction

The permanent progress of electronic industry and the increasing use of electrical appliances involve an increase in chronic exposure of people to extremely-low-frequency electromagnetic field (ELF-EMF) of various intensities and forms. Popular media and scientists have raised concerns about possible health hazards of environmental exposure to EMF, especially to 50 and 60 Hz. There are speculations that ELF-EMF can act as promoter or co-promoter of cancer. To date, numerous contradictory results regarding the carcinogenic potential of EMF have been reported in the literature. Although data from various epidemiological studies indicate that exposure to ELF-EMF may lead to an increased risk of certain types of adult and childhood cancer, including leukemia, cancer of central nervous system, and lymphoma [1–4], others [5–7] have failed to find such an association. A critical point concerning epidemiological studies, which are always retrospective investigations, is the lack of data regarding the exact dose of exposure.

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Several in vitro investigations on the biological consequences of EMF exposure effects were performed to estimate the risk of genotoxicity of EMF and to elucidate the underlying mechanisms. Knowledge concerning any genotoxic potential of EMF is an important basis for the assessment of EMF-induced cancer risk. Genotoxic effects of EMF may occur directly by damage to chromosomes or by damage to DNA repair mechanisms. Indirect genotoxic effects may arise by various processes like the generation of oxygen radicals or impairment of radical-scavenging mechanisms. Controversial results have been reported [8–11] with genotoxic endpoints as sister chromatid exchange (SCE), micronuclei (MN), chromosomal aberrations (CAs) and assessment of DNA strand breaks at exposure levels ranging from 1 μT to 10 mT. The majority of these studies did not show any EMF-related genotoxic effects. The few positive reports have not or could not be independently reproduced. An exposure–response relationship could not be demonstrated as well. However, these positive results must not be neglected.

Different in vitro exposure conditions, exposure setups, used methods and investigated targets make it difficult to compare the results for risk evaluation. Due to these facts, the REFLEX (risk evaluation of potential environmental hazards from low energy EMF exposure using sensitive in vitro methods) project was started, funded by the European Union, involving 12 independent research groups. To enable experiments under controlled and standardized conditions and to verify positive results, each of these groups was supplied with the same exposure setup, for ELF or radio frequency (rf), respectively.

Our aim of the project is to investigate possible genotoxic effects due to ELF-EMF exposure using the comet assay. To discriminate between DNA single-(SSB) and double-strand breaks (DSB), the neutral comet assay (detection of DSB) was used in addition to alkaline comet assay analysis (detection of SSB + DSB).

2. Materials and methods

2.1. ELF-EMF exposure conditions and cell culture

Human diploid fibroblasts (IH-9: 28 years of age, female; ES-1 male, 6 years of age) initiated from skin biopsies from healthy donors were maintained in culture in Dulbecco's modified Eagle's medium (DMEM, Gibco, Vienna, Austria) supplemented with 10% fetal calf serum (FCS), 20 mM Hepes buffer, 40 μg/ml neomycin, 2 mM L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin (Gibco, Vienna, Austria). Cells were grown in 175 cm^2 culture flasks at 37 °C in a humidified atmosphere in the presence of 5% CO_2 and were supplied with fresh culture medium every 48 h. Twenty-four hours before the start of the ELF experiments, fibroblasts were seeded into 35 mm Petri dishes at a density of 5×10^4 cells/3 ml.

The exposure system was built and provided by the IT'IS-foundation (Foundation for Information Technologies in Society, ETH, Zurich, Switzerland) within a study funded by the European Union. The setup generating a vertical EMF consisted of two four-coil systems (two coils with 56 windings, two coils with 50 windings), each of which was placed inside a μ-metal box. The currents in the bifilar coils could be switched parallel for field exposure or non-parallel for control (sham-exposure). Both systems were placed inside a commercial incubator (BBD 6220, Kendro, Vienna, Austria) to ensure constant environmental conditions (37 °C, 5% CO_2, 95% humidity). In addition, the temperature was monitored at the location of the dishes during exposure and was maintained at 36.5–37.5 °C. The temperature difference between the chambers did not exceed 0.3 °C, so possible thermal effects could be ruled out. A current source based on four audio amplifiers (Agilent Technologies, Zurich, Switzerland) allowed magnetic field up to 2.3 mT. The field could be varied in the frequency range from dc—1.5 kHz by a computer controlled function generator. To enable blinded exposures, the computer randomly determined which of the two the exposure setup was. The fields as well as all sensors in each chamber were continuously monitored. All experiments were performed at a frequency of 50 Hz sinusoidal for 24 h. To study the influence of different intermittences, the magnetic flux density was maintained at 1 mT, for studying dose–response effects, the magnetic flux density was varied between 20 and 2000 μT at a definite intermittence (5 min field-on/10 min field-off).

After exposure the fibroblasts were detached by trypsin, suspended in fresh culture medium. After

comet assay analysis, results were decoded. Each exposure level was tested in duplicate.

2.2. Comet assay analysis

We followed the technique described by Östling and Johanson [12] with minor modifications by Singh et al. [13,14]. Fully frosted microscopic slides (CMS, Houston, USA) were used.

ELF- and sham-exposed cells (10,000–30,000) were mixed with 100 μl low melting agarose (0.5%, 37 °C) to form a cell suspension, pipetted onto 0.5% normal melting agarose pre-coated slides, spread using a cover slip, and maintained on a cold flat tray for about 10 min to solidify. After removal of the cover slip the third layer of 0.5% low melting agarose was added and solidified. The slides were immersed in freshly prepared cold lysis solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, pH 10, 1% sodium sarcosinate, 1% Triton X-100, 10% DMSO, pH 10) and lysed for 90 min at 4 °C. Subsequently, the slides were drained and placed in a horizontal gel electrophoresis tank side by side, nearest the anode. The tank was filled with fresh electrophoresis buffer (1mM Na_2EDTA, 300 mM NaOH, pH 13 in case of alkaline comet assay, and 100 mM Tris, 300 mM sodium acetate, 500 mM sodium chloride, pH 8.5 in case of neutral comet assay) to a level approximately 0.4 cm above the slides. For both, alkaline and neutral comet assays, slides were left in the solution for 40 min for equilibration and unwinding of the DNA before electrophoresis. Electrophoresis conditions (25 V, 300 mA, 4 °C, 20 min, field strength: 0.8 V/cm) were the same for neutral and alkaline comet assay. All steps were performed under dimmed light to prevent the occurrence of additional DNA damage. After electrophoresis the slides were washed three times with Tris buffer (0.4 M Tris, pH 7.5) to neutralize, then air-dried and stored until analysis. Comets were visualized by ethidium bromide staining (20 μg/ml, 30 s) and examined at 400× magnification using a fluorescence microscope (Axiophot, Zeiss, Germany). One thousand DNA spots from each sample were classified into five categories corresponding to the amount of DNA in the tail according to Anderson et al. [15] with modifications. The proposed classification system provides a fast and inexpensive method for genotoxic monitoring. Due to the classification to different groups by eye, no special imaging software is required. The different classification groups are not weighted equally, due to the fact that they do not represent equal grades of damage. Moreover, the technique becomes more sensitive, because many cells can be scored in a short time (1000 cells instead of 50–100 cells with image analyzing). The subsequent calculation of a "comet tailfactor" allows quantifying DNA damage as a single figure, which makes it easier to compare results. Due to the scoring of 1000 cells in one experiment, which are 10-fold the cells processed with image analyzing, standard deviations are very low. Reproducibility has been thoroughly checked.

Results were expressed as "comet tailfactors", calculated according to Diem and Rüdiger [16]. All analyses were performed by the same investigator. Fig. 1 shows the five classification groups with the group averages and the microphotograph.

Tailfactors were calculated according to the following formula:

$$\text{tailfactor (\%)} = \frac{AF_A + BF_B + CF_C + DF_D + EF_E}{1000}$$

where A is the number of cells classified to group A, F_A the average of group A (2.5), B the number of cells classified to group B, F_B the average of group B (12.5), C the number of cells classified to group C, F_C the average of group C (30), D the number of cells classified to group D, F_D is the average of group D (67.5), E the number of cells classified to group E, and F_E the average of group E (97.5).

2.3. Statistical analysis

Statistical analysis was performed with STATISTICA V. 5.0 package (Statsoft Inc., Tulsa, USA) and SPSS 10.0 package (SPSS Inc., Illinois, USA). All data are presented as mean±standard deviation (S.D.). The differences between exposed and sham-exposed, as well as between different exposure conditions were tested for significance using independent Student's *t*-test or one-factorial ANOVA with post-hoc Bonferroni correction. A difference at $P < 0.05$ was considered statistically significant. Correlations were assessed by multiple regression analysis using linear regression.

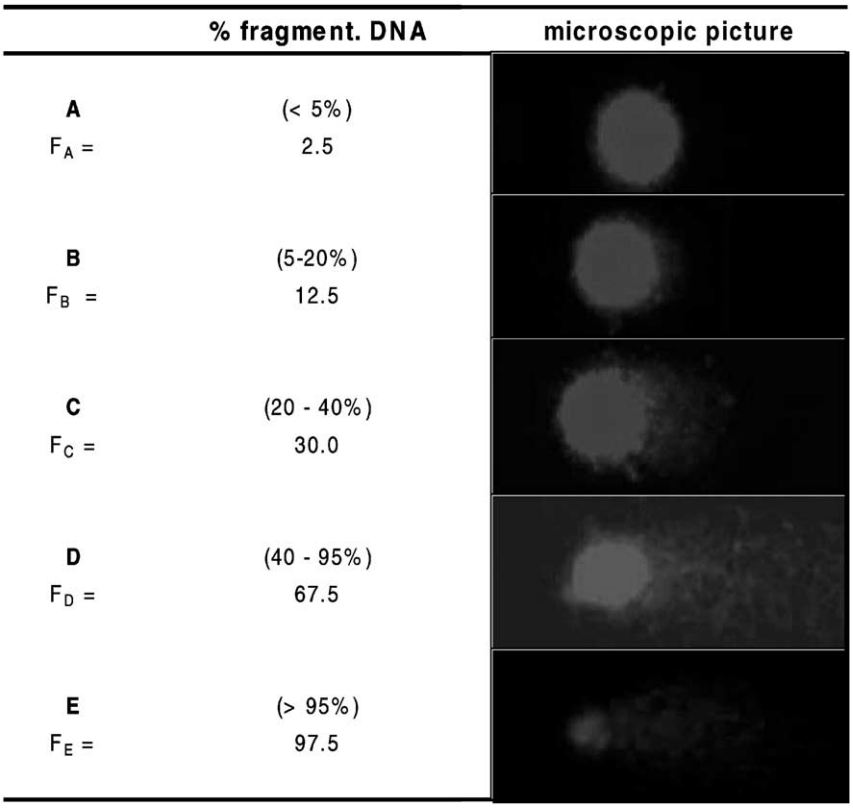

Fig. 1. Classification groups and respective microscopic appearance (cell line ES-1).

3. Results

In the first set of experiments fibroblasts were continuously exposed to ELF-EMF at 1000 μT 24 h. In these experiments we did not find any significant differences between exposed and sham-exposed cells (Tables 1 and 2) in the alkaline and neutral comet assays. As Nordenson et al. [17] reported positive genotoxic effects applying intermittent field exposure, our next experiments concentrated on exposures at different intermittence conditions. Intermittences of 5/5 and 15/15 min revealed an increase of both, alkaline and neutral comet assay levels, compared to sham-exposed cells, whereas 5 min field-on/25 min field-off failed to reproduce these results (Tables 1 and 2). On the basis of these findings, we tried to figure out the optimal exposure conditions for maximal effects on DNA strand break levels.

We started with a fixed field-on time of 5 min and varied field-off times from 5 to 25 min. These experiments indicated that DNA strand break levels (SSB and DSB) culminated at an off-time of 10 min and reached control levels at extended off-times (Fig. 2). Significant differences ($P < 0.01$) between exposed and sham-exposed cells were found at 5/5, 5/10, 5/15 and 5/20 intermittences for alkaline comet assay and at 5/5, 5/10 and 5/15 intermittences for neutral comet assay, but not at 5/25 for both assays.

Subsequently, a fixed off-time of 10 min was chosen and on-times have been varied from 1 to 25 min. Again, the highest level of DNA strand breaks was obtained at an intermittence of 5 min field-on/10 min field-off (Fig. 3). Tailfactors of exposed and sham-exposed cells differed significantly at each on-time in alkaline comet assay and at 3–15 min field-on in the

Table 1
Mean values of alkaline comet assay tailfactors at different exposure conditions ($n = 2$), cell line IH-9

Different exposure conditions	Exposed		Sham	
	Comet tailfactor (%)	±S.D.[a]	Comet tailfactor (%)	±S.D.
Continuous exposure (24 h)	4.29	0.02	4.27	0.03
15/15 on/off	6.47*	0.14	4.23	0.05
5/5 on/off	6.98*	0.04	4.41	0.16
5/10 on/off	7.47*	0.13	4.48	0.05
5/15 on/off	6.68*	0.17	4.42	0.03
5/20 on/off	5.90*	0.12	4.38	0.12
5/25 on/off	4.27	0.04	4.23	0.03
1/10 on/off	5.89*	0.19	4.21	0.14
3/10 on/off	6.60*	0.06	4.19	0.22
10/10 on/off	6.91*	0.07	4.24	0.07
15/10 on/off	6.56*	0.15	4.11	0.08
25/10 on/off	5.37*	0.05	4.21	0.04

[a] Indicates standard deviation.
* Indicates significant differences ($P < 0.05$) exposed vs. sham.

neutral comet assay. Significant differences between the tailfactors of exposed cells at various intermittence conditions are presented in Table 3 for the alkaline comet assay and in Table 4 for the neutral comet assay. Solely the alkaline comet tailfactors of 5/10, 5/25 and 25/10 EMF-exposed cells differed significantly to the other applied intermittence conditions. Since an intermittence of 5/10 was able to induce the highest levels of DNA strand breaks in both alkaline and neutral comet assays, further experiments were performed at 5/10.

Testing two different cell lines (IH-9, ES-1; 1000 μT, 24 h, 5/10), again, significant differences ($P < 0.00001$) in alkaline and neutral comet tailfactors between exposed and sham-exposed cells (Table 5, Fig. 4) as well as between exposed cells of these two strains could be found. Sham-exposed IH-9 and ES-1 cells did not differ significantly, indicating that inter-individual differences in ELF response may exist. Figs. 5 and 6 show the distribution of cells with different grades of damage in alkaline and neutral comet assays, respectively.

Table 2
Mean values of neutral comet assay tailfactors at different exposure conditions ($n = 2$), cell line IH-9

Different exposure conditions	Exposed		Sham	
	Comet tailfactor (%)	±S.D.[a]	Comet tailfactor (%)	±S.D.
Continuous exposure (24 h)	4.20	0.03	4.17	0.05
15/15 on/off	5.72*	0.01	4.25	0.04
5/5 on/off	6.09*	0.02	4.31	0.08
5/10 on/off	6.21*	0.01	4.35	0.07
5/15 on/off	5.66*	0.06	4.23	0.13
5/20 on/off	4.52	0.16	4.50	0.21
5/25 on/off	4.25	0.05	4.34	0.07
1/10 on/off	4.16	0.15	4.16	0.13
3/10 on/off	5.94*	0.05	4.20	0.06
10/10 on/off	6.19*	0.11	4.11	0.11
15/10 on/off	6.02*	0.03	4.21	0.10
25/10 on/off	5.44	0.01	4.15	0.01

[a] Indicates standard deviation.
* Indicates significant differences ($P < 0.05$) exposed vs. sham.

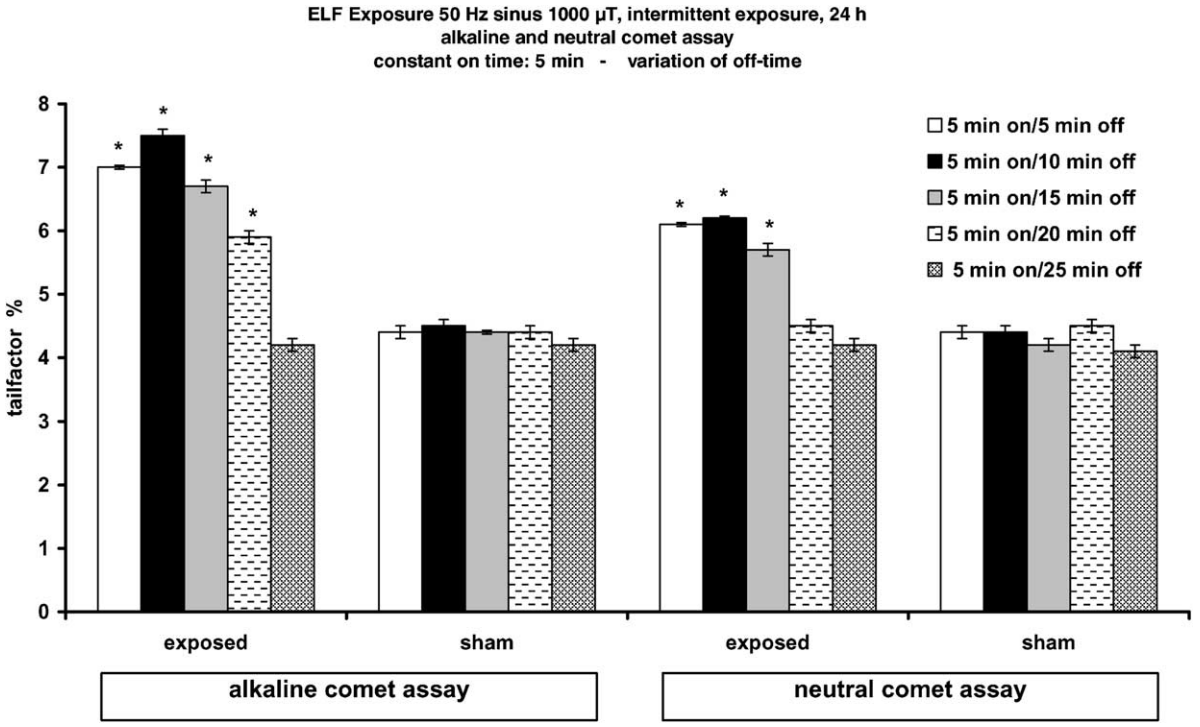

Fig. 2. Alkaline and neutral comet assay tailfactors of ELF exposed fibroblasts (cell line IH-9, 50 Hz sinus, 24 h, 1000 μT, intermittent) variation of off-time. *$P < 0.01$ exposed vs. sham-exposed.

To test the exposure–response relationship, magnetic flux density was varied between 20 and 2000 μT (at 24 h, 5/10). Even a magnetic flux density as low as 70 μT produced significantly elevated ($P < 0.01$) alkaline and neutral comet assay levels as compared to sham-exposed controls (Table 6, Fig. 7). Two plateau-like sections between 100 and 500 μT and between 1000 and 2000 μT could be revealed, which

Table 3
Significant ($P < 0.05$) differences between different intermittence conditions—alkaline comet assay

	P-values at various on/off times (min)									
	5/5	5/10	5/15	5/20	5/25	1/10	3/10	10/10	15/10	25/10
5/5	–	0.02	N.S.	<0.001	<0.001	<0.001	N.S.	N.S.	N.S.	<0.001
5/10	0.02	–	<0.001	<0.001	<0.001	<0.001	<0.001	0.007	<0.001	<0.001
5/15	N.S.	<0.001	–	<0.001	<0.001	<0.001	N.S.	N.S.	N.S.	<0.001
5/20	<0.001	<0.001	<0.001	–	<0.001	N.S.	0.001	<0.001	<0.001	0.021
5/25	<0.001	<0.001	<0.001	<0.001	–	<0.001	<0.001	<0.001	<0.001	<0.001
1/10	<0.001	<0.001	<0.001	N.S.	<0.001	–	0.001	<0.001	0.001	0.021
3/10	N.S.	<0.001	N.S.	0.001	<0.001	0.001	–	N.S.	N.S.	<0.001
10/10	N.S.	0.007	N.S.	<0.001	<0.001	<0.001	N.S.	–	N.S.	<0.001
15/10	N.S.	<0.001	N.S.	<0.001	<0.001	0.001	N.S.	N.S.	–	<0.001
25/10	<0.001	<0.001	<0.001	0.021	<0.001	0.021	<0.001	<0.001	<0.001	–

N.S.: not significant, post-hoc compared Bonferroni corrected.

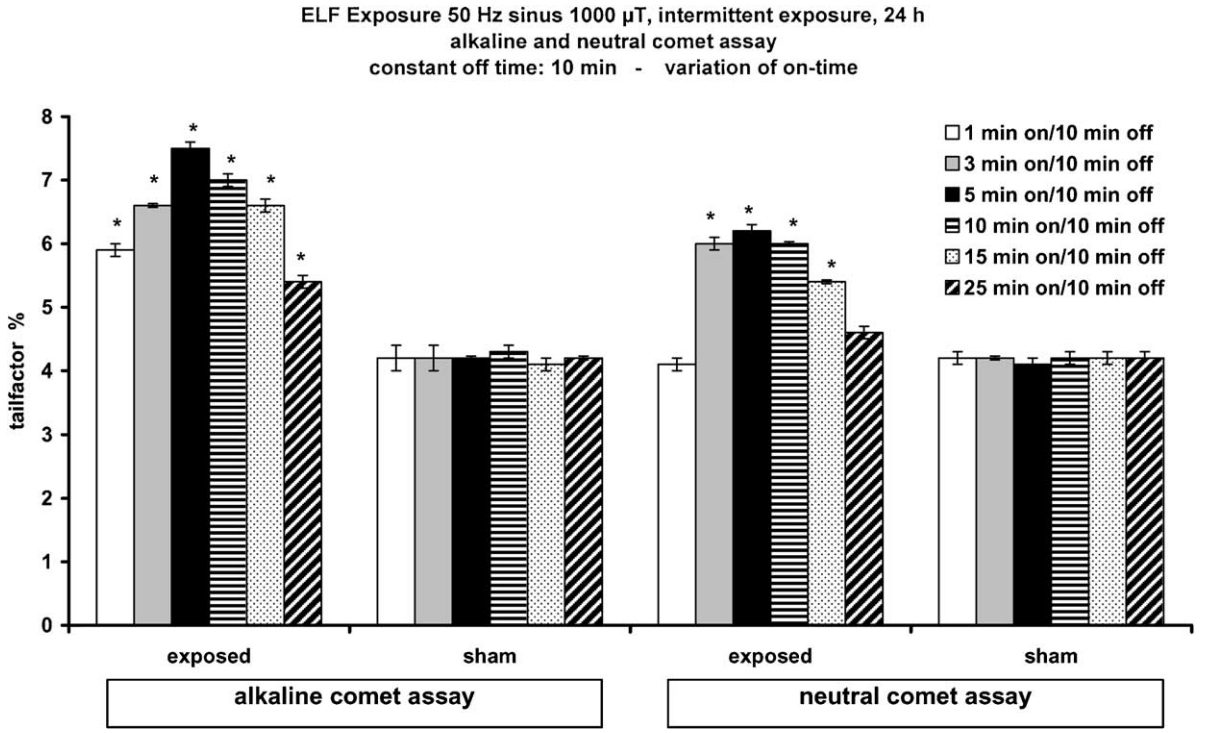

Fig. 3. Alkaline and neutral comet assay tailfactors of ELF exposed fibroblasts (cell line IH-9, 50 Hz sinus, 24 h, 1000 µT, intermittent) variation of on-time. $^{*}P < 0.01$ exposed vs. sham exposed.

might point to a biphasic mechanism. In Fig. 8 the alkaline and neutral tailfactors are plotted versus log (magnetic flux density in µT), showing an exponential dose–response relationship. Using regression analysis, a significant correlation between comet tailfactors and applied magnetic field (alkaline comet assay: $r = 0.843$, $P = 0.004$; neutral comet assay: $r = 0.908$, $P = 0.0007$), as well as between alkaline and neutral comet assays could be found ($r = 0.974$, $P = 0.00001$).

Table 4
Significant ($P < 0.05$) differences between different intermittence conditions—neutral comet assay

	P-values at various on/off times (min)									
	5/5	5/10	5/15	5/20	5/25	1/10	3/10	10/10	15/10	25/10
5/5	–	N.S.	N.S.	<0.001	<0.001	<0.001	N.S.	N.S.	<0.001	<0.001
5/10	N.S.	–	0.007	<0.001	<0.001	<0.001	N.S.	N.S.	<0.001	<0.001
5/15	N.S.	0.007	–	<0.001	<0.001	<0.001	N.S.	N.S.	N.S.	<0.001
5/20	<0.001	<0.001	<0.001	–	N.S.	N.S.	<0.001	<0.001	<0.001	N.S.
5/25	<0.001	<0.001	<0.001	N.S.	–	N.S.	<0.001	<0.001	N.S.	N.S.
1/10	<0.001	<0.001	<0.001	N.S.	N.S.	–	<0.001	<0.001	<0.001	N.S.
3/10	N.S.	N.S.	N.S.	<0.001	<0.001	<0.001	–	N.S.	0.013	<0.001
10/10	N.S.	N.S.	N.S.	<0.001	<0.001	<0.001	N.S.	–	0.002	<0.001
15/10	<0.001	<0.001	N.S.	<0.001	N.S.	<0.001	0.013	0.002	–	<0.001
25/10	<0.001	<0.001	<0.001	N.S.	N.S.	N.S.	<0.001	<0.001	<0.001	–

N.S.: not significant, post-hoc compared Bonferroni corrected.

Table 5
Mean values of alkaline and neutral comet assay tailfactors of different cell strains (IH-9, ES-1) at intermittent ELF exposure (5/10 on/off, 1000 μT, 24 h) ($n = 10$)

	Alkaline comet assay				Neutral comet assay			
	Exposed		Sham		Exposed		Sham	
	Tailfactor (%)	±S.D.[a]	Tailfactor (%)	±S.D.	Tailfactor (%)	±S.D.	Tailfactor (%)	±S.D.
ES-1	6.50*	0.12	3.98	0.12	5.62*	0.16	3.95	0.08
IH-9	7.44*	0.09	4.12	0.12	6.53*	0.08	4.06	0.12

[a] Indicates standard deviation.
* Indicates significant differences ($P < 0.05$) exposed vs. sham.

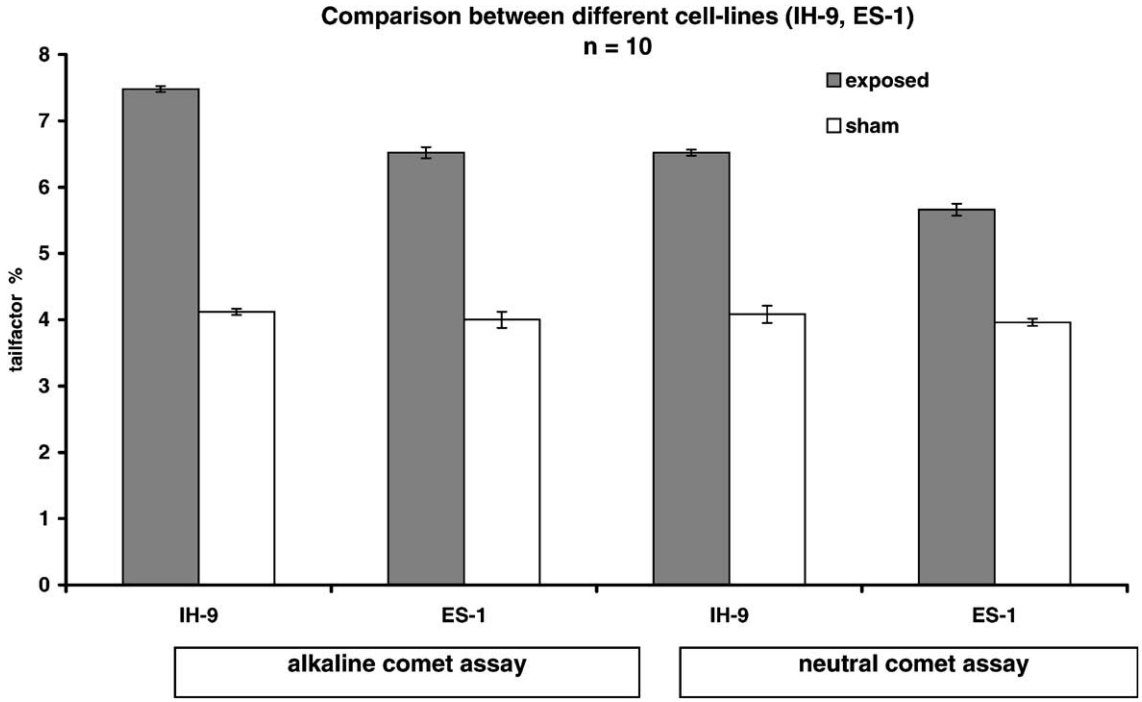

Fig. 4. Alkaline and neutral comet assay tailfactors of ELF exposed fibroblasts (50 Hz sinus, 24 h, 1000 μT, intermittent 5/10) comparison between different cell lines.

4. Discussion

Our results show, that intermittent exposure to a 50 Hz magnetic field causes a reproducible increase in DNA strand breaks in cultured human diploid fibroblasts. The majority of the studies investigating genotoxic effects of 50/60 Hz [8,9] EMF were performed at continuous exposure. Nearly all have reported a negative outcome on genotoxicity of ELF-EMF. Our results from tests with continuous exposure of fibroblasts to EMF corroborate these findings. Subjecting cells continuously to a constant field probably may induce adaptive mechanisms, protecting the genome from harmful influences. A regular change of environmental conditions might interfere with such mechanisms and lead to DNA impairment. The extent of damage would depend on the duration of exposure and the time of recovery. A proper mechanism by which magnetic fields

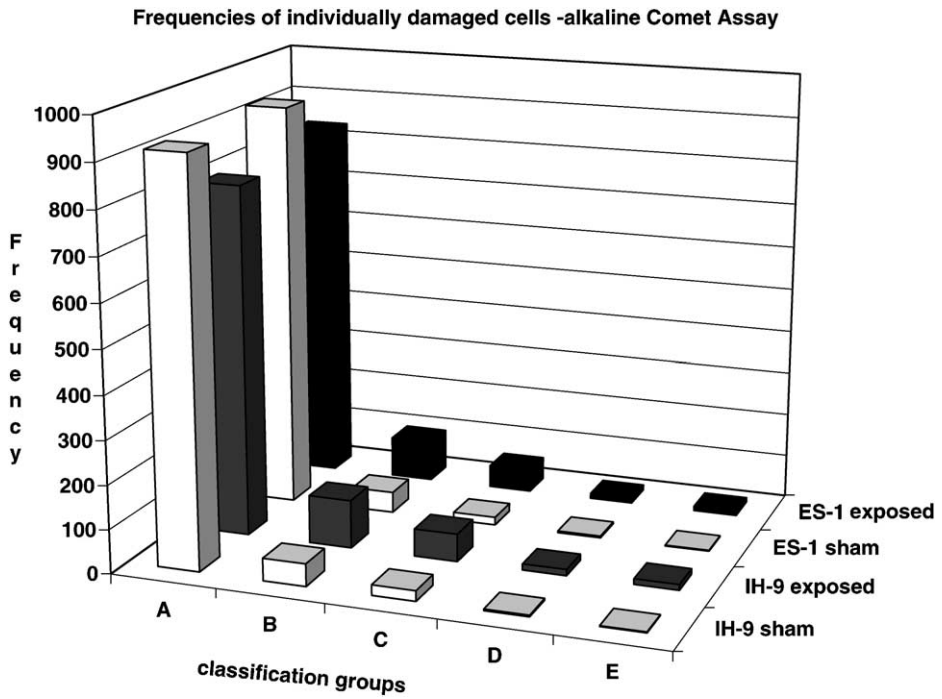

Fig. 5. Alkaline comet assay frequencies of individually damaged cells of sham- and ELF-exposed fibroblasts (50 Hz sinus, 24 h, 1000 μT, intermittent 5/10) comparison between different cell lines.

may interact with biological systems has not been found as yet. Several hypotheses like induction of electric currents [18], increased free radical activity [19] or acceleration of electron transfer in different enzymes and proteins [20] have been proposed. Although all of these theories are mostly speculative, i.e. the latter, the interaction of EMF with moving charges seems to have a plausible biophysical basis. Recent observations

Table 6
Mean values of alkaline and neutral comet tailfactors at intermittent ELF exposure (5/10 on/off, 1000 μT, 24 h) ($n = 2$) dose response, cell line ES-1

Magnetic flux density (μT)	Alkaline comet assay				Neutral comet assay			
	Exposed		Sham		Exposed		Sham	
	Tailfactor (%)	±S.D.[a]	Tailfactor (%)	±S.D.	Tailfactor (%)	±S.D.	Tailfactor (%)	±S.D.
20	4.16	0.02	4.21	0.13	3.63	0.01	3.60	0.08
50	4.16	0.06	4.20	0.12	3.70	0.16	3.72	0.03
70	4.87*	0.03	4.28	0.02	3.99*	0.01	3.71	0.01
100	5.25*	0.06	4.28	0.05	4.32*	0.00	3.73	0.04
250	5.31*	0.02	4.25	0.07	4.24*	0.06	3.60	0.02
500	5.52*	0.01	4.22	0.01	4.48*	0.02	3.79	0.05
750	6.17*	0.08	4.26	0.11	5.08*	0.08	3.67	0.10
1000	6.50*	0.18	4.27	0.10	5.71*	0.01	3.79	0.16
2000	6.62*	0.01	4.13	0.04	5.79*	0.05	3.70	0.01

[a] Indicates standard deviation.
* Indicates significant differences ($P < 0.05$) exposed vs. sham.

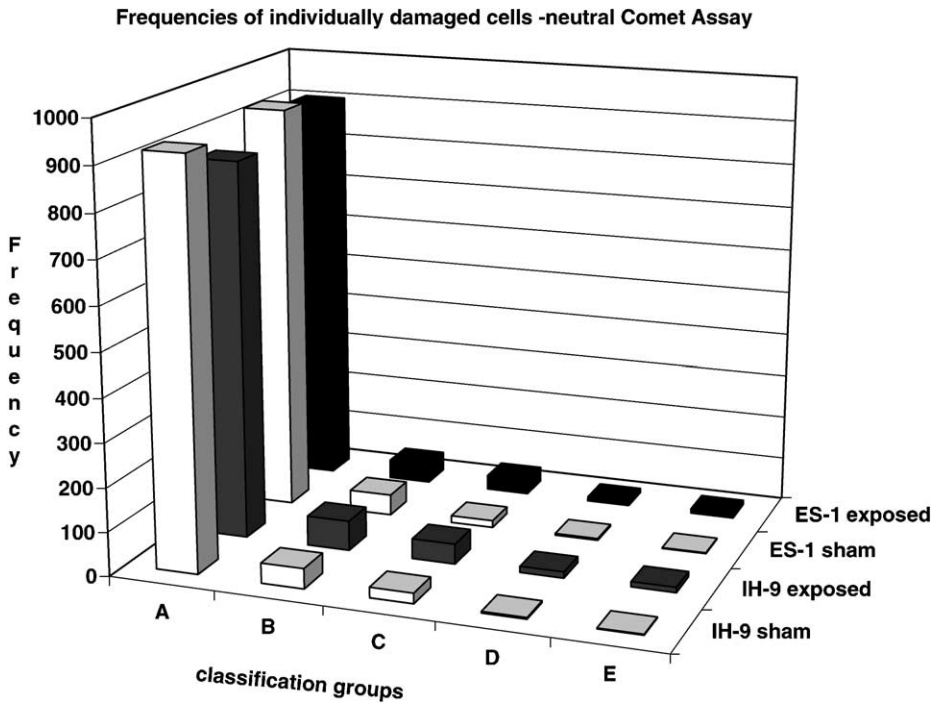

Fig. 6. Neutral comet assay frequencies of individually damaged cells of sham- and ELF-exposed fibroblasts (50 Hz sinus, 24 h, 1000 μT, intermittent 5/10) comparison between different cell lines.

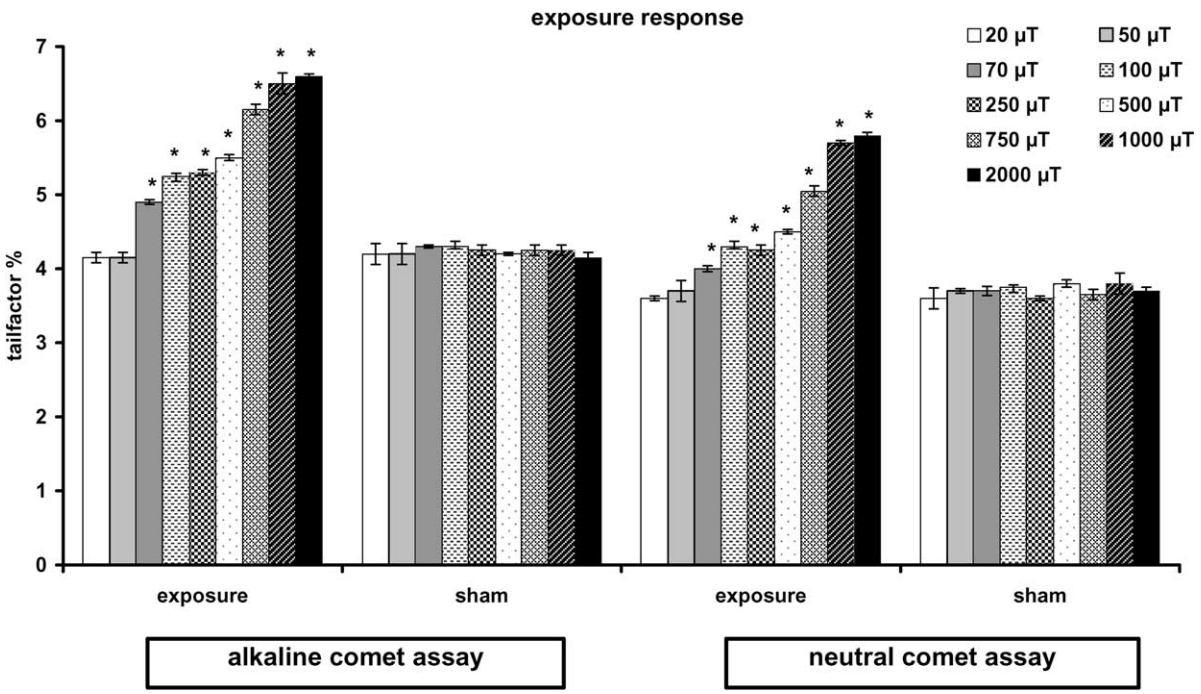

Fig. 7. Alkaline and neutral comet assay tailfactors of ELF exposed fibroblasts (ES-1, 50 Hz sinus, 24 h, 1000 μT, intermittent 5/10) exposure response. $^{*}P < 0.01$ exposed vs. sham-exposed.

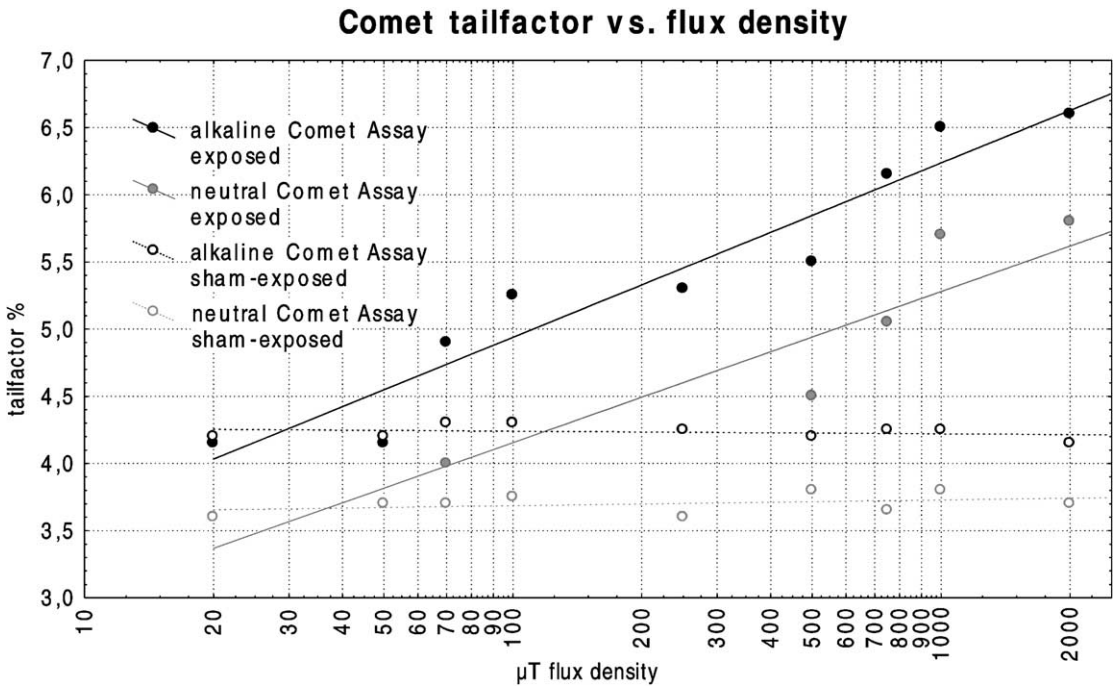

Fig. 8. Alkaline and neutral comet assay tailfactors of ELF exposed fibroblasts (cell line ES-1, 50 Hz sinus, 24 h, 1000 µT, intermittent 5/10) exposure response.

show, that DNA can transfer electrons within its base pairs [21,22]. These studies suggest that EMF may initiate transcription by interacting with moving electrons in DNA [23] by generating repulsive (Lorentz) forces causing chain separation at specific DNA sequences (*n*CTCT*n*) [24]. As within the state of transcription DNA presents a vulnerable target to genotoxic influences, the on/off switching at intermittent ELF-EMF exposure may lead to DNA disruption, causing DNA strand breaks. In our experiments the relation on- to off-time appeared to be crucial for the extent of damage caused. An intermittence of 5/10 was able to produce the highest levels of DNA strand breaks, whereas at 5/25 no significant difference to the concurrent control was found. We suppose, that at extended off-times (>15 min) ELF-induced damage could be removed by DNA repair mechanisms. This explanation is speculative and requires further assessment, e.g. evaluation of repair kinetics. However, a study conducted by Miyakoshi et al. [25] demonstrated that ELF exposure (continuous exposure, 5–400 mT, 30 min) alone did not induce DNA strand breaks in human glioma cells, but enhanced X-ray-induced DNA damage, by affecting DNA repair. These findings suggest, that ELF exposure seems to have a negative impact on DNA repair mechanisms. Therefore at intermittent exposure an extended off-time would give time for recovery.

Since we found significant differences in ELF-EMF response in different donors, individual factors like sex, or age might play an important role in the susceptibility of DNA to EMF. Regarding the distribution data (Figs. 5 and 6) in different cell lines after ELF exposure, the observed effect does not seem to be present in all cells. It is possible, that there may exist an "ELF-sensitive" fraction (e.g. cells in a specific stage of cell cycle) and that this portion of cells might vary individually.

At our experimental settings (24 h, 1000 µT) we observed, that an intermittent exposure of 5 min field-on/10 min field-off was most effective to produce DNA strand breaks in human fibroblasts. Environmental exposure to continuous ELF-EMF is rather exceptional. Different electrical household devices (hair dryer, razor, vacuum cleaner) reaching peak values up to 1 mT are often used for a short period of time (5–10 min), producing a variety of exposure

levels. To date, we could make out only one study dealing with genotoxic effects of ELF-EMF at intermittent exposure. This was done by Nordenson et al. [17], who found a significant increase of CAs in human amniotic cells (50 Hz, 30 μT, 20 s on/off). However, these results have not been corroborated by other studies as yet.

Due to the highly significant correlation between alkaline and neutral comet assays, we suggest, that predominantly non-repairable DNA DSBs are generated by ELF-EMF exposure. The observed increase in strand breaks (4.2–7.5%) after ELF-EMF exposure is quite similar to the levels found in a previous study, regarding age dependent changes in alkaline comet tailfactors in lymphocytes of healthy subjects [16] and therefore does not constitute a drastic effect. However, our data are reproducible and significant. In addition, we were able to confirm the results obtained by Lai and Singh [26], Singh and Lai [27], who performed in vivo ELF-exposure experiments (60 Hz, 0.1–0.5 mT, 2 h) and found an increase in DNA strand break levels in rat brain cells.

Moreover, due to the software controlled exposure setup, we could demonstrate a dose dependent relationship between alkaline and neutral comet assay tailfactors and applied magnetic flux density, which is unique. The guidelines of the International Commission on Non-Ionizing Radiation Protection (ICNIRP) [28] are 500 μT during workday for occupational exposures and 100 μT for 24 h per day for the general population. The on-set of genotoxic effects in our tests was at a magnetic flux density as low as 70 μT (24 h, intermittent), being well below these proposed threshold values. Moreover, the ICNIRP guidelines are dealing with continuous EMF exposure. No proposal how to handle intermittent exposures has been made as yet.

In conclusion, the outcome of this study strongly indicates a genotoxic potential of intermittent EMF. This points to the need of further studies in vivo and consideration about environmental threshold values for ELF exposure.

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