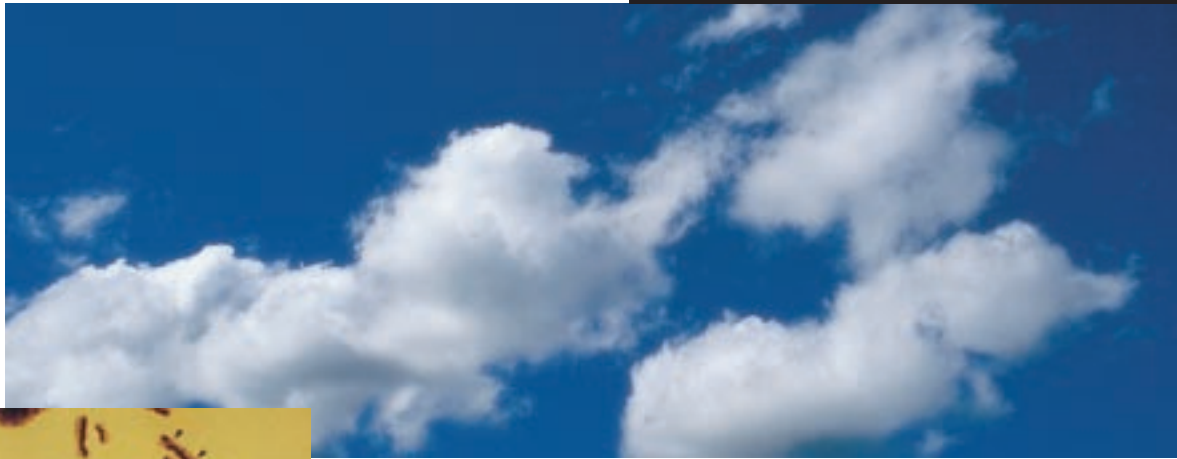


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Cell Proliferation, Sister Chromatid Exchanges, Chromosome Aberrations, Micronuclei and Mutation Rate of the HGPRT Locus

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Forschungsgemeinschaft Funk

Dear readers:

The Forschungsgemeinschaft Funk e.V. (FGF) was founded in 1992 with the clear mandate to enable and support research on the biological effects of radio-frequency electromagnetic waves on human beings and the environment. It is of absolute importance to us to offer only scientifically-accurate and objective information in this area to the general public. As well as the well-known FGF "Newsletter" which reports on the entire area of electromagnetic compatibility (EMC) and of electromagnetic compatibility with the environment (EMCE), a new edition of "Edition Wissenschaft" (Research Report) will inform readers of the current status of scientific research on these topics.

Numerous research contracts have been commissioned by the FGF to independent research groups. The results of the individual projects will be informally presented to our readers in the new "Edition Wissenschaft". The projects commissioned so far deal with the biological effects of radio-frequency electromagnetic fields as used in cellular mobile radio. The conclusions of the studies to this date are that no indication of cancer-initiation or cancer-promotion effects has been found.

The first four contributions presented here should not be taken as "final results" from the FGF. It is far more usual that the research results reported here are taken as a base for further studies. For this reason the FGF will continue to inform readers in future editions of "Edition Wissenschaft" on the progress made in this particular field.

Gerd Friedrich

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Investigations of the "Biological Effects of Radio-Frequency Electromagnetic Fields".

1st issue: Growth response of human leukaemia cells (promyelocytes) under the influence of radio-frequency electromagnetic fields (900 MHz and 1.8 GHz, each pulsed with 217 Hz) to investigate any potential cancer-promotion effect.

2nd issue: The influence of radio-frequency electromagnetic fields at cellular mobile radio frequencies on the calcium homeostasis of heart-muscle cells and lymphocytes.

3rd issue: Exposure equipment.

4th issue: Cell proliferation, sister chromatid exchanges, chromosome aberrations, micronuclei and mutation rate of the HGPRT locus as a result of the exposure of human peripheral lymphocytes to radio-frequency electromagnetic fields (440 MHz, 900 MHz and 1.8 GHz).

Biological Effects of Radio-Frequency Electromagnetic Fields

**Prof. Dr.-Ing. E.h. Karl Brinkmann,
Prof. Dr.-Ing. Rudolf Elsner**

Cellular mobile radio systems subject both their users and the general public to electromagnetic fields. Precautions need to be taken to ensure the prevention of any physical injury. The manufacturer therefore has to ensure that his product conforms to the relevant safety standards. These standards are based on the knowledge of the thermal effect of radio-frequency electromagnetic fields. It is known that at a specific absorption rate (SAR) of 4 W/kg the temperature of animal tissue, and therewith presumably also human tissue, increases by 1 °C [1]. To protect the general public against such effects, an SAR limit of 80 mW/kg has been specified. If this limit is complied with, thermal effects will certainly be insignificant.

Recently, however, it has been asserted that radio-frequency electromagnetic fields could also give rise to athermal effects. The aim of the present research project is to identify whether such effects do in fact occur for the biological systems considered..

It is the conventional procedure to try to observe any such effect by investigating the biological

behaviour of animal or human cells. For this purpose, the cells are inserted in a nutrient solution and exposed to a radio-frequency electromagnetic field for a suitable time duration. To eliminate environmental influences, a comparable sample is observed at the same time in the absence of the radio-frequency electromagnetic field. Any different behaviour of the cells between the cases of exposure and sham-exposure is then taken to be caused only by the influence of the electromagnetic field. This investigation approach leaves open the question as to whether these effects also cause cellular or organic damage, since this would require experiments on the entire organism. Yet it seems clear that no damage arises if no or just negligible effects occur. The biological systems examined were: lymphocytes of healthy human donors (working group Eberle, Braunschweig), cardiac-muscle cells and lymphocytes (working group Meyer, Bonn) and human leukemia cells (working group Fitzner, Berlin). The exposures were at 440 MHz (C-net), 900 MHz (D-net) and 1800 MHz (E-net). In the D- and E-frequency bands pulsed radio-frequency signals were used. The nutrient solutions

were kept at a constant temperature of 37 °C ± 0.1 °C. The sample holders (incorporating cells, nutrient solution, and white oil for keeping the temperature constant) had dimensions of 10 cm (groups of Eberle and of Fitzner) and of only 1 cm (Meyer group).

In the absence of test objects, the electromagnetic fields should be as homogeneous as possible within the sample holders. Therefore, suitable waveguides (working group Elsner/Neibig, Braunschweig) were provided. These waveguides consist of metallic tubes which suppress any interaction between the inner radio-frequency fields and unwanted external fields.

Due to the properties of the nutrient solution, only a small fraction of the power fed into the wave-guide is absorbed by the nutrient solution. The field inside the nutrient solution and therefore inside the cells cannot be measured. However, with a knowledge of the electromagnetic characteristics of the nutrient solution and of the material of the sample holder, the electric and magnetic field strengths in the nutrient solution can be calculated. Using the values of the electrical



Insertion of the sample holder, with samples, into the TEM cell. On the left side of the picture, equipment for the generation and measurement of radio-frequency electromagnetic fields is depicted. Both the measurement procedure and the recording of the field-strength values are carried out under computer control.

permittivity and conductivity, the SAR value, which represents the power absorbed per unit mass, can be computed at each point within the nutrient solution. Some model simplifications had to be applied for that purposes, resulting in calculated values that can differ from the true ones by a factor of at most two. The SAR-values were specified to be 80 mW/kg (equivalent to 80 mW/cm³ for a mass density of 1 g/cm³) for all experiments. This could in practice be obtained only approximately, as the calculations were carried out while the biological investigations were already running. Most of the calculated SAR values were below the limit values given in the safety standards.

For the radio-frequency electric and magnetic field strengths, the limit values are 100 V/m and 0.265 A/m (corresponding to a magnetic flux density of 0.3 mT), respectively, according to DIN VDE 0848 Part 2. Further calculations revealed that in the nutrient solutions used the magnetic field strength within the waveguide is varied by only a very small amount by the insertion of the test object, whereas the electric field

strength is considerably decreased. This happens also when the human body is exposed to radio-frequency electromagnetic fields.

In the following four reports on parts of this work, the biological basics, the experimental set-up and the results are described. Note that no athermal effect has been identified in the investigations performed, as listed immediately below.

- Report on part of the work: Dipl.-Ing. Uwe Neibig, Technische Universität (Technical University) Braunschweig "Exposure Equipment"
- Report on part of the work: Dr. rer. nat. Susanne Diener, Prof. Dr. rer. nat. Paul Eberle, Technische Universität Braunschweig "Cell Proliferation, Sister-Chromatid Exchanges, Chromosome Aberrations, Micronuclei and Mutation Rate of the HGPRT-Locus as a Result of the Influence of Electromagnetic Radio-Frequency Fields (440 MHz, 900 MHz and 1.8 GHz) on Human Peripheral Lymphocytes"

- Report on part of the work: Dr. rer. nat. Rainer Meyer, Universität Bonn "Influence of Radio-Frequency Electromagnetic Fields of Mobile Communication on the Calcium Homeostasis of Cardiac Muscle Cells and Lymphocytes"
- Report on part of the work: Dr. med. R. Fitzner, E. Langer, Freie Universität Berlin "Growth Behaviour of Human Leukemia HL-60 Cells Influenced by Radio-Frequency Electromagnetic Fields (1.8 GHz and 900 MHz, pulsed with 217 Hz) for the Investigation of Cancer-Promotion Effects"

Acknowledgement:

The series of research work listed above was supported by the Forschungsgemeinschaft Funk e.V.

Reference:

- [1] Heinrich Baggenstoës: "Dosimetrische Untersuchungen zum Mobilfunk", Kleinheubacher Berichte, Book 37 (1993), P. 589.

Final report on part of the Project:

Cell Proliferation, Sister Chromatid Exchanges, Chromosome Aberrations, Micronuclei and Mutation Rate

of the HGPRT Locus following the Exposure of Radio-Frequency Electromagnetic Fields (440 MHz, 900 MHz and 1.8 GHz) on Human Peripheral Lymphocytes

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Collaborators: Dr. Susanne Diener, Dr. Martina Erdtmann-Vourliotis, Dipl.-Biol. Hans-Günter Finke, Dipl.-Biol. Bettina Löffelholz, Dipl.-Biol. Anette Schnor and Dipl.-Biol. Mechthild Schröder

1. Introduction

To ascertain whether or not the 440 MHz, 900 MHz and 1.8 GHz radio-frequency electromagnetic fields typical of cellular mobile radio represent a health risk, we conducted experiments on human donor blood. In so doing, reference is made to verified findings in cancer and mutation research, according to which certain mutations in genes and chromosomes, and also changes in cell proliferation, may cause cancerogenesis. On the other hand, non-genotoxic cancerogenes do exist that may act as cofactors. The study concept chosen does not take into account the functionality of the entire human body. Nevertheless, no particular restrictions on the resulting findings of the experiments

are seen, as the results of X-ray pathology do not suggest any significant differences between considering the body as a whole and at the cellular level.

The test systems used form part of the battery of tests for mutagenity recommended by international commissions (e.g. OECD Guidelines). They are suitable for different levels of sensitivity of the genetic structures. The chromosome aberration rate accounts for DNA damage on the DNA double-strand level with respect to chromatids and chromosomes, the SCE frequency reflects DNA damage on the DNA single-strand level and the genetic mutations of the HGPRT locus reveal adverse changes in an X-chromosomal hereditary disposition.

The micronucleus frequency indicates primarily whether the chromosome distribution over the daughter nuclei follows the normal pattern; an additional distinction can be made between damaged and intact chromosomes. The cell proliferation rate on the other hand is an indicator of the cell division rate. Deviations in the proliferation rate could suggest a change in the effectiveness of the DNA repair system, which in turn could result in a changed mutation rate. Also, a number of changes might have to be expected to occur in the immunobiological sector, in that the cell-growth promoting effect, in the sense of the promotion of potential tumor cells, presently is of particular significance. The immunobiological consequences of a changed cell



Fig. 1: Metaphase chromosomes of a human peripheral lymphocyte with a diploid chromosome set in the second mitotic cycle. The effect of adding bromodesoxyuridine to the culture medium and its consequent introduction into the DNA enables the subsequent differential staining of the chromatids. This permits the occurrence of sister chromatid exchanges to be detected (see arrows in figure).

proliferation can, however, go much further as the entire system of immune-competent cells can be affected in such a way that the elimination rate of mutated cells is changed and the outbreak and course of numerous so-called immunodiseases are adversely affected.

For reasons of time, the scope of our experiments had to be considerably restricted. It was, for instance, not

possible to extend the HGPRT test to all test groups or to generally implement the combination with 1 mT/50 Hz magnetic fields. This study is of particular importance, because in the performance and evaluation of the experiment experts within the relevant areas (radio-frequency engineering and cytogenetics) worked closely together. This should ensure that the experiments are reproducible to a high level.

2. Materials and Methods

All the tests used the blood of healthy male donors (non-smokers, 20 to 33 years old), obtained by extraction from the veins. This was to provide a homogeneous sample with respect to age, hormonal and health status as well as any possible nicotine-related mutagenic predisposition. The degree of a

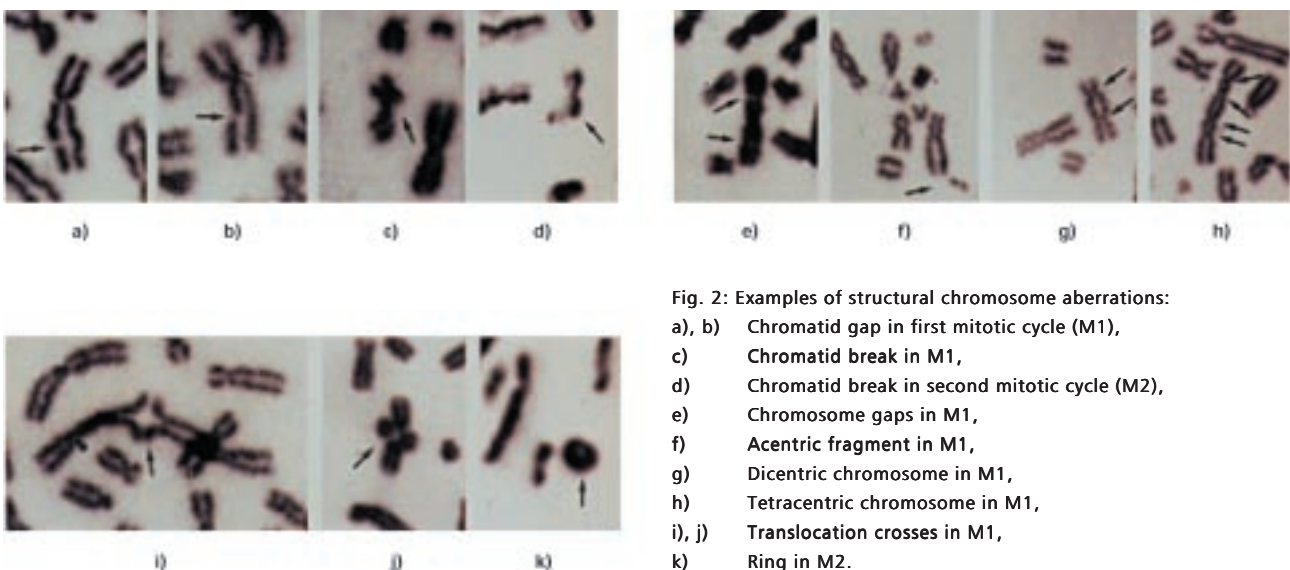


Fig. 2: Examples of structural chromosome aberrations:
a), b) Chromatid gap in first mitotic cycle (M1),
c) Chromatid break in M1,
d) Chromatid break in second mitotic cycle (M2),
e) Chromosome gaps in M1,
f) Acentric fragment in M1,
g) Dicentric chromosome in M1,
h) Tetracentric chromosome in M1,
i), j) Translocation crosses in M1,
k) Ring in M2.

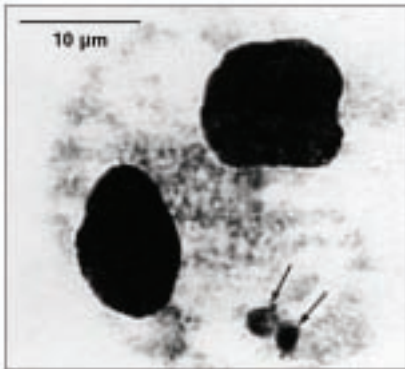


Fig. 3: Binuclear human peripheral lymphocyte with two micronuclei and intact cytoplasm.

possible predisposition is known to have an effect on the response of the immune system. For an analysis of the cytogenetic test parameters proliferation index (PI), sister chromatid exchange (SCE) frequency and chromosome aberration rate (CA), the lymphocyte cultures were cultivated for a total of 72 hours [1]; for demonstration of micronuclei (MN) the cultivation time was 51 hours [2]. For determination of the mutation frequency of the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) locus the cultivation duration was 40 hours [3]. For all the batches, a permanent cultivation temperature of $37\text{ }^{\circ}\text{C} \pm 0.1\text{ }^{\circ}\text{C}$ was kept constant

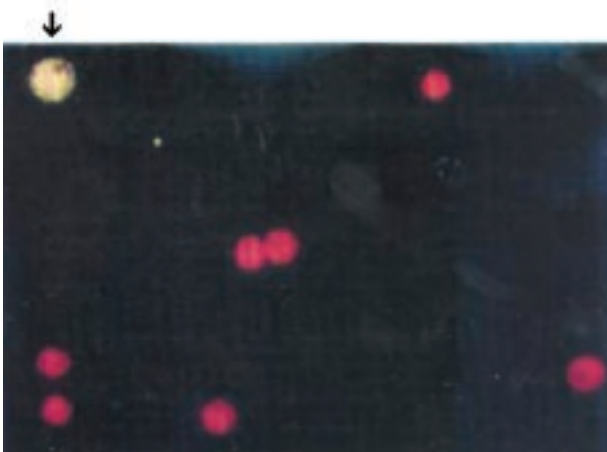


Fig. 5: Mutant detection using indirect immuno-fluorescence staining of a human peripheral lymphocyte culture after exposure at 440 MHz (67 nT). The arrow indicates a cell nucleus with at least one mutation in the HGPRT locus.

over the entire cultivation duration. Standard methods were used for preparing the reagents for the chromosome and micronucleus analyses and for staining the coded slides (for details see [1, 2]). For the determination of the SCE frequencies as well as their median and standard deviations, 30 diploid bromodeoxyuridine (BrdU) labelled metaphases of the second division cycle were analyzed (Fig. 1).

The findings for the chromosome aberrations were also derived from these 30 metaphases, and also from 50 metaphases of the first division cycle (Fig. 2).

For the calculation of the chromatid and chromosome break rate, the gaps are given a weight value of 'zero', breaks a weight of 'one', and translocations, and ring and dicentric chromosomes a weight of 'two'. For determination of the micronucleus frequency, 1000 and 2000 binucleate cells, respectively, were analyzed (Fig. 3).

Figures given for the cell proliferation index are based on a minimum of 200 BrdU-labelled metaphases of the first (M1), the second (M2), and the third and higher division cycles (M3) (Fig. 4).



Fig. 4: Metaphases (46, XY) in different mitotic cell cycles from a human lymphocyte culture (top: M1, centre: M2, bottom: M3).

Mutations of the HGPRT locus in lymphocytes were verified by means of a modified preparation and indirect immunofluorescent staining (Fig. 5).

Each culture was completely checked for mutants; the number of lymphocytes was extrapolated following an aliquot count; for determination of the mutation frequency, the number of mutants was divided by the total number of cells [3].

The 440-MHz electromagnetic fields were generated using a transverse-electromagnetic (TEM) cell. The cell is an electrically shielded chamber. It can be considered as an laterally-

Methods



Fig. 6: TEM cell used in the experiment, with one side wall opened to permit a view into the cell interior. In the lower part of the cell interior, the sample holder with nine culture containers can be seen. Above this is the isolated suspended septum, which is the extension of the inner conductor of the coaxial feed line, present on the figure's right-hand side. The thermostatically-controlled bath is located outside the TEM cell.

extended coaxial line. The outer wall serves as the outer conductor; the inner conductor ("septum") physically separates the chamber into an upper and a lower part and is supported by dielectric studs. The magnetic field lines have elliptical form around the septum and are perpendicular to the electric field lines (see Fig. 6).

Within the TEM-cell, above or below the septum, a maximum of nine culture tubes can be accommodated in a plexiglass sample container. During the tests they are exposed to a reproducible radio-frequency electromagnetic field. The power fed into the cell is 2 Watts resulting in an electric field strength of 20 V/m at 440 MHz. In the empty TEM cell the corresponding magnetic flux density is 67 nT.

White oil flows through the sample container. The oil is kept at a constant temperature of $37\text{ }^{\circ}\text{C} \pm 0.1\text{ }^{\circ}\text{C}$ by means of a thermostatically-controlled bath and flows through thermally-insulated hose lines to and from the sample container.

The experiment is fully PC-controlled by means of dedicated software.

The container holding the control cultures is a hollow iron cylinder, closed at the bottom and provided with a tightly fitting, removable top cover as well as openings through which the hoses for the circulating water are passed. Placed into the iron cylinder is a plexiglass container that houses a temperature probe and a maximum of eight

culture tubes that are almost perfectly shielded from (alternating) magnetic fields ($< 10\text{ nT}$). The temperature of the control cultures is also maintained at $37\text{ }^{\circ}\text{C} \pm 0.1\text{ }^{\circ}\text{C}$ by means of a thermostatically-controlled bath.

The 900-MHz and 1.8-GHz electromagnetic fields were generated in a G-TEM cell (Gigahertz TEM cell) that has a design similar to that of the TEM cell and which subjects the cultures to an exposure in similar containers (number of culture tubes is 6). Both units were made available by the Institute for Telecommunications Technology of the Technical University of Braunschweig (for detailed description of the instrumentation see "Edition Wissenschaft", No 2/95, Appendix).

The exposure of the lymphocyte culture followed the requirements of the test system in question, as is

Exposure at:	Parameters investigated	Exposure duration (hours)	Number of donors
440 MHz	PI, SCE, CA	70	12
	MN	50	10
	GM	39	10
440 MHz and $1\text{ }\mu\text{T}/50\text{ Hz}$	PI, SCE, CA	30 and 40	9
	MN	30 and 40	10
$1\text{ }\mu\text{T}/50\text{ Hz}$ and 440 MHz	PI, SCE, CA	30 and 40	9
900 MHz	PI, SCE, CA	70	6
	MN	50	6
900 MHz and $1\text{ }\mu\text{T}/50\text{ Hz}$	PI, SCE, CA	8 and 16, total 70	3
	MN	8 and 16, total 50	3
1.8 GHz	PI, SCE, CA	70	6
	MN	50	6

Table 1: Overview of experiments.

Legend:

PI	Proliferation index
SCE	Sister chromatid exchanges
CA	Chromosome aberrations
MN	Micronuclei
GM	Genetic mutations of the HGPRT-locus

Donor	RF field	SCE / Cell ± S.D.	Median	Percentage of cells in			PI	Chromosome aberrations (CA) in			CA [%] in	MN [%]
				M1	M2	M3		M1	M2	M1		
a	-	6.30 ± 2.83	6.75	15.0	14.5	70.5	255.5	3 Br'	-	6	-	1.4
a	+	5.83 ± 2.59	6.17	11.0	13.5	75.5	264.5	1 Br''	-	2	-	1.4
b	-	6.03 ± 2.65	6.30	7.5	15.0	77.5	270.0	2 Br'	-	4	-	1.5
b	+	5.87 ± 2.52	6.25	10.0	10.5	79.5	269.5	1 Br'	-	2	-	1.5
c	-	6.80 ± 3.44	7.58	14.0	21.0	65.0	251.0	1 Gap'	-	-	-	1.3
c	+	8.77 ± 3.05*	9.25	15.0	23.5	61.5	246.5	1 Br'	-	2	-	1.6
d	-	6.83 ± 6.92	6.92	16.5	25.5	58.0	241.5	1 Br'	-	2	-	1.1
d	+	7.00 ± 3.54	6.63	16.0	21.5	62.5	246.5	2 Br', 1 Br''	-	6	-	1.1
e	-	8.10 ± 4.37	7.30	20.0	21.0	59.0	239.0	1 Br'	-	2	-	2.7
e	+	7.23 ± 2.06	7.63	18.0	22.5	59.5	241.5	-*	-	-	-	2.3
f	-	7.87 ± 3.23	7.75	21.0	21.0	58.0	237.0	1 Gap', 3 Br'	-	6	-	2.3
f	+	6.63 ± 2.34	7.17	18.5	23.0	58.5	240.0	2 Br'	2 Br'	4	6.66	2.4
g	-	6.60 ± 2.77	6.90	21.5	17.5	61.0	239.5	1 Br'	-	2	-	1.4
g	+	8.00 ± 3.06*	7.93	15.5	25.0	59.5	244.0	1 Gap', 1 Br'	-	2	-	1.8
h	-	7.07 ± 3.28	7.50	14.5	28.0	57.5	243.0	1 Br'	-	2	-	2.2
h	+	7.47 ± 2.49	8.13	15.5	33.5	51.0	235.5	1 Gap', 1 Gap''	-	-	-	2.2
i	-	5.77 ± 2.42	6.08	12.0	11.5	76.5	264.5	2 Br'	-	4	-	2.1
i	+	7.13 ± 2.49*	7.38	11.5	21.0	67.5	256.0	2 Br'	1 Br'	4	3.33	2.1
j	-	7.40 ± 2.86	7.63	10.0	16.0	74.0	264.0	2 Gap'	-	-	-	n.e.
j	+	7.37 ± 3.23	8.07	11.0	21.0	68.0	257.0	3 Gap', 2 Br'	-	4	-	n.e.
k	-	6.57 ± 3.32	6.83	7.5	19.0	73.5	266.0	1 Br'	-	2	-	n.e.
k	+	6.53 ± 3.25	6.50	9.0	10.5	80.5	271.5	2 Br'	-	4	-	n.e.
l	-	7.27 ± 3.08	7.83	12.0	11.0	77.0	265.0	1 Br', 1 Br''	-	4	-	n.e.
l	+	8.67 ± 2.50*	8.69	9.5	14.5	76.0	266.5	5 Br', 1 Gap', 1 Gap''	-	10	-	n.e.
m	-	n.e.			n.e.				n.e.			2.5
m	+	n.e.			n.e.				n.e.			2.2

Table 2: Sister chromatid exchanges (SCE), Proliferation index (PI), Chromosome aberrations (CA) and Micronuclei (MN) of human peripheral lymphocytes from 13 donors after an exposure of 70 hours or 50 hours for MN to a 440-MHz radio-frequency electromagnetic field in a TEM cell. To determine the SCE frequency and its standard deviation (S.D.), 30 diploid cells undergoing the second division cycle (M2) were investigated. The degree of aberration in these 30 cells was examined together with that of 50 cells undergoing the first division cycle (M1) and the results expressed as percentages. The specifications of the cell proliferation kinetics are with respect to 200 cells in the M1, M2, and M3 (i.e. cells in the third or higher division cycle). For the determination of micronucleus frequency, 2000 binucleate cells were evaluated.

Legend:
 Br Break, ' Chromatid, '' Chromosome,
 * Significant difference from the control, using the t-test at the 5 % significance level,
 • Only 30 metaphases in first cycle were evaluated,
 n.e. Not evaluated

In column 2 of Tables 2–8, the + symbol indicates the presence of the field (exposure), and the – symbol the absence of the field (sham-exposure).

usual for cytogenetic investigations, i.e. for a period of 70, 50 or 39 hours [1, 2, 3].

Radio-frequency exposure at 440 MHz and 900 MHz was also sequentially combined with 1 mT/50 Hz exposure in several different ways (a: 30 h 440 MHz, then 40 or 20 h 1 mT/50 Hz; b: 30 h 1 mT/50 Hz, then 40 h 440 MHz; c: alternating between 8 h 900 MHz (day) and 16 h 1 mT/50 Hz (night) for a total of 50 or 70 hours; see also Table 1). The 1 mT/50 Hz exposure was implemented in a Helmholtz coil made available by the Institute for High-Voltage Engineering of the

Technical University of Braunschweig (for a description of the instrumentation see [4, 8]). Both the exposed and the sham-exposed (control) biological material was investigated the same procedures. Prior to that the control cultures were kept in a magnetically shielded chamber limiting the mean internal magnetic flux density to less than 50 nT/50 Hz.

3. Results

Should it turn out that the electromagnetic fields have an influence on the biological cultures examined, the change in the cyto-

genetic test parameters would have to meet the following conditions in order to be classified as biologically relevant (this applies also if a statistical evaluation were to furnish proof of statistically-significant differences at an earlier stage):

- 1) The SCE frequency, the chromosome aberration rate, the micronucleus frequency and the mutation rate of the HGPRT locus would have to differ from the values of untreated cultures by at least a factor of two, and
- 2) The proliferation index of a completely-analyzed cell culture

Results

Donor	RF field	Total number of cells in aliquots	Multiplication factor	Total number of cells in culture	Number of mutated cells	Mutation frequency [$\times 10^{-5}$]	Relative difference [%]
a	-	5286	25	132150	5	3.78	25.1
a	+	4228	25	105700	5	4.73	
b	-	9882	25	247050	29	11.74	-45.1
b	+	10554	25	263850	17	6.44	
c	-	18903	25	472575	840	177.75	-34.7
c	+	16828	25	420700	488	116.00	
d	-	9964	25	249100	1	0.40	45.0
d	+	6865	25	171625	1	0.58	
e	-	14516	25	362900	5	1.38	4.3
e	+	11092	25	277300	4	1.44	
f	-	10453	25	261325	145	55.49	-69.0
f	+	13272	25	331800	57	17.18	
g	-	20398	25	509950	132	25.88	42.4
g	+	20620	25	515500	190	36.86	
h	-	24325	25	608125	37	6.08	74.3
h	+	20381	25	509525	54	10.60	
i	-	26790	25	669750	70	10.45	31.6
i	+	31424	25	785600	108	13.75	
j	-	13957	25	348925	71	35	-86.6
j	+	10280	25	257000	7	2.72	

Table 3: Mutation frequency of the HGPRT locus and relative difference (with respect to the controls) after exposure of human peripheral lymphocytes from 10 donors to a 440-MHz radio-frequency electromagnetic field.

Donor	RF field	SCE / Cell \pm S.D.	Median	Percentage of cells in			PI	Chromosome aberrations (CA) in		CA [%] in		MN [%]
				M1	M2	M3		M1	M2	M1	M2	
a	-	7.60 \pm 4.16	7.38	12.0	22.5	65.5	253.5	3 Gap'', Br'	-	4	-	2.0
a	+	6.97 \pm 3.01	6.94	11.0	13.5	75.5	264.5	2 Gap'', 1 Br', 1 Br''	2 Gap', 1 Br'	4	3,33	2.5
b	-	7.63 \pm 3.73	7.83	15.5	21.0	63.5	248.0	2 Gap'', 1 Br'	1 Gap'	2	-	1.4
b	+	7.37 \pm 2.77	8.07	16.5	11.0	72.5	256.0	3 Br'	1 Gap'	6	-	1.7
c	-	6.37 \pm 2.98	6.75	11.5	19.5	69.0	257.5	2 Gap', 1 Br'	2 Gap'	2	-	2.5
c	+	6.57 \pm 2.85	6.50	13.5	18.0	68.5	255.0	1 Gap', 1 Gap'', 1 Br'	1 Gap'	2	-	2.1
d	-	6.70 \pm 3.72	6.88	56.5	30.5	13.0	156.5	2 Br'	1 Gap'	4	-	1.6
d	+	8.13 \pm 3.27	8.70	44.0	36.5	19.5	175.5	1 Br', 1 Br''	-	4	-	2.0
e	-	7.37 \pm 2.81	7.50	24.5	37.0	38.5	214.0	3 Br'	1 Gap'	6	-	1.6
e	+	7.33 \pm 3.34	7.63	21.0	31.5	47.5	226.5	1 Gap', 2 Br'	3 Gap'	4	-	1.4
f	-	8.53 \pm 4.38	8.75	8.5	17.0	74.5	266.0	1 Br'	-	2	-	1.7
f	+	7.43 \pm 3.69	7.63	4.5	20.5	75.0	270.5	2 Gap', 1 Gap'', 1 Br''	1 Gap'	2	-	2.0
g	-	6.50 \pm 3.00	6.88	20.0	45.5	34.5	214.5	1 Gap', 1 Br'	4 Gap'	2	-	2.3
g	+	6.80 \pm 3.28	6.75	24.0	33.0	53.0	239.0	3 Gap', 2 Br'	-	4	-	1.7
h	-	8.13 \pm 3.86	8.38	5.5	13.5	81.0	275.5	2 Gap''	-	-	-	n.e.
h	+	9.10 \pm 4.47	9.17	7.0	12.5	80.5	273.5	- 2	Gap''	-	-	n.e.
i	-	7.57 \pm 3.34	8.10	11.5	26.0	62.5	251.0	- 2 Gap',	1 Gap''	-	-	n.e.
i	+	6.73 \pm 3.73	7.17	11.0	25.0	64.0	253.0	3 Gap'', 1 Br'	3 Gap'	2	-	n.e.
j	-	n.e.				n.e.		n.e.				1.7
j	+	n.e.				n.e.		n.e.				2.0
k	-	n.e.				n.e.		n.e.				1.7
k	+	n.e.				n.e.		n.e.				1.6
l	-	n.e.				n.e.		n.e.				2.3
l	+	n.e.				n.e.		n.e.				1.8

Table 4: Sister chromatid exchanges (SCE), Proliferation index (PI), Chromosome aberrations (CA) and Micronuclei (MN) of human peripheral lymphocytes from 12 donors after an exposure of 30 hours to a 440-MHz radio-frequency electromagnetic field in a TEM cell, followed by an exposure of 40 hours or 30 hours for MN to a 1 μ T/50 Hz electromagnetic field in a Helmholtz coil. To determine the SCE frequency and its standard deviation (S.D.), 30 diploid cells undergoing the second division cycle (M2) were investigated. The degree of aberration in these 30 cells was examined together with that of 50 cells undergoing the first division cycle (M1) and the results expressed as percentages. The specifications of the cell proliferation kinetics are with respect to 200 cells in the M1, M2, and M3 (i.e. cells in the third or higher division cycle). For the determination of micronucleus frequency, 2000 binucleate cells were evaluated.

Br = Break, ' = Chromatid, '' = Chromosome
n.e.: Not evaluated

Donor	RF field	SCE / Cell ± S.D.	Median	Percentage of cells in			PI	Chromosome aberrations (CA) in		CA [%] in	
				M1	M2	M3		M1	M2	M1	M2
a	-	7.27 ± 2.99	8.07	8.5	12.5	79.0	270.5	1 Br'	3 Gap'	2	-
a	+	8.03 ± 3.36	8.50	23.0	40.0	37.0	214.0	2 Gap', 3 Br'	1 Gap'	6	-
b	-	5.67 ± 1.77	6.25	11.0	9.5	79.5	268.5	1 Br'	1 Br''	2	3.33
b	+	7.17 ± 3.14*	7.50	9.5	7.5	83.0	273.5	2 Br'	1 Gap', 1 Br'	4	3.33
c	-	6.83 ± 3.14	6.92	18.0	22.0	60.0	242.0	2 Br'	2 Gap'	4	-
c	+	6.40 ± 3.14	6.17	11.0	19.0	70.0	259.0	-	-	-	-
d	-	4.70 ± 2.05	5.19	10.0	10.5	79.5	269.5	-	1 Gap''	-	-
d	+	5.90 ± 2.92*	6.25	13.0	16.5	70.5	257.5	1 Br'	2 Gap'	2	-
e	-	5.93 ± 3.27	6.75	4.0	8.0	88.0	284.0	1 Gap', 1 Gap''	-	-	-
e	+	6.00 ± 3.64	5.50	8.5	9.5	82.0	273.5	-	2 Gap'	-	-
f	-	5.30 ± 2.82	5.30	10.5	10.0	79.5	269.0	-	1 Gap'	-	-
f	+	7.20 ± 3.86*	7.13	7.5	8.0	84.5	277.0	1 Gap', 2 Gap''	1 Gap', 1 Gap''	-	-
g	-	6.20 ± 3.09	5.81	11.5	10.5	78.0	266.5	1 Gap', 1 Br'	1 Gap', 1 Gap''	2	-
g	+	6.93 ± 2.78	6.90	17.5	15.0	67.5	250.0	1 Br'	1 Gap', 1 Br'	2	3.33
h	-	7.13 ± 2.99	7.50	8.5	18.5	73.0	264.5	2 Gap', 2 Br'	1 Br''	4	3.33
h	+	7.50 ± 3.71	7.75	11.0	13.5	75.5	264.5	2 Gap', 1 Gap'', 1 Br''	2 Gap'', 1 Br''	2	3.33
i	-	7.00 ± 3.42	6.90	11.0	19.0	70.0	259.0	-	-	-	-
i	+	5.87 ± 3.44	6.38	15.5	21.5	63.0	247.5	-	-	-	-

Table 5: Sister chromatid exchanges (SCE), Proliferation index (PI), Chromosome aberrations (CA) and Micronuclei (MN) of human peripheral lymphocytes from 9 donors after an exposure of 30 hours to a 1 T/50 Hz electromagnetic field in a Helmholtz coil, followed by an exposure of 40 hours to a 440-MHz radio-frequency electromagnetic field in a TEM-cell. To determine the SCE frequency and its standard deviation (S.D.), 30 diploid cells undergoing the second division cycle (M2) were examined. The results of the degree of aberration in these 30 (M2) cells were collected together with aberration results from 50 cells undergoing the first division cycle (M1) and expressed as percentages. The specifications of cell proliferation kinetics are with respect to 200 cells in the M1, M2, and M3 (i.e. cells in the third or higher division cycle). Br = Break, ' = Chromatid, '' = Chromosome

***: Significant difference from the control sample, using the t-test at the 5% significance level**

would have to be outside a -3 % and +6 % spread, as determined at our institute [9]. The values obtained in the experiments discussed here follow empirically from the limited size of samples. Also, the findings for each donor would have to tend to satisfy these criteria.

3.1 Experiments at 440 MHz

The blood of 13 donors was exposed to a radio-frequency (RF) field of 440 MHz and tested for the frequencies of SCE, CA and MN as well as the proliferation index (see Table 2). No influence of the RF field on the SCE frequency, the micronucleus frequency, or the proliferation index could be established. Chromosome aberrations were observed almost

exclusively in metaphases of the first division cycle, and this applied for both the exposed and the non-exposed cultures. For two donors (h and i), the PI was found to be slightly below the -3 % value. No influence of the RF field was observed for the size of the samples used here.

Ten different test procedures, each using the blood of various donors, were used to determine the mutation frequency of the HGPRT locus. Following 440-MHz exposure, no effect of the RF field on the mutation frequency of the HGPRT locus, despite considerable inter-individual fluctuations, could be observed (see Table 3). Statistical evaluation using the variate difference method at the 5 % level confirmed this, although for two of the donors (f and j) the mutation

frequency of the HGPRT locus was reduced by more than half.

3.2 Experiments at 440 MHz and 1 mT/50 Hz and vice versa

The blood of 12 donors was exposed to an RF field of 440 MHz for a period of 30 hours, followed by a 40-hour exposure (20-hour exposure for the micronucleus test) at 1 mT/50 Hz. The SCE, CA and MN frequencies as well as the PI were determined (see Table 4). No effect of the electromagnetic fields on the SCE and micronucleus frequency was observed. For two of the 9 donors (d and g) show a distinct PI increase following field exposure. Except for one case, CA were detected

Results

Donor	RF field	SCE / Cell ± S.D.	Median	Percentage of cells in			PI	Chromosome aberrations (CA)		CA [%]		MN [%]
				M1	M2	M3		In M1	In M2	In M1	In M2	
a	-	7.77 ± 3.78	7.50	10.75	12.75	76.00	264.25	-	2 Gap', 1 Gap''	-	-	0,8
a	+	7.83 ± 3.72	7.69	9.75	10.50	79.75	270.00	-	2 Gap', 1 Gap''	-	-	1,0
b	-	7.37 ± 3.55	7.25	10.25	24.50	64.75	254.00	1	Gap', 3 Br'	6	3.33	1,3
b	+	8.03 ± 3.77	8.63	7.50	10.75	81.75	274.25	1	Gap', 1 Gap'', 1 Br'	2	-	1,2
c	-	7.00 ± 3.42	6.90	11.25	19.25	69.50	258.25	-	-	-	-	1,1
c	+	7.40 ± 3.47	7.50	11.50	17.25	71.25	259.75	4	Gap', 3 Br'	6	9.99	0,9
d	-	6.10 ± 2.66	7.07	10.50	16.00	73.50	263.00	1	Gap'	-	6.66	1,1
d	+	8.40 ± 3.62*	8.75	14.75	20.00	65.25	250.50	-	-	-	-	1,3
e	-	8.57 ± 4.18	8.70	28.00	18.75	53.25	225.25	1	Gap', 1 Gap'', 1 Br'	2	-	0,7
e	+	7.87 ± 3.18	8.17	25.25	22.50	52.25	228.00	2	Gap'	-	-	0,9
f	-	8.27 ± 3.33	8.42	10.25	12.75	77.00	266.75	1	Gap', 2 Br'	4	3.33	1,3
f	+	8.40 ± 3.10	8.63	8.50	13.50	78.00	269.50	1	Gap', 1 Br'	2	-	1,1

Table 6: Sister chromatid exchanges (SCE), Proliferation index (PI), Chromosome aberrations (CA) and Micronuclei (MN) of human peripheral lymphocytes from 6 donors after an exposure of 70 hours or 50 hours for MN to a 900-MHz radio-frequency electromagnetic field, pulsed at 217 Hz (0.577 ms in 4.615 ms period, 5 W, 66 V/m) in a GTEM-cell. To determine the SCE frequency and its standard deviation (S.D.), 30 diploid cells undergoing the second division cycle (M2) were inspected. The results of the degree of aberration in these 30 (M2) cells were collected together with aberration results from 50 cells undergoing the first division cycle (M1) and expressed as percentages. The specifications of cell proliferation kinetics are with respect to 400 cells in the M1, M2, and M3 (i.e. cells in the third or higher division cycle). For the determination of micronucleus frequency, 1000 binucleate cells were evaluated.

Br = Break, T = Translocation, ' = Chromatid, '' = Chromosome

*: Significant difference from the control sample, using the t-test at the 5% significance level

Donor	RF field	SCE / Cell ± S.D.	Median	Percentage of cells in			PI	Chromosome aberrations (CA)		CA [%]		MN [%]
				M1	M2	M3		In M1	In M2	In M1	In M2	
a	-	8.93 ± 4.23	8.50	14.00	17.50	68.50	254.50	1	Br''	2	-	0.9
a	+	9.57 ± 4.31	9.75	19.50	21.25	59.25	239.75	-	-	-	-	1.1
b	-	7.63 ± 3.23	7.70	20.00	21.50	58.50	238.50	1	T	4	-	0.8
b	+	8.07 ± 2.65	8.25	18.25	17.50	64.25	246.00	-	-	-	-	0.9
c	-	8.00 ± 3.87	7.90	24.50	21.25	54.25	229.75	1	Gap'	-	-	1.1
c	+	7.07 ± 3.14	7.50	14.75	16.00	69.25	254.50	1	Gap'	-	-	0.9

Table 7: Sister chromatid exchanges (SCE), Proliferation index (PI), Chromosome aberrations (CA) and Micronuclei (MN) of human peripheral lymphocytes from 3 donors after an exposure of 70 hours or 50 hours for MN to a 900-MHz radio-frequency electromagnetic field, pulsed at 217 Hz (0.577 ms in 4.615 ms period, 5 W, 66 V/m) in a GTEM-cell, followed by exposure to a 1 μ T/50 Hz electromagnetic field in a Helmholtz coil. The magnetic-field cultures were placed in the RF field for eight hours during daytime. To determine the SCE frequency and its standard deviation (S.D.), 30 diploid cells undergoing the second division cycle (M2) were inspected. The results of the degree of aberration in these 30 (M2) cells were collected together with aberration results from 50 cells undergoing the first division cycle (M1) and expressed as percentages. The specifications of cell proliferation kinetics are with respect to 400 cells in the M1, M2, and M3 (i.e. cells in the third or higher division cycle). For the determination of micronucleus frequency, 1000 binucleate cells were evaluated.

Br = Break, T = Translocation, ' = Chromatid, '' = Chromosome

only in M1 metaphases.

The CA did not reveal any field influence.

For testing with a 30-hour exposure at 1 mT/50 Hz followed by a 40-hour exposure at 440 MHz, the blood of 9 donors was used and analyzed for SCE, CA and PI (see Table 5). As regards the SCE frequency, no field influence could be detected. CA are observed mainly in M1; however, no RF effect could

be ascertained. Six of the 9 donors revealed a changed PI of the exposed cultures beyond the previously-mentioned spread determined by us, where one value being was higher (donor c) and five values were lower (donors a, d, e, g and i).

3.3 Experiments at 900 MHz

Investigation of possible effects of exposure at 900 MHz, pulsed at

217 Hz (0.577 ms pulse duration, 4.615 ms pulse repetition time, 5 W, 66 V/m) used the blood of 6 donors, which was tested for SCE, CA and MN frequencies and the proliferation index (see Table 6). No influence on either the SCE frequency or the micronucleus frequency was found. CA were sporadically observed in both M1 and M2 and both for the exposed and for the non-exposed cultures. Changes in PI following field exposure were found for two of

Donor	RF field	SCE / Cell ± S.D.	Median	Percentage of cells in			PI	Chromosome aberrations (CA)		CA [%]		MN [%]
				M1	M2	M3		In M1	In M2	In M1	In M2	
a	-	8.83 ± 4.40	8.88	15.00	13.75	71.25	256.25	-	-	-	-	1.5
a	+	9.87 ± 3.03	10.58	11.50	13.00	75.50	264.00	2 Gap', 1 Gap''	2 Gap'	-	-	1.3
b	-	8.00 ± 4.16	8.63	7.25	12.00	80.75	273.50	1 Br'	-	2	-	0.7
b	+	8.23 ± 2.99	8.83	8.00	13.75	78.25	270.25	1 Gap'	1 Br''	-	3.33	0.9
c	-	9.23 ± 3.34	9.50	9.00	10.75	80.25	271.25	-	-	-	-	0.8
c	+	8.70 ± 3.44	9.08	19.50	30.00	50.50	231.00	2 Gap'	1 Br'	-	3.33	0.9
d	-	6.17 ± 2.46	6.10	13.75	14.50	71.75	258.00	-	-	-	-	1.4
d	+	7.57 ± 2.91*	7.90	14.00	15.75	70.25	256.25	-	1 Gap'	-	-	1.0
e	-	7.53 ± 3.01	8.38	13.25	11.25	75.50	262.25	1 Gap', 1 Gap''	-	-	-	0.9
e	+	6.47 ± 2.97	7.25	15.25	17.75	67.00	251.75	-	-	-	-	1.1
f	-	6.17 ± 2.55	6.70	14.00	18.75	67.25	253.25	2 Br'	-	4	-	1.1
f	+	6.87 ± 3.57	6.69	14.75	20.25	65.00	250.25	-	-	-	-	1.2

Table 8: Sister chromatid exchanges (SCE), Proliferation index (PI), Chromosome aberrations (CA) and Micronuclei (MN) of human peripheral lymphocytes from 6 donors after an exposure of 70 hours or 50 hours for MN to a 1.8-GHz radio-frequency electromagnetic field, pulsed at 217 Hz (0.577 ms in 4.615 ms period, 5 W, 66 V/m) in a GTEM-cell. To determine the SCE frequency and its standard deviation (S.D.), 30 diploid cells undergoing the second division cycle (M2) were inspected. The results of the degree of aberration in these 30 (M2) cells were collected together with aberration results from 50 cells undergoing the first division cycle (M1) and expressed as percentages. The specifications of cell proliferation kinetics are with respect to 400 cells in the M1, M2, and M3 (i.e. cells in the third or higher division cycle). For the determination of micronucleus frequency, 1000 binucleate cells were evaluated.

Br = Break, ' = Chromatid, '' = Chromosome

*: Significant difference from the control sample, using the t-test at the 5% significance level

the six donors, with one increased value (donor b) and one decreased value (donor d).

3.4 Experiments at 900 MHz and 1 mT/50 Hz

The blood of 3 donors was exposed to an RF field of 900 MHz, pulsed at 217 Hz (0.577 ms pulse duration, 4.615 ms pulse repetition time, 5 W, 66 V/m), for a duration of eight daytime hours, followed by a nocturnal duration of 16 hours at 1 mT/50 Hz exposure, with a total exposure duration of 70 hours or 50 hours for MN, respectively. The blood was tested for the SCE, CA and MN frequencies as well as for the PI (see Table 7). For neither the SCE frequency nor the micronucleus frequency could an influence of the electromagnetic fields be observed. Magnetic-field-related CA did not occur. The blood of donor (a) revealed a reduced PI in the exposed culture, that of donor (c) an

increase; for donor (b) the PI was within the normal spread. Hence, there was no indication of a field effect.

3.5 Experiments at 1.8 GHz

Investigation of possible effects of exposure at 1.8 GHz, pulsed at 217 Hz (0.577 ms pulse duration, 4.615 ms pulse repetition time, 5 W, 66 V/m), over a duration of 70 hours or 50 hours for MN, respectively, used blood from 6 donors, which was analyzed for the SCE, CA and MN frequencies as well as for the PI (see Table 8). For neither the SCE frequency, nor the micronucleus frequency, nor the CA could an influence of the RF field be detected. For two of the donors (c and e) a field-induced change in the PI was observed, in as much as a reduced cell division rate was found.

3.6 Remarks on the proliferation index

The experiments revealed PI deviations in as much as the PI exceeds the given -3 % and +6 % spread interval. The statistical uncertainty results from the fact that for the PI determination just a small number of samples could be used. Deviations in both directions were found, suggesting that no influence of the field occurred.

4. Summary

To investigate the possible existence of detrimental effects of the radio-frequency electromagnetic fields of mobile radio systems on genetic structures, we exposed human peripheral lymphocytes to RF fields at 440 MHz, 900 MHz and 1.8 GHz. Following exposure, the lymphocytes were analyzed with respect to different cytogenetic test parameters. The test parameters, the

number of cells analyzed, and the number of donors for each individual experiment were as follows:

1. Sister chromatid exchanges (SCE; 30 cells in M2, 45 donors)
2. Chromosome aberrations (CA; 50 cells in M1 and 30 cells in M2, 45 donors)
3. Micronuclei (MN; 1000 and 2000 binucleate cells, 35 donors)
4. Proliferation index (PI; 200 and 400 cells, 45 donors)
5. Mutation of the HGPRT locus (GM; all cells; evaluated at 440 MHz only, 10 donors)

A total of 90 individual tests was evaluated. For SCE, CA and PI, the exposure time was 70 hours, for MN 50 hours and for GM 39 hours. The total culture durations were 72, 51 and 40 hours, respectively. Standard methods were used for preparation and staining of the coded samples. Some testing was done at 440 MHz and 900 MHz separately; other testing alternated exposure at each of these two frequencies individually with 1 mT/50 Hz [a: 30 hours at 440 MHz, followed by 40 hours or 20 hours for MN at 1 mT/50 Hz; b: 30 hours at 1 mT/50 Hz, followed by 40 hours at 440 MHz; c: alternating between 8 hours at 900 MHz (day) and 16 hours at 1 mT/50 Hz (night) for a total of 70 hours or 50 hours for MN].

In summary, the results obtained in this study do not suggest the existence of any field-induced changes of the test parameters SCE, CA, MN, PI and GM.

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Research Project “Biological Effects of High-Frequency Electromagnetic Field”

Cytogenic Studies fo the Effects of Mobile Telephone Radio Waves

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This research project was designed to determine whether high-frequency electromagnetic fields, such as those that are generated during the use of mobile telephone technology, have a carcinogenic effects. Therefore, we examined whether mutations occurred in genes and chromosomes and whether there were any changes in cell growth.

Blood taken from healthy non-smoker male donors ranging in age from 20 to 33 was used as the object of the study. Lymphocytes were taken from this blood and exposed at temperatures of 37°C to high-frequency fields of 450 MHz, 900 MHz and 1.8 GHz, which are typical in current mobile telephone service (in the German C, D, and E networks, for example). Exposure time ranged from 39 to 70 hours.

TEM or GTEM cells were used to examine the samples; these cells operate according to the principle of an „expanded coaxial line“. They were provided by the Institute for Communications Technology of the Technical University of Braunschweig

(see the report entitled „Experimental set-up for studying electromagnetic alternating fields“). To exclude interference from outside, the cell cultures used as controls were placed in a space that was specially shielded against magnetic alternating fields.

The test parameters used are given in the following list:

Chromosome aberration rate - CA: This parameter is a measure of interference in the double-strand system, in which two pairs of chromatids are linked together in an intertwining helix.

Sister chromatid exchange - SCE: The sister chromatid exchange sequence records damage to the molecular structure of the gene (DNA)

Hypoxanthine guanine phosphoribosyltransferase - HGPRT: The mutation frequency of the hypoxanthine guanine phosphoribosyltransferase site is a measure of the frequency with which harmful modifications occur in the gender-related X-chromosomes.

Micronucleus rate - MN: The micronucleus frequency is related to the distribution of the chromosomes and provides a measure of how many of the chromosomes are damaged.

Cell proliferation - PI: With the cell proliferation index, one can record the cell division rate, which is closely related to malignant tumors. Modifications in the proliferation rate can be caused by many factors, such as problems with the internal cell system with which DNA damage is repaired, or problems in the immune system. These problems have various serious effects on the cells, in that mutated cells may no longer be effectively eliminated, or immunological diseases may occur more frequently.

Standard methods were used in examining the preparation.

There were no indications whatsoever that the processes recorded in the test parameter were effected by the high-frequency electromagnetic fields.

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