TRADESCANTIA MICRONUCLEUS BIOASSAY FOR DETECTING MUTAGENICITY OF GSM-FIELDS

H. Lehmann*, M. Urech**, C. Pickl***
*Environment and Electromagnetic Compatibility, Swisscom Ltd., Innovations, CH-3050 Bern, Switzerland
**puls Umweltberatung, Mühlemattstrasse 45, CH-3007 Bern, Switzerland
***ÖkoTox GmbH, Wollgrasweg 49, D-70599 Stuttgart, Germany

(Principal contact: martin.urech@pulsbern.ch)

Abstract: Electromagnetic fields generated by the GSM mobile phone systems are applied to the Tradescantia micronuclei bioassay. Some details of the exposure system and assessment of the experimental conditions are presented. Verification experiments in the set-up showed that, in the given experimental conditions, the bioassay works. Preliminary results of the first exposure experiment are also given.

1. Introduction

From a physical point of view, the quantum energy of a photon in the microwave region is orders of magnitude too low to damage bounds of molecules or ionising an atom. Nevertheless, there are some studies, which show evidence of DNA-breaks in brain cells of rat [1]. However, these results have not yet been reproduced in other laboratories [2]. Vijayalaxmi et al. [2] considered in their work also the micronuclei (MCN) frequency of human blood cell cultures and did not observe evidence of an increase in the number of MCN. In a recent work of D'Ambrosio et al. [3] an increase of micronuclei formation in human blood lymphocytes has been observed, but only by using a GMSK modulated 1800 MHz signal. This few examples evince the necessity of further work to clarify the contradictory situation.

The Tradescantia micronucleus bioassay is commonly used for the detection of genotoxity of chemical agents [4]. Haider et al. [5] applied this method to determine whether electromagnetic fields at short-wave frequencies induce an increase of MCN formation and show thus a mutagenic behaviour. It is the aim of the present study to apply this highly sensitive bioassay to detect a possible mutagenicity of electromagnetic fields generated by the two mobile phone standards GSM and UMTS.

2. Materials and Methods

2.1 Tradescantia plants

The Tradescantia micronuclei bioassay (Trad-MCN) uses the clone (# 4430) of the Tradescantia plant. As this hybrid can not reproduce sexually, the genetic material of the plants should not vary during the different experiments. Therefore, the inherent variability of the biological system diminishes to a possible minimum. However, in order to be sure that the plants react in the foreseen manner, the performance of the plants has to be monitored all the way through the experiment by using reference substances with a known genotoxic effect on Tradescantia.

2.2 Test procedure

In order to monitor whether the plants have a standard MCN frequency at the background level a so-called background control is taken directly from the greenhouse culture. The experiment is only valid, if the result of this test shows normal MCN frequencies. For the experiment the Tradescantia plants are cut in the greenhouse and placed in Hoaglands nutrient solution. During the experiment a negative as well as a positive control are used to monitor the experimental conditions during the whole experiment. As positive control maleic hydrazide (MH), a chemical agent known to induce mutagenic effects [4], is used. Therefore, the positive control should show a higher MCN frequency. After the experiment the buds are chemically fixed, slides of the pollen cells are prepared and analysed under the optical microscope. To exclude a possible bias of the results through the experimenter, the counting of the MCN number is done under double blind conditions.

2.3 Experimental set-up for the GSM experiments

The experiments are performed at ÖkoTox GmbH near Stuttgart. As the greenhouse culture is also located nearby this prevents long transports during which the experimental conditions can not be controlled [6]. A schematic view of the set-up is given in Fig. 1.

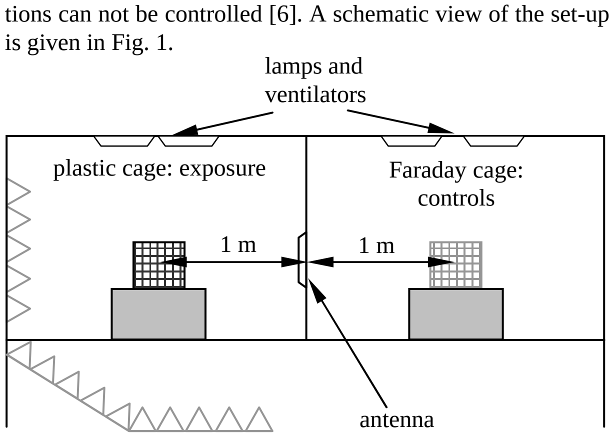

Fig. 1: Experimental set-up realised in Stuttgart. The black structure is composed out of wood whereas the absorber materials are given in grey. The two cages for the controls and the exposure are posed on plastic boxes.

The set-up is designed for maximum simplicity and easy access without neclecting full control over the exposure system. The exposition probe is placed in a plastic cage whereas a Faraday cage of identical size shields the negative and positive controls from the electrical field. The difference in the GSM field strength between exposed and unexposed probes is greater than 40 dB.
The 50 Hz magnetic field generated mainly by the lamps of the exposure set-up is at both experimental locations below 0.5 μT. This intensity should not be problematic, as in a standard Trad-MCN test the intensity of the 50 Hz magnetic field is higher. In such a laboratory situation no increase in MCN frequency is observed.
The light with an adapted spectral composition has in both cages identical intensities of about 2800 Lux. As the plastic cage and the Faraday cage do not have exactly the same optical properties − even in keeping the mesh width for the two used grids identical – the external illumination of the two boxes has to be adjusted for the same luminosity of both sets of plants. In this way it is possible to guarantee that both sets of plants are hold in the same luminosity. Also the temperature and the air flux generated by the ventilators are kept the same at the two locations.
Fig. 2 gives the details of the RF set-up for the GSM exposition. A Rhode&Schwarz signal generator in combination with a pulse generator from the University of Wuppertal, which modulates the high frequency GMSK modulated signal of 940 MHz with the low frequency pulse generated by the GSM frame structure [7], is used for signal generation. With the gain of the chosen antenna, the amplifier of a maximal power of 80 W should allow for a maximal electrical field of about 100 V/m in approximatly 1 m distance from the antenna. The quite small GSM antenna having a maximal dimension of 0.2 m has been chosen in order to have far field conditions already at small distances from the source.

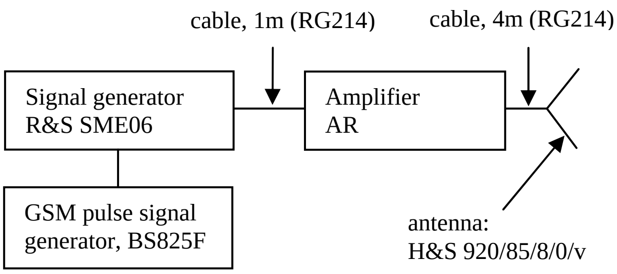

Fig. 2: RF-scheme of the GSM exposure system.

In principal the RF set-up for the UMTS-exposure system will be similar. However, the single components will have to be changed.

Table 1: Exposure conditions in terms of the electric field strength in the restricted exposure volume. The measurement was performed without the plants.

Power level [dBm]	**Average electrical field [V/m]**	**Maximal electrical field (deviation from the average field [%])**	**Minimal electrical field (deviation from the average field [%])**
-10	87.4	22	-18
-16	46.4	21	-18
-36	4.8	20	-17

2.4 Exposure assessment

The above described exposure set-up was validated by measuring the electrical field strength in the exposure region. The measurement was performed with a W&G EMR20 with a 100 kHz to 3 GHz probe (type 11). Before the geometry of the experimental set-up was fixed, the ideal distance between antenna, exposure region as well as the best installation of the absorbers was determined. This situation with the antenna in vertical polarisation is illustrated in Fig. 1.
For three different power levels the electric field distribution in the restricted exposure volume (20 x 10 x 6 cm in the symmetry axes of the set-up) has been determined. The results of these measurements performed without the plants are shown in Table 1. As is shown by Table 1 the electrical field strength at the exposure location lies within a quite small window of ±1.7 dB. Therefore, it was decided that the experimental series could be started.

2.5 Test of the environmental exposure conditions

In order to test whether the environmental conditions in the present set-up are adequate for the plants, a test without the application of the electrical fields but with all equipment switched on was performed. In this case the MCN frequency of the negative control should be identical to the number of MCN in the unexposed plants at the exposure location. The chemotoxical positive control should be working also in this situation. The results of this experiment are illustrated in Fig. 3. As the above mentioned condition was fulfilled, it was concluded that the Trad-MCN works in the conditions of the present experimental set-up.

3. First exposure experiments

Until now only one experiment with a real exposure to a GSM field has been performed, namely for the highest power level given in Table 1. The MCN-frequencies with the respective standard deviations are given in Fig. 3. Besides the effects of the reference substance (positive control), the differences have not been significant (Mann-Whitney U-test, confidence level at p=5%).

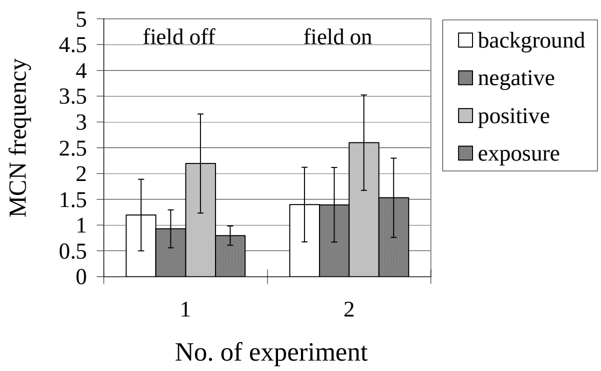

Fig. 3: Results of the two Trad-MCN experiments in the experimental set-up described in this contribution. The first experiment served as verification test for the experimental conditions, whereas the second set of data has been obtained by applying a GSM field at the power level –10dBm. The average electrical field stregth for this power level was 87.4 V/m (see also Table 1).

4. Conclusion and Outlook

The designed experimental set-up allows for a well characterised exposure of the Tradescantia plants and thus the application of the Trad-MCN bioassay for the determination of possible mutagenicity of electromagnetic fields generated by the GSM standard.

The first valid experiment shows no significant increase in the MCN frequency. Further experiments using different intensities, polarisations and modulations (without GMSK modulation, without GSM pulsation) are planned in the near future. Moreover, the experimental method will also be applied to UMTS signals.

5. Acknowledgements

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6. References

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