

**Answer to comments by A. Lerchl on “Radiofrequency electromagnetic fields (UMTS, 1950 MHz) induce genotoxic effects *in vitro* in human fibroblasts but not in lymphocytes” published by C. Schwarz et.al. 2008.**

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## **Abstract**

Genotoxic effects induced *in vitro* by the third generation mobile communication standard UMTS have recently been described by Schwarz et. al. 2008. These findings which may have considerable significance for environmental health have been commented upon by Lerchl 2008 (this issue). These comments which are invalid in part have to be set right. The objected inconsistencies are largely based on the author's incomplete and superficial consideration of published data in the field. Moreover, the statistical points being made cannot cast doubts on the validity of the experimental data reported by Schwarz et. al., and may not change the principal conclusion of *in vitro* genotoxic action of UMTS signals.

## **Introduction**

A genotoxic action of radiofrequency electromagnetic fields (RF-EMF) has been demonstrated *in vivo* and *in vitro* by a large number of investigators and using various genetic endpoints and a wide variety of cells and tissues (table 1). The majority of these studies has been conducted using exposure to Global System for Mobile Communication (GSM) signals. The study of Schwarz et.al. 2008 demonstrates, that genotoxic effects are observed *in vitro* after exposure to Universal Mobile Telecommunication System (UMTS) radiation as well, and reports consistent effects in human cultured fibroblasts at exposure intensities of 0.05 W/kg with both endpoints Comet assay and micronucleus assay. Since the validity of their data has been questioned by Lerchl 2008 it is the aim of this communication critically to deal with the objections being raised by this author. The subsequent response is structured according to the arrangement used by this author.

## **Areas of concern**

In his introductory and concluding statements the author [Lerchl 2008] claims that “vast majority of scientific publications do not indicate biological effects of RF-EMF”. This allegation is outdated and therefore meaningless, since meanwhile – even when confined to genotoxic effects only – there are more than 30 positive publications (table 1). There is no doubt that no-effect papers have been published also and that positive findings could frequently not be reproduced in repetition studies. However, the two experimental papers cited by the author to substantiate this, do in fact not represent *in vitro* studies but deal with the induction of lymphoma in laboratory animals. Furthermore, the author’s suggestion that biological effects of RF-EMF may

be irreproducible in general because the results of Repacholi et.al 1997 could not be reproduced, seems dubious.

The author's statement that "no biophysical mechanisms have been identified..." to explain genotoxic interactions is surprising. There is published evidence, that genotoxic EMF effects may be mediated via oxidative processes [Friedman et.al. 2007]. This makes sense, since an SAR of 0.05 W/kg does not provide enough energy directly to break a chemical bond in DNA. Thus an indirect mode of genotoxic action becomes likely, e.g. radicals [Lai and Singh 1997; Tkalec et.al. 2007], or alteration of DNA repair mechanisms [Sykes et.al 2001]. An influence of microthermal effects has also been postulated [Holtze et.al. 2006]. Moreover, the well documented alterations of gene expression – although not per se genotoxic – indicate an interaction of RF-EMF with the genetic material [Leszczynski et.al. 2002; Nylund and Leszczynski 2006; Pacini et.al. 2002; Lee et.al. 2005; Belyaev et.al 2006; Zhao et.al 2007].

The author's brief description of the comet assay procedure used by Schwarz et.al. is correct, but (deliberately?) omits an essential methodological point, namely that a twofold blinding procedure had been applied here:

- i. The controlling unit of the exposure setup randomly determined which of the two waveguides in the same incubator was exposed. This setting which could neither be controlled nor ascertained by the experimenter was stored in an encoded file and uncovered by the ITIS foundation in Zürich via E-mail in exchange with the transmission of the results. This implicates, that

in case of doubts all original experimental data may be independently assessed hereafter.

- ii. All microscopic evaluations have been done with coded slides and by the same investigator (E.K.).

The strictly and double blinded conditions used by Schwarz et.al. must be taken into consideration, when their low standard deviations of comet tail factors (CTF) are used to cast doubts on the solidity of the published data. Low standard deviations have consistently been obtained and published by the Vienna group [Diem et.al. 2002; Ivancsits et.al. 2002a; Ivancsits et.al. 2002b; Ivancsits et.al. 2003; Diem et.al. 2005; Ivancsits et.al. 2005], using a visual classification of the comets. A comparison of this method with a computer aided densitometric classification has revealed that visual classification is faster, more sensitive and probably more accurate, but otherwise corresponds with the computer based analysis [Kobayashi et.al 1995].

The high coefficient of variation ( $CV > 50\%$ ) of E-type comets is expected and is neither “surprising” nor “implausible”. E-cells (which contain  $>95\%$  of DNA in their comet’s tail) do occur only sparsely in unexposed controls, and the CV of  $>50\%$  is therefore due to their low and variable frequency. This may also explain that a higher total CTF was observed in unexposed cells as compared to positive controls and EMF exposed cells.

Even more, the author considers low CV’s of the CTF between 1.2 and 4.1 to be “utterly impossible”. He does not present own comet assay results and has not published any, instead he refers to a CV “in the order of 30 and 40%” published by Speit et.al. 2007, obtained by densitometric evaluation of 50 cells per assay. In contrast,

published data from others gave an intra-assay variability of 4.1 - 21.3% [Holz et.al 1995], 0.5 – 4% [Hughes et.al. 1997], and 1.5 – 4.8% [Panayiotidis and Collins 1997]. In addition, the low CV's published by Schwarz et al are not "in sharp contrast to previously published results". Here the author refers to a systematic study [Diem et.al. 2002] into the intra- and inter-individual variability of CTF in blood lymphocytes (not in cultured fibroblasts) which investigated the influence of donor's age, smoking habits and the influence of genotoxic compounds *in vitro*. In 80 individuals tested the inter-individual CV for the comet assay was 30.7% but was much lower intra-individually, for example 6.2% without and 0.3% in the presence of 80µM H<sub>2</sub>O<sub>2</sub>. For the Micronucleus assay a CV of 30.1% inter-individually and of 5.4% intra-individually was published [Diem and Rüdiger 1999]; Thus the author's conclusion of "incomprehensibility" of the published data is unfounded.

The basis of the author's statistical calculations is difficult to extract. It is not always clear how he gets his numbers (e.g. "differences up to 14.6 cells"), and there are no figures and tables in his MS. One would expect, that a serious scientist would request the raw data of Schwarz et al before doing calculations, surprisingly this has not been done. The author's considerations, that Student's t-test would have been possible here, and that this statistical test would have resulted in even higher significances than the non-parametric Mann-Whitney test is unnecessary and cannot be regarded to question the results of the Schwarz et.al. paper, nor can this modify its conclusions. Thus, the author mentions "calculation errors" in one header and in the penultimate sentence, but he does not tell what calculation errors have been made. Sentences such as "for several reasons the extremely low standard deviations are far too low for this kind of experiment..." require a detailed listing of the biological rea-

sons. Above all this pertains the micronucleus data, being rated in the single lump-sum sentence: "Many of the arguments would be valid for the analysis of the micronucleus data too".

The demonstration of genotoxic effects far below the current safety limits defined by the International Commission for Non-Ionizing Radiation Protection [ICNIRP 2004] cannot be used to cast doubts on the *in vitro* data by Schwarz et. al. since these limits have been delineated from thermal effects only without considering other mechanisms being responsible for a genotoxic action.

**In conclusion**, the critical comments concerning the publication by Schwarz et.al. are largely based on an incorrect and perfunctory consideration of relevant publications in the field. The statistical points being made do neither give reason to doubt the validity of the data nor to modify the conclusions of the paper by Schwarz et.al.

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