

NEWS

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Final workshop report held on the subject:

**Genetic and cytogenetic aspects
of RF-fields effects**

Löwenstein, Germany, 24-27. November 2002

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Brief Summary

The primary goal of the workshop was to address the question whether or not genetic or cytogenetic abnormalities can be induced in cells and animals in experiments exposing them to radio frequency radiation (RF), as used in today's modern wireless communication technologies, at in-

tensities which do not cause a measurable increase in temperature. Published literature was critically reviewed and some of the techniques that are currently being used in genome analysis were presented by the guest speakers. On the one hand, the discussion that followed helped to evaluate the scientific and statistical significance of the experimental findings and on the other hand it also helped to evaluate the biological consequences of the findings.

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It became clear that some of the findings presented in the literature could not be validated in later experiments. A general reason for the controversy could be the use of different test materials, (cell types, animals, human probands), the methods, (comet assay, micronuclei-tests, sister chromatid exchange), as well as the experimental conditions and the duration of RF-exposition (frequency, modulation, specific absorption rate). Moreover, some of the investigations did not guarantee a homogenous RF exposure, and therefore, "hot spots" (viz., points in irradiated material with a higher field intensity and temperature gradients), might have been created leading to a convection heating of the test material. Moreover, some of the effects that were measured could only be just a simple reaction to the activated system of thermoregulation, without there being any measurable alternations in core or surface temperatures of the animal or human volunteers.

The workshop participants generally agreed that there is no conclusive scientific evidence supporting the supposition regarding human health that exposing cells or animals to RF-fields at intensities which do not cause a measurable increase in temperature, induces cytogenetic alterations of any biological significance and consequences to human health. To resolve the controversial data published in various reports, the participants of the workshop proposed a multi-centric collaborative study to be conducted involving experienced cytogeneticists from many different countries examining human peripheral lymphocytes with identical methods in order to guarantee statistical significance of the findings obtained. In addition, it was recommended for the cytogenetic evaluations that cultivated human fibroblasts, as a second cell type, be exposed to similar RF-exposition conditions. This was a positive outcome of the workshop.

Background and Goals of the Workshop

The following questions were the main focus of the workshop: (1) can humans exposed to electromagnetic RF-radiation at the frequencies used in wireless communication and at intensities where there is no increase in temperature lead to genetic alternations and to an increase in the incidence of cancer and (2) do the existing maximum exposure limits guarantee that modern technology used in wireless communication systems is safe?

It is generally well-known that the quantum energy of RF-fields used in wireless communication is many orders of magnitude below 12eV, and this is regarded as the boundary for breaking covalent bonds by ionising radiation. This means that the quantum energy of RF-fields, in contrast to x-rays order gamma-rays is not able to disrupt covalent bonds in DNA and hence it is not directly able to induce any alternations to genetic material. Moreover, it is known that the stability of genetic material in living organisms is protected with a complex system of repair mechanisms. Therefore, any interference with this repair system can lead to an increase in genetic defects.

In the past decades many experimental studies have been carried out investigating whether or not any genetic or cytogenetic effects are caused by RF-fields. Some of the published data suggests that cells and animals exposed to RF-fields resulted in an increase in DNA strand breaks (using comet-assay) and in the incidence at micronuclei. These results, however could not be validated in later investigations. An international "Genotox Expert Panel" decided in 1998: "*The data from over 100 studies indicate that RF-radiation is not directly mutagen, and that adverse effects on organisms from high frequency exposure and high power intensities is predominantly the result of hyperthermia; albeit there could be subtle indirect effects*

on gene replication and/or transcription of genes under relatively limited exposure conditions." (Brusick et al., 1998). This conclusion is correct even today! Nevertheless, a number of questions remain unanswered, which were extensively discussed during the workshop. These include:

(1) What could be the possible reasons for "subtle indirect effects on gene replication and/or transcription while under relatively restricted exposure conditions?"

(2) Could low intensity RF-fields induce an effect on inherent biological repair mechanisms?

(3) Are the observed genotoxic effects merely a result of thermal heating?

(4) To what extent are the methods applied relevant?

(5) Is there a misinterpretation of experimental data?

(6) What are the confidence limits of the statistical methods used?

(7) What kinds of biophysical mechanisms could trigger genetic or carcinogen effects of RF-fields?

(8) What biological significance do RF-induced genetic alternations have, if there is any significance at all?

(9) Is there a threshold for RF-intensities when genetic effects are induced, i.e. the observed effects were only induced at intensities that are well beyond the recommended limits of maximum exposure?

(10) How is animal and human life affected by the genetic effects induced by RF-fields?

(11) Is it necessary in view of the latest experimental data on cytogenetic and genetic effects from RF-fields, to revise the existing safety limits?

Conference Venue and Programme

Sponsorship and Attendance

The workshop was organized and financed by the Forschungs Gemeinschaft Funk e.V. (FGF) and the European "Cooperation in Science and Technology

281" (COST 281), in cooperation with the Ministerium für Umwelt und Verkehr, Baden-Württemberg, (Ministry for the Environment and Transportation) and Berufsgenossenschaft für Elektrotechnik und Feinmechanik (Professional Association for Electro-Technology and Fine-Mechanics).

The conference took place in the scenic area of Löwenstein, Germany. Forty-four scientist from different countries took part in the workshop (Austria, Canada, Belgium, Germany, Finland, France, Hungary, Israel, Italy, USA). All of the participants received an abstract book and a leaflet containing information on the scientific programme. The guest speakers critically reviewed the data from published reports. Various techniques used for evaluating genetic alternations were presented. Reports were given on the combined effects of RF-fields and other environmental agents. The main subject of the discussion was the biophysical background of possible effects posed by RF-fields. The limited number of participants made it possible that plenty of time was allotted for fruitful discussions. The results of the whole workshop were reviewed by rapporteurs. A positive result of the workshop was the joint recommendation to resolve the controversial cytogenetic observations published in the literature by initiating a multi centric collaborative study, whose framework should encompass experienced cytogeneticists from different countries, who would investigate human peripheral lymphocytes and human fibroblasts using identical methods, which will guarantee that the results are of statistical significance.

The social part of the conference consisted of: (1) the participants having dinner in a restaurant which is typical for the region of Baden-Württemberg, (regional wine tasting); the wine tasting session was held by a wine grower and (2) a tour given by the owner of the Guttenberg Castle, the tour was followed by a medieval banquet

in the castle restaurant with live music and entertainment.

Opening Speeches

Gerd Friedrich, Forschungsgemeinschaft Funk e.V. welcomed the participants.

Oskar Grözinger, Minister for the Environment and Transportation for the state of Baden-Württemberg, held the opening speech. He came to the conclusion that the public discussion on the aspects of modern radio applications is still controversial. The recommendations of the German Commission for Radiation Protection are a valuable contribution to an objective debate on exposure value limits and precautionary measures. The German exposure value limits are in accordance with the levels recommended by the ICNIRP. Even though currently emerging new scientific findings have revealed no evidence that electromagnetic fields pose any health risks, these kinds of effects cannot be completely ruled out. Therefore, it is of utmost importance that controversial findings be resolved with further research. A lack of explicit information on possible health risks for humans posed by electromagnetic fields from mobile phone base stations often lead to public apprehension. He emphasized how urgent it is that the public be provided with objective information on the development, the function and the effects of modern mobile radio applications.

Lawrence Goldstein, WHO, presented an outline on the World Health Organization's RF-projects. He emphasized how important it is to differentiate between effects on health and biological effects. The effects that have been seen in cells, in animals and in human probands do not necessarily bring about health risks. Just as important is the difference between high exposure on the job and exposure to the general public. Children are probably the



most sensitive and therefore, should be regarded as a critical population. He spoke about the “precautionary principle”, which is a controversial issue at the European Commission.

Norbert Leitgeb, coordinator of the European project COST 281, reviewed the research activities of his organization and praised the activity of the workshop. He made reference to the thematic parallels of the COST 281 conference on “Subtle Thermal Effects” which had recently taken place in London. During the discussions at the London conference the question was also addressed as to whether or not the effects on cells and animals from weak RF-field exposure could also be the result of a subtle increase in temperature.

Presentations and Discussions

1. Do RF-fields effect genes?

A critical review

This topic was the central focus on the first day of the workshop. The chairman Jürgen Kiefer (Gießen, Germany) chaired both the morning and afternoon sessions. The speakers were: Isabelle Lagroye (Pessac, France), Vijayalaxmi (San Antonio, USA), Wolfgang-Ulrich Müller (Essen, Germany), Rafi Korenstein (Tel Aviv, Israel),

Maria Rosaria Scarfi (Neapel, Italy), and Dariusz Leszczynski (Helsinki, Finland).

Isabelle Lagroye presented data material from different studies that were done in collaboration with Joe Roti Roti, St. Louis, USA. The goal was to determine the extent of DNA single-strand breaks in C3H 10T 1/2 cells (*in vitro*) and in the brain cells of Sprague-Dawley rats (*in vivo*) exposed to RF-radiation, 1.2-5W/kg SAR. The RF frequencies that were employed were: 835.62 MHz (FDMA), 847.74 MHz (CDMA), 900MHz (GSM) and 2450 MHz (continuous or pulsed waves). As a positive control cells/animals were used and exposed to 4-Gy gamma-rays, cis-plation or ethyl methane sulfonate. Some of the studies were primarily aimed to reproduce the experimental conditions used in experiments carried out by Lai and Singh (1995, 1996). They reported in their findings an induction of DNA-strand breaks in the brain cells of rats exposed to 2450 MHz. Therefore, the same alkaline comet assay was used as a validation method. The results showed no induction of DNA damage after RF-exposure, neither *in vitro* nor *in vivo* (Li et al., 2001). On the other hand, cells used in the positive control demonstrated a significant increase in DNA dam-

age. The discussion which followed her talk included the data from Malyapa et al. (1998), who in similar experiments found no induction of DNA damage in RF-exposed cells.

Vijayalaxmi gave a review of the literature published on cytogenetic damage in mammalian cells when irradiated at different RF-fields and SARs. In an earlier study Sarkar et al. (1994) isolated DNA from testis and brain cells, of a total of 4 controls and 6 RF-exposed Swiss albino mice (2450 MHz RF, SAR 1.18 W/kg, 1 mW/cm² power; 2 mice were exposed for 120 days, 2 mice 150 days and 2 mice 200 days) and subjected to agarose-gel electrophoresis. When compared to the control mice the DNA of all of the irradiated mice clearly showed altered band patterns in the 7-8 kd region. Therefore, the data suggested rearrangements in the DNA of RF irradiated mice. Subsequent studies used the comet assay to investigate single and double strand breaks in the DNA of animals/cells which were exposed to RF. Out of the 11 reports on DNA strand breaks, three studies done by Lai and Singh (1995, 1996 and 1997) showed an induction of DNA single - and double strand breaks in brain cells of rats under RF exposure at

2450 MHz, whereas, the findings from a subsequent study (Phillips et al., 1998) documented a presence as well as a absence of RF induced DNA strand breaks in cultivated mammalian cells. The data from seven other studies (Malyapa et al., 1997a, 1997b, 1998; Maes et al., 1997; Vijayalaxmi et al., 2000; Li et al., 2001; Tice et al., 2002) could not establish any RF induced strand breaks in *in vitro* and *in vivo* experiments. Vijayalaxmi commented if DNA strand breaks are caused by RF-induced free radicals and/or because an impairment of the DNA repair process (as Lai and Singh suggested) then such an effect must be able to be observed immediately and at 4 hours after RF exposure. Data from studies published later could not verify such effects. Malyapa et al. (1998) also discussed the importance of the method used to sacrifice the animals (guillotine verses carbon dioxide) and the time factor in which the rats were sacrificed for the collection of the brain tissue for the comet assay. Similar contradictory results can be found in the literature pertaining to cytogenetic damage assessed in the form of chromosomal aberrations, micronuclei, and sister chromatid exchange. RF-induced cytogenetic damage was reported on in 5 publications, whereas, there were 13 other studies which could not establish such an effect. With regard to the damage established by Tice et al. (2002), where a significant increase in the number of micronuclei in RF-irradiated lymphocytes was demonstrated, Vijayalaxmi pointed out that they themselves in their study mentioned a limitation, namely that the temperature was measured at the location of the cells, but it actually could be that highly localized higher temperatures could have been produced. Moreover, in some experiments SAR (10 W/kg) was used, which is several-fold higher than the amount recommended as value limits. In addition Vijayalaxmi also presented data from two chronic animal exposure studies (RF-irradiation for

several hours a day, 5-7 days a week, from 1.5-2 years). The first study revealed a statistically significant increase in genetic damage (in the form of micronuclei), although there was no correlation to an increase in cancer genesis. The second study could not establish an increase in the frequency of genetic damage and cancer. Vijayalaxmi concluded by pointing out the problem of the difference between statistical significance and biological significance.

Wolfgang-Ulrich Müller gave a detailed presentation on the different methods used in determining cytogenetic damage. The presentation included the evaluation of unstable chromosomal aberrations, reciprocal translocations, micronuclei tests, sister chromatid exchange, premature condensation of chromosomes and comet assay. In addition, he spoke about the utility of DNA assays. The technical details and about the advantages and disadvantages of the various methods. Müller emphasized, how important it is to take artefacts into account which can occur during microscopic slide preparations and biological cell response including repair processes, mitosis-activities and heterogeneity of cell populations. Each and every misinterpretation could either lead to an overestimation or an underestimation of induced genetic effects. He reiterated that estimating genetic damage after acute exposure is by no means easy and after chronic and fractionated exposure it is even more complicated. Müller explained the advantages and the disadvantages of each method.

Rafi Korenstein described the “non-thermal induction of genomic instability in human lymphocytes after RF-irradiation” Lymphocytes from human blood were exposed *in vitro* for 72 hours to 830-MHz (CW, SAR 1.6 – 8.8 W/kg). By means of the FISH technique, a significant SAR dependent increase in aneuploidy of chro-

mosome 17 was found (Mashevich et al., 2003). Korenstein supports the supposition that epigenetic alternations are involved in the SAR-dependent genomic instability. The discussion included comments on the problem of RF-dosimetry, the lack of homogeneity of RF-field distribution and potential increases in temperature during a three-hour session of RF-irradiation at 3 – 21 W/kg SAR. Korenstein explained that the temperatures registered during the experiments were between 34.5 and 37.5 degrees. In control cells which were exposed to the same temperatures no genetic or epigenetic alterations were observed at all. Glaser emphasized that heating caused by RF-fields could not be equated with the heating conditions of conventional thermostats. Between these two forms of energy input there must be differences concerning the temperature gradients that occur. Korenstein expressed his doubts as to whether such temperature gradients could cause genetic effects and formulated the hypothesis that the genetic effects that were observed could be the result of the RF-field influence on the structure of bound water in the functional centres of cell proteins. Foster and Gimsa expressed doubts concerning this hypothesis. Obe and Vijayalaxmi, on the other hand, pointed out that in the case of increases in aneuploidy, as demonstrated in the data, the cells should be full of micronuclei. This however could not be interpreted in the published studies.

Maria Rosaria Scarfi spoke about the topic “The evaluation of genotoxic effects (MN induction) in human lymphocytes after being exposed to RF-irradiation: recent findings from our research group” (see. d’Ambrosio et al., 2002). Several RF-signals (CW, GSM, GMSK) with SARs from 0.2 – 5 W/kg were used in the *in vitro* exposure of human lymphocytes. The duration of exposure was between a minimum of 15 minutes to a maximum of 44

hours. The kinetics of cell proliferation was not affected after RF-exposure to either CW or GSMK fields. Exposure with unmodulated fields had no effect on the number of micronuclei, whereas, after phased modulated exposure a statistically significant increase was observed. Then the problem of RF-dosimetry was discussed. Scarfi summarized that thermal effects cannot be ruled out, even when in experiments calorimetric probes were held in nine different positions in the exposure system (this temperature control was better than in the previous publication by d'Ambrosio et al., 1995). In addition, Scarfi talked about some of the on-going combination experiments (RF +/- chemical mutagen).

Dariusz Leszczynski discussed the question as to whether or not induced gene expression posed by mobile radio radiation could be dependent on cell genotype. During his talk he referred to his current publication (Leszczynski et al., 2002) on human endotheloid cells (EA.hy926), which were exposed to 900 MHz, GSM-modulation for 1 hour at an average SAR of 2.2 W/kg. It can be interpreted from the data that RF exposure results in a temporary increase in hsp27 phosphorylation, which was negated by SB203580, a specific inhibitor of p38 mitogen-activating protein kinases (p38MAPK). In addition, transient changes in the protein expression levels of hsp27 and p38MAPK were observed. Since during RF-exposure the cells experienced no increase in temperature, it can be concluded that many of the alterations observed were not caused by a thermal effect. Leszczynski formulated the hypothesis that mobile telephone radiation induces the activation of hsp27. This could result in two things: (a) facilitate the development of brain cancer by inhibiting the cytochrome-c/capase-3 apoptotic pathway, and (b) the permeation of the blood brain barrier is increased

through the stabilization of endotheloid cell stress fibres. The speculation also includes other brain damaging factors concerning the effects induced by mobile phone radiation. Leszczynski presented a schematic diagram illustrating a series of pathways from hsp-induction to brain cancer to apoptosis. This presentation resulted in a lively discussion. Kiefer mentioned that every induction by hsp has to result in the same effect, provided that a causal relationship between hsp-activation and damage to the blood brain barrier (and brain tumour) exist. Hsp induction is generally regarded as a physiological cell response to an increase in temperature. Quite a number of other forms of stress such as extreme stimuli will elicit similar hsp reactions in cells. Kiefer asked the question whether all of these types of hsp reactions have to be consequently regarded as harmful. Vijayalaxmi inquired: how many of the intermediary steps (in the schematic diagram) have been investigated in order to determine a correlation between mobile telephone radiation and brain tumours. Korenstein wanted to know to which degree the transformed cell lines that are used in experiments represent normal body cells. Vijayalaxmi also provided the information that many animal studies on chronic RF-irradiation (including two of her own studies where mice and rats were exposed to RF fields several hours per day for 1.5 – 2 years) found no conclusive proof that there was an increase in the incidence of cancer in all of the tissue examined. Glaser asked if any local temperature changes took place in the petri dish where the cells were irradiated (not once not even at the SAR of 5 W/kg). Leszczynski reported that an increase in temperature did not occur due to the water jacketing used for the exposure system. Korenstein wanted to know if a threshold temperature exists in the cells for hsp-27 expression. Leszczynski responded that it was not measured.

The **general discussion** which took place on the first day after the reports mainly focused on the following topics:

(1) Can the term "statistical significance" be equated with "biological significance"? The presence or the absence of a genetic effect is in itself a statistical event, and even if genotoxic effects really exist, they could be relatively rare events in relation to the surviving cells. Stephan maintained on the basis of his own data, which was presented the next day, that it is necessary to observe at least 1000 mitotic cells in order to evaluate with a high degree of certainty chromosomal aberrations, and to reliably determine "subtle" differences between RF-irradiated, sham-irradiated or non-irradiated cells. Vijayalaxmi reminded us about the recommendation of the regulatory agencies that the classification of test agents as genotoxic or non-genotoxic is not to be based on a single assay but on "a battery of tests", (e.g. mutation tests in salmonella and drosophila, rodent micronucleus, chromosomal aberration, and micronuclei in human blood lymphocytes, *in vitro* and *in vivo*). A test agent is regarded as "non-genotoxic" only when all or the majority of the tests indicate that there is an absence of a significant effect.

(2) Relevance of genotoxicity data from *in vitro* experiments to *in vivo*? Müller is of the opinion that to draw conclusions and to transfer *in vitro* findings to *in vivo* conditions is very complicated. Korenstein pointed out the necessity of using synchronised cells in *in vitro* experiments because of the various types of cell responses at different positions in the cell cycle. This is especially valid for the apparently more sensitive cells in the S-phase (DNA syntheses). The participants discussed technical and statistical methodologies, for example, the applicability of Giemsa staining method and the Fisher test. Leszczynski made a point by questioning if the methods applied in microscopic analysis are

sensitive enough to reveal weak genetic alterations. Kiefer mentioned that the “classic” chromosomal aberration-analysis is indeed used as a biological dosimeter for ionising irradiation exposure. Alterations in genetic material is also of importance when they lead to carcinogenesis. Glaser commented that unfortunately, the molecular mechanism that triggers chromosomal mutations or DNA protein alterations are not fully understood. Several participants also discussed the methods used for statistical analyses.

(3) It seems that the question regarding the relevance of chromosomal aberration and cancer genesis has not yet been answered. Obe reported that a large-scale study conducted in northern countries and Italy indicated that there is a correlation between chromosomal aberration and cancer. Kiefer gave the participants something to reflect upon when he said that there could be a genetic predisposition which corresponds to the results of chromosomal aberration tests and with the incidence of cancer without one being the reason for the other.

(4) What are the means to resolve the contradictory data in the literature on cytogenetic effects caused by RF-exposure? There was a strong suggestion that cy-

togeneticists, who have experience with the advantages and disadvantages of the various methods applied in estimating genetic damage must work more closely together. In the field of RF-research this seems to be a major problem. Why are experiments which assert genotoxic effects not being exactly reproduced by other researchers? Kiefer remarked that nobody likes to conduct experiments which are exactly the same as those of previous researchers, especially when it is known that the methodology was weak or mistakes were made. Repeat studies are not regarded as “exact reproduction experiments” when the conditions are correspondingly modified. The majority of the participants agreed with this. Vijayalaxmi suggested initiating a large-scale collaborative study (ideal RF-exposition conditions and temperature controls) with several experienced cytogeneticists taking part. This idea was resonated and was recommended for discussion in the final session of the workshop.

2. Are there synergy effects with other influences?

This question was the subject of the afternoon session on the second day of the workshop. Günter Obe, (Essen, Germany)

chaired the session. Luc Verschaeve (Belgium), Günter Stephan (Oberschleißheim, Germany) and Myrtil Simko (Rostock, Germany) were all speakers during this session.

Luc Verschaeve presented data on “In vitro and in vivo genetic effects posed by RF-radiation in conjunction with other environmental factors”. In the meantime, the public’s reservations are mostly focused on possible synergy-or additive effects from RF-radiation and environmental mutagens.

A literature search indicates that all experiments dealing with simultaneous RF-exposure and the use of a mutagen produced negative results. The same is valid for cases when RF-exposure was posterior to mutagen exposure. However, if RF-exposure was prior to mutagen exposure, the genetic damage was sometimes higher than when the cells were treated with the mutagen alone (Verschaeve 2001). In some investigations the RF-irradiation strengthened the effect of a chemical mutagen in the blood cells of one donor, whereas in the blood of another donor a decreased mutagen induced effect was determined (Maes et al., 2000). There were no indications of an increase in genotoxic effects in



rats who were exposed to RF-radiation from mobile telephones and to the chemical carcinogen MX (3-Chloro-4 (dichloromethyl) -5-hydroxy-2(5H)-furanone), when compared to the chemical alone. This was demonstrated with the alkaline comet assay test and with the micronucleus test in blood from rats. The exposure regime tested was 0.3 and 1 W/kg, 900 MHz RF (2 hours/per day) and 19mg/ml MX, which was continuously present in the drinking water. To date, exposure time periods for investigations have been from 3 - 6 months. Müller asked, if there is a difference between a single mutagen exposure in *in vitro* experiments and chronic mutagen exposure in a *in vivo* experiment. Obe, Vijayalaxmi and Kiefer asked if MX can be used at all as a genotoxic agent in combination studies, since MX alone did not induce genetic damage.

Günter Stephan spoke on the topic "Chromosomal damage in human lymphocytes after RF-exposition - methodological aspects". Despite methodological problems in the evaluation of chromosomal aberrations, Stephan highlighted how valuable lymphocytes from human blood were for studies on cytogenetic effects from RF-radiation. Cell cycle controlled cultures make it possible to distinguish between direct and indirect effects of RF-radiation on chromosomes. The published data do not present sufficient evidence that RF-signals were directly mutagenic. Reasons for the contradictory findings with regard to RF-induced chromosomal damage could be: methodological shortcomings, inadequate dosimetry, inability to eliminate potential thermal effects, or poor biological design.

Myrtill Simkó spoke on "Synergy effects after NF-EMF-exposition and other effects". Her talk was based on the latest publications on investigations of EMF-effects (Lange et al., 2002; Richard et al.,

2002; Simkó et.al., 2001). Embryonic cells from Syrian hamsters, human amniotic cells or human skin carcinoma cells were irradiated for 24 - 72 hours at 50 Hz EMF (1 mT), in combination with paracetamol, benzo(a)pyrene, TPA and asbestos fibres. The data showed that the orientation of the magnetic field, with respect to the surface of the culture medium, was decisive whether or not there was an induction of micronuclei. After exposure to gamma-rays +/- EMF, the cell cycle distribution of these cells was examined. After gamma irradiation alone, a dose dependent transient delay in G₁ and G₂ cell phases was observed, whereas with a combined exposure no evolution of this effect was determined. After this presentation a hefty discussion took place. Kiefer threw the following question out to the group: could this, excluding the aspect of dependency on the orientation of the magnetic field, mean that the magnetic field is not responsible, but rather the induced current is much more responsible for the observed effect? Korenstein stressed that the data do not support such a hypothesis, since the eddy currents in the exposure dish should be minimal. Müller expressed his doubts regarding the statistical analysis of the data and asked if perhaps methodical artefacts could have lead to the observed results? Obe wanted to know why asbestos fibres were used in the experiment and what the underlying hypothesis of the study was.

During the general discussion the participants talked about the best agents for investigating the effects of combined exposure. Obe asked why salmonella bacteria, one of the most thoroughly researched and understood microbiological genetic test systems, was not more exploited to investigate the genetic effects of RF-fields. At this point Vijayalaxmi's suggestion to initiate a large-scale collaborative study (ideal RF conditions and temperature controls) with experienced cytogeneticists participating was again met with wide approval.

3. What forms of biophysical interactive-effects are feasible?

This was the discussion topic during the afternoon session on the second day of the workshop. The chairman for this session was Jan Gimsa (Rostock, Germany). Reports were given by Roland Glaser (Berlin, Germany) and Ken Foster (Philadelphia, USA).

Roland Glaser asked the question: "What are non-thermal effects?": As a rule, an observed effect which is not accompanied by a measurable increase in temperature (in an exposure culture dish, animal and human) is described as a "non-thermal" effect. However, this does not correspond to the biophysical definition: (1) a mechanism is non-thermal when the effect is immediately produced by the interaction of electric or magnetic RF-fields with charges or dipoles of the system, and (2) independent of the production of heat. Typical non-thermal mechanisms of RF-fields are dielectrophoreses and electrorotation (Glaser, 2000), which only occur at strong field strengths. Glaser reminded the group that in the last century in the 60s and 70s studies were carried out showing that thermoregulation systems can be achieved at RF-field intensities without a measurable increase in body temperature taking place (for summary, see Adair, 1983). From this it can be concluded that many of the effects which are described as "non-thermal" could actually be subtle thermal effects, which should be ascribed to normal physical reactions to minimal diathermal heating. However, *in vitro* experiments must be carefully evaluated to find out whether or not temperature gradients were triggered by inhomogenous RF-irradiation. Foster pointed out that molecular and biophysical mechanisms in thermo-sensitive cells or nerve endings and thermoreceptors of maximal 0.002 degrees are not fully understood. Therefore, we do not know how these thermo receptors integrate with

RF-fields. Gimsa remarked that the heterogeneity of RF-field absorption can lead to local temperature differences, which cannot be measured experimentally.

Ken Foster asked the question “Is there a biophysical mechanism that might produce genetic effects induced by RF-energy?” Any correct biophysical theory must take into account both the strength of the interaction with the RF-field as well as the dynamics of the biological system; including the presence of thermal noise and dissipative mechanisms. If this happens then there are no other established mechanisms other than thermal by which RF-fields, at typical environmental levels, can produce visible biological effects; not to mention the effects which are linked to genetic damage. Nonetheless, many reports in scientific literature can be found on biological effects which can not be explained by the mechanism which is being currently discussed. Either the theory is incomplete or the experimental data in one way or another was misinterpreted. In scientific circles there is an argument that says when something is “impossible” it doesn’t count for much in science. In this respect a lack of a plausible biophysical mechanism from RF-effects speaks for the necessity of a careful investigation of the biological data in order to find another explanation for the results.

In the general discussion the question came up concerning the problem of biophysical mechanisms, could it be that the RF-effects can be somewhat neglected, simply because there is no biophysical interpretation. The participants all agreed that that this of course was not the case. An example is the above-mentioned extremely high sensitivity of thermoreceptors, whose mechanism(s) are still not known. On the other hand, surprising biophysical experimental results should always be carefully reproduced and examined for any possible artefacts.

General Discussion and Conclusions

The general discussion on the third day of the workshop was chaired by Jürgen Kiefer (Gießen, Germany). The results of the reports and the discussions of the first day were that there is no conclusive scientific proof for genetic alterations (or epigenetic mechanisms) induced by RF-exposure within the current range of limits. Some positive findings, which were published years ago, could not be validated in the most recent repeat studies. Nevertheless, the controversial data on cytogenetic effects of RF are unsatisfactory. What is behind this? Are we using the proper methods? Are we asking the right questions? Are there possible genetic effects that are just too difficult to demonstrate?

(1) What sample size is necessary in order to achieve significant results?

Stephan established the fact that the number of cells used in the majority of RF-experiments was much too small for cytogenetic evaluations, especially when taking into account the rarity of stochastically occurring effects.

(2) How effective and how reliable is the temperature control during experiments? What artefacts can be found in exposure systems with inhomogeneous field distribution and with SAR gradients?

Glaser reminded the group about the question concerning possible inhomogeneous RF-field absorption and SAR-distribution as well as dielectric differences in *in vitro* exposure cultures and in *in vivo* experiments in animal studies. On the one hand, such non-homogeneities could lead to an underestimation of the RF-field intensities and on the other hand, to a local temperature gradient with undesirable consequences. Korenstein emphasised that this problem has neither been experimentally nor theoretically adequately investigated and he suggested that this aspect receive more attention in future studies.

(3) What could be the receptor of weak RF-fields in biological systems?

This question is of course completely open, especially in view of the report given by Foster, who pointed out that with the exception of thermal effects there are no established biophysical mechanisms for RF-fields at typical environmental levels.

(4) What effect does RF-exposure have on cells during the different phases of the cell cycle?

The reports and the discussion showed that cells in different phases of the cell cycle probably react to RF-field intensities. Therefore, synchronised cells are especially desirable for experiments. Kiefer stressed that suitable cultured cells have to be selected according to the particular problem being investigated.

(5) Which genetic assays should be recommended?

According to Korenstein the classic methods used in cytogenetic analysis are not sufficient. Instead new molecular techniques with a higher degree of evaluation specification should be employed. In principle, Obe and Vijayalaxmi agreed fully with his position but added that the method should be selected depending on what subject is being concretely investigated. As far as the induction or the promotion of mutations or cancer is concerned, the currently used classical genotoxic assays are sufficient.

(6) What role do *in vitro* systems play in the evaluation of *in vivo* effects?

Kiefer stressed the necessity of always beginning with the simplest test system which is able to predict clear effects and draw conclusions on interaction mechanisms. In this context Kiefer recommended the utilisation of the expertise and experience of cytogeneticists who are familiar with statistical analysis, applicability of *in vitro* experiments to *in vivo* situations and the specification of exposure value limits for ionising irradiation exposure be utilized. Then a suggestion made

by Vijayalaxmi was taken up again, namely establishing a large-scale multi-centric collaborative study on the possible genotoxic effects posed by RF-exposure.

After an in-dept consultation with participants who are cytogeneticists Vijayalaxmi presented a tentative protocol for a cooperative study.

(1) Human blood samples from at least 6 healthy male probands (blood from female probands cannot be considered because of hormonal influences) will be RF irradiated *in vitro* (only one frequency which is yet to be decided and 0, 0.2, 2.0 and 5.0 W/kg SAR) in a reputable laboratory, under optimal exposure conditions, with excellent temperature controls and validated dosimetry.

(2) Positive control cells will be exposed *in vitro* to an acute dosage of 0.5c Gy ionising radiation (for chromosomal aberration and micronuclei) or mitomycin-C (for sister chromatide exchanges).

(3) In a central cytogenetics laboratory lymphocytes will be cultivated and the slides will be prepared.

(4) Coded slides will be disturbed among the participating cytogeneticists for evaluation.

(5) Every participating researcher will collect data on the following: (a) 1000 cells in their first mitose-division for chromosomal aberrations, (b) 2000 binuclear cells for micronuclei, and (c) 100 cells in their second mitose division for sister chromatide exchanges. The essential advantage of this procedure is that several cytogenetic experts working on the investigation will be involved in the examination of the same RF- and sham exposed cells and pooled data will be rock-solid to resolve the issue. The goal is that all of the above-mentioned points will be managed to be completed within 1.5 – 2 years after the start of the study.

(6) Similar investigations will be carried out using cultured human fibroblasts.

(7) At the end of the data collection the

results will be decoded by all of the participating scientists.

(8) The cost of the proposed collective study was estimated.

The proposal gave rise to a series of questions and useful advice: Are the 6 selected human probands really representative of the entire population?

Why aren't children, disabled and older people included? What about electro-sensitive individuals? Why aren't other cell types being investigated along with lymphocytes? To reach the intended conclusions are genotox assays proposed sufficient enough?

All of the participants agreed that this, of course, was the beginning of the proposed project. It is not intended to answer all of the questions raised in the discussion. The proposal will definitely not convince or satisfy everyone. However, all of the participants had, in principle, agreed to the initiation of the investigation proposed by Vijayalaxmi. Nevertheless, a great effort must be taken to provide for the funds that are necessary to get the project started.

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