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Genotoxic Effects of Radiofrequency Fields – Lessons from the Conflicting Results.

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Abbreviations

AM. amplitude modulated
Avg. average
B-field. magnetic field
BfS. Bundesamt für Strahlenschutz at the Federal Office for Radiation Protection Germany
°C. **degrees centigrade**
CA. chromosome aberration
COST. European Cooperation in the Field of Scientific and Technical Research
COST Action 281. Potential Health Implications from Mobile Communication Systems 2002-2007
CW. continuous wave
DNA. Deoxyribonucleic acid
dsb. double strand breaks of DNA
DTX. Discontinuous transmission (DTX) is a method of momentarily powering-down
E field. electric field
ELF. extremely low frequency, electric and magnetic fields
EMF. electromagnetic field
FGF. Forschungsgemeinschaft Funk**,** Bonn, Germany
FYI. for your information
GSM. Global System for Mobile Communications. A digital cellular phone technology
Gy gray (unit), unit of absorbed ionizing radiation
HL-60 cells. Human promyelocytic **cell** line
Hz. Hertz unit of frequency
ICNIRP. International Commission on Non-Ionizing Radiation Protection
IEC International Electrotechnical Commission
IEEE. Institute of Electrical and Electronics Engineers
IR. infrared radiation
IT'IS Research on Information Technologies in Society, Zurich
MHz megahertz
MN micronuclei
MNT micronuclei test
MMP. metalloproteinases
mT. milli Tesla
QLK4-CT Quality of Life &Management of Living Resources Key Action 4 "Environment and Health" Contract
REFLEX. Potential health hazards of mobile telephones. This research project is part of an EU-funded (Quality of Life) action.
RF. radio frequency EMF
Rms. root mean square
ROS reactive oxygen species
SAJ. Sheila A Johnston
SAR. specific absorption rate
SD. standard deviation
SCE sister chromatid exchange
SEM standard error of the mean
S Phase. synthesis phase, interphase, between G1 phase & G2 phase in cell cycle.
ssb .single strand breaks of DNA
sXc systems. in vitro RF & ELF exposure chambers built by IT'IS
TEM cells. transverse electromagnetic (TEM) device for exposing cells in vitro.
UMTS. universal mobile telecommunications system
UV. ultraviolet radiation
VERYC Madrid. Investigacion Bioelectromagnetismo. Hospital Ramon y Cajal.
W/kg. Watt per kilogram

Executive Summary.

- The central issues in this meeting were the lack of clear communications between biological and physical scientists resulting in confusion about the exposure calibration methods used by the REFLEX researchers and the confusion concerning the results and inconclusive lessons for future research.
- Calibration of the Kuster group's [IT'IS], sXc systems in situ, using positive biological controls is a necessary first step in the experimental design that is lacking in Rüdiger's research and possibly lacking in other REFLEX laboratories using the sXc system.
- A full review of the REFLEX Report research is justified to clarify many possible dosimetric questions and problems relating to the sXc chambers used by the partners as elucidated at this FGF meeting, at the BfS Mechanisms meeting May 9-10, 2007, at the FGF Rostock meeting Sept 2006, and by inaccuracies in the REFLEX Report 2004.
- There appear to be serious dosimetric and biological methodological problems with the Rüdiger group's results. Both the ELF and RF positive results of the Rüdiger group have failed independent replications several times.
- Dr Kuster hypothesized: if you get effects with CW exposures, they are definitely due to a thermal mechanism; if there are no effects for CW exposures at levels below the guidelines, but there are effects at the same levels for modulated fields, these effects must be due to a mechanism other than thermal. But, there is no biophysical reason to expect a more likely biological non thermal effect of RF with an AM modulated signal rather than a CW signal at the same exposure level, below guideline limits [Foster and Repacholi, 2004].
- Dr Kuster also hypothesized that CW positive effects in vitro suggest their results are due to thermal effects of sXc exposures above guideline limits.
- We need to find out if these sXc RF CW exposures were indeed above 2 W/kg and if the effects reported were caused by heating.
- Speit et al., 2007 report no effect of RF (mean ± SEM of three independent experiments) in the comet assay and MNT with human fibroblasts exposed to 1800 MHz, [SAR 2 W/kg, CW signal, intermittent signal: 5 min on / 10 min off] over 1, 4 or 24 hours. They failed to replicate the results of Diem et al., 2005.
- A Speit co-experiment [1950 MHz, SAR 2 W/kg, CW signal, intermittent 5 min on / 10 min off] with Diem in Vienna was abandoned because in the MN phase of the experiment there was unexplained evidence of contamination and cell death.
- Dr Schär showed us a situation of an RF effect with 1 cell line and not with another but he is not talking about genetic damage. His results are not published.
- The summary discussion of the REFLEX comet assay results suggests conflicting evidence even if it doesn't tell us very much. They are events in the S phase related shift and we should not over interpret them.
- With the comet assay nobody understands what they are looking at. In the comet the strand breakage is not proven as genotoxic.
- You have to look at molecules to see a genotoxic effect. You need evidence such as chromosome aberrations and sister chromatid exchanges to identify DNA damage.
- Dr Pollet reported that due to the relevance to human health of potentially adverse effects on DNA integrity, three new projects are underway to investigate possible

genotoxic effects of GSM signals on different in vitro models [1. lymphocytes; 2. enucleated and retinospheroid eye models: slides; 3. fibroblasts: final discussion].

- The multicentered lymphocyte study [Project FM 8823] is funded by the German Federal Office for Radiation Protection to determine possible genotoxic effects of RF-fields generated by telecommunications equipment on DNA, at the chromosomal and genomic levels. Project FM 8823 follows a recommendation by COST Action 281 [Löwenstein 24-27 Nov 2002].
- The waveguide setup for Project FM 8823 is designed by IT'IS.
- Calibration of the waveguide setup in situ using a positive biological control is required before experimentation for validated and reliable dosimetry.
- The second new BfS project of isolated and reconstructed vertebrate retinae as in vitro models will test if there are genotoxic effects of GSM signals in cells.
- They need to take into consideration the lack of blood flow in their two eye models when extrapolating any results to human exposures.
- With no established EMF effect we have nowhere to start on a mechanism theory.

Introduction

Twenty-five invited scientists attended this one day meeting [See List, Appendix A; M Simkó was absent]. There were 5 presenters: Niels Kuster, Hugo Rüdiger, Günter Speit, Primo Schär, and Dieter Pollet. Questions after each speaker were followed up at the end of the speaker session by a general concluding discussion lead by Dr Jürgen Kiefer.

The central issue in this meeting was the lack of clear communications between scientists resulting in confusion about the exposure calibration methods used by the REFLEX researchers and confusion concerning the results. The repercussions of that were that we did not know in detail what procedures were followed in the REFLEX experiments. The Speit replications of Diem et al., 2005 were discussed. When the presenters were questioned they often could not answer key questions about specific details for various reasons. Two new projects underway were presented by Dr D Pollet to address gaps in the knowledge of RF effects in cells. During the final discussion, Dr Pollet mentioned a third BfS research project undertaken which aims to clarify whether or not the effects reported by the REFLEX program in human fibroblasts (DNA strand breaks and induction of micronuclei) can be reproduced independently [01.09.2007-28.02.2010]. We ended the meeting with a lively and open discussion of the implications of the conflicting results for future genotoxic RF research led by Dr Kiefer.

1. Dr Niels Kuster's presentation: 'Slow progress in evaluation of genetoxic effects of RF because biologists do not like to talk to engineers.' [Summary derived from N. Kuster's slides and SAJ's conference notes]

Niels Kuster noted that REFLEX biologists had not communicated with the physicists/engineers and had compromised the interpretation of their in vitro results. He began with his conclusion slides and repeated those slides at the end of his presentation. He concluded: 'If the biologists would talk to the engineers we would be several steps ahead now, not discussing results based on faulty hypothesis or evaluating the meaning of non-relevant replications.'

Dr Kuster suggested his REFLEX in vitro exposure chambers [sXc-systems] are 'fool-proofed to guarantee proper functioning' but proper usage/application is not [fool-proofed].

His working hypothesis is that 'non thermal continuous wave (CW) [radiofrequency (RF) induced] biological effects are very unlikely [at or below 2W/kg]' 'and amplitude modulated (AM) RF is only different than CW if it is detected by non-linear electromagnetic elements' [Excerpted from Dr Kuster's presentation slides].

'Induced RF fields are a function of frequency, polarization, field impedance, dielectric parameters, shape and volume of the biological sample. Electric fields, magnetic fields, temperature, potential or currents can interfere with the biological process. Interference is a strong function of the time course of the stimuli. SAR or B-Fields are only a substitute for a complex exposure matrix (but a poor one)' [Excerpted from Dr Kuster's presentation slides].

The 4 RF REFLEX exposure setups include: the sXc-1800 MHz, designed by IT'IS that was used by 5 partners; the sXc UMTS designed by IT'IS that was used by 1 partner; the 900 MHz wire-patch setup designed by Laval et al., 2000, that was used by 1 partner and the STUK resonator designed by Toivo et al., 2001, that was used by 1 partner [Excerpted from Dr Kuster's presentation slides].

The 3 extremely low frequency [ELF] REFLEX exposure setups include: the coil system designed by IT'IS used by 4 partners, the coil system designed by VERYC Madrid, used by 1 partner and the coil system designed in Bologna used by 1 partner [Excerpted from Dr Kuster's presentation slides].

RF sXc Systems:
The sXc 1800 MHz, RF signals were continuous wave with no modulation or GSM mainframe with 217 Hz components. For sXc 1800 MHz AM, there was GSM basic with additional 8 Hz components; DTX [discontinuous transmission] with additional 2 Hz and 8 Hz components; and GSM talk, made up of 34% DTX and 66% GSM basic (statistics were based on measurements).
For sXc UMTS there was the UMTS constant power signal [i.e. no TPC]; or a UMTS TPC power controlled UMTS signal with a maximized low frequency content [1 Hz harmonics] [Excerpted from Dr Kuster's presentation slides].

ELF sXc Systems:
For ELF they use a 50 Hz undistorted sinusoidal signal.
For the IT'IS coil only: they used an arbitrary sinusoidal undistorted signal with arbitrary frequency of 3 Hz- 1000 Hz or a 50 Hz power-line signal with a maximal accepted distortion for power systems by IEC with frequency components up to the 25th harmonic present. [International Electrotechnical Commission (IEC)] [Excerpted from Dr Kuster's presentation slides].

The sXc Dosimetric Concepts included: numerical optimization of the field distributions with respect to maximal uniformity, efficiency and minimized uncertainties; numerical evaluation of field conditions (Avg/max/min/SD); assessment of secondary field exposures; verification of simulations by free field and dosimetric measurements; determination of temperature load and other artifacts; and uncertainty and variation analysis.
The experimental verification of the SAR was done with a 1 mm E-field probe. The RF experimental assessment of the thermal load was done by a one point measurement at the temperature maximum in the Petri dish. Time constants of this measurement were dependent upon the liquid height, fan speed and signal strength [Excerpted from Dr Kuster's presentation slides].

Dosimetric Conclusions
The sXc systems provide well controlled exposure conditions, numerically optimized and experimentally verified. They have a high dynamic range. [RF: 0.001-200 W/kg CW; ELF: 0.02 -3.6 mTrms at 50 Hz]. They have a uniform field distribution [RF: SD/Avg SAR < 30%; ELF: SD/Avg B-field < 1 %]. The signals for RF are CW, 217 Hz GSM Basic and DTX, Talk; for ELF the signal is 50 Hz: power-line, arbitrary frequency or arbitrary intermittency of exposure.
The possible sources of artifacts evaluated for RF were temperature change of < 0.03 °C/W/kg and for ELF were maximum acceleration 0.008g_{Gr} (V1) 0.09 g_{Gr} (V2).
The exposure conditions were blinded and monitored [excerpted from Dr Kuster's presentation slides].

The REFLEX CW effects reported with his exposure sXc systems according to Kuster's hypothesis are thermal artifacts of RF exposure at SAR's that must have been above guideline limits and the above the highest designed experimental exposure of 2 W//kg.

Dr Kuster concluded, as he began stating that the Speit et al., 2007 replication of the CW results of Diem et al., 2005 was a meaningless replication of a useless experiment with a faulty genetic hypothesis.

<u>Comment SAJ [Excerpted from conference notes]</u>

The RF sXc Systems: Thermal and Sham DTX effects?

Thermal' CW RF Effects in REFLEX sXc Systems?

The Rüdiger group: Vienna
According to Kuster's hypothesis as outlined above, for instance, in the Diem et al., 2005 results, Kuster maintained that their CW effects on DNA strand breaks are thermal artifacts of RF exposure at SAR's that must have been above guideline limits and above the experimental exposure design [specifically of the CW signal continuously on at 1800 MHz at SAR 2 W/kg and the CW intermittent signal (5 min on/10 min off) 1800 MHz, at the SAR 2 W/kg (Diem et al., 2005)]. Dr Kuster

maintained he was not consulted before publication of this paper and if he had been he would have advised the Rüdiger group that their exposure equipment was over exposing the tissue cultures and needed to be recalibrated and the experiment redone before publication. They should not have published the CW effects which were 'controls' to verify the exposure calibration according to Dr Kuster. ['If the biologists would talk to the engineers we would be several steps ahead now, not discussing results based on faulty hypothesis or evaluating the meaning of non-relevant replications.'] Kuster is not a co-author of their paper [see Diem et al 2005 reference below]. If the exposures were indeed above 2 W/kg [on the basis of Kuster's hypothesis, above] should the paper be withdrawn by the authors **[**Diem et al., 2005**]** because of the possible need for recalibration of the RF 1800 MHz exposures?

The Tauber Group: Berlin
Another example of published CW RF effects include the unpublished results from the group of Tauber and Fitzner of Berlin reported in the QLK4-CT-1999-01574 / REFLEX / Final Report: quoted from section 6.2: "6.2 Experiments with the human promyelocytic cell line HL-60: ...PIERS 2002 (1800 MHz radiofrequency exposition of human HL-60 cells induces DNA strand breaks as measured by the alkaline comet assay)," and "in preparation: Schlatterer K., Gminski R., Tauber R., Fitzner R. (2004) 'Radiofrequency (1800 MHz) electromagnetic fields cause DNA strand breaks and micronuclei formation in HL-60 human promyelocytic cells."
The authors report that these CW effects occurred with exposures below 2 W/kg.
Also see the paper [Remondini et al., 2006] with their positive CW exposure results where they report 'in .. HL-60 leukemia cells we found between 12 and 34 up- or down-regulated genes', where the CW exposures were 1.3 W/kg.

In this published paper [Remondini et al., 2006], Dr Kuster is named as a co-author [see the full reference below]. Are the Tauber and Fitzner group's positive results [unpublished and published] at CW exposures an indication that their sXc RF equipment also needed to be re-calibrated because their CW exposure levels are evidently above 2 W/kg, as Kuster's hypothesis suggests?
In that case [on the basis of Kuster's hypothesis, above] should the papers [Remondini et al., 2006; and in preparation: Schlatterer et al., 2004**]** have been withdrawn by the authors because of possible need for recalibration of the RF 1800 MHz exposures?

Sham DTX Effects
The Simkó Group: Rostock
Simkó's group in Rostock [http://www.cost281.org/documents.php?node=141&dir_session=] has reported exposures effects with sham DTX exposures. They tested four different sets of RF exposure conditions:
Continuous wave RF [CW]:
1. continuously on (1800 MHz, SAR 2 W/kg), CW
2. intermittent (5 min on/10 min off, 1800 MHz, SAR 2 W/kg), CW
Modulated wave RF [AM]:
3. pulse modulation (5 min on/10 min off, 1800 MHz, SAR 2 W/kg, amplitude 217 Hz)

4. and talk modulation (continuous 1800 MHz, SAR 1.2 W/kg, DTX 66%, GSM basic 34%).
These modulations are characteristic for the normal use of mobile phones. Different exposure durations were applied: 4, 16 and 24 hours. In this functional study after 1.8 GHz RF field exposure in different immune relevant cells, "her aim was to investigate the effects of continuous and pulsed 1.8 GHz wave RF exposures on changes in homeostasis with free radical release using several immune relevant cells, including human Mono Mac 6 cells and analyse the free radical production".

During Dr Myrtill Simkó's presentation for the Rostock FGF meeting in September 2006 and again during her presentation for the BfS Mechanisms meeting in Ismaning May 9-10, 2007, we learned that her published results show that the GSM-DTX signal at 2 W/kg induced a significant increase of free radicals if data are compared to that sham exposure. But the frequency modulation of the GSM-DTX signal induces a "sham effect". With Dr Kuster's group they tested for the source of the apparent sham effect in the chamber. Results were negative for statistical differences between controls, medium evaporation, temperature increase, pH differences and background ELF influence. The reason for the DTX sham exposure effect was not found.

The Wobus Group: Gatersleben
A similar GSM-DTX signal induced "sham effect" may have occurred in the results of the Wobus group in Gatersleben [Czyz et al., 2004] as reported to me [SAJ] privately in personal communication from an exchange between Dr T. Nikolova and Dr M. Simkó in Rostock in Sept 2006. The question was raised by Dr Myrtill Simkó privately because in Rostock in September Dr T Nikolova did mention the DTX exposure in her presentation of methods but did not mention the DTX results: [see Table 1 inserted below the reference here FYI. See also Dr Nikolova's presentation pdf at the COST 281 weblink: http://www.cost281.org/documents.php?node=141&dir_session= : Table 'exposure parameters' page 3 and also see Table 1 in the paper, Czyz et al., 2004.]

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TABLE 1. Exposure Parameters for the Applied GSM Modulation Schemes; SAR Values have been Determined with Simulations and Measurements and Represent Voxel Average Values Over the Area of the Cells

Exposure parameters	GSM-217 Hz	GSM-Talk (33% GSM-Basic 67% GSM-DTX)	GSM-DTX
Cultivation in ‘hanging drops’:			
Slot SAR	12 W/kg	8 W/kg	8 W/kg
Time-averaged SAR	1.5 W/kg	0.4 W/kg	0.11 W/kg
Exposure duration	6 hr 48 hr	6 hr 48 hr	6 hr
ON/OFF cycles	5/30 min	5/30 min s	5/30 min Continuous
Cultivation in suspension			
Slot SAR	16 W/kg		
Time-averaged SAR	2 W/kg		
Exposure duration	72 hr (22 hr, 40 hr[1])		
ON/OFF cycles	5/30 min		

The uncertainty for the average values was assessed to be 50% for hanging drops and 20% for suspension cultivation.
[1]Monolayer.
” [Czyz et al., 2004].’

It is notable that the authors did not report the results of their DTX exposure within the published paper although they did mention in the published methodology that they used this exposure [see Table 1 in Czyz et al., 2004 above]. All results should be reported. It would be relevant if this ‘sham DTX effect’ were reported and confirmed directly as it could indicate a possible ‘sham effect’ in another sXc system for the RF 1800 GHz GSM DTX exposure in a second laboratory setting.
From the results of Lantow et al., [2006a, b, c] and Czyz et al., [2004] it is evident sham/sham controls are essential at each exposure to rule out significant results due to as yet unexplained and uncontrolled experimental sham effects. Full reporting in publication of these sham/sham results is equally essential.

Co-author Responsibility
Although Dr Kuster suggests that the biologists have not communicated with the engineers [who designed the exposure systems] for the REFLEX project, it seems that in relation to Dr Simkó there was extensive direct communication about exposure problems with Dr Kuster’s group. In addition Kuster is a coauthor in papers of Simkó,

Wobus and Tauber groups [see references below]. Co-authorship includes co-responsibility.

Incomplete Methods and Results
From the discussions and presentations and publications reviewed above it is evident that effective communication and full open discussion among the co-authors and co-investigators did not occur sufficiently to give a clear understanding of the need for biological in situ calibration of the exposure chambers before experimentation, or the need for full understanding and reporting of the results [published and unpublished] of the 4 research groups, Rüdiger, Tauber, Wobus and Simkó. Consequently a clear evaluation of this research is not possible.

Failure to Calibrate ELF and RF sXc Systems in Situ
The calibration of the sXc system within each laboratory before experimentation required a co-operation of the biologists and the engineer partners. Positive and negative biological controls needed to be run to verify the equipment was calibrated correctly within that laboratory before experimentation. Kuster has suggested this in his statement that his 'REFLEX in vitro exposure chambers [sXc-systems] are 'fool-proofed to guarantee proper functioning' but proper usage/application is not [fool-proofed]'. Also see in the abstract of Prise et al., 2008 at the end of this summary: 'Calibration of the assays was done by exposing cells to X-rays to generate dose-response curves.'

The uncertainty of the RF exposure varies more with cells in suspension than cells in a monolayer in the Petri dishes [Schuderer et al., 2004]. The position is different for suspension and monolayer Petri dishes within the exposure chamber [Kuster presentation, May 10 2007]. Specific types of cells vary in size and polarity; that impacts on their absorption of RF [Simeonova and Gimsa et al., 2005]. Unless specific cells are modeled in the culture medium in the Petri dish in the sXc chamber during test exposures before the real experimental exposures we will not know the amount of exposure and exposure uncertainty of the cells of that in vitro experiment. Positive biological controls are one way to look at this [Prise et al., 2008].

This biological calibration check is not evident in the published research of the Rüdiger laboratory for either the ELF or the RF sXc systems. Positive controls are missing in Diem et al., 2005 and Ivancsits et al., 2003.

It would be difficult to assess which partners did calibrations of the sXc equipment with positive controls before experimentation and which did not from the final REFLEX Report as essential details appear to be missing and/or incomplete [i.e. see below 2.1.1 and 2.1.2] but this would be an essential first step in a much needed review of the validity and reliability of the REFLEX results overall.
According to the REFLEX Final Report [10: Kuster]:
' 2.1.1: A novel ELF setup was developed, and four copies were installed in the laboratories of Participants 3, 4, 7 and 11.' [Namely Drs Rüdiger, Wobus, Kolb, and Clementi.]

And ‘2.1.2: A novel RF setup (GSM) was developed, and four copies were installed in the laboratories of Participants 2, 5, 6, and 8’. [Namely Drs Tauber, Trillo, Leszczynski and Lagroye]. This is inaccurate as RF exposure was carried out in [3] Dr Rüdiger’s laboratory as well. The Reflex Report 2004 is inconsistent with the details given in Dr Kuster’s presentation May 11, 2007: ‘the sXc-1800 MHz, designed by IT’IS that has 5 partners’ [see page 3 above].

Dr Kuster’s Hypothesis

There is nothing in the established RF literature to support Dr Kuster’s hypothesis that AM modulated signals are the likely source of non-thermal, non-linear biological effects below 2 W/kg. There is plenty of established evidence that CW and AM modulated RF bioeffects are due to the same thermal mechanism above exposure limits [reviews ICNIRP 1998; Foster and Repacholi, 2004; IEEE C95.1-2005]. Please note that thermal cellular tissue damage occurs when the cell temperature is raised to a temperature above 40-41 °C [Lepock, 2003; 2005].

There is no biophysical reason to expect a more likely biological non thermal effect of RF with an AM modulated signal rather than a CW signal at the same exposure level, below guideline limits. An amplitude modulated RF field is still an RF field, with a spread of frequencies about the carrier that is equal to the modulation frequency. We know of no plausible biophysical argument why such modulation should be important. There are no nonthermal effects or mechanisms established for RF fields at ordinary field levels [below ICNIRP guideline or IEEE standards exposure limits] that are biologically important [Foster and Repacholi, 2004].

Dr Kuster is hypothesizing that if you get CW RF exposure effects they are definitely due to thermal effects and in that case your AM RF exposures with the same sXc system would also be due to heat**.** And if you get no CW RF effects he suggests that then you know the sXc system is operating correctly [i.e. CW and AM RF exposures are at or below the guideline limits]. In that case if you get AM modulated RF effects they would be due to non linear AM interactions with the living cell that would be unique to only AM RF and would not occur with CW RF exposure at the same level. He said he used the CW for a control exposure to test the sXc exposure level.
This CW positive control is faulty because as explained above there is no biophysical scientific reason to suppose AM RF interacts with cells any differently than CW RF at exposures at or below 2 W/kg. Positive controls should have a range of exposures and a known dose-response curve of the known biological effect [see Prise et al., 2008]. There is no known dose-response curve for nonlinear RF effects. We have not identified any established non-linear RF effect. His CW control is also faulty because calibration needs to be carried out before the experiments begin not during experimentation. We need to verify if the Rudiger and Tauber sXc RF exposures were indeed above 2 W/kg and if the effects reported could be caused by heating.

CONCLUSION [SAJ]

- Dr Kuster maintained that there is slow progress in evaluation of genotoxic effects of RF because biologists do not like to talk to engineers.

- The IT'IS sXc-systems for REFLEX in vitro exposure chambers were 'fool-proofed to guarantee proper functioning' but proper usage/application is not' fool-proofed.
- The REFLEX CW effects reported with the sXc exposure systems according to Kuster's hypothesis are thermal artifacts of RF exposure at SAR's that must have been above guideline limits and the above the highest designed experimental exposure of 2 W/kg.
- Kuster concluded that replication of the CW results was a meaningless replication of a useless experiment with a faulty genetic hypothesis.
- In relation to 'thermal effects' of the Rüdiger group Kuster maintained they published without proper consultation with his group and erroneously published CW effects that showed the 'thermal effects' of overexposure and that their exposure chambers required further adjustment for correct calibration. A similar conclusion could be drawn on the CW results of the Tauber group.
- He said he used the CW for a control exposure to test the sXc exposure level.
- This CW positive control is faulty because there is no biophysical scientific reason to suppose AM RF interacts with cells any differently than CW RF [ICNIRP 1998; Foster and Repacholi, 2004; IEEE C95.1-2005].
- Controls require a range of RF exposures and a known dose-response curve of the known biological effect. There is no known dose-response curve for nonlinear AM RF effects. We have not identified any established non-linear RF effect.
- His CW control is also faulty because calibration needs to be carried out before the experiments begin not during experimentation.
- We need to verify if these sXc RF CW exposure effects [of the Rudiger and Tauber groups] were indeed above 2 W/kg and if the RF effects reported were caused by heating.
- The Simkó group reported 'sham effects' on the DTX exposures with the RF sXc chambers and Wobus group failed to report their similar DTX exposure results.
- These problems undermine the experimental results reported by the Rüdiger, Tauber, Simkó and Wobus research groups using their sXc exposure systems in partnership with the Kuster group.
- It requires reciprocal collaboration of the engineer and biologist partners to biologically, and biophysically calibrate the exposure chambers in situ before valid and reliable experimentation can be done.
- A lesson for future research is that using positive biological controls during calibration is one way to derive this necessary biophysical 'in situ' calibration [Prise et al., 2008].
- The assessment of biological in situ calibration of sXc exposures is a first step to assess the validity and reliability of the REFLEX results.
- This biological calibration check is not evident in the published research of the Rüdiger laboratory for either the ELF or the RF sXc systems.
- It may be difficult to assess which partners did in situ biophysical calibrations of their sXc equipment with positive controls before experimentation and which groups did not because parts of the REFLEX Final Report and some of the REFLEX group publications appear incomplete and/or inaccurate.
- In order to assess the validity and reliability of REFLEX research it may be necessary to setup an independent review panel of expert scientists in relevant

disciplines to write a full report based on invited fully shared verbal and written disclosure of methods and results and difficulties by researchers involved in calibrating and using the ELF and RF sXc systems.

- Recent in vitro published results from RF exposures by independent groups should also be taken into consideration for an updated evaluation of the weight of the evidence on the bioeffects of RF exposures on cells [i.e. Scarfi et al., 2006; Hirose et al., 2006; Hirose et al., 2007a, b; Sakuma et al., 2006; Takashima et al., 2006][summary 2006/11 Joint Workshop on Radio Frequency and Health, Tokyo, COST281: J Miyakoshi]; Sanchez et al., 2007; Zeni et al., 2007a, b, c].
- Recent failed replications [Dawe et al., 2006; Tattersall, Rostock 2006] of published positive bioeffects of RF CW exposures [Tattersall et al., 2001; de Pomerai et al., 2000] suggest these positive results were due to thermal artifacts and should also be taken into account.

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2. Hugo Rüdiger

Summary [Excerpted from his slides and SAJ conference notes]

Dr Rüdiger presented results from Diem et al., 2005 as well as their replication of that work in cooperation with Speit's group in Ulm [Speit et al., 2007]. In addition he presented his group's results with the UMTS exposure system designed by Kuster's group. The error bars in the bar graphs [Diem et al., 2005] were lower than expected and he was asked whether each experiment's values were the result of a single experiment or the averages of several replicated experiments. Dr Rüdiger could not answer the question. Generally, at least 3 replication experiments of preliminary results within a laboratory are suggested to verify if their results are reliable before publication.

Dr Rüdiger presented slides of joint unpublished results of the replication between his group in Vienna and Dr Speit's group in Ulm with the 1800 MHz exposure conditions of Diem et al., 2005 showing some similar control values for sham exposures suggesting consistency of results. But the exposure results of Diem et al., 2005 were not replicated by Speit's group [Speit et al., 2007]. Results from the Diem 2006 replication are unpublished [see bar graph below: 'Human fibroblasts in culture..']. Results from the Rüdiger group on UMTS were also significant [including CW exposures] and showed a dose dependency over the duration range of exposure peaking at 20 hours and at an exposure of 0.1 W/kg; the effects were higher over intermittent than continuous UMTS exposures. It was unclear if the UMTS results were from a single experiment or several replications.

Dr Rüdiger made no comment on the point of Dr Kuster that the CW effects were an indication that the exposure levels were higher than designed for and that the CW exposures were put in the experiment for a control to test the exposure levels. At that point Dr Rüdiger seemed to confuse his CW results with continuously on exposure results, but, his presentation slides clearly indicate CW effects, with an intermittent signal, in the Diem et al., 2005 replication in 2006 by his laboratory using 2 W/kg over 24 hours exposure on the comet assay, tail factor with human fibroblasts [see bar graph below: 'Human fibroblasts in culture..' Excerpted from Dr Rüdiger's slide presentation May 11, 2007]

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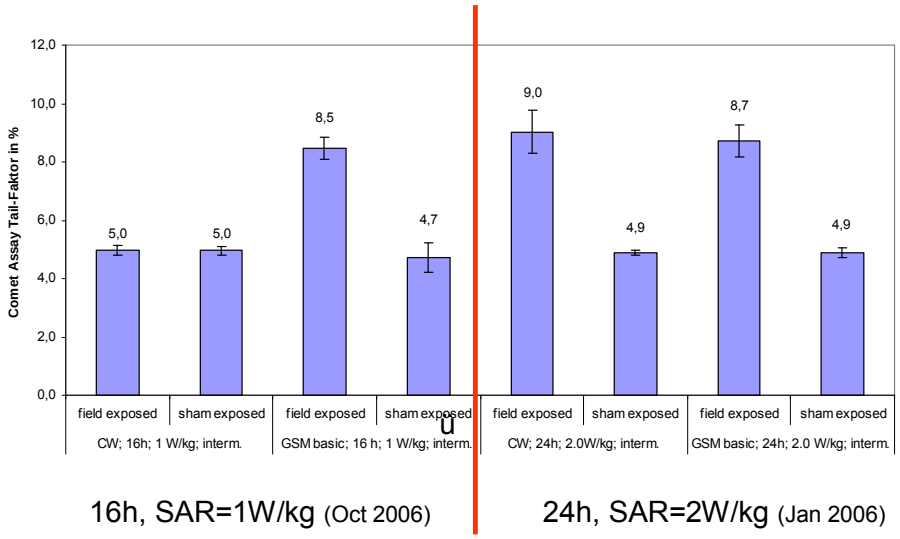
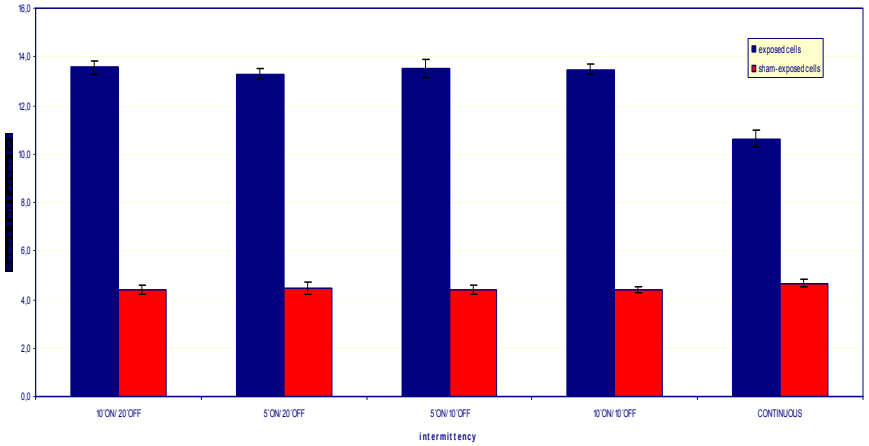


Human fibroblasts in culture
Comparison between CW signal and GSM basic signal

In his group's unpublished results with the new UMTS exposure system designed by Kuster's group once again the error bars in the UMTS bar graphs are much smaller than expected in biological results and he was asked again whether each experiment's values were the result of a single experiment or the averages of several replicated experiments. Again Dr Rüdiger could not answer the question. In the UMTS results the continuous exposure showed effects although slightly lower than the intermittent signal effects [see the bar graph entitled 'Different modes of intermittency' below]. (See Kuster page 4: sXc UMTS: there was the UMTS constant power signal [i.e. no TPC]; or a UMTS TPC power controlled UMTS signal with a maximized low frequency content [1 Hz harmonics]).

Different modes of intermittency



UMTS Bar Graph: Different modes of intermittency. The values on the y axis are the comet tail factor [λ: 0-16]; on the X axis are ratios of intermittency [on/off [10 on/20 off; 50 on/20 off; 50 on/10 off; 10 on/10 off or continuous]. The red bars are sham-exposed cells and blue bars are UMTS exposed cells. [Excerpted from Dr Rüdiger's slide presentation May 11, 2007]

Discussion SAJ [excerpted from conference notes and Vijayalaxmi et al., 2006]
Drs Vijayalaxmi, G Obe, MR Scarfi, I Lagroye questioned the results of Dr Rüdiger at length. Firstly, continuously growing cultured cells with cell cycle durations of 24-30 h (except human blood cells) were used in these studies. During prolonged RF and ELF-exposures (especially 24 h) a number of 'normal' cells would have entered into DNA synthesis (S-phase). 'Normal' S-phase cells displaying increased comet tail length could mimick 'damaged' cells and could be classified as category E [most damaged] [A,B,C,D, E categories, were based on increasing levels of DNA damage.] The absolute differences in tail factors between exposed and sham groups reported in Diem et al., [2005] and Ivancsits et al., [2005] are small. The fact that freshly isolated, non-dividing cells (human lymphocytes and monocytes) did not display changes in the tail factor [Ivancsits et al., 2005] lends credence to the possibility that such cells may be a significant confounding variable for the derivation of the 'tail factor' parameter. Since the number of S-phase cells in each exposure condition was not determined in either of these two reports, the absence of the actual numbers of cells classified into groups A-E raises considerable uncertainty/doubt about the comet data and the conclusions [partially excerpted from Vijayalaxmi et al., 2006].
The second major confounder with the use of 'tail factor' relates to the possible inclusion of apoptotic cells in the comet data. This has serious implications for the 'tail factor' since apoptotic cells, which also exhibit extensive DNA fragmentation, would be visually classified into category E. An apoptotic cell will appear as a totally

damaged cell with only a small amount of DNA remaining in the comet head and almost all or most of the DNA in the comet tail. In the reports by Diem et al. [2005] and Ivancsits et al. [2005] there was no mention of the criterion used to exclude apoptotic cells in the comet data. As with the potential changes in cell cycle mentioned above, for every 1% difference in the incidence of apoptotic cells between exposed and sham groups, the tail factor would change by a value of 1.0 [partially excerpted from Vijayalaxmi et al., 2006].

Third: Their concern also relates to the statistics applied in these studies. In the study by Diem et al. 2005, the data presented in Figs. 1 and 2 (SSB), 3 and 4 (DSB) show negligible standard deviations. Indeed, it is surprising that such small standard deviations were presented in Diem et al. [2005] while in the technical document describing the 'tail factor' transformation technique, the standard deviations reported by Diem et al. [2002] were ~25% that of the mean. Most researchers would consider the use of standard error of the means (S.E.M.) to be the appropriate variance estimator used for statistical analysis and the data from a minimum of at least three independent experiments.

Therefore, future replication and/or confirmation investigations should focus on each of these three endpoints. It is also suggested that DNA damage assessment be performed with more quantitative techniques. Since the numbers of these confounding cells were not determined in exposed and sham groups the validity of 'tail factor' data is questionable [partially excerpted from Vijayalaxmi et al., 2006].

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3. Günter Speit 'Attempts to reproduce genotoxic effects of RF-EMF reported by the REFLEX project' Universität Ulm Institut für Humangenetik, D – 89069 Ulm (Germany), guenter.speit@uni-ulm.de

Summary [Excerpted from Dr G Speit's abstract, slide presentation and SAJ's conference notes.]

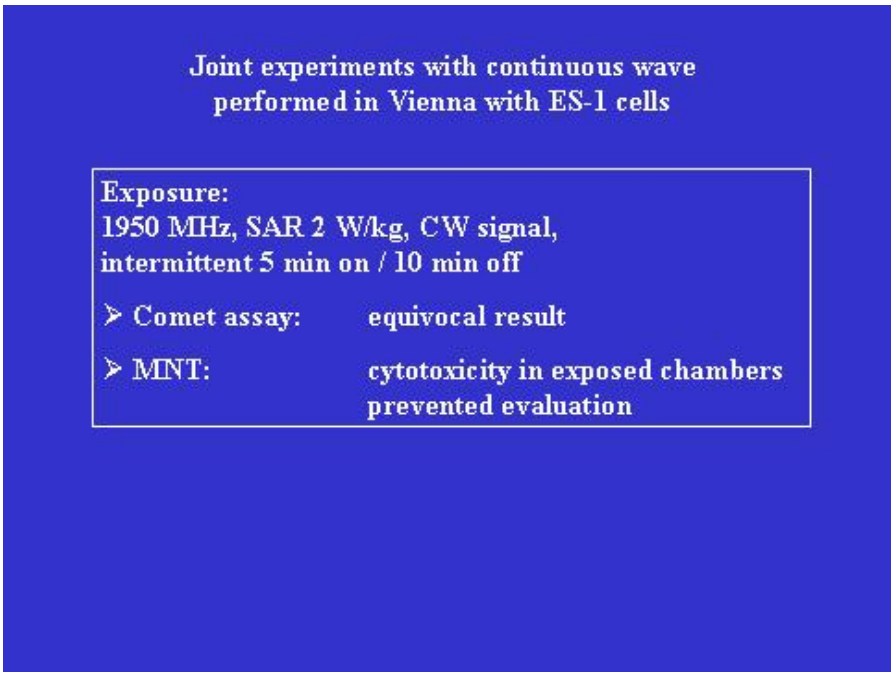
Because of the ongoing discussion on the biological significance of the REFLEX observed effects, it was the aim of this study to independently repeat the results of Diem et al., 2005 using the same cells, the same equipment and the same exposure conditions.

In an independent replication in Ulm, they exposed human fibroblast cells to RF (1800 MHz; SAR 2 W/kg, continuous wave with intermittent exposure) for different time

periods and then performed the alkaline comet assay and the micronucleus test. 'The experiments with ES1 cells were independently performed three times and the differences between mean values were tested for statistical significance ($P < 0.05$) using Student's *t*test [Speit et al 2007]. For both tests, clear negative results were obtained in three independently repeated experiments.' [Speit et al, 2007 report no effect of RF-EMF in the comet assay with human fibroblasts exposed to a 1800 MHz, SAR 2 W/kg, CW signal, intermittent 5 min on / 10 min off (mean ± SEM of three independent experiments) exposed over 1, 4 or 24 hours].
They also performed these RF experiments with V79 cells, a sensitive Chinese hamster cell line which is frequently used in genotoxicity testing and also did not measure any genotoxic effect in the comet assay and the micronucleus test. Experiments with V79 cells were confirmed in a second independently performed test and results are presented as the mean of the two tests. Appropriate measures of quality control were considered to exclude variations in the test performance, failure of the RF exposure or an evaluation bias [Speit et al., 2007].
Additional attempts to clarify the conflicting results were undertaken to check and compare the function of the exposure unit to exchange coded slides for independent evaluation, to exchange of scientists / technicians and to perform joint experiments in Vienna and Ulm with CW exposure of human fibroblast cells. The experiment was done in the same way; there was only one experiment done. The exposure: was 1950 MHz, SAR 2 W/kg, CW signal, intermittent 5 min on / 10 min off. For this co-experiment Dr Speit's wife went to Vienna for one week, the comet assay was done and did not appear to replicate the human fibroblast cells effects of Diem et al., 2005. The results were reported to be 'equivocal'. The Speit group wanted to be open but they did not see a comet assay tail moment effect. Dr Speit said that E Diem claimed she could tell the RF exposed cells from the sham exposed before the results were unblinded in Vienna [but not in Ulm] because she saw changes in the morphology. But, the micronuclei co-experiment could not be evaluated. The micronuclei test failed in Vienna due to cytotoxicity in the exposed chambers that prevented evaluation. The micronucleus test cells died for an unknown reason. The cells were damaged in Vienna and could not proliferate. The co-experiment was discontinued and is unpublished.
Dr Speit said that it was not clear what happened in these experiments in Vienna. There is no MN data from Vienna, it was not possible. The Ulm group stopped working with the Vienna group.

Discussion SAJ [from conference notes]
Participants asked again how many replications were performed in Vienna and also how many replication experiments were done in Berlin [Dr Fitzner was present] and Ulm. Was there only 1 exposure per data point for HL 60 cells and fibroblasts? This was a recurring important question that was not definitively answered.

Dr Speit was asked to explain what he thought happened to the cells in the Vienna co-replication. He said the cells were exposed or sham exposed to 1950 MHz, CW then the cells died [See below, 'Joint experiment with continuous wave..'. Excerpted from Dr Speit's presentation May 11, 2007]. Was it the exposure? It is equivocal? 'OK, maybe somebody spit in the culture.'



Dr Speit said something unknown had happened so the experiment could not continue. There was a strong indication of chromosome aberrations [CA] and these CA were very unusual. It was very implausible how such an effect could occur, and it was not clear what happened in these experiments.

There was further discussion about the 'equivocal' results in Vienna, and Berlin but the details were unclear and no clear conclusions could be made since these results are not published and the technicians who did the experiments were not there to answer specific questions of methodology.

Perhaps, as Kuster continued to suggest, the continued discussion around the need for replication of the CW [REFLEX Project. 2004] results of Diem et al., 2005; Speit et al., 2007 and the Berlin group's HL60 results was a meaningless discussion of replication of useless experiments with a faulty genetic hypothesis? But even this remained to be demonstrated. We need to find out if RF exposures were indeed above 2 W/kg and if the effects reported were caused by heating.

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REFLEX [Risk Evaluation of Potential Environmental Hazards from Low Energy Electromagnetic Field (EMF) Exposure Using Sensitive in vitro Methods], Final Report QLK4-CT-1999-01574, 31 May 2004. http://ec.europa.eu/research/quality-of-life/ka4/pdf/report_reflex_en.pdf

Speit G, Schutz P, Hoffmann H. Genotoxic effects of exposure to radiofrequency electromagnetic fields (RF-EMF) in cultured mammalian cells are not independently reproducible. Mutat Res. 2007 Jan 10;626(1-2):42-47.

4. Primo Schär 'Genotoxicity of EMFs: Exploring DNA directed effects and experimental discrepancies' Centre for Biomedicine, DKBW, University of Basel, Mattenstrasse 28, CH-4058 Basel, Switzerland. primo.schaer@unibas.ch
Dr P Schär is a molecular geneticist. Although he had preliminary unpublished results from RF exposures of cells as far as I know he did not provide the FGF with an abstract of his presentation or his slide presentation and disallowed any digital photos of his presentation to be taken, and asked that his preliminary results not be made public. It is not possible to present his results here. Presently he has no publications on the topic of genotoxicity of EMF's on pub med in his 44 listed publications.

5. Dieter Pollet 'Possible genotoxic effects of GSM signals on different in vitro models.'
Anja Heselich[1,2], Paul G. Layer[1], Dieter Pollet[2], Petra Waldmann[3]
[1]Darmstadt University of Technology, Germany; [2]University of Applied Sciences, Darmstadt, Germany; [3]Incos GmbH, Nieder-Olm, Germany.

Summary [Excerpted from Dieter Pollet's abstract, slide presentation and SAJ's notes] 'Non-thermal' exposure to RF-EMF below the limits defined by current safety guidelines does not lead to DNA or chromosomal damage according to established scientific evidence [ICNIRP, 1998; IEEE C95.1-2005]. However, recent studies from the REFLEX project report on DNA strand-breaks and chromosomal mutations induced by experimental RF exposure. Due to the relevance to human health of potentially adverse effects on DNA integrity, two new projects are now underway to investigate genotoxic effects.

1. Project FM 8823: The effect of GSM 1800 MHz -signals on isolated human blood lymphocytes: genotoxicity.

Due to the relevance to human health of potentially adverse effects on DNA integrity, a multicentered study [Project FM 8823] is now funded by the German Federal Office for Radiation Protection to determine possible genotoxic effects of RF-fields generated by mobile telecommunication equipment on DNA, at the chromosomal and genomic levels. The design of this study follows a recommendation by COST Action 281 [see Löwenstein 24-27 Nov 2002: http://www.cost281.org/events.php; and http://www.cost281.org/activities/Gentox-recomm-090304AW.doc]. Thus, PHA-stimulated [phytohemagglutinin, a mitogen, promotes cell division] peripheral lymphocytes from 20 donors (adults and children) are being exposed to GSM signals. DNA strand breaks, chromosomal aberrations, micronuclei and sister chromatid exchanges are being investigated as genotoxicological endpoints. The blinded samples are being scored by three different laboratories. The blood donors are healthy, non-smoking males from two age groups, 10 teachers (50-60 years) and 10 school boys (16-17 years). Whole blood stimulated with PHA in appropriate medium is exposed to a generic GSM 1800 MHz signal (intermittent 5 min on / 10 min off) SARs of 0.2, 2, 10 W/kg (plus concurrent sham-irradiated and positive controls).

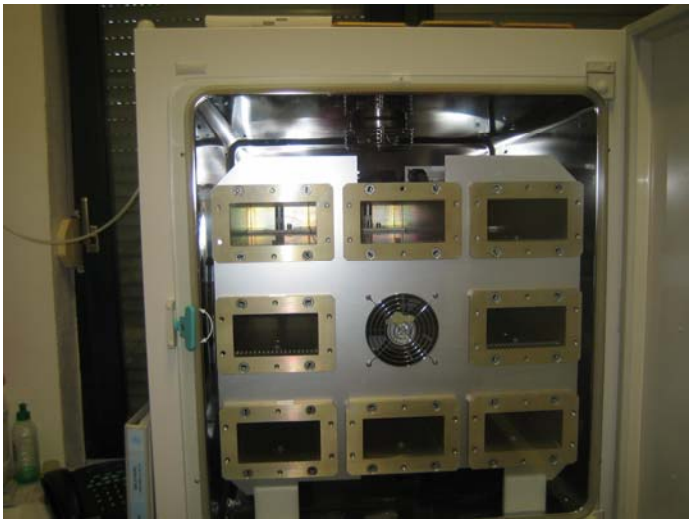
The 4 Assays:
The four genotoxicological endpoints assessed in this project are:

1. Chromosome aberrations [CA]. The CA analysis is done at 1st mitosis, of 1000 cells by each laboratory.
2. Sister chromatide exchanges [SCE]. The SCE analysis is done at 2nd mitosis, of 50 cells by each laboratory.
3. The micronucleus test [MNT]. The MNT analysis is of 2000 binucleated cells, scored by each of the 3 laboratories.
4. The alkaline comet assay. Each of the 3 laboratories will assess 100 cells each.

Analysis and statistics:
The scoring of blinded slides is being done in 3 different laboratories. The exposure/assays of the slides are carried out in different laboratories too. The statistics will be performed by an experienced biostatistics group.
The groups involved include:
1. INCOS: Research Group for Molecular Mechanisms of Environmental Gentoxicity (AMMUG/ INCOS GmbH) **[culture and PHA stimulation; exposure, tests and staining]**
2. *IMBEI*: Institute of Medical Biostatistics, Epidemiology & Informatics, University of Mainz [**donors, questionaire, anamnesis blood sampling]**
3. *IT'IS*: Foundation for Research on Information Technologies in Society, Zürich [**Exposure Waveguide setup. Encoding/Decoding slides]**
4.DBZ, 5.HD, 6. RCC: [Encoding: Comet Assay, Micronucleus Test, Chromosome Aberrations, Sister Chromatide Exchange: Statistical analysis and reporting].
4. *DZB*: Division of Molecular Cell Biology, Dermatology Center, Buxtehude.
5. *HD*: Department of Biotechnology, University of Applied Sciences, Darmstadt.
6. *RCC*: RCC Cytotest Cell Research GmbH, Roßdorf.

The waveguide setup [see photo below] is by IT'IS, Zurich, for GSM 1800 MHz exposure of cell cultures with defined conditions with respect to homogeneity of the EMF, and minimum variation of SAR and temperature. The 8 waveguides are arranged in 4 separately controlled exposure units (placed within one CO_2-incubator). And 9 dishes (Ø 35 mm) can be placed in 1 waveguide. There will be 18 dishes/dose and 72 dishes/experiment.

The Waveguide Setup [Excerpted from Dr Pollet's presentation May 11, 2007]



The graphed time-course of exposures for each test: [Excerpted from Dr Pollet's slide presentation May 11, 2007]

Sister Chromatid Exchange [SCE]							
0 h	→	20 h	→	48 h	→	72 h	74 h
+ PHA + BrdU			*exposure*			*+colcemid*	

Chromosome Aberrations [CA]							
0 h	→	20 h	→	48 h	→	50 h	
+ PHA			*exposure*	*+colcemid*			

Micro Nuclei Test [MNT]							
0 h	→	20 h	→	48 h	→	68 h	
+ PHA			*exposure*	+CB after 44 h			

Comet Assay							
0 h	→	20 h	→	48 h			
+ PHA			*exposure*				

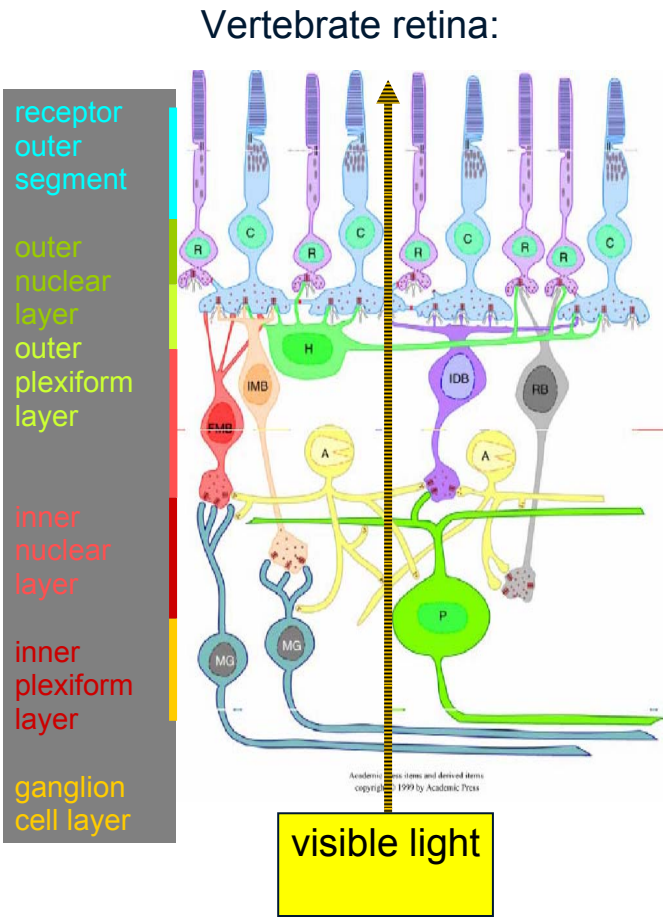
The FM 8823 Project Outline:
For each donor, there are 4 assays, 5 treatment groups and 400 slides in each of 3 labs. The blood sampling will proceed with from 2 - 3 donors per month. Thus the project is projected to end in August 2008.

FM 8823 Current Project Status: May 2007:
The pretest is finished, scoring parameters are harmonized and most of the problems with the waveguide setup are solved. The blood samples of 2 donors have been exposed and slides are ready for scoring.

Project 2. Reconstructed Vertebrate Retina as a Potential in vitro Model for Genotoxicity Testing. Joint project of the Technical University and the University of Applied Sciences, Darmstadt.

2A: Effects of GSM exposure on freshly enucleated bovine eyes. [See the 2 Vertebrate retina diagrams below. Excerpted from Dr Pollet's presentation May 11, 2007]

Vertebrate retina:

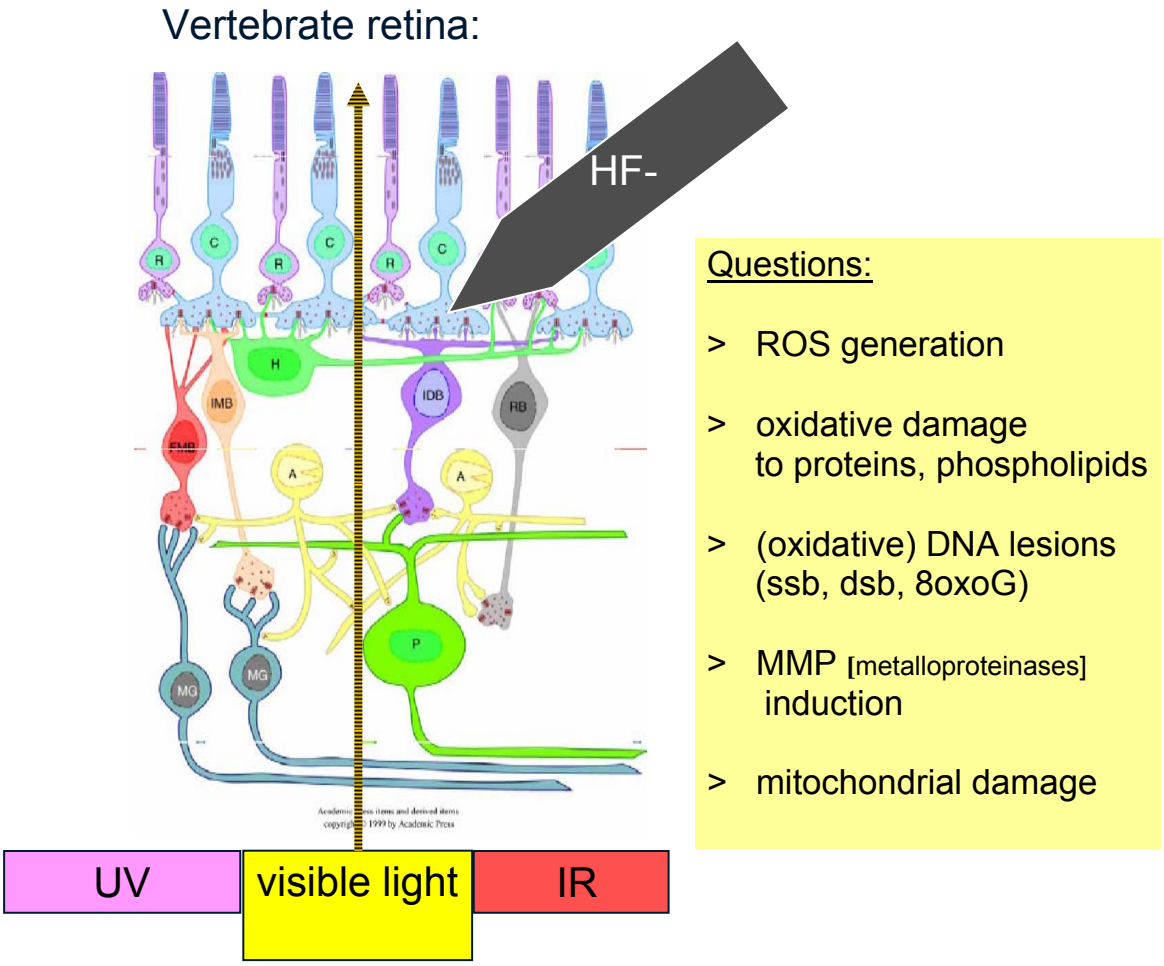


Vertebrate retina is a system of structured layers of different cell types:

horizontal cells, bipolar cells, amacrine cells, ganglion cells, photoreceptors

3 layers of cell bodies and 2 plexiform layers with synaptic connections

structure is similar to a 3-layered neuronal net



Questions:

> ROS generation

> oxidative damage to proteins, phospholipids

> (oxidative) DNA lesions (ssb, dsb, 8oxoG)

> MMP [metalloproteinases] induction

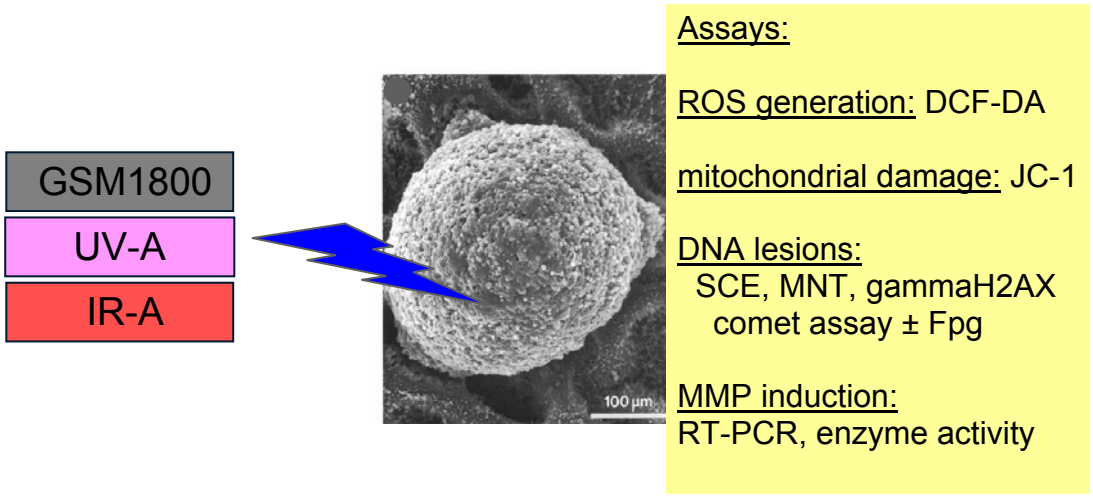
> mitochondrial damage

2B: Possible adverse effects of GSM exposure on retinospheroids from embryonic animal retinae *in vitro* [See the 2 retinosphere diagrams below. Excerpted from Dr Pollet's presentation May 11, 2007]

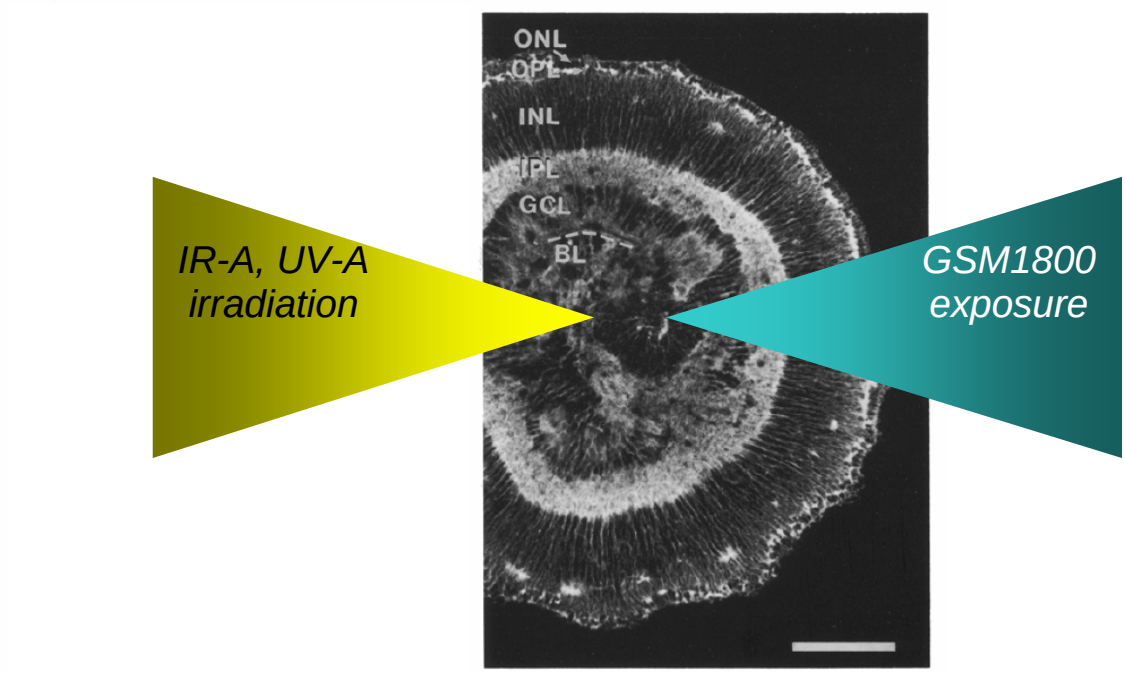
Fully dissociated cells of the embryonic chick or mouse retina can reconstitute different types of spheres with a more or less complete arrangement of retinal layers. These retinospheroids show correctly laminated retinal spheres with photoreceptors forming a regular outer nuclear layer.

The system has already been used as a pharmacological and toxicological test system and for molecular and neurogenetical studies. Topics of interest are the influence of non ionizing radiation such as Ultraviolet [UV], infrared [IR] and RF on the organotypic development of the retinospheres and their regenerative potential. Generation of reactive oxygen species [ROS], changes in enzyme activities and expression profiles of genes related to proliferation and cellular stress responses as well as DNA damage are further endpoints.

"Retinospheres": reconstructed vertebrate retina as model system



"Retinospheres": reconstructed vertebrate retina as model system



The objective of this multifaceted experimental approach is to investigate possible adverse effects of non-ionizing radiation including GSM 1800 MHz signals on the retina as a functionally most complex and delicate tissue of the visual system and to elucidate possible underlying mechanisms of action.

Comment SAJ

One limitation of these retinal preparations is that they have no blood supply or blood circulation. Blood supply reduces heating by up to 30% in live animals [Flyct et al., 2007; Hirata, 2007; Hirata et al., 2006; 2007; Wainright et al., 2007]. This will need to be taken into consideration in the exposure bioheating calculations.

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Final discussion lead by Dr J Kiefer [The content below is from SAJ's conference notes done to the best of my ability. NB: These notes should be checked for accuracy against other persons' notes from this conference.]

Dr Kiefer lead off the discussion by suggesting we first address the conflicting results then turn to mechanistic biophysical theories.

Dr Kuster said with this approach we will never end; 'replication of the CW results was a meaningless replication of a useless experiment with a faulty genetic hypothesis'.

Dr Kiefer focused us on the conflicts among the results of Vienna [Diem et al, 2005], Schär and Speit since they do not have the same experimental results when attempting the replications. Why are they different?

Dr Speit in his published paper only reports on CW intermittent exposures with the comet assay and MN and he sees no effect. The co-replication [Ulm & Vienna groups] experiments with 1950 MHz, SAR 2 W/kg, CW signal, intermittent 5 min on / 10 min off exposures were never finished because during the experiment the cells were

contaminated and died in the Vienna laboratory and the experiment of necessity was abandoned.

Dr Kiefer asked: There were no visible morphological changes reported after RF/sham exposures seen by scientist in Ulm or Berlin but Diem reports after exposures in Vienna that she could see changes/differences in the cells on the slides of the exposed versus the sham exposed before breaking the blind code. Were the differences due to different exposure chambers? The Vienna group has a different exposure chamber from Berlin [Fitzner] and Ulm [Speit] that can also produce the higher frequency of UMTS as well as 1800 MHz.

Dr Kuster said that the higher carrier frequency should play no role; the problem is with CW results. If you see something with CW then it is a thermal effect, then the GSM effects are thermal too. The exposure needed to be adjusted. Dr Kuster suggested his REFLEX in vitro exposure chambers [sXc-systems] are 'fool-proofed to guarantee proper functioning' but proper usage/application is not [fool-proofed].

Dr Kiefer asked if the CW effect reported was a genotoxic one with CW. Since Diem saw a morphological difference then the investigator knew which was exposed & not exposed before scoring? If not blinded, if the difference is so low then personal opinion could multiply the differences. Why can Diem see that the sample has been exposed? When she sees this in the plates, what does she see? Diem says she could see in the water drop, a clear difference in Vienna but not in ULM. What does that mean? There is a question in the experimental system; the problem is something bigger; the confounder could be massive for instance apoptosis is easy to detect. Because the cells died after exposure, as reported by the Speit group when co-working in Vienna, it raises the question? Can that happen, can something go wrong in the exposure system? Since they randomly select chamber 1 or 2, they can't find the problem with exposures; the temperature was checked. They did the comet assay then cultured for MN. Did something go wrong before?

Kuster said the air flow enhances evaporation; the fans were running at the same time and velocity for sham and exposures. The only difference was RF exposure or not .

[Kiefer] Was the position of the cell culture sitting on shelf maybe different from the sham to experimental? Variable conditions in the laboratory could increase by 1.4 but it should have no biological relevance. There is not much discrepancy between Vienna & Berlin on the RF dose effect 2.1W/kg and 1.3 W/kg respectively. According to Dr Kuster, the uncertainty, the variation between the exposure setups is 5% and within a setup is 20 %. The CW effect was in the same [dose] region by all modulations and forms in Berlin as in Vienna.

Then in Berlin the effects are all thermal as well since no CW effects could occur except thermally according to Dr Kuster?

Dr Kiefer asked: Do the positive results identify DNA damage?

Forget the neutral comet assay for double strand breaks; it is not specific [Vijayalaxmi, G Obe]. The Vienna group reported the same effects on neutral and alkaline assays [Diem et al., 2005]. You need evidence of chromosome aberrations; the comet is nonsense for identifying DNA damage. It is nonsense to conclude DNA damage from the comet assay [Vijayalaxmi.]. You have to look for molecules to see an effect. With the comet assay nobody understands what they are looking at. In comet the strand breakage is not proven as genotoxic.

Dr Kiefer: The summary of the comet assay results appears to be conflicting evidence even if doesn't tell us very much. They are events in the S phase related shift and we don't have to over interpret them. Miss Diem could not replicate something in Speit's laboratory that she saw in the Vienna laboratory. Do we need to resolve this?
Gunter Obe sees a contradiction in the Diem results in Vienna versus Ulm. Why, were they positive in Vienna and negative in Ulm? What is the reason? Imperfect work, the comet is not enough, to see DNA effects.

Dr Kiefer suggested: We must discuss more on what must be done in the future. There is uncertainty with the scientists' results; there is no clear statement here. On the surface there is nothing, more experiments are required. Schär showed us a situation of an effect with 1 cell line and not in another. Is that depending on how you cultivate cells that you get different effects? Do different procedures give different results?

N Kuster said the ELF sXc chamber results are a different point. Historically Vienna started with the ELF exposures first. They checked the exposure chambers in very a systematic way, and eliminated vibrations. The Vienna ELF results should hold up.

[Dr Scarfi] But ELF effects [Ivancsits et al., 2003] repeated by the Scarfi et al., replication with the same exposure system protocol did not see effects with the comet assay or MN test [Scarfi et al., 2005 see reference below]. She failed to replicate the work of Ivancsits et al., 2003. Dr Scarfi agreed with Dr. Isabelle Lagroye who said different results are evident in different laboratories, not just in the RF exposures. GTEM cell exposure systems are totally different. Maria Scarfi pointed out they found no effect at all with ELF even when varying the B field exposures [Scarfi et al., 2005]. 'She said 'Stronger, less stronger, nothing!' I expect a variation a qualitative difference but did not get one. This I cannot explain.'

Comment SAJ: Yes, there are other failed replications with the ELF sXc exposures as well [See also Prise et al., 2008 attached below]. The ELF sXc exposure system of Kuster was used by Prise et al., 2008 [see abstract below]. They did in situ checks and calibrations on the ELF chamber before running replications of the Vienna experiment [Ivancsits et al., 2003] in the UK. The Prise replications showed no effects of the same ELF exposures on cells [see Prise et al., 2008 attached conference abstract, paper in progress; as well see above Scarfi et al., 2005 failed to replicate the results on ELF of the Rüdiger group]. Are there also problems with the ELF sXc calibration in the Rüdiger group? N Kuster said they checked the exposure ELF sXc chambers in very a

systematic way, and eliminated vibrations but there is no evidence of positive biological controls in ELF research of the Rüdiger group [Ivancsits et al., 2003].

Dr Kuster said, 'the RF chambers were handled missing the systematics'. What was he referring to? He said the RF primary data are not very strong with Vienna; he can believe something has gone wrong. [See Miyakoshi J. 2006, failed replication of Vienna RF comet assay results]

Dr Kiefer: You must know the genetic damage fundamentals of biology before you discuss genetic damage. Dr Schär is not talking about genetic damage; the exposure reproduces no effects. There is nothing after 4 hr; MN effects are not proven to be genotoxic. With this type of result we would have to probe, and look into a mechanism of RF interaction.

Dr Adlkofer agreed he might have gone too far, saying there is DNA damage. But something happens if the comet assay is positive.

Dr Kiefer stated, in relation to MN test results with every type of modulation, MN is not auto genetic damage; it is not DNA damage. OCD guidelines specify 2 validation studies. MN claustogenicity is something but not DNA damage but one should test the effect further. There is something; we have conflicting results of the comet assay without supporting evidence. With MN there are clear conflicting results. ULM has a problem to find positive results.

The reason is something I cannot solve. [Speit, Scarfi]

Lessons

[Dr Kiefer] Replicate before it gets to the public; but there are no replications before publication with the Vienna group or with the Berlin group. The Darmstadt program, [see D Pollet summary] we ask, do you think that might be a useful approach. We need a technical look for chromosome aberrations [CA]. We should use ionizing radiation as a positive control for a DNA effect [High doses 6 Gy. You see results at 0.2 Gy]? Harmonizing scoring could be a hard test.

Dr Adlkofer said with lymphocytes, we are sure that they won't see anything.

FGF take responsibility to take that over and chose the study design [Dr Gerd Friedrich].

[Dr Kiefer] Are fibroblasts in pipeline with GSM exposures now supervised by BfS? Yes. Will the results be reliable? In relation to the Rüdiger group's work, the general principle is how to expose cells to ELF magnetic fields. They didn't do enough; they used one donor, no statistics, no publications of CA. We need replications of the fibroblast results [Pollet et al., http://www.emf-forschungsprogramm.de/akt_emf_forschung.html/bio_HF_001.html].

First we must sort out conflicts in experiments. In relation to future research should we chose fibroblasts against lymphocytes for future research? And then we should move on to discuss mechanisms.

For repair processes we look at lymphocytes, Schär.

Dr Speit, Nucleotides?- very few. Cells are very good in strand break repairing. We have no consistent evidence of DNA damage from 1940 to 2007, no lymphocytes, fibroblasts etc. If we have no established effect how can you go into a mechanism? There is no scientific basis for these results; the fibroblast results could not be replicated.

Dr Monica Asmuss said BfS waited for the COST 281 recommendations. PHA stimulated lymphocytes are a good model. BfS took the lead from COST281/FGF. It is a very important step. Lymphocytes are standard with ionizing radiation. Other cells react, repairing. With lymphocytes you get chromosome aberrations. You don't get synchronizing. They looked at a few cells, including fibroblasts. At end BfS can say yes to lymphocytes. If this type of studies come out negative we can conclude, and it would let us stop the research. Then we will have good data: it has to be good. To compare DNA breaks versus chromosome gaps is necessary. With weak forces we look for indirect effects such as changes in active oxygen species. If the lymphocytes papers would be positive, we could look at mitochondria of the lymphocytes. If there is a direct to DNA effect what will induce oxygen? But we can only look at the mechanism when we have effects. An international research project could put a lid on it. Wait next year, they will RF expose 20 different blood samples over the whole cell cycle. Induction or no induction should come out from this study. There would be no end to studies if they used diff cell types.

Vijayalaxmi has a very simple plan, a 200,000 euro experiment done by researchers with expertise in cytogenetics. Ray Tice had hot spots in the TEM cells. We couldn't find anything in 6 different cell types.

The Vienna data is from only 1 experiment per data point -Speit. How do we interpret CW results in the light of other data available, if these are faulty [thermal] results? How reliable are the ELF results? Berlin RF results would be faulty too; we have to look at the thermal aspect of this.

Dr Kuster said the air flow in the sXc RF chambers had nothing to do with the temperature of the cells; we have to calculate the difference in SAR. We have too look at the strong discrepancies in a rationale way.
Dosimetry is a number one issue.

References re: discussion

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David Lloyd: Summary for EMFBRT Workshop Jan 10, 2008: 'To determine whether intermittent *in vitro* exposure to ELF EMF causes DNA and chromosomal damage in human cells'. **Kevin M**. Prise[1], Kai Rothkamm[2], Melvyn Folkard[2], Susanne Burdak-Rothkamm[1], Gaurang Patel[1], Pat Hone[3] and David Lloyd[3]

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The perceived risk associated with human exposure to environmental electromagnetic fields has proved difficult to quantify despite considerable effort and a range of epidemiological and experimental studies. From experimental studies, the overall consensus has been that exposures to domestic 50 or 60 Hz fields are not genotoxic. A common feature of virtually all of the previous experimental studies has been that continuous exposures to EMF fields have been used typically of 1 – 24 hours in duration. Also, many of the field strengths studied have been very high typically in the range of 1 – 5mT. This may not be representative of environmental situations where fields may vary in both time and space and are significantly lower than those used in experimental studies.

In a series of studies, carried out as part of the EU REFLEX programme, Ivancsits *et al*, reported that intermittent exposures with 50Hz EMF induced DNA strand breakage in cultured human fibroblasts whereas continuous exposures did not. The finding has attracted significant interest and, if verified, has considerable implications because DNA damage, particularly double strand breakage, is known to be a fundamental step in the processes leading to cancer. It was also reported that intermittent but not continuous exposure of fibroblasts induced a tenfold increase in dicentric chromosomal aberrations.

We have performed a series of studies aimed at replicating the Invancsits *et al* data. Using primary human fibroblasts, exposed in both actively growing and stationary phases, we determined whether intermittent exposures induce damage in the form of ssb, dsb and chromosomal aberrations. Intermittent fields (alternating 5 min on/10 min off) were maintained for 15h and compared with 15h sham and continuous EMF exposures. For DNA damage measurements we used the alkaline comet assay and changes in the phosphorylation of histone H2AX. The chromosomal assays were for unstable aberrations and sister chromatid exchanges in metaphases and micronuclei in cytokinesis blocked binucleate cells. For the DNA assays the cells were exposed to 100 and 1000μT and for the chromosomal damage to 50,100 and 500μT. Replicate exposures were made with the EMF source at the Gray Cancer Institute and a Trust owned facility installed at HPA Chilton. Calibration of the assays was done by exposing cells to X-rays to generate dose-response curves.

In a series of repeat studies using both exposure facilities, no significant effect of the EMF, in either continuous or intermittent modes has been observed for any of the DNA or chromosomal damage endpoints.

Appendix A

Participant list: FGF Workshop, May 11, 2007 at the Federal Office for Radiation Protection in Germany (BfS) Oberschleißheim (Neuherberg), near Munich. Genotoxic Effects of Radiofrequency Fields – Lessons from the Conflicting Results.

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