

Workshop

“SUBTLE THERMAL EFFECTS OF RF-FIELDS *IN VITRO* AND *IN VIVO*”



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Abstracts

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AIM OF THE WORKSHOP

The question whether there exist "non-thermal" effects of weak RF-fields still remains controversial. There are a number of publications claiming to have found "non-thermal" effects in experiments with cells, as well as with animals or human volunteers. These authors call an effect "non-thermal", if irradiation intensity in the experiment is so low that changes in temperature are unlikely to occur physically or if no significant change in temperature has been measured in the body or in the experimental vessel during exposure. But how accurate could temperature be controlled in fact during experiments? At best, the accuracy of measurements is given as ± 0.1 K. In some cases, the assumption of temperature constants during irradiation is based on calculations. Only to a limited extent those calculations can take into account the dielectric inhomogeneity and processes of heat transport on a microscopic level.

What do we know today about thermoreceptors and their physiological interactions in cells, humans and animals? What does "non significant temperature change" in this context really mean? Specialized thermoreceptors in some animals respond to temperature elevations of some hundredths or some thousandths of a degree. Over the past years a number of detailed investigations provided new insight into the molecular mechanisms of thermosensation. Thermosensitive molecules were found not only in specialized nerve cells but also in a large variety of different cells, including keratinocytes of the skin. There are thermosensitive "riboswitches" in cytoplasm, controlling RNA-activities, and therefore specific processes of protein expression. Thermosensitive transport proteins in membranes are able to induce various cellular signal pathways. Interestingly, the mechanism of thermosensation of these proteins consists in "melting" of specialized helical components, occurring only inside a narrow temperature window of several degrees, typical for each of these protein.

Such data induced a number of new insights and rises new questions. What does "molecular temperature" mean? What are the ways of information processing starting from a thermosensitive molecule up to the hypothalamus? What kinds of physiological reactions are triggered on this pathway by possibly small and local changes of the temperature? How does this correspond with temperature elevations in the skin, the ear, and the brain while using a mobile phone? Are there differences in the response to CW fields as compared to pulsed fields? Shouldn't the problem of "microdosimetry" be discussed again by taking into account all those new findings?

The aim of this workshop is to bring together specialists of the fields mentioned and to focus the attention of RF-EMF-researchers to the mentioned new subjects. Biological, physiological and health significance of the reported "subtle thermal effects" will be reviewed in the workshop.

PROGRAM AND TOPICS OF THE WORKSHOP:

1. Thermoregulation: molecular and physiological aspects

(Thermoreception by specific transport proteins; molecular aspects of HSP-expression and phospholipid-adaptation; thermoreceptors in animals; peripheral thermoreceptors and neuronal aspects of thermoregulation in humans.)

2. Subtle thermal effects of in-vitro experiments with RF-fields

(Summary of MMF/FGF mechanism program findings; Feasibility and limitations of temperature measurement in *in-vitro* experiments and micro-dosimetric calculations; "temperature" in dimensions of biomacromolecules: what does this mean? dielectric heterogeneity and possible differences in heat generation on cellular level)

3. Possibility of generation of subtle thermal effects in RF-experiments with animals and human volunteers

(Heat generation by RF-fields and metabolic energy dissipation in animals and humans; Temperature- versus SAR-profiles in human head during use of a mobile phone.)

4. General discussion:

(Are effects of low-level RF-exposure in cells, animals, and humans in fact subtle-thermal? Are there differences in the stationary temperature profile or in the time course of temperature variation between continuous and pulsed fields? Are there dose-parameters which reflect biological effects better than SAR? What is the health significance of the reported "subtle thermal effects" based on the current scientific knowledge?)

CHARACTER OF THE WORKSHOP

The workshop foresees presentations of invited speakers representing research groups that played a significant role in research performed in the corresponding fields. Additionally, a limited number of presentations will be selected from submitted abstracts by a scientific committee. An especial aim of the workshop is to bring together specialists working purely on molecular processes of thermoreception and thermoregulation with those doing RF-EMF research. The workshop will provide enough time for discussion. A report from the workshop summarizing results of presentations and discussions will be prepared and published.

THERMOREGULATION: MOLECULAR AND PHYSIOLOGICAL ASPECTS

THERMOREGULATORY MECHANISMS IN HUMANS

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The systemic temperature is meticulously regulated to 37-37.5 °C. This regulation is controlled in the hypothalamus by comparing the actual value with the nominal value and probably other thermo-sensors in the core of the body are included. To regulate the temperature two major mechanisms exist, first perfusion increase of the skin (to increase heat transfer) in conjunction with sweat production; and second muscle trembling to increase temperature.

Under normal conditions for an immobile person the metabolic heat rate is about 1-2 W/kg, which is provided by the inner organs (60 %), muscle and skin (20 %) and brain (20 %).

Depending on the special demands three organ systems are competing for the blood perfusions shoring the total cardiac output of 5.6 l/min.

- a) The skin is regulating the heat transfer to the surroundings. During intensive physical work the energy turn-over in the muscle can be as 10 times higher approaching amounts of 300 W, which is now 90 % of the total metabolic rate (depending on the training status). Then the perfusion in the periphery can be nearly 10 times higher than the basal values (rising from 0.3 l/min to more than 3 l/min). Locally the perfusion can even increase from a few ml/100 g/min up to 100-200 ml/100 g/min.
- b) The digestive system is fed by three large arteries: the upper and lower mesenteric artery and the truncus coeliacus with 1.3 l/min in the basal state (covering intestine, stomach, liver and spleen). The perfusion can be increased 4-5 times during the digestive process (rising from 0.8 l/min to more than 5 l/min) as characterized by the flow through the portal venous system).
- c) The muscles (approximately 30 kg in a normal human body) can also increase the perfusion from approximately 1 l/min to more than 6 l/min, if required and achievable from the cardiac fitness. During top performance sports the energy production in the muscles can be increased from 30 to 250 W (a factor of 8) and the systemic temperature can be shifted to 40, even 41 °C.

Clearly, these organ systems (skin, digestive system, muscles) cannot increase the perfusion at the same time. However, the human organism has a high potential to adapt to specific requirements such as thermal regulation, physical activity or digestion.

While the regulation of the systemic temperature (37.5 °C) is quite strict, the tolerance and regulation potential with respect to local heat is much more flexible.

We know from laboratory studies, that cells are killed in 90 %, if exposed to 43 °C for 60 min. Equivalent effects are approximately achieved for exposures to 44 °C in 30 min or to 45 °C in 15 min. Therefore, a tissue damage for short term expositions (in the range of minutes) is only possible for temperatures above 50 °C.

Therefore, we can apply heat treatments for therapeutic purposes in cancer therapy, where we induce local tissue temperatures in the range of 40-45 °C for 30-60 min. Thermo-ablation with irreversible destruction of tissue (preferably tumors) is performed with temperatures > 50 °C for 10 min or so.

During local hyperthermia (with heated volumes < 1 l) specific absorption rates (SAR) of 100-200 W/kg are achieved and reactive perfusion of 20-40 ml/100 g/min. Then the temperature is increased to 42-43 °C in the tumor, but less in the normal tissue. Normally no side effect or damage in the normal tissue, such as muscle or skin, have been seen (Wust 1996).

During regional hyperthermia SAR of 30-40 W/kg are found in heated volumes of 10 l or more. The temperatures observed in tumor-related measurement points are 41-42 °C (maximum values). Then the reactive perfusion is 6-9 ml/100 g/min (mean value 8 ml/100 g/min) (Tilly 2001).

During non-invasive MR-monitoring (Gellermann 2005) of sarcoma heating we have seen even higher SAR but as well higher corresponding perfusions in specific regions of the muscles showing the high regulatory potential of the normal tissue, esp. muscles. We know that the local temperature is under normal conditions regulated to values of not more than 40-41 °C. For these temperatures no damages in normal tissues have been found after regional hyperthermia in hundreds of patients.

We conclude, that the thermoregulatory potential for the whole body or large body regions is limited by the heart, which can double the output from 5 to 10 l/min. Even higher is the potential to tolerate a power deposition and to regulate to a temperature in the range of 40 to 42 °C in smaller volumes. Here the perfusion can be increased from the basal value of 2-4 ml/100 g/min to 10-20 ml/100 g/min, e.g. the local perfusion can be 5-10fold increased.

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THERMAL AND PHOTOMECHANIC INFRARED RECEPTORS IN FIRE-SEEKING BEETLES

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Only very few animals are equipped with special infrared (IR) receptors. In vertebrates some night-hunting snakes make use of IR receptors to pinpoint warm-blooded prey. In insects three species of so-called pyrophilous beetles use IR receptors for the detection of remote forest fires. All IR sensitive animals are poikilotherm and, therefore, are unable to keep their receptors at a constant temperature. Also it is impossible for the animals to cool their receptors for the suppression of thermal noise. Additionally, the biological receptors do not have IR transmitting lenses which could concentrate incoming IR radiation onto the absorbing surfaces. Compared to sensitive technical IR sensors, these inabilities seem to be disadvantageous. However, the biological IR receptors have been shaped by millions of years of evolution to perfectly fulfill their tasks.

The IR receptors of snakes function according to the principle of a microbolometer: incoming IR radiation is absorbed by the specialized surfaces of the receptors and the corresponding increase in temperature is measured by sensitive thermoreceptors. An IR-sensitive pit viper is able to detect a mouse by its IR emission from a distance of about 100 cm. This corresponds to an intensity of IR radiation at the receptor of about 10 – 20 $\mu\text{W}/\text{cm}^2$. Although lenses are missing, recent investigations of Japanese biologists have shown that the IR organs of pit vipers are image-forming because the receptor components build up a pinhole camera (R.C. Goris, pers. communication).

In two of the pyrophilous beetles, IR receptors also function like a microbolometer. In the third species, however, IR radiation is measured in a way which has not yet utilized in technical sensors. In beetles of the genus *Melanophila*, IR radiation is absorbed by cuticular microspheres. Each sphere is innervated by a highly sensitive mechanoreceptor which measured the expansion of the sphere due to IR absorption (so-called photomechanic mechanism). In the meantime there is evidence that this mechanism may have some advantages compared with the bolometer principle, e. g. shorter latencies and a higher spectral sensitivity. Therefore, in a biomimetic approach, we established a simple prototype of a technical photomechanic IR sensor. In first preliminary tests we determined a noise equivalent power (NEP) of $1.5 \times 10^{-5} \text{ W}$ and a normalized detectivity (D^*) of $4 \times 10^6 \text{ cm} \times \text{W}^{-1} \times \text{Hz}^{1/2}$.

TEMPERATURE DEPENDENCE OF ION CHANNELS, MEMBRANES, CELLS AND CNS TISSUE

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Biological membranes are close to two dimensional thermodynamical systems with complex structure and behaviour. The temperature dependence of functions of such systems is first discussed according to the behaviour of the lipid structures and consequences of these for ion transport parameters of simplified transport systems (models) like alamethicin and gramicidin (ion pore) and valinomycin (carrier), as well as on bare membrane permeability. This part of the presentation will include a presentation of some significant temperature effect given especially in the range of phase transitions in the membrane.

In the next step the general gating behaviour of ion-channels is introduced in some detail and its temperature dependence, as known from classical examples (Na⁺-channel AChR) is discussed. Following that, structural details about a special group of ion-channels, the TRP-group are presented, and then the physiological functions of these channels are discussed in more detail, including pharmacological and physical aspects, and here especially the involvement of these channels in temperature sensing.

The next, central part of the talk will then be dealing with the extremely high temperature dependence (Q_{10}) of parameters of some members of this group of ion-channels and the consequences for cellular function of these. Some mechanistical aspects of channel structure and its relation to function will be included.

At the end, shortly a CNS-tissue model will be presented, demonstrating the temperature dependence of a complex system. Here it will be shown that the molecular responses of ion-channel to temperature changes in a complex system can result in a reaction to extreme small stimuli (here temperature changes), possible even done to threshold zero. It will be finally shown that the direction of the response of such a complex system is not always predictable from the molecular data.

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THERMOSENSOR CONTROL IN THE DNAK CHAPERONE SYSTEM

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Heat shock proteins protect all cells of all organisms against the consequences of high temperature. Most of them are molecular chaperones, proteins that prevent the aggregation of unfolded proteins and assist the folding of proteins. The most important chaperone in *Escherichia coli* is DnaK, an evolutionary relative of eukaryotic Hsp70 (heat shock proteins with a molecular mass of 70 kDa). DnaK and its co-chaperones DnaJ and GrpE are necessary for normal cell growth at 25 - 40 °C; their overexpression underlies the resistance of cells to temperatures above 40 °C.

Exposure to high temperature elicits a twofold response of *E. coli* cells: First, the DnaK system will directly and instantaneously modulate its functional properties in such a way as to cope better with the heat shock (direct heat shock response); secondly, the cell will synthesize more DnaK, increasing its concentration within minutes about twofold (σ^{32} -mediated heat shock response).

σ^{32} -mediated heat shock response - How does the *E. coli* cell sense the heat shock and induce the synthesis of DnaK? The expression of DnaK is controlled at the transcriptional level by the concentration and the activity of an alternative σ factor (σ^{32}), a subunit of RNA polymerase, which directs the polymerase to transcribe the set of heat shock genes. The expression of σ^{32} on its part is enhanced by decreased negative supercoiling of the DNA due to thermal inhibition of topoisomerase II and by melting of secondary structure elements in its mRNA that prevent binding of the mRNA to the ribosome and block the initiation codon. Thus, both DNA and σ^{32} -mRNA act as thermosensors. The cellular level of σ^{32} is further regulated by the DnaK system itself: DnaK binds σ^{32} and competes with RNA polymerase for binding of σ^{32} . Under normal growth conditions, σ^{32} is for the most part sequestered by DnaK and shows a short half-life of about 1 min. Upon exposure to a heat shock, unfolded proteins are generated and compete σ^{32} away from DnaK. Released σ^{32} now binds to RNA polymerase which then starts to synthesize more mRNA of DnaK and other Hsp. Thus, DnaK acts as a sensor for unfolded proteins.

Direct heat shock response - How does the DnaK system sense the heat shock and immediately react to it? In the ATP-driven chaperone cycle, DnaK alternates between two states, low-affinity ATP·DnaK with fast binding and release of the substrate and high-affinity ADP·DnaK with slow kinetics. The substrate, an unfolded protein, is fed into the chaperone cycle by binding to ATP·DnaK, which is then converted to high-affinity ADP·DnaK through DnaJ-triggered hydrolysis of DnaK-bound ATP. In ternary ATP·DnaK·substrate·DnaJ complexes, in which chaperone and co-chaperone are bound to the same substrate molecule, efficient *cis*-interaction of DnaJ and DnaK greatly facilitates the ATPase-triggering action of DnaJ. Thus, DnaK and DnaJ act together as a sensor for unfolded proteins, accelerating the intake of substrate into the chaperone cycle. GrpE, the second co-chaperone facilitates the exchange of DnaK-bound ADP with ATP and reconverts ADP·DnaK·substrate to low-affinity ATP·DnaK·substrate, from which the substrate is released. GrpE acts as the thermosensor of the system, undergoing a reversible change in conformation at heat-shock temperatures. While the rate of the DnaJ-triggered low-to-high affinity conversion of DnaK follows an Arrhenius temperature dependence, the rate of the GrpE-dependent high-to-low affinity conversion increases less and less with increasing temperature and even decreases above 40 °C. Stabilization of the long NH₂-terminal helix pair of the GrpE dimer with an engineered inter-subunit disulfide bond (R40C) indicated that the helix pair is responsible for the thermal responsiveness of the co-chaperone. The

differential thermal behavior of DnaJ and GrpE results in a shift of DnaK from its low-affinity to its high-affinity state and therefore in a higher fraction of substrate being sequestered by DnaK. We compared the performance of wild-type GrpE with intact thermosensor as a component of the chaperone system with that of GrpE R40C with desensitized thermosensor. The chaperone system with wild-type GrpE yielded not only a higher fraction of refolding-competent substrate protein at the end of a heat shock but also protected the protein more efficiently against inactivation during a heat shock. Thus, the direct thermal response of the DnaK/DnaJ/GrpE system together with the unfolded-protein sensor of DnaK/DnaJ are essential for efficient chaperone action during heat shock and complement the slower σ^{32} -mediated heat shock response.

**SUBTLE THERMAL EFFECTS
OF *IN VITRO*
EXPERIMENTS WITH RF-FIELDS**

REQUIREMENTS, DOSIMETRY AND PERFORMANCE COMPARISON OF DIFFERENT SETUPS FOR THE EXPOSURE OF CELLS AT 900 AND 1800 MHz USED IN CURRENT STUDIES

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OBJECTIVES: For a sound interpretation of RF-*in vitro* bio-experiments, reliable exposure units exhibiting a well-controlled SAR and temperature distribution are needed. The technical requirements are high peak and time averaged exposure of the cells with a minimal temperature rise for the cell cultures, flexible modulation schemes, high uniformity and low variability of exposure as well as support of blinded protocols. Therefore, the performance of different setups used in current studies (e.g. REFLEX, Perform-B) is compared with respect to these parameters. Published results of the dosimetric analysis are reviewed and summarized. These include the exposure systems based on waveguide resonators [1,2,3,4], the Wire-Patch cell [5] and a transverse electromagnetic (TEM) cell [6].

METHODS: The methodology for the evaluation, optimization and characterization of the exposure setups used is summarized. FDTD simulation platforms were applied in order to compute the SAR distributions. Based on the calculated SAR distribution the temperature distribution during exposure was evaluated. For the simulations, high-resolution models including details such as precise meniscus models at the solid/liquid interface as well as all relevant plastic parts were generated. The numerical results were verified using appropriate experimental tools equipped with E- and H-field as well as dosimetric field and temperature probes.

RESULTS: Different *in vitro* exposure setups have been compared for their performance with respect to uniformity of SAR, temperature rise, SAR efficiency and exposure and environmental control. For the exposure of cell monolayers the key parameters of the waveguide setup [1][2] are the low non-uniformity of SAR (< 30%), the low temperature rise for the cells (< 0.03°C per W/kg) and the high SAR efficiency (> 50 W/kg per W input power at 1800 MHz), while providing full exposure and environmental control. Reasonable performance is achieved with the resonator and TEM cell [3][4][6].

Uniformity of SAR is a fundamental issue for cell suspensions. A non-uniformity of less than 30% was not achieved by any of the setups. The best performance for cell suspensions is given by the TEM cell [6] with a non-uniformity of SAR of 46% and a temperature rise of 0.05°C per W/kg. Acceptable performance is also achieved with the waveguide system [1][2] and resonator [3][4]. The Wire-Patch [5] cell for the investigated configuration without cooling cannot be recommended, because of its high temperature rise. However, the improved design including water-cooling and SAR monitoring exhibits a much better performance.

The temperature rise is an issue of concern for all *in vitro* setups. Measures have to be taken in order to suitably control the temperature rise and to maintain equal temperature profiles between exposure and sham.

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DIELECTRIC PROPERTIES OF CELL MEMBRANE MOLECULES AND THEIR INFLUENCE ON THE SUBCELLULAR RF-FIELD-DISTRIBUTION

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The electric field distribution at the cellular and subcellular levels is based on various mechanisms that depend on electric surface, interfacial, bulk and molecular properties [1]. Cells are compartmentalized and consist of complexly arranged various media with very different frequency-dependent properties. Compartmentalization is the reason for the dominating structural dispersions. Nevertheless, structural dispersions can strongly be modulated by molecular properties. Up to GHz-range frequencies both contributions dominate the frequency dependencies of the field distribution and the local absorption at the subcellular level.

Field distribution and energy absorption have been considered for two model systems, the human red blood cell (HRBC) [2, 3] and lipid vesicles [4]. The frequency-dependent properties of the most abundant molecules were obtained from literature studies and own experiments, respectively. For HRBCs the cytoplasmic properties are mainly determined by the properties of hemoglobin (Hb) and cytoplasmic water. For modeling, the lipid phase of the membranes their structure can be taken into account by a sandwich structure of regions of different frequency-dependent behavior, i.e. bound water, hydrophilic lipid headgroup, and hydrophobic lipid chain-regions as well as orthogonal segments formed by transmembrane proteins. We found that the anisotropic properties of the lipid headgroup region has a strong influence on the subcellular field distribution, leading to a local energy absorption increase by one order of magnitude.

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MORE HEAT THAN LIGHT: THE DIFFICULTY OF SEPARATING SUBTLE THERMAL EFFECTS OF MICROWAVES FROM SUBTLE THERMAL ARTIFACTS

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Separating "thermal" from "non-thermal" effects has long been controversial in radiofrequency (RF) bioeffects research. For generations, scientists have conducted studies in search of effects of RF exposure at low levels, looking for "non-thermal" effects. Numerous "non-thermal" effects have been reported over the years -- frequently from exploratory or otherwise preliminary research projects. This talk will examine several aspects of this issue.

There are a variety of well-established mechanisms for thermal effects of RF energy. Biochemical reactions typically vary in rate by about 3 %/°C. Thus, even modest temperature increases, particularly if they persist over appreciable times, can elicit biological effects. There are, in addition, examples of exceptional temperature sensitivity in biological systems. For example, thermal receptors in the human body or infrared receptors in some species of snake respond to very small changes in temperature, as little as a few hundredths of a degree or less, regardless of whether the temperature changes are induced by RF energy or other forms of heating. Pulsed microwave energy will generate acoustic transients in tissue due to the abrupt but very small (microdegree) increases in tissue temperature with a consequent abrupt (but very small) thermal expansion. These transients, under appropriate exposure conditions, can be perceived by human subjects as "clicks", the so-called microwave auditory effect. The human body has an exquisite system for thermoregulation, with temperature sensors located in the hypothalamus, skin, and other regions. Small changes in temperature of these receptors, at levels that might be considered "insignificant" with respect to thermal injury, can activate the system of thermoregulation, and may be responsible for some effects in vivo as well as in vitro, which have previously been considered "non-thermal". These effects are unequivocal examples of RF exposures providing sensory, and hence informational, input to the organism in the absence of "significant" temperature increase. However, these effects require very specific exposure conditions, which are typically very different from encountered with wireless communications, and consequently their implications are extremely limited with respect to possible biological effects of mobile communications signals at ordinary levels of exposure.

The temperature sensitivity of biological systems has, however, led to immense difficulties over the years in separating "thermal" from "non-thermal" effects, the latter being considered to arise from direct interaction of the electric or magnetic field with the biological system. In countless studies starting in the mid-1950s, investigators have exposed biological preparations to RF energy and compared various measured endpoints with those from unexposed controls, reporting any changes that were observed in the absence of what the investigators considered to be significant temperature change as "non-thermal" effects. However, many of these reported effects have been subject to unsuccessful attempts by other investigators to confirm their existence, frequently in studies that were better designed and better conducted than the original studies that reported the "effects". Difficulties include inadequate exposure assessment (which is even now a problem with many studies), inadequate temperature control, and lack of a mechanistic hypothesis which allows the investigator by which the significance of potential artifacts can be judged. Since absorption of RF energy is necessarily associated with temperature increases at some level in the exposed preparation, and such temperature changes can be extraordinarily difficult to predict, measure and control, this is an obvious potential source of experimental artifact.

This talk will conclude with a discussion of several examples of the difficulty of adequate temperature control that have been documented in previous bioeffects studies. These include the difficulty of controlling temperature when exposure apparatus is located within thermostatically controlled incubators, and high-SAR studies involving small biological preparations inside thermostatically controlled exposure chambers.

MICROWAVE SAR AT THE DNA-SCALE

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Based upon existing data of dielectric properties of cells and molecules, we recently evaluated the relative SAR value between the frequencies of 300 MHz and 3 GHz at the scale of the nucleic acid (NA) molecules (Vanderstraeten and Vander Vorst, 2004, *Bioelectromagnetics* 25: 380-389). At the microscale, the SAR ratio between the nucleus and the cytoplasm has been found to depend on the nuclear NA concentration and to be above 1 for > 30 mg/ml. At the nanoscale, in the immediate vicinity of NA molecules, but with the exception of areas where proteins are bound to NA, the SAR value has been estimated to be one order of magnitude (up to 20 times) above its value in tissues as a whole when these are considered at the millimeter scale. This means that under common circumstances of microwave (MW) exposure, the SAR value around DNA may locally be up to nearly two order of magnitude above its averaged value over 10 g tissue (Van Leeuwen et al, 1999, *Phys Med Biol* 44: 2367-2379).

The explanation of it lies in the higher conductivity of the immediate surroundings of free DNA compared to the solution, which is due to the particularly high counterion and bound water concentration at this place. These last elements are respectively responsible for ohmic losses and for dielectric losses in the considered frequency range.

If confirmed, this observation could have biological relevance if, instead of the temperature distribution, one considers the local process of intermolecular thermal energy transfer. Considering indeed a SAR hot spot in tissues and a continuous MW exposure (in a first instance), it appears that a given temperature rising in tissues does not reflect what the thermal energy is at the particular (molecular) level of that hot spot or in the immediate vicinity of it. This concept has been underlined and validated by Hamad-Schifferli and colleagues in 2002 (*Nature* 415: 152-155). This consideration is here worth while because of the immediate proximity of the areas of maximal MW absorption with the DNA side polar chains with, whether or not we also consider the still debated possibility of a coupling between the absorbing solvent and reacting solute which is put forward in "MW-assisted" chemistry (Kalhori et al, 2002, *J Phys Chem A* 106: 8516-8524)?

Could those considerations give a new insight into the currently debated concept of "athermal" gene activation, where thermo-activated genetic processes have been reported to be activated for lower temperature thresholds under MW exposure than under conventional heating? The question to be here addressed is that of the DNA dynamics because of the presumable role of these in DNA recognition and reactivity (Michalczyk and Russu, 1999, *Biophys J* 76: 2679-2686), and perhaps that of the heat shock factor 1 (HSF1) activation too, knowing that this notably occurs among condensed DNA (Jolly et al, 2002, *J Cell Biol* 156: 775-781).

Finally, the question here addressed is that of the possible relevance of the DNA-scale for the SAR quantification under microwave exposure.

“SUBTLE THERMAL” EFFECTS OF INTERACTION OF NON-IONIZED RADIATION WITH DNA CRYSTALS: A QUANTUM MECHANICAL APPROACH

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Results of the EU project “THz-Bridge” QLK4-CT2000-00129 among other findings verified that both genotoxic and epigenetic effects were induced in lymphocytes following exposure to CW 100 GHz radiation at 0.05 mW/cm² when the exposure period exceeds one hour. Although the reported effects have been observed on cells directly exposed to non-ionizing radiation, without the shielding effect of the human body, they occurred at a relatively low intensity when compared to the exposure limits set by the ICNIRP guidelines (1mW/cm² for general public exposure and 5mW/cm² for occupational exposure). These and other findings could be explained taking into consideration that the interaction and the coupling of non-ionizing radiation with biological matter is taking place via “subtle-thermal” effects of interaction of radiation with matter which are well described quantum mechanically. In this communication we report on the following “subtle thermal” conditions:

- 1) Quantum mechanical calculations predict that weak magnetic fields could be amplified to high levels under specific flow conditions and experimental evidences verified that the state of crystallization (symmetry) of inorganic nano-composites is strongly modified in the presence of magnetic fields [1]. Such findings could have profound biological effects on blood under flow conditions in the presence of weak electromagnetic fields.
- 2) By applying quantum mechanical calculations it is verified that rotational states of DNA crystals and its bases, strongly interact with electromagnetic radiation in the spectral region ~ 1 GHz. This type of interactions could enhance the probability of DNA bond breaking in the ground electronic states in a similar way as ionizing radiation does in excited electronic states. The theoretical predictions were supported by experimental evidences by applying ultra high vacuum mass spectroscopy of DNA crystals and its bases irradiated at ~ 1GHz using the same experimental procedure as for the exposure with the ionizing radiation case [2].

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MICROWAVE RADIATION AND TEMPERATURE EFFECTS ON THE GREEN FLUORESCENT PROTEIN

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The green fluorescent protein (GFP) and its genetic mutants have been widely used as in-vivo biological markers. Fusion of GFP to another protein does not alter the function of the protein¹. The exceptional stability of the GFP allows for studying cell dynamics and cell development. As this protein is sensitive to its local pH and temperature² environment, it has been utilized as a biological sensor.

Following several reports suggesting a "non-thermal" microwave effect^{3,4,5}, we have studied the effect of microwave radiation on the fluorescence of solution-based Enhanced Green Fluorescent Protein (EGFP) and wild type green fluorescent protein (wt-GFP) and have compared that to conventional heating. We measured the spectrum of fluorescence under 488 nm excitation and in the temperature range 20-40°C. Upon increasing temperature, the intensity of fluorescence changes and the spectrum of fluorescence becomes red-shifted. The red shift gives a measure of the "local temperature" of the protein.

We found that the microwave radiation has a larger effect on EGFP fluorescence as compared to conventional heating. In particular, while thermally heating the EGFP in a buffer solution results in a ~ 1% decrease in fluorescence for every 1°C rise (at T < 40°C), a 250 mW microwave irradiation at 8.5 GHz can decrease the EGFP fluorescence by up to 3-10% with an accompanying temperature rise of only 1-2°C. This looks as if, under microwave exposure, the "local temperature" of the fluorescent molecule increases by 3-10°C while the temperature of the solution increases only by 1-2°C. As such, the "local temperature" of the protein" is distinguishable from the macroscopic temperature of the solution. We believe that bound water around the chromophore of the EGFP results in the enhanced microwave absorption and is, therefore, responsible for the enhanced microwave effect on fluorescence. This may suggest that the microwave radiation effect on this protein is mostly thermal yet it is different from conventional heating.

A similar study was performed on wt-GFP. However, the temperature increase and the microwave effects were found to be indistinguishable. Moreover, in the temperature range 20 - 40 °C, the fluorescence linearly increases with temperature and microwave irradiation, in contrast to the behavior of EGFP where fluorescence decreases with temperature. We attribute this to the higher quantum efficiency of the wt-GFP fluorescence. In other words, the wt-GFP molecule is more rigid which precludes quenching of fluorescence here as compared to EGFP. The increase of fluorescence of wt-GFP with temperature probably points to a thermally-activated process.

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⁴ D. de Pomerai, A. Dawe, N. Vasic, and D. Thomas, *Cost281*, Helsinki, Finland, p.24, (2004).

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**POSSIBILITY OF GENERATION
OF SUBTLE THERMAL EFFECTS IN
RF-EXPERIMENTS WITH
ANIMALS AND HUMAN VOLUNTEERS**

NUMERICAL AND EXPERIMENTAL DETERMINATION OF SAR AND TEMPERATURE DISTRIBUTION FOR 'IN VIVO' BIO-EXPERIMENTS INVOLVING RADIO FREQUENCY EXPOSURE

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The determination of the electromagnetic field distribution and specific absorption rates (SAR) for experiments concerning radio frequency (rf) exposure of humans or animals are extensively discussed in the literature for the last decades. Usually, the computation of field and SAR distribution is performed by application of numerical methods, e.g. Finite-Difference Time-Domain (FDTD) method. In the FDTD the tissue distribution of real bodies is approximated by models consisting of small tissue-cuboids (voxels). The coupling of the incident field to the body depends on various parameters, e.g. frequency, spatial distribution of the incident field (whole body or partial body exposure), shape and dimension of the body.

In order to validate the computed SAR distributions by measurements samples of the temperature can be taken in simplified homogeneous phantoms or cadavers. For this purpose the temperature increase at specified locations during a short time span after switch-on of the rf-exposure, when heat transfer modes like conduction and convection are negligible, is recorded.

In the literature there are only a few contributions available dealing with the temperature distribution inside human or animal bodies, especially as a result of absorbed rf-energy. Measurements of the body core temperature of rodents as function of the whole body SAR showed that it is essential to take into account the thermoregulatory system of the respective species, since the increase of core temperature of mice and rats are different for the same whole body SAR.

One approach for the numerical computation is to utilize the so-called bio-heat equation. This equation takes into account the blood flow in tissue as well as heat generation due to metabolic processes. It can easily be implemented in a Finite Difference scheme which makes it possible to use the same tissue model as for the field computation. Further, the SAR distribution obtained from the field computation can directly be impressed as source without data conversion. With this procedure - and provided that proper thermal tissue parameters are available - it is possible to compute the time-dependent temperature distribution inside humans and animals as function of SAR, but without consideration of the thermoregulatory system. A first approach to model the thermoregulatory system was applied for the calculation of temperature increase inside the human body due to rf-exposure. Amongst other modifications, thermoregulatory mechanisms like sweating and vasodilatation as function of the temperature in specific parts of the human body were considered in addition to the temperature computation with the bio-heat equation. If the main mechanisms of the thermoregulatory system of a test animal are described in a similar way, these mechanisms can be taken into account for the realistic calculation of the temperature distribution in rf-exposed animals.

INCREASE IN SKIN TEMPERATURE DURING MOBILE PHONE CALLS. THE EFFECT OF RF EXPOSURE AND OTHER FACTORS EXPLORED IN EXPERIMENTAL AND THEORETICAL STUDIES

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Some people complain about the sensation of warmth on or around the ear when using their mobile phone. In a Norwegian-Swedish epidemiological investigation warmth sensation was the most common complaint attributed to the use of mobile phones, and in particular it was a result of long mobile phone calls (6). People experiencing the warmth sensation tend to focus on the radio frequency (RF) exposure as the reason for heating. In this presentation, also the contributions to change in skin temperature from two more factors are addressed. The first is the thermal insulation provided by the phone box. The second is the heating of the phone box caused by energy dissipation when currents are drawn from the battery.

Both theoretical and experimental studies have been conducted. Among the theoretical studies one has explored the effect of all three factors (3), two have included the combined effect of insulation and phone heating as well as calculated the effect of RF exposure (2, 4), while two publications have considered the effect of RF fields alone (12, 13). In all theoretical studies the temperatures have been calculated by applying a mathematical head model, using a bio-heat equation, and the finite-difference time-domain method. The resulting peak temperature in each of the actual tissues has been presented.

Experimentally the skin temperature has been measured either by using an infrared camera (5, 8, 9, 10), infrared thermometer (7), or temperature probe in contact with the skin during (11, 12.) or immediately after (1) the exposure. In two of these studies (7, 9) the effect of both the insulation, the phone heating and the RF exposure has been investigated. In most of the experimental studies a GSM phone operating at approximately 900 MHz has been used, and similarly, in most of the theoretical studies exposure to frequencies around 900 MHz has been considered.

The skin area that is heated the most during mobile phone use is the ear. When holding a GSM900 phone that is switched off in the normal talking position, the average skin temperature in this region may increase about 1.5 °C after long time exposure; but not all experimental data demonstrate an increase in temperature by insulation alone. When switching the phone on (without RF exposure), experimental results suggest that the heating of the phone due to energy dissipation causes a further increase in temperature of less than 1 °C, while theoretical calculations indicate almost no further temperature increase. Both experimental and theoretical data show that the additional effect by RF exposure is negligible; calculated to be less than 0.05 °C. When applying RF exposure alone (no insulation and phone heating), the temperature rise is slightly higher than due to RF exposure added to the other factors, but still it is very low (less than 0.14 °C). When applying higher frequencies (1800 – 1900 MHz), a relatively higher portion of the transferred RF energy is absorbed by the pinna. However, since the maximum average output power of a GSM1800 phone is lower than that of a GSM 900 phone, the heating caused by the RF exposure is not indicated to be higher.

The values given above are results from mobile phones operating at maximum output power and with exposure times sufficient to reach (or almost reach) the steady state temperatures. Therefore, in practice the resulting temperature increases are often lower. Also other factors, such as antenna and mobile phone design and the position of the phone, as well as the initial skin temperatures and various anatomical and physiological factors influence the increase in skin temperature. The overall results, however, indicate that the main reason for

the increase in skin temperature after long duration calls is the heat insulation caused by the phone box. The power dissipation causing a heating of the phone may increase the skin temperature somewhat further, while the contribution from the RF exposure seems to be insignificant compared to the contribution from the insulation and the phone heating.

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SAR AND TEMPERATURE ELEVATIONS IN THE HUMAN HEAD DURING RF EXPOSURE

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The resulting absorption during RF exposure of the human head is under investigation since years. In recent years the rapidly increasing performance of hardware as well as software tools for numerical RF dosimetry enabled increasingly detailed insights into the detailed spatial distribution of power absorption and corresponding temperature elevations in the tissue.

Provided proper source modelling, today the uncertainty in solving the electromagnetic problem (i.e. calculation of spatial SAR distribution for a considered exposure scenario) is mainly caused by the limited spatial resolution of the body models, the available hardware as well as software resources, the tolerable computation times and individual variations of anatomical and dielectric tissue parameters. However concerning the thermodynamic problem (i.e. calculating the tissue temperature elevations caused by the given SAR distribution), there are several additional methodological sources of uncertainties. Due to the thermally active and complex behaviour of living tissue the physical and mathematical models currently applied must be seen as more or less coarse approximations of real situations. Most of the work published so far in the context of exposure to RF communication devices is based on the Pennes' Bio Heat Transfer Equation (BHTE) which considers the different body tissues as a continuum model and which takes into account (at least in a static and linear manner) heat transport by blood perfusion and a metabolic heat generation. More sophisticated models are known from the field of diathermy, e.g. the Discrete Vasulature (DIVA) Model published by a group from Utrecht in 1996 and 1999 [1-2], respectively, which allows to consider also the impact of discrete larger blood vessels.

Concerning the maximum temperature elevation in the brain due to mobile phone usage several papers (e.g. [3-5]) were published in recent years. Depending on the considered exposure situation (source model, source power, distance to the head, head model) and the details of thermal modelling the reported values ranged between approximately 0.12°C and 0.5°C, for a 1g averaged spatial peak SAR of 1.6 W/kg in the brain.

In this talk the expected temperature elevation in the head and brain of human test subjects of recently finished provocational studies with different exposure setups [6-7] will be discussed.

Furthermore an overview of currently ongoing work concerning RF absorption in anatomical small head structures (eye, inner ear organs) will be given.

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EFFECTS OF NON-THERMAL EMF (GSM; UMTS) ON HAMSTERS AND MICE: IMPLICATIONS FOR THE DEFINITION OF "NON-THERMAL"

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With regard to the biological effects of non-ionizing electromagnetic fields (EMF) between 10 MHz and 10 GHz, the definition of "non-thermal" is based on the assumption that temperature effects below a certain threshold are considered harmless (ICNIRP 1998). In humans, exposure levels of approximately 4 W/kg SAR for 30 min. cause temperature rises > 1°C. Thus, maximum occupational exposure (OE) levels were set to 0.4 W/kg (safety factor 10), and maximum exposure levels for the general population (GP) to 0.08 W/kg (safety factor 50). To study the long-term (days to years) effects of exposure to EMF close to GP or even OE levels, animal studies were performed and are under way. Own studies in hamsters (Lerchl et al., 2000) and mice (Sommer et al., 2004; UMTS study in preparation) have shown no adverse biological effects of exposure at 383 MHz (TETRA), 900 MHz and 1800 MHz (GSM) at SAR values of 0.08 W/kg (hamsters) and 900 MHz (GSM) and 1966 MHz (UMTS) at SAR values of 0.4 W/kg (mice). However, both the 383 MHz and 900 MHz studies in hamsters and the 900 MHz study in mice revealed significantly (p between < 0.01 and < 0.001) increased body weights in the animals, while the 1800 MHz and UMTS exposure did not affect the body weight significantly. These effects are consistent with the hypothesis (Sommer et al., 2004) that EMF exposure may lead to metabolic shifts, i.e., decreased utilization of food for heat production in exposed animals. This hypothesis is also supported by recent findings in rats (Koyu et al., 2005) showing markedly decreased levels of TSH, T3 and T4, thus a decreased thyroid function which is an indicator of lowered metabolism and heat production.

Conclusion: Absorbed EMF energy may not cause thermal, but metabolic effects, especially in thermo-regulating mammals which try to keep their body temperature constant. Thus, instead of defining biological effects of EMF exposure only via changed or unchanged temperatures, one should consider "non-metabolic" or "metabolic" effects of EMF exposure as well, e.g., as reflected by changes in the physiological heat production. However, one must also take into account the fact that any dose-response relations (SAR vs. metabolic effect) may not be linearly transferable to humans because the metabolic rates are non-linearly related to the body mass.

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PRACTICAL SUGGESTIONS ON THE USE OF THERMAL THRESHOLDS IN ANIMAL STUDIES ON ELECTROMAGNETIC FIELDS (EMF)

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When performing animal studies on potential adverse effects of EMF, energy is added to biological systems. As a consequence of this, any addition leads to thermal reactions in animals, and there is no real "athermal" range. Attempts were made to introduce such a threshold at the energy flow density that leads to an increase in rectal body temperature of 1 °C, but this appears to be rather artificial.

In order to give practical guidance for study directors, it was suggested to use as a high "dose" energy flow densities high enough to ensure the application of appropriate safety factors. The basis for such a comparison could be the exposure assessment of humans, produced by base stations and/or mobile phones using a worst case scenario. The use of lower dose group(s) is strongly recommended in order to find potential dose-response relationships as well as (threshold) "doses" without effects.

When testing chemicals, normally a safety factor of 100 (10 for interspecies differences, 10 for differences in susceptibility in humans) in extrapolation from animal studies to the human exposure has proven to be protective. It must be further discussed whether this safety factor should be the same when testing EMF. On one hand, the safety factor of 100 is applied when a broad spectrum of guideline studies in at least two species can serve as a basis. This situation is not given in the case of EMF, suggesting a higher safety factor. On the other hand, the safety factor partly consists of metabolic differences between the animal models and humans, which does not play a role for EMF. Moreover, the "geometry" of the used animal models may be considered an "intrinsic safety factor". The latter two facts would suggest the use of a lower safety factor.

When performing these animal studies, the body temperature of the animals as well as the cage temperature should be measured in an appropriate way, either during the study or in pilot investigations. For the (rectal) body temperature, this could either be done towards the end of the daily exposure period in order to determine an expected increase in temperature or soon after switching off the exposure in order to determine a transient decrease in body temperature in response to stopping the external administration of energy. By doing this, it should be shown that the selected exposure range does not cause "excessive" heating (e.g. more than 1 °C in body temperature during exposure).

With an exposure like this and using a wide spectrum of study types (based on toxicity testing) but including modified endpoints (more functional ones), either no effects will be observed or it will be possible to find (reproducible) effects. In the first case, this indicates the (relative) safety of the exposure, while in the latter it is appropriate to check whether these effects are (solely) produced by heating and/or if these effects are species specific or can be extrapolated from animals to man. But even when it can be shown that they are thermal ones, but their occurrence in humans cannot be excluded, then the effects still remain relevant. Since the endpoints investigated are based on (modified) toxicity test guidelines, it can be assumed that any deviation from "normality" (i.e. the concurrent control group) will be regarded as adverse unless the opposite is proven. A final assessment will often only be possible after investigation of the observed effects in more detail, i.e. in collaboration with basic researchers.

The thus established experimental data base can then be used to determine the no observed adverse effect level (NOAEL) for the given type of exposure. By applying the above mentioned safety factors, a safe exposure for humans can be assessed.

The suggested approach could serve as a basis for discussion within a (FGF) workshop (Testing EMF for adverse effects: similarities and differences from chemical testing).