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Knowledge Gaps in Genotoxicity Studies for RF EMF Risk Assessment

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Knowledge gaps in genotoxicity

Knowledge gap 1: Is there a mechanism, which enables genotoxic effects of RF-EMF below the current exposure limits?

Knowledge gap 2: The design of suitable exposure setups for experiments on genotoxic effects of RF-EMF does not seem to be limited by knowledge gaps.

Knowledge gap 3: Are methodological shortcomings or real effects responsible for the controversial reports on RF-EMF induced DNA-strand breaks detected by the Comet assay?

Knowledge gap 4: What are the reasons for the controversial results on the classical genotoxicity parameters like chromosome aberrations, micronuclei and sister chromatid exchanges?

Knowledge gap 5: Can the controversial results on genotoxic action of RF-EMF be explained by different sensitivity of various cell types?

Knowledge gap 6: Is it possible to perform investigations on genotoxic effects in humans based on a reliable dosimetry?

Knowledge gap 7: Are RF-EMF able to act genotoxically inside a living human body?

Knowledge gap 8: Do RF-EMF and chemical mutagens like mitomycin C act synergistically?

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1 Introduction

Radiofrequency electromagnetic fields (RF-EMF) are used in an increasing number of technical devices. Although RF-EMF have been applied in TV- and radio-transmission as well as also in medical treatment since decades, concern about health risks caused by these fields has gained public awareness mainly since the worldwide introduction of cell phone networks. A major concern is an increased risk of cancer primarily tumors of the head. According to the actual hypotheses cancer is initiated by mutations of the DNA in the genome. If RF-EMF were able to induce genomic mutations, they would act genotoxically and they could initiate tumors. Such a link would have numerous consequences concerning the use of RF-EMF in technical devices and the respective exposure limits.

Up to now an impressive amount of investigations on genotoxic effects of RF-EMF has been performed. There is a number studies who found genotoxic effects of RF-EMF, however the majority of studies in this field where negative. The aim of this evaluation is to identify knowledge gaps in this field. Future experiments may thus be planned to answer the open questions. However, as present exposure limits are in accordance with international standards it is not necessary to report health relevant influences of RF-EMF above the actual exposure limits.

As aim of this review is to identify knowledge gaps concerning genotoxic action of RF-EMF, only publications will be discussed, which investigate the mutagenic effects of RF-EMF directly. Studies on tumor initiation or promotion are not included because they provide only indirect evidence for genotoxic effects of RF-EMF. Many investigations in this field have been performed during the past 25 years, therefore most questions have been addressed by more than one study and there are often controversial results. Does that mean that the different results are based on a knowledge gap or on studies of different quality? This question may explain that the identification of a knowledge gap or the denial of one represents the personal opinion of the author. It is important to mention that this assessment is not designed to give a complete review of the literature, aim is to identify knowledge gaps by identification of controversial issues.

A number of actual reviews in the field have been screened (Heynick et al., 2003; Meltz, 2003; Vijayalaxmi und Obe, 2004; Verschaeve, 2005) as well as the cited original research publications.

2 Interaction mechanisms between RF-EMF and the genome

Electromagnetic fields of different wavelengths interact in specific ways with biological organisms. At low frequencies between 1 and 10000 Hz the most relevant changes are caused by magnetic fields which induce electrical currents in the living tissue. These currents are able to elicit action potentials in excitable cells, like neurons, skeletal muscle or cardiac muscle cells. Magnetic stimulation of the central nervous system used in therapeutic treatment is based on this effect. At higher frequencies in the range of RF-EMF (10^5 – 10^{10} Hz) the fields are absorbed in the aqueous compartment of tissues, leading to oscillations of the water molecules. These oscillations cause heating of the water and thus the tissue. At higher frequencies, starting in the range of UV light, absorbed photons have sufficient energy to break chemical bonds in molecules or to ionize chemical elements. Fields of these frequencies are thus called ionizing radiation. Ionizing radiation acts genotoxically. The three mentioned interaction mechanisms are well understood and the present exposure limits are based on their health relevant effects. In case of RF-EMF exposure limits warrant that a temperature rise of biological tissue is kept below 1°C to avoid hazardous heating. Therefore the amount of heat is limited by defining the specific absorption rate (SAR) measured as W/kg. Due to the frequency dependent interaction mechanisms it is not possible to extrapolate the genotoxic action of ionizing radiation to the RF-EMF frequency range. However, as there are publications in the literature which have reported genotoxic damage caused by RF-EMF fields, the question remains, whether there are unknown interaction mechanisms between RF-EMF and biological tissue, which are able to damage the genetic material of a cell at field strength below the exposure limit. One hypothesis which has been proposed is the increased formation of radical oxygen species (ROS) in the presence of RF-EMF (c.f. chapter 4 Lai and Singh, 1997; European Union 2004 a). Interestingly two studies (Lantow et al., 2006; Simko et al., 2006) which were carried out on a Monocyte cell line failed to demonstrate an increased ROS production due to RF-EMF exposure at 1800 MHz and SAR up to 2 W/kg.

> Knowledge gap 1: Is there a mechanism, which enables genotoxic effects of RF-EMF below the current exposure limits?

3 Exposure setups

The reliability of experiments depends highly on the quality of the exposure setups. Exposure setups, which produce higher field strength than expected, may lead to false positive results or in case of lower field strength than expected false negative results may be obtained. False positive results may be misinterpreted as a knowledge gap, therefore the quality of exposure setups is discussed here. In general there are different approaches to test the influence of RF-EMF on the DNA:

1. Living animals are exposed to the field, and afterwards possible changes due to genotoxic action of RF-EMF are investigated in various tissues.
2. Cell cultures or isolated cells are exposed to RF-EMF fields, and later on checked for mutations.

There are some important properties, which an exposure device, should have. The field strength or the SAR inside the specimen should be well defined and be as homogeneous as achievable. In addition heating of the specimen should be avoided as this may cause adverse effects. In case of *in vivo* experiments the comfort of the living animals should be taken into account, in order to avoid stress induced changes. In the last years sophisticated exposure setups have been developed for *in vivo* as well as *in vitro* experiments.

In case of *in vivo* studies animals may be exposed either freely moving in their cages or in restrainers. Restrainers have the advantage that the RF-EMF is constant and can be easily modelled. This leads to a good reproducibility. However, animals are not used to stay in a restrainer and they may experience this as stress, which by itself may have an effect. From biological point of view exposure setups, which allow the animals to move within their cages, are superior, usually this is accompanied by a changes in the absorbed field depending on the location of the animal in the cage. In a setup by the group of Hansen the two contradictory demands of the well being of the laboratory animals on the one hand and the reproducibility and homogeneity of the RF-EMF have been joined to a good compromise (Hansen et al., 1999). The setup consists of a radial waveguide of 4 m in diameter, which is closed by an absorber. Near to the absorber up to 24 cages are placed. The standard deviation of the SAR inside the body of the mice employed in a study on the effects of RF-EMF was ± 40% (Sommer et al., 2004). The mice stayed inside the waveguide for 24 h daily for 280 days except the times when the cages were cleaned and the animals were inspected.

In case of *in vitro* experiments the most widely used setup of the last years shall be introduced as example. The group of Kuster designed a setup which is based on a rectangular waveguide, which is short-circuited by a terminating plate, thus leading to a standing wave (Schönborn et

al., 2000). Petri dishes with cell cultures are placed in the maxima of the standing wave. The original setup is suitable for frequencies 1.2 to 1.7 GHz. In a version for the frequency range around 900 Hz and one for the frequency range around 1800 Hz this setup became a reference in the REFLEX project (Schuderer et al., 2004a, b; European Union, 2004) and was also applied in further *in vitro* studies on the influence of RF-EMF fields emitted from mobile phones.

Although improvements of exposure devices are still desirable, good exposure devices have been designed for *in vivo* as well as *in vitro* studies The necessary know how for the construction of such devices has been published. From a biologist´s point of view the construction of appropriate exposure devices does not seem to be limited by knowledge gaps, however, it is intellectually demanding and expensive.

Knowledge gap 2: The design of suitable exposure setups for experiments on genotoxic effects of RF-EMF does not seem to be limited by knowledge gaps.

Older studies may suffer from insufficient exposure setups, this makes it very difficult to decide retrospectively whether a study is reliable or not. One example not taken from studies on genotoxicity, but on gene regulation, shall explain this problem. In 2000 de Pomerai et al. published a non thermal induction of heat shock protein 16. The experiments were performed in a transversal electromagnetic cell (TEM-cell). After re-evaluation of the TEM-cell a temperature rise of 0.2°C during RF-EMF exposure was shown (Dawe et al., 2006). Changes in the TEM-cell reduced the temperature rise to 0.1°C without changing the RF-EMF. In the improved TEM-cell the RF-EMF dependent rise in hsp16 disappeared and the original publication had to be retracted (de Pomerai et al., 2006)

4 Do RF-EMF cause DNA strand breaks detected by the Comet assay?

In the past years the Comet assay has been applied in many studies which focus on genotoxic effects of RF-EMF. The Comet assay is suited to prove single and double strand breaks of the DNA. The advantage of the Comet assay is, that it is relatively easy to execute, but it is sensitive to artefacts and the results may thus be misinterpreted easily (Tice et al., 2000).

In the middle of the ninetieth a series of publications from Lai and Singh (1995; 1996a; 1996b; 1997) demonstrated DNA strand breaks by the alkaline Comet assay in the brain of rats after exposure in 2450 MHz fields SAR 0.6 or 1.2 W/kg for 2 h. Surprisingly the DNA strand breaks were more distinct if the animals were sacrificed 4 h after exposure than 2 h

after exposure. As these results are important considering the situation, that using a mobile phone the highest SAR-values are found in the head, they received high scientific and public attention. However, the observation that DNA strand breaks are more distinct after 4 h than after 2 h does not seem logical as repair mechanisms should have eliminated some of the strand breaks already (Williams, 1996). Lai and Singh (1996b) replied that this phenomenon had already been observed by others. In their latest publication they tried to elucidate the mechanism by which RF-EMF may cause DNA-strand breaks in the rat brain, they published that an injection of scavengers like melatonin reduces the RF-EMF induced damage (Lai and Singh, 1997). Although the results gained very high attention, Lai and Singh stopped working on this topic, however, their findings stimulated a number of new investigations. In one study of Malyapa et al. (1998) the exposure regimen of Lai and Singh was reproduced. To get better insight into the mechanisms, Malyapa et al. (1998) sacrified the rats either by CO_2 intoxication like Lai and Singh or by decapitation. In general, they could not reproduce the findings of Lai and Singh, but in the group of CO_2 intoxication the data scatter was much higher. Malyapa et al. (1998) suspect that this may be a reason for the increased DNA-strand breaks in the studies of Lai and Singh. However, this is not more than a hypothesis, more important is, that Malyapa et al. (1998) were not able to reproduce the findings of Lai and Singh.

In an *in vitro* study Malyapa et al. (1997a) tested the same topics on human glioblastoma cells of the line U87MG. The cells were exposed at 2450 MHz with a 0.7 and 1.9 W/kg SAR for 2 h and were allowed to rest for 4 h after exposure. In addition also murine fibroblasts C3H 10T½ were investigated using the same exposure regimen. The analysis performed with the alkaline Comet assay did not reveal an effect of the exposure. This study was complemented by a study testing cell telephone signals of 835 MHz at 0.6 W/kg SAR on the same cells for 24 h (Malyapa et al. (1997b). Again no RF-EMF induced changes could be detected.

There is an interesting discussion on the findings of Lai and Singh in the review of Vijaylaxmi and Obe (2004), which takes more literature into account than the works mentioned here. But they cannot solve the problem of the findings of Lai and Singh. Some papers published after the review of Vijaylaxmi and Obe (2004) deserve to be mentioned here in this context. An important publication is that of Lagroye et al. (2004a) which was designed as a reproduction of the Lai and Singh studies and tested also differences in the Comet assay protocol between the studies of Malyapa et al. (1998) and Lai and Singh (1995). The exposure setup consisted of waveguides comparable to that of Lai and Sing as well as Malyapa et al. (1998). The exposure regimen followed that of Lai and Singh 2450 MHz 1.2 W/kg SAR for

2 h and the animals were sacrificed 4 h later. No DNA damage could be detected independently of the protocol of the applied Comet assay. This reproduction study clearly failed.

Lagroye et al. (2004b) performed a methodologically important attempt to explain the differences between the studies of Lai and Sing and Malyapa, which are discussed above. There are two variants of the alkaline Comet assay one with the use of proteinase k dependent digestion of the specimen and one without. The experiments were performed *in vitro* on cells of the murine fibroblast line 10½, which were exposed to RF-EMF of 2450 MHz at 1.9 W/kg for 2 h. Two positive controls were executed with the chemical mutagen cisplatinum or with γ-rays. However, the 2-h exposures did not induce measurable alkali-lable DNA damage nor DNA-DNA or DNA-protein crosslinks. Thus the methodological differences between the studies of Malyapa et al. (1997a, b; 1998) and those of Lai and Singh (1995; 1996a; 1997) are not responsible for the conflicting results.

As the extensive efforts to reproduce the results of Lai and Singh failed, one may judge them as being artificial. But this does not answer the question, whether RF-EMF is able to cause DNA strand breaks. Therefore further studies are presented.

Recently Paulraij and Behari (2006) have published an *in vivo* study on rats, which is not a reproduction study of Lai and Singh but focuses on the developing rat brain. Young rats (35 d) were exposed by a horn antenna in an anechoic chamber to 2450 MHz and 16.5 GHz at 1.0 and 2.01 W/kg SAR, respectively, daily for 2 h 35 days. The repetitive exposure and the age of the rats differ clearly from the design of Lai and Singh. After the end of the exposure period brain tissue was analysed by alkaline Comet assay and a highly significant increase in single strand breaks was found.

As part of the REFLEX project the group of Rüdiger investigated the influence of RF-EMF on human fibroblasts and rat granulosa cells (European Union, 2004a; Diem et al., 2005). The *in vitro* exposure was performed in the described exposure setup of (Schuderer et al. 2004b) with 1800 MHz modulated in various ways at 1.2 or 2 W/kg SAR for up to 24 h. The authors detected an increase in all investigated parameters like DNA-strand breaks monitored by neutral and alkaline Comet assay as well as chromosome aberrations (CA) and micronuclei (MN) at 2 W/kg after 4 and 24 h exposure. The results were criticised by Vijayalaxmi et al. (2006) especially because of the visual evaluation of the comets. An independent reproduction of the results of Diem et al. (2005) was tried by using the same cells and the same exposure setup in another lab (Speit et al., 2007). In addition, also V79 cells, a Chinese hamster cell line, which is frequently used in genotoxicity testing, was included in the study.

Neither in the human fibroblasts nor in the hamster cells any genotoxic influence of RF-EMF could be detected. The findings of Diem et al. (2005) became thus less plausible.

Two further studies also performed as part of the REFLEX (European Union, 2004a) project deserve to be discussed here. One interesting study has not been published in a scientific journal yet. Participant 2 Tauber exposed HL-60 (human leukaemia line) cells at 1800 MHz pulsed in various ways at 0.2 to 3 W/kg SAR for 2 to 72 h. The investigated biological endpoints were MN, cell cycle, comets and reactive oxygen species (ROS). The main result is a SAR dependent increase in MN and comet tail moment. The increases did not follow a simple dose response relationship, but they were limited to intermediate SARs of 1.3, 1.6 and 2 W/kg. Lower and higher SARs did not cause changes, thus the result represents a so called “window effect”. The authors attribute the RF-EMF influence to an elevated ROS production as the effects could be reduced by addition of ascorbic acid.

The results gathered in the REFLEX-project by participant 4 (Wobus) were compiled in two publications Czyz et al. (2004) and Nikolowa et al. (2005). Both publications deal mainly with the influence of RF-EMF on gene expression, however there are also some experiments included, which were performed with R1 murine stem cells to monitor CA, sister chromatid exchanges (SCE), proliferation, differentiation and DNA-strand breaks. The cells were exposed at 1710 MHz at various pulsation patterns with SARs between 0.1 and 1.5 W/kg in the exposure setup of Schönborn et al. (2000) described in chapter 3. In the neutral but not in the alkaline Comet assay the authors detected an increased comet tail factor after 6 h exposure at 1.5 W/kg.

It should be mentioned here, that studies discussed in chapter 8 like Maes et al. (1997), Baohong et al. (2005) Stronati et al. (2006), and Verschaeve et al. (2006) could not prove DNA strand breaks caused by RF-EMF.

This summary on experiments performed with the Comet assay does not review the complete literature, further information is found in Heynick et al. (2003), Meltz (2003), Vijayalaxmi and Obe (2004), and Verschaeve (2005). However, the discussed examples demonstrate that the majority of experiments on DNA-strand breaks are negative, but positive findings appear regularly, therefore the reason for this diversity should be clarified.

Knowledge gap 3: Are methodological shortcomings or real effects responsible for the controversial reports on RF-EMF induced DNA-strand breaks detected by the Comet assay?

5 Do RF-EMF cause chromosome aberrations, micronuclei or sister chromatid exchanges?

Studies dealing with the question whether RF-EMF cause CA, MN or SCE have been performed on many different cell types. They have been discussed extensively in the four mentioned reviews (Heynick et al., 2003; Meltz, 2003; Vijayalaxmi und Obe, 2004; Verschaeve, 2005). An evaluation of the new publications by the German radiation protection committee is going to appear in 2007. Many of these studies are also presented in the other chapters of this survey, therefore they are not demonstrated here again. The authors of the different reviews report positive and negative studies. The most comprehensive review of Vijayalaxmi and Obe (2004) presents 58% negative studies and 23% with an increase in damaged cells due to RF-EMF exposure, 19% studies were judged to be inconclusive. Thus there is a controversy in the literature about the different findings. Although positive studies are presented in all reviews, the authors do not interpret these as a "reproducible scientific valid experimental basis" (Heynick et al., 2003). Vijayalaxmi and Obe (2004) list a number of methodological problems like insufficient temperature control or shifts in the pH, which may be the reasons for the conflicting results. But this does not disprove the conflicting results, it only offers possible explanations.

Knowledge gap 4: What are the reasons for the controversial results on the classical genotoxicity parameters like chromosome aberrations, micronuclei and sister chromatid exchanges?

Interestingly the reviews offer a solution to the problem Vijayalaxmi and Obe (2004) as well as Verschaeve (2005) recommend a well coordinated, large scale, multicenter, collaborative research project to solve the question.

6 Do different tissues or cell types exhibit diverse sensitivity to RF-EMF?

Different cell types have been used in experiments to test the genotoxic potential of RF-EMF. The most frequently investigated cells were human peripheral lymphocytes. These were used e.g. in the studies of Baohong (2005), Maes et al. (1995, 1996, 1997, 2000, 2001, 2006); Stronati et al. (2006) or of Vijayalaxmi et al. (1997, 2000, 2001a, 2001b, 2006). Fibroblasts have been investigated e.g. by Diem et al. (2005), Lagroye et al. (2004b), Malyapa et al. (1997a), Speit et al. (2007), and others. Glioblastoma cells have been tested by Malyapa et al. (1997a) and stem cells by Czyz et al. (2004) as well as Nikolova et al. (2005). Many more cell

types have also been investigated. Of course there are differences between the cells. Isolated peripheral lymphocytes have to be stimulated to initiate cell division, when they shall be tested for cytogenetic changes, whereas fibroblasts, cell lines or stem cells proliferate spontaneously. Cell lines have usually lost proliferation control and are thus different from cells in an intact organism.

Differences in cell type are often discussed as an explanation for controversial results. As long as we do not know, whether there is a non-thermal interaction of RF-EMF with the nucleus of a cell, which may lead to genomic changes, nor know by which interaction mechanism this could happen, one cannot exclude cell specific effects. They could be due to difference in DNA repair mechanisms. However, it is complicated to find out, whether different cell types differ in their sensitivity to RF-EMF, as long as one does not know whether there is a genotoxic interaction in the non-thermal SAR range at all.

Knowledge gap 5: Can the controversial results on genotoxic action of RF-EMF be explained by different sensitivity of various cell types?

7 Do RF-EMF fields cause genotoxic effects in humans?

If genotoxic changes due to RF-EMF exposure could be demonstrated in *in vitro* experiments or in *in vivo* animal experiments, this would not mean that RF-EMF could have the same effects also in humans. Therefore it is desirable to monitor such changes also in cells of persons who have been exposed to RF-EMF in comparison to control persons. Such explorations are, however, difficult to perform, as instruments for quantitative dosimetry in the general population are not available. Epidemilogic studies have the same problem, which is extensively discussed in the contribution of Kundi in this project, c.f. exposure indicators there.

Knowledge gap 6: Is it possible to perform investigations on genotoxic effects in humans based on a reliable dosimetry?

However, a few studies on genotoxic effects of RF-EMF have been performed despite of the mentioned problems. One possibility is to investigate people who are occupationally higher exposed than the average population like radio-linemen (Garson et al., 1991). In this study 38 radio-linemen were compared to 38 controls by means of counting CA in stimulated lymphocytes. No difference could be detected. In another study of Fucic et al. (1992) MN

were tested and the authors state that "microwaves posses some mutagenic characteristics typical of chemical mutagens". Garaj-Vrhovac (1999) presented a study on 12 persons employed on radar and antenna system service and 12 controls. The exposed persons worked in fields of 10 µW/cm^2 to 20 mW/cm^2 in the frequency range of 1250-1350 MHz. The exposed persons showed a higher frequency of MN. In a study of Gadhia et al. (2003) 24 mobile phone users were compared to 24 individuals who did not use mobile phones. In the exposed group as well as in the control group two subgroups were formed one of smokers and alcohol drinkers and one of non smokers and non drinkers. The blood was investigated for CA and SCE. Mobile phone users had more CA than non users in both groups. After mitomycin C (MMC) treatment of the blood cultures dicentric and ring chromosomes were increased more among mobile phone users. In a study of Maes et al. (2006) lymphocytes of 49 professionally exposed individuals was compared to 25 subjects from the general population. Blood samples were also treated by MMC in order to investigate possible combined effects of RF-EMF and MMC. Maes et al. (2006) found neither an influence of RF-EMF alone nor a cooperative action with MMC.

As mentioned above, all these studies suffer from poor dosimetry, nevertheless this is also true for epidemiologic studies. A final answer whether RF-EMF can cause genotoxic changes in a human organism can be gained by such experiments, if they are conducted in a very careful way. This not possible today (see knowledge gap 6), however, there is a knowledge gap in this field.

Knowledge gap 7: Are RF-EMF able to act genotoxically inside a living human body?

8 Do RF-EMF act synergistically with other mutagens?

According to physics RF-EMF is theoretically not able to ionize chemical elements or to break chemical bonds, c.f. knowledge gap 1. Therefore it has been suggested already before 1990 that RF-EMF may facilitate the mutagenic action of other mutagens. Two investigations were performed on murine leukaemia cells (line L5178Y; Meltz et al., 1989) and on hamster ovar cells (CHO; Ciaravino et al., 1987) which were exposed for 4 h in a 2450 MHz field of 30 W/kg SAR with or without mitomycin C (MMC). In these studies neither the field alone did cause mutations nor did it increase those induced by MMC.

Starting in 1996 the group of Verschaeve investigated also the combined effects of RF-EMF and MMC on isolated human lymphocytes exposed by an antenna of a base station at

954 MHz and 1,5 W/kg SAR for 2 h (Maes et al., 1996). The RF-EMF exposure alone did not increase sister chromatid exchanges (SCE). MMC, however, increased the SCE in a dose dependent manner, and this increase was enhanced, if the specimen had been exposed to RF-EMF prior to MMC treatment. In a second study human blood cultures were exposed in a TEM cell at 935.2 MHz (CW) 0.3-0.4 W/kg SAR for 2 h (Maes et al., 1997). One part of the cells was treated by MMC after RF-EMF exposure. CA, SCE, and proliferation index as well as alkaline Comet assay were evaluated in lymphocytes. RF-EMF did not change any of the evaluated parameters. But the cells exposed to RF-EMF and MMC developed slightly more SCE than those treated by MMC alone. In a third study of this series human blood cultures were exposed to RF-EMF of 455.7 MHz with 6.5 W/kg SAR for 2 h transmitted by an antenna in 5 cm distance. Again RF-EMF alone did not change CA and SCE. Some specimens were treated by MMC or X-rays after the RF-EMF exposure. There were no consistent differences between those treated by RF-EMF and MMC or X-ray and those treated by MMC or X-ray alone. The authors conclude that the data do not support the hypothesis that RF-fields act synergistically with chemical or physical mutagens. In a next study the same authors exposed blood cultures in a TEM cells to 900 MHz 0.4, 2.0, 3.5, 5.5, and 10 W/kg SAR for 2 h (Maes et al., 2001). Three different signal modulations (CW, GSM-talk and GSM stand by) were tested. Parts of the specimens (2 and 3.5 W/kg) were additionally treated by MMC or X-rays. Again CA and SCE were evaluated. The authors conclude in their discussion that “the investigation was not able to give supporting evidence of 900 MHz microwaves alone or in combination with a chemical or physical mutagen”.

Short time later a study of Zhang et al. (2002) appeared, which demonstrated synergistic effect of RF-EMF 2450 MHz field 5.0 mW/cm^2 for 2 h and MMC on human blood. They evaluated MN and DNA strand breaks by the Comet assay. Verschaeve (2005) classifies this study as confirming the Maes et al., (1996) results, although the later studies of his own group failed to do so.

In 2005 another study appeared which also investigated co-genotoxic actions of RF-EMF on human lymphocytes (Baohong et al., 2005). The blood samples were exposed a setup of the type described in chapter 3 (Schuderer et al., 2004b) at 1.8 GHz and 3 W/kg SAR for 2 h. Bleomycin (BLM), MMC, methylmethansulfonat (MMS), and 4-Nitrochinolin-1-oxid (4NQO) were applied as chemical mutagens in 4 concentrations either before or after the field exposure. DNA strand breaks were investigated by the alkaline Comet assay. RF-EMF alone did neither change tail moments nor tail length. All chemical mutagens led to concentration

dependent changes in the Comet assay. The mutagenic effects of MMC and 4NQO were significantly increased by RF-EMF

The controversial findings concerning the synergistic action of RF-EMF and other mutagens initiated a study as part of the PERFORM B project (European Union, 2004b). The results of this part have been gathered in Stronati et al. (2006). As PERFORM B was designed to conduct reproduction studies, the experiments were carried out in two independent laboratories under strictly controlled conditions. The exposure setups were identical with the one described for cell cultures in chapter 3 (Schuderer et al., 2004a). The blood samples were exposed at 935 MHz SAR 1 and 2 W/kg for 24 h. Blood samples were obtained from 14 donors, which is a big sample size compared to most other studies, e.g. Zhang et al. (2002) had one female and one male donor and Baohong et al., (2005) only one male donor. As physical mutagen X-rays of 1 Gy were applied before or after RF-EMF exposure. DNA strand breaks were documented with the alkaline Comet assay, in addition, MN, CA, and SCE were monitored. No effects of RF-EMF alone could be detected for any of the endpoints. In addition RF-EMF did not modify any measured effects of the X-rays.

A very interesting *in vivo* study concerning a possible synergistic action of RF-EMF and chemical mutagens has appeared recently (Verschaeve et al., 2006). Female rats were exposed in a radial waveguide as described in chapter 2.2 at 900 MHz amplitude modulated at 217 Hz SAR 0.3 or 0.9 W/kg 2 h daily 5 times a week for 2 years. The chemical mutagen and carcinogen 3-chloro-4(dichlormethyl)-5-hydroxy-2(5H)-furanone (MX) was added to the drinking water throughout the whole time. Animals were divided in four groups of 72 individuals each: 1. Cage control, 2. MX-sham exposure, 3. MX+0.3 W/kg, 4. MX+0.9 W/kg. Samples of blood, liver, and brain were investigated for DNA damage by alkaline Comet assay and blood was checked for MN in addition. Only in brain tissue MX caused a significant increase in the DNA content of the comets. This was not further increased by the additional RF-EMF exposure. MN were not increased by MX alone, nor in combination with RF-EMF. This extensive study did not show any co-genotoxic effects, however the tested mutagen did not act very strongly.

In the chapter 6 two studies are presented, which also follow the question of synergistic action of RF-EMF and chemical mutagens (Gadhia et al. 2003; Maes et al. 2006). Gadhia et al. is in favour of such an effect, but the authors call this study preliminary and therefore it shall not be taken into account further here. Maes et al. could not demonstrate a cooperative action of RF-EMF and MMC.

Taken together there are only three studies pointing to synergistic genotoxic action of chemical mutagens and RF-EMF, these are in contrast to a higher number of more extensive and more carefully planned studies. However, in the most extensive study of Stronati et al. (2006) chemical mutagens like MMC were not tested. Thus, the results of this study may not be fully applicable on studies working with chemical mutagens, therefore there is still a knowledge gap in this field.

Knowledge gap 8: Do RF-EMF and chemical mutagens like mitomycin C act synergistically?

9 Conclusions

In this study some knowledge gaps have been identified. The question remains, do these knowledge gaps urgently ask for further studies. Using knowledge gap 1 as example the problem shall be discussed. Is it possible to design studies to identify an unknown interaction mechanism of RF-EMF, as long as there is no reproducible genotoxic effect of RF-EMF in the relevant field-strength range? This seems to be impossible. Therefore it is important first to identify a reproducible effect and then to try to think about a mechanism. There are many studies of questionable quality, therefore it does not help to perform new studies executed by single labs. For further progress in this field on should follow the proposals of Vijayalaxmi and Obe (2004) as well as Verschaeve (2005) who recommend a well coordinated, large scale, multicenter, collaborative research project.

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10. References

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