

„Knowledge gaps in cancer animal studies for RF EMF risk assessment“

Jochen Buschmann

1. Introduction

2. Present Situation

2.1. Carcinogenesis

2.2. Toxicity (Carcinogenicity) testing

3. Research gaps

3.1. Exposure assessment

3.2. Mode of Action

3.3. Susceptibility

3.4. Extrapolation

4. Proposed Research Strategy

4.1. Interaction between "apical" tests and hypothesis driven studies

4.2. Thermal vs. non-thermal effects

4.3. Role of "Omics" Studies

1. Introduction

The present paper is mainly focussed on the results of the discussion during the IMBA meeting held in Berlin, October 12-13, 2006. More related information and literature data can be obtained from a review article "In vivo studies on the biological effects of high frequency electromagnetic fields: a survey and future prospects" by Dr. Thomas Tillmann and Dr. Jochen Buschmann (2003, available at: http://www.fgf.de/english/fup/fgfpub/editionmat/Edition_Wissenschaft_Nr19_e.pdf).

Since the author is a practical toxicologist, one of the focuses of this paper is the application of principles from toxicological testing which are applied in the process of registration of chemicals and pharmaceuticals in order to minimize the risks during their future use.

Although the attempt was made to detect some specific knowledge gaps, the intention is mainly to suggest **a practicable research strategy** which could result in valid data required for a sound risk assessment. Such an approach is assumed to be more successful and cost effective rather than closing single knowledge gaps by adding more studies to the existing patchwork of data.

2. Present Situation

General knowledge gap: is there a causal link between RF EMF exposure and cancer

General knowledge gap: current animal research has its methodological limitations in terms of risk assessment. These are partly, but by far not exclusively, the result of poorly conducted research, but rather a consequence of a lack of a valid research strategy. The availability of lifetime exposure bioassays in more than one species after exposure to relevant frequencies including the investigation of the complete spectrum of organs (comparable to guideline studies performed under GLP conditions) could add significant information. This information, however, is not suitable to show the total absence of a carcinogenic effect of EMF. Should these studies not give evidence on carcinogenic effects in animals, this would, however, allow the conclusion, that the uncertainty is acceptable to the same degree as it is for chemicals of ubiquitous use.

2.1. Carcinogenesis

Carcinogenesis (i.e. the development of cancer) is a complex, multi-stage process. Through the phases of initiation, promotion and progression after a latency of years to decades, the process of carcinogenesis leads from the initial transformation of normal cells to a clinically manifest tumour. The first stage of this development (initiation) is characterized by direct damage of the cell genome (e.g. caused by ionizing radiation) or indirect alterations provoked by metabolites (e.g. radicals). If repair processes fail, immunological control mechanisms can lead to cell death (apoptosis, necrosis), or the genetic damage/alteration (in the cell) is permanent (mutation). Initially, the damaged cell is still controlled by the normal tissue, but already during the promotion phase, proliferation by clonal multiplication of the transformed cell(s) is stimulated. The changed genome thus disseminates to the daughter cells (preneoplastic foci) and, at first, so-called benign tumours develop. The term 'progression phase' stands for the progressive transformation of benign tumours into malignant ones which are characterized, among other things, by progressive autonomy and an increased metastatic potential. A scheme of this process, taken from the "Roche Lexicon", is provided in Fig. 1.

2.2. Toxicity (Carcinogenicity) testing

Knowledge gap: can the mechanism of chemical carcinogenesis be applied to investigate potential carcinogenic or cocarcinogenic effects of EMF ?

Knowledge gap: is the application of a test strategy for the investigation of a potential carcinogenicity risk of EMF comparable to the one used in chemical risk assessment ?

As a prerequisite for a sound risk assessment of chemicals in the process of their registration, amongst other investigations, toxicity studies have to be performed. These studies are regulated by a complex framework of guidelines (e.g. OECD, OPPTS). For the estimation of a carcinogenicity risk, the main information is delivered by a lifetime-exposure test (e.g. OECD Test Guideline 451: Carcinogenicity Studies. Available: http://www.oecd.org/document/22/0,2340,en_2649_34377_1916054_1_1_1_1,00.html) in two rodent species (rat, mouse), supporting evidence can be collected from mutagenicity tests. The application of a comparable test strategy for the investigation of a potential carcinogenicity risk of electromagnetic fields (EMF) appears to be possible and should be considered.

Some in vivo animal studies focussing on the determination of carcinogenic effects from EMF are already published. However, many of them do not fulfil the criteria which are applied to standard guideline studies. Consequently, some methodological conclusions for the performance of future investigations can be drawn from a survey of existing experimental EMF studies. These primarily refer to the inclusion of a sham-treated and a non-treated cage or shelf control, and the simultaneous use of several groups subjected to different exposure levels (dose groups), a procedure that is well-established in toxicological routine tests on chemicals, pharmaceuticals, etc. This guarantees the detection of possible dose/effect relations. Also, the inclusion of extensive histological diagnostics (according to the IARC/WHO) seems urgently required for covering the whole spectrum of neoplastic changes and e.g. an improved evaluation of shifts in the dignity of neoplastic changes. Moreover, advantages and disadvantages of using freely moving versus restrained animals for exposure are to be considered in planning and performance of such studies.

Worldwide, numerous 'new' animal experimental studies have been initiated, as well as replication studies for a (re-) evaluation of available study results. As an example of these studies, the research project PERFORM-A already launched in March 2000 within the Fifth Framework Program (FP 5) of the EU, and the CEMFEC (Combined Effects of Electromagnetic Fields with Environmental Carcinogens) project that began in May 2001, shall be mentioned here. They are aimed at clarifying whether radiofrequency EMF have carcinogenic or co-carcinogenic effects on the animal species of rats and mice.

Within the European study project PERFORM-A, two carcinogenicity studies were performed using B6C3F1 hybrid mice as well as Wistar rats over two years to radiofrequency EMF of 902 MHz and 1747 MHz, resp. In a third part project, the influence of a daily exposure to 902 MHz performed over six months was examined in female Sprague-Dawley rats, in which mammary tumours were previously induced with DMBA (7.12-dimethylbenz(a)anthracene). In this animal model, the influence of radiofrequency EMF on tumour growth, incidence, latency and dignity will be explored. The fourth part project is aimed to reproduce the results of the Australian mouse study performed by Repacholi and colleagues who – already in 1997 – described a two- to three-fold increase in lymphoma rates in Pim1 transgenic mice after 18-month daily one-hour exposure to an EMF of 902 MHz.

The results of the whole PERFORM-A project will be available later in 2007 and it is expected that they will close some of the knowledge gaps currently still existing.

3. Research gaps

Knowledge gap: lack of a sufficient set of lifetime exposure bioassays in more than one species after exposure to relevant frequencies including the investigation of the complete spectrum of organs (comparable to guideline studies performed under GLP conditions).

In summary, it can be concluded that currently only a small number of chronic EMF studies have been published and that there still are few studies using characteristic (pulsed) GSM mobile radio signals. Long-term experimental studies investigating the GSM signal technology widely spread in Germany and Europe, are still rare. 'Early' studies were not always satisfactorily performed or published so that their contribution to an assessment of human health risks from chronic RF EMF is limited. Especially incomplete, non-standardized histopathological evaluations found in carcinogenesis studies and the partially unclear exposure technology (EMF signal) minimize the relevance of these investigations.

Based on such data, a sound scientific assessment of potential cancer risks from RF-EMF is not possible; however, concrete evidence for such health concerns to date has not been found.

The main factors limiting the relevance of currently existing studies on carcinogenicity of EMF are

- the absence of any reproducible effects.
- the absence of guideline type studies covering the whole organ spectrum, using standardized histopathology, a sufficiently high number of animals and more than one "dose" group.
- the academic type of studies so far published, using models which are often not enough standardized and, therefore, of limited value for a sound risk assessment.
- The investigation of different frequencies, where it is nearly impossible to extrapolate from one frequency range to another (in terms of toxicology each different frequency would be considered a different compound requiring specific testing).

3.1. Exposure assessment

Knowledge gap: data are missing on "worst case" exposures that could occur for humans under the conditions of the existing and intended use of EMF.

Knowledge gap: what is the specific situation for the application of safety factors for EMF compared to chemicals ?

In the case of toxicity testing in chemicals, the sequence of events is to apply in animal studies doses close to the maximum tolerated ones in order to determine the no observed adverse effect level (NOAEL). Applying a safety factor (see 3.4), a safe dose for humans is determined based on the NOAEL from animal studies. Consequently, one of the core principles in toxicity testing in animals is overdosing. This principle is strongly limited if EMF are tested in animal models, since overdosing would result in heating of the organism, which should be omitted. This significantly compromises the above described procedure for chemicals.

Unlike in the case of chemicals, for pharmaceuticals normally the intended therapeutical dose is known before testing, allowing the determination of the doses to be tested using the opposite procedure: applying a safety factor to the intended human use would determine the doses used in the experiment.

Applying this approach for EMF testing could solve the problem of producing thermal effects in the experiments. To be able to do this, however, we need more data on worst case exposure scenarios that could occur for humans under the conditions of the existing and intended

use of EMF. Only if these data are known, a research strategy as described in 4.2 can be applied.

3.2. Mode of Action

Knowledge gap: the lack of any reproducible data on a potential mode of action of EMF (e.g. genotoxic vs. tumour promoting effects) limits a sound risk assessment. However, this knowledge gap is better addressed in cellular research than in animal experiments.

Due to the complex nature of the process of carcinogenicity, different modes of action could contribute to the eventually observed effect of an increased tumour rate. Consequently, should such effects be observed reproducibly in valid studies, then the investigation of the underlying mechanism is important in terms of risk assessment: if the nature of the effect allows to determine a threshold for the observed effect (mostly applicable in the case of a co-carcinogenic effect), then the above described normal procedure of risk assessment can be applied. In the contrary situation (e.g. genotoxic mechanism of tumour induction), if no threshold of the effect can be assumed, this procedure cannot be applied.

Since guideline studies can normally not provide such information, it is important to better interlink basic and applied research, should data suggest carcinogenic effects due to EMF (see 4.1)

3.3. Susceptibility

Knowledge gap: the lack of dosimetry and absorption models for different animals species (and different ages) strongly limits the extrapolation from animal studies to human risk assessment.

The susceptibility of the currently used rodent models for carcinogenicity has been shown for many chemical compounds. The striking differences in life time between the model animals and the target organism (human) is outweighed by differences in the repair mechanisms so that the process of carcinogenesis is comparable in these species. The question is what the situation is in the case of EMF. As will be discussed later, for a sound data extrapolation a good dosimetry is of high priority. While relatively good models for the absorption of EMF in humans are available, it is highly important to have such models for experimental animals, too. This also includes the availability of such models for the developing organisms. The predictive value of animal experiments will significantly benefit if these models can be used in order to compare the exposure situation in the target organs of animals and humans (see also 3.4)

3.4. Extrapolation

Knowledge gap: based on the uncertainties described under 3.1 through 3.3, is it possible to apply a risk assessment for EMF comparable to that for chemicals ?

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.

4. Proposed Research Strategy

In order to get more information, which can be better used in the process of risk assessment, the adoption of similar procedures to those of toxicological standard tests applied for the registration of chemicals, drugs or pesticides appears possible and sensible.

Since these methods have been developed and applied for a long time and fixed in permanently improved guidelines, they are scientifically well-based and widely accepted for a successful risk assessment of chemical substances. Consequently, these animal models are known to be predictive for humans, and the applied methods are well standardized and validated.

When planning this type of studies, the following points should be considered beforehand:

- selection of the best suitable and sensitive species/strain
- selection of relevant field strengths as an analogon to dose ranges applied in toxicity testing
- consideration of the advantages and disadvantages of the use of free moving versus restraint animals.

Although the performance of complete carcinogenicity studies in two species is complex and expensive, such studies should deliver essential information for risk assessment. The upcoming publication of the results of the PERFORM-A program will be of high value in this context.

4.1. Interaction between "apical" tests and hypothesis driven studies

When comparing the different scientific approaches applied in the different studies accessible so far, two basic groups of studies can be distinguished: studies aiming at validating a specific hypothesis (hypothesis driven studies), and studies mainly serving as a means for 'global' risk assessment (toxicology type of studies). The former are strongly academic attempts at basic research level, often of high scientific value, and are performed using very sensitive model systems. Extrapolation to the human target organism often is limited, and it is very difficult (above all in case of negative results from the verification of single hypotheses) to draw conclusions on 'general' health risks to humans. The second study group uses a markedly toxicological approach in the sense of applied research: Different test methods are used to examine various groups of test animals exposed to different field strengths, including as many potentially health relevant endpoints as possible. This approach, best described as 'apical', due to the large number of test parameters allows for a fairly good extrapolation of results to humans and, therefore, is highly suitable for an assessment of health risks. However, such studies, due to their 'broad spectrum' characteristics, often are less profound than those of the first group, thereby not always being sensitive enough for exploring specific health risks.

A promotion of the dialogue between both groups of researchers could produce significant synergies: If, on one hand, results achieved in basic research can be reproduced in applied

research and, on the other hand, findings obtained by applied studies can be confirmed by mechanistic approaches, the framework of risk assessment will be considerably improved.

4.2. Thermal vs. non-thermal effects

Principal knowledge gap: is it possible to apply the principles of toxicity testing (incl. necessary overdosing) for the investigation of the risk assessment of electromagnetic fields, where overdosing may cause thermal effects, which is a different quality of action.

One basic principle of toxicity testing is overdosing in animal studies in order to be able to apply safety factors in the process of extrapolation from the model (animal species) to the target organism (human). 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 future studies, it is 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 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 study type which is based on carcinogenicity testing, 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 secondary to 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 indication for an increase in tumour occurrence compared to the concurrent control group will be regarded as an adverse effect. A final assessment will often only be possible after investigation of the observed effects in more detail, i.e. collaboration with basic researchers as described in 4.1. will add significant information.

The thus established experimental data base can then be used to determine whether there is any indication of a carcinogenic or co-carcinogenic effect and whether a no observed adverse effect level (NOAEL) for the given type of exposure can be determined. By applying the above mentioned safety factors, a safe exposure for humans can be assessed.

4.3. Role of "Omics" Studies

As described above, carcinogenesis is a complex, multi-stage process. Assessing individual stages of this process may be of high relevance in terms of basic research. However, in terms of risk assessment the application of this approach is (currently) of very limited value. The "omics" approach investigates the effect of a given factor on the gene expression (and further downstream processes like proteins etc.). Thus, it can also be applied to predict genotoxic effects of a given stimulus, which play an important, but not the only role in carcinogenesis. In this situation, "omics" methods can currently be used to either determine the mechanism of an effect observed on the level of the whole organism (see 3.2.) or as a screening tool to determine sort of a "relative risk" (e.g. compound A is more likely to be a carcinogen than compound B). While the former is currently limited by the lack of such (reproducible) effects, the latter could deliver useful information, if in a first step the different frequency ranges (as an analogon to different compounds in the case of chemicals) could be screened using these methods in order to set priorities for further investigations (i.e. to get some information about what frequency range is most likely to cause health effects). The results of such studies could then be used for planning further studies investigating the "critical" frequency range (should there be one) with higher priority.

Fig. 1: Scheme of the process of chemical carcinogenesis (taken from the "Roche Lexicon")

