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Review article:

Brain tumour growth in rats exposed to electromagnetic fields used in wireless cellular communication.

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ABSTRACT

In 1996 there was no convincing laboratory evidence that EMFs used in wireless communication could cause tumour promotion at non-thermal exposure levels. Therefore we then performed a study of the effects from exposure to such electromagnetic fields in the rat brain glioma model we were using in our research for brain tumour therapy. By stereotaxic technique rat glioma cells (RG2 or N32) were injected into the head of the right caudate nucleus in 154 pairs of Fischer 344 rats in both exposed and matched controls. Starting on day 5 after inoculation, the animals were exposed for 7 hours a day, 5 days a week during 2 - 3 weeks. Rats of both sexes were exposed to electromagnetic fields in the microwaves frequency range 915 MHz both as continuous waves (1 W), and as pulse-modulated at 4, 8, 16 and 217 Hz in 0.57 ms long pulses and 50 Hz in 6.67 ms pulses, all with a maximum power amplitude of 2 W per pulse. The animals were kept un-anaesthetized in well-ventilated TEM cells during 7 hours a day for 5 days a week for 2-3 weeks. Their matched controls were kept in identical TEM cells without EMF exposure. At the end of the exposure period the rat brains were examined histopathologically. The tumour size was measured with a calliper and the volume estimated as an ellipsoid.

Our study of the 154 matched pairs of rats did not show any significant difference in tumour volume between animals exposed to 915 MHz microwaves, and those not exposed. Thus our results did not support that daily exposure to EMF promotes tumour growth when given from the fifth day after the start of tumour growth in the rat brain until the sacrifice of the animal 16 days later.

In the present review our results published 1997 have been re-evaluated in terms of SAR dependence of tumour volume observed ratio (exposed / control). We thus surprisingly found that the shape of tumour volume-OR versus SAR response was of bath-tube pattern, similar to that found in our parallel studies of albumin leakage through the blood-brain barrier. Since the SAR varies between most other animal studies reviewed and human epidemiological studies this SAR dependence might explain the controversy in rendering the results.

Keywords: Brain tumour, RG2, N32, Fischer rats, electromagnetic fields 915 MHz, CW, pulse

modulated: 4, 8, 16, 50, 217 Hz , SAR.

1   Introduction

Radiofrequency fields in the frequency range 300 MHz - 300 GHz are called microwaves used in microwave ovens (2450 MHz) and in radar equipment (1-100 GHz). In cellular mobile communication the frequency band $0.8 - 8$ GHz is frequently used. In the present study the health hazards of microwave radiation of 915 MHz was particularly considered. When we started our project in 1996 there existed only a few epidemiological studies of the cancer incidence from electromagnetic fields exposure (Knave 2001; NRPB. 1992). In a study of naval personnel and radar operators it was found that workers in this occupation had a higher risk for brain cancer than the general population (Robinette, et al. 1980). Mortality from cancer was reported to be increased in people living close to air force bases with proximity to radar installations (Lester & Moore 1982). That study was, however, criticised on the grounds of faulty data collection, absence of physical measurements and failure to investigate potential confounders (Polson & Merritt 1985). A possible association between chronic exposure to microwaves and polycythemia and myeloid leukaemia has been found in a study of only a few cases (Friedman 1981). During a 12-year period, 340 American police officers were studied, and in those who regularly were exposed to hand-held speedometers operating through radar waves, incidence of testicular cancer in 6 cases was found (Davis & Mostofi 1993).

There have been some lawsuits launched in the US alleging that some instances of brain tumours were the result of cellular phone use (Elmer-Dewit 1993). But none of these cases brought forward any evidence other than the anecdotal history of the subject's mobile telephone usage. Recently, however, the Supreme Court of Italy has ruled in favour for a link between mobile phone radiation and brain tumour development (Marcolini 2012).

Early *in vitro* studies have indicated that, low level, low frequency-modulated microwave radiation may affect intracellular activities of enzymes involved in neoplastic promotion without measurable influence on the overall DNA synthesis. Already in the late 1980[ies] evidence was found that microwaves have an effect on intracellular levels of ornithine-decarboxylase (ODC) which is an enzyme implicated in cell growth, and which is increased by tumour promoters (Byus, et al. 1988). They noticed higher frequency of transformed cells with increasing SAR values (SAR from 0.1 to 4.4 W/kg) for certain modulations of the carrier wave exposed cells to 120-Hz-modulated microwaves followed by treatment with a phorbol-ester tumour promoter (Balcer-Kubiczek & Harrison 1989). Thermal effects in lymphocyte transformation was found after exposure of the cells to pulsed or continuous radiofrequency radiation (Czerska, et al. 1992; Stodolnik-Baranska 1967). Still another interesting observation was that 120-Hz-modulated 450 MHz microwaves inhibit gap junction cell-to cell communication in cultured hamster ovary cells, that play an essential role in regulation of cell growth which may lead to unregulated growth with tumour formation (Adey, W. R. 1990a; Adey, W. R. 1990b). Several other in vitro model systems have

been developed that examine the effects of EM field exposures combined with a chemical tumour promoter but with no conclusive results (Cain, et al. 1994; Marino 1994) .

A number of animal *in vivo* investigations have also been performed, and of particular interest is the study conducted by Szmigielski et al. (1982) who observed faster development of benzpyrene induced skin tumours in mice exposed for some months to sub-thermal 2450 MHz microwaves (Szmigielski, et al. 1982). Of great interest is also another study of 100 rats (aged 2 to 27 months) exposed to pulsed microwaves (0.4 W/kg), where the exposed group had a significant increased frequency of primary malignant lesions as compared to the control group when lesions were pooled regardless of their location in the body (Kunz, et al. 1985).

In 1996 we found no <u>*in vivo*</u> study of brain tumour development during exposure to radiofrequency radiation. Therefore we decided to study the effects of exposure to electromagnetic fields in the rat brain glioma model which we for several years were using in our laboratory for developing new tumour therapy modalities. This same model was used in the 1996 - 1997 study to examine the effect of 915 MHz microwaves on the growth of the inoculated tumour cells in the rat brain. Both continuous waves and waves pulse-modulated with 4, 8, 16 Hz and 217 Hz with 0.57 ms pulse lengths and 50 Hz with 6 ms pulse lengths were used. We studied the effects of electromagnetic fields upon the promotion of growth of tumours from two different rat glioma cell lines, RG2 and N32, inoculated in the brains of Fischer 344 rats. No promotions of tumour growth due to the EMF could be demonstrated in large series of animals as compared to their matched controls. (Salford, L. G., et al. 1997; Salford, L.G., et al. 1993).

We continued our studies of the effects of real GSM modulated microwaves upon the incidence of tumours in a newly generated "knock-out" mouse model (Pekny, M.,1996; Pekny, M., et al. 1995). By the use of gene targeting, Pekny et al. (1995) had created mice deficient for glial fibrillary acidic protein (GFAP), which is an astrocyte-specific protein that forms intermediate filaments in the cytoskeleton of astrocytes. The GFAP-deficient mice utilized by us develop spontaneous tumours, both lymphoblastic (non-Hodgkin) lymphomas and anaplastic rapidly growing tumours diagnosed as malignant Schwannomas. The GFAP-mice were exposed in the TEM Cells for about 8 hours a day during 147 days from November16 1995 to July 9 1996. The total time of exposure was 1143 hours (47.62 days).

In male GFAP-mice we found tumours in all 6 controls but only in 4 out of 6 exposed mice. We found no tumours in FM controls and in seven exposed FM mice we found only 1 tumour. Microwave exposure thus revealed no significant increase on tumour evolution in the GFAP-mice (Salford, L.G., et al. 2012).

Presently, in 2013, there is a substantial body of animal <u>*in vivo*</u> experimental studies as well as human epidemiology studies of the incidence and growth of brain tumours due to microwave exposure from wireless phones and base-stations. The results of the principal human epidemiology studies suggest an increased risk of glioma, at the highest exposure levels, for ipsilateral exposures (Cardis, E., et al. 2010). No risk of tumour incidence was, however, found in most recent animal

in vivo experimental studies presented at the end of this review (Cardis, E., et al. 2010; Little, et al. 2012; Repacholi, et al. 2012).

In the present review we also present a re-evaluation of our results of the 1996-97 study. An interesting pattern in the SAR-dependence was found that might explain the discrepancies between epidemiological and *in vivo* experimental animal studies.

2 Material and methods

2.1 Animal model

The rat glioma cell-line RG2, originates from an *N-ethyl-N-nitrosourea*-induced rat tumour, which grows very well in infinite cell culture cycles since more than four decades, and produces malignant astrocytoma-like tumours when inoculated in the brain of Fischer 344 rats (Wechsler, et al. 1969). When at least 1000 RG2 cells are inoculated, all rats within 3 weeks well-delineated tumours with a diameter of about 3 - 6 mm (Aas , et al. 1995). Fischer 344 rats of both sexes, weighing 150 - 250 g, were used in all the experiments. The animals had free access to water and pellets (SAN-bolagen, Malmö, Sweden). By stereotaxic technique 5000 RG2 cells in 5 µl nutrient solution were injected with a Hamilton syringe into the head of the right caudate nucleus in a total of 218 rats. To avoid growth of extra-cranial tumour, the injection site was cleaned with 70% ethanol after injection, and the bore hole sealed with wax.

A new *N-ethyl-N-nitrosourea* induced rat glioma cell line has been developed, that produces glioma of malignant astrocytoma type with only half the growth rate of the RG2 cells (Siesjö, et al. 1993). This N32 cell line was used in 90 rats that received 15 000 N32 cells in the head of the right caudate nucleus. Groups of 2 - 8 animals were inoculated at each instance with cells harvested immediately before the procedure.

At inoculation, every animal to be exposed with electromagnetic fields was matched to a control animal. The matched control was inoculated with identical tumour cells immediately before the animal to be exposed. The exposure was started on day 5 after inoculation. In all 154 of the 308 animals in the series served as controls and the other 154 animals were exposed to electromagnetic fields.

2.2 Electromagnetic fields

The GSM (Global System for Mobile communication) phone at the 900 MHz band has a peak output power of 2 W. With a duty factor of 1/8 (switched "on" once every eight second) this leads to a time average of 0.25 W output power leaving the antenna. The maximum power absorbed is thus of the order of one tenth of a Watt.

In the present study we expose the whole animal in a TEM-cell using continuous wave (CW) and pulsed (or modulated) 915 MHz microwaves. These fields can be accurately generated in the TEM-cell without the distortion that is typically introduced when conventional antennas are used to establish impulse test fields.

The TEM-cell is enclosed in a wooden box that supports the outer conductor and central plate. The outer conductor is made of brass-net and is attached to the inner walls of the box. The centre plate, or septum, is constructed of aluminium and is held up by Teflon braces which are attached at the inner side walls. To allow access to the inside of the TEM-cell both ends can be removed. The inside of the cell is ventilated through 18 holes (diam. 18 mm) in the side walls and top of the box and the brass-net allows air to circulate. These holes are also used for ocular examination of the interior during exposure. Probes for monitoring temperature inside the cell or test object are inserted through these holes.

The test system consists of four TEM-cells. A microwave power generator (MCL model 15 222) is used for feeding the TEM-cells. A power splitter divides the power from the RF generator into two equal parts that are fed two of the four cells. The output from the cells is terminated in a 50 Ohms dummy load. Both forward and reflected powers are measured, with a Bird model 43 power meter, at the inputs and outputs of the TEM-cells. The output from the RF generator can be pulse modulated from an external source by applying a positive pulse of 5 volts with a pulse width of 5.0 microseconds minimum. The rise- and fall time of the RF pulses used in the experiments has been 0.04 ms and 0.81 ms respectively.

Calculations of SAR-distributions in the brain of rats exposed to three-dimensional electromagnetic fields have been performed for our TEM-cell using the Finite Difference Time Domain (FDTD) method (Martens, et al. 1993; Vanhese, et al. 1992). The SAR values were determined experimentally by measuring the input power and the transmitted power by power-meters on each side of the TEM cell with and without rat load.

Experimental absorbed power measurements of reflected and transmitted power were performed with rats in the TEM-cell and at an input power of 1 W continuous wave. The experimental average SAR value of 1.4 ± 0.3 W/kg per 1 W input power is in good agreement with the theoretical, calculated value of 1.67 W/kg per 1 W input power (Malmgren 1998; Nittby, et al. 2008).

The exposed animals were kept un-anaesthetized in TEM cells producing 915 MHz continuous or modulated microwaves (see table 1). Exposure was started on day 5 after inoculation. The animals were exposed during 7 hours a day for 5 days a week. All animals were given a half hour break for feeding after 4 hours of exposure.

It is noteworthy that the animals in this type of experiments, when given a chance, rush back into the TEM cells where they evidently feel at home. The controls were kept two of the identical TEM cells without EMF exposure. The TEM cells were well ventilated. The rat rectal temperature was recorded with an optical temperature device (LUXTRON 2000) before exposure, and immediately after 4 hours and 7 hours of exposure.

2.3 Histopathological examination

When the exposed animal or its matched control started to develop neurological signs of tumour growth, both animals were sacrificed by perfusion-fixation of the brains under chloral-hydrate anaesthesia. All brains were blindly examined histopathologically by one of the authors (A.B.). Five coronal slices from each animal were paraffin embedded, sectioned at 5µm and studied microscopically in cresyl violet staining. In this way the entire telencephalon was covered except the frontal and occipital poles.

For tumour size measurements the slice with the largest tumour extension was measured and chosen as the long semi-axis (a) of an ellipsoid, and its perpendicular axis was chosen as the small semi-axis (b). The number of slices at 1 mm apart was chosen as the diameter (c) of the ellipsoid. The tumour volume was estimated by the ellipsoidal volume calculated from the following equation:

$$V = \frac{\pi}{6} \cdot \left(a \times b \times c \right)$$

2.4   Statistical evaluation

Student's t-test for paired samples as well as Wilcoxon´s matched pairs' test was used for the statistical evaluation.

3 Results and discussion

The exposure parameters for the various groups of experiments are shown in **Table 1**. The number of exposure periods was from 9 to 15 which in average resulted in around 80 hours of exposure in the TEM-cells. The animals showed no signs of stress from the exposure with electromagnetic fields. They returned spontaneously into the TEM cells after break for feeding. The rectal temperature of the animals recorded before exposure, at the break after 4 hours and directly after the end of the daily exposure did not show any significant variation.

After inoculation, all 154 exposed animals as well as their matched controls developed polycyclic tumours, rounded with well-defined boundaries. On the histo-pathological examination the tumours were usually found to be solid with minor necrotic areas without correlation to treatment, tumour size or time from inoculation to death. In most animals, a sharp clean demarcation was seen between the tumour and the surrounding brain. In some animals, the tumours had a slightly blurred border and minor satellite tumour foci or nests of migrating cells were revealed in the surrounding brain. There were no signs of brain damage outside the tumour areas, neither necrosis, gliosis nor inflammatory changes that could be ascribed to the EMF exposure.

Table 1. Exposure scheme

Modulation frequency (Hz)	Number of days of exposure	Pulse length (ms)	Peak power in pulse (W)	Duty cycle	SAR (W/kg)
0 (CW)	10-15	-	1	1	1,67
4	10	0.57	2	0.002	0.0077
8.33	10	0.57	2	0.005	0.016
16	13	0.57	2	0.009	0.030
50	9-13	6	2	0.3	1.00
217	9	0.57	2	0.12	0.4

Table 2 shows the results of the tumour volume measurements for groups of rats exposed to different modulation frequencies. The frequency distribution of tumour-volume-difference between exposed and matched control animals are shown in **Figure 1** for the whole population of 154 pairs of rats. The results for the different types of tumours and various modulation frequencies are shown in **Tables 2, 3 and 4** with p values based on both Wilcoxon´s matched test and Student´s paired t-test.

Table 2. Brain tumour volume of all tumours (RG2+N32) in EMF exposed rats and controls

Mod. freq. (Hz)	Number of pairs	Tumour Volume exposed (mm^3)	Tumour Volume Controls (mm^3)	Wilcoxon matched pair test p	Students matched pair t-test p
CW	15	29±22	18±13	0.17	0.10
4	12	21±15	15±12	0.53	0.43
8.33	32	22±17	28±21	0.18	0.17
16	24	21±13	20±17	0.90	0.92
50	31	21±20	19±19	0.97	0.61
217	40	18±20	18±15	0.37	0.88
All	154	21±19	20±18	0.97	0.73
All PW	139	20±18	20±18	0.56	0.79
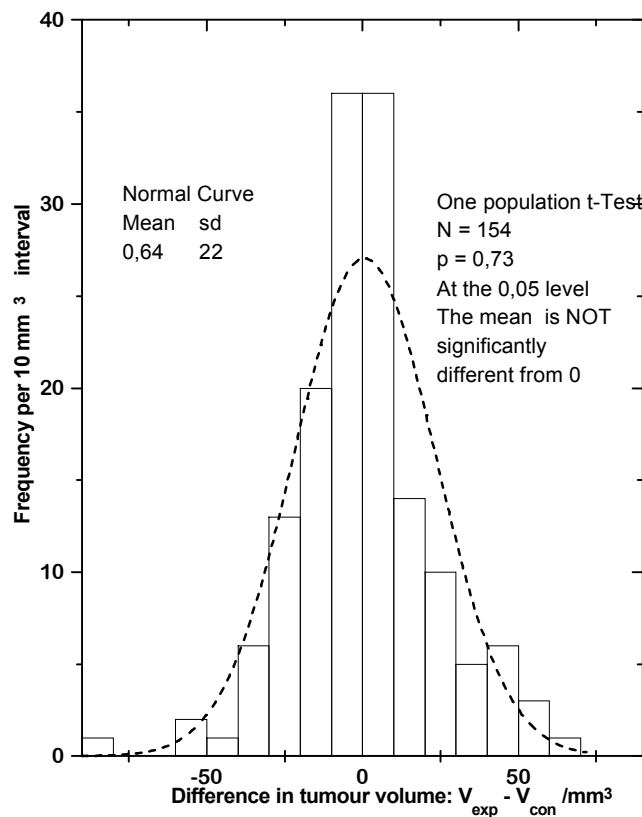
Figure 1   Frequency distribution of the matched difference
                    between the tumour-volume of exposed and control.
                    The mean value 0.64 is not significantly (p<0.05)
                    different from zero.

Our study does not show any significant difference in the tumour growth between animals exposed and those not exposed. The standard deviation is large, but this is the result of the large individual variation in the model where the status of the inoculated cells as well as that of the recipient animal is influential.

From the results in **Tables 2, 3 and 4** no significant differences can be seen between the animals exposed to CW and the animals exposed to pulse modulated fields.

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Table 3. Brain tumour volume of RG2- tumours EMF exposed rats and controls

Mod. freq. (Hz)	Number of pairs	Tumour Volume exposed (mm^3)	Tumour Volume Controls (mm^3)	Wilcoxon´s matched pair test p	Student´s matched pair t-test *p*
CW	15	29±22	18±13	0.17	0.10
4	12	21±15	15±12	0.53	0.43
8.33	28	19±13	24±21	0.28	0.21
16	24	21±13	20±17	0.90	0.92
50	19	26±21	20±17	0.52	0.33
217	11	11±8	15±12	0.25	0.19
All	109	21±17	20±17	0.56	0.56
All PW	94	20±15	20±17	0.99	0.96

Table 4. Brain tumour volume of N32 tumours in EMF exposed rats and controls

Mod. freq. (Hz)	Number of pairs	Tumour Volume exposed (mm^3)	Tumour Volume Controls (mm^3)	Wilcoxon´s matched pair test p	Student´s matched pair t-test *p*
CW	-	-	-	-	-
4	-	-	-	-	-
8.33	4	43±24	51±9	0.71	0.66
16	-	-	-	-	-
50	12	15±18	19±23	0.39	0.34
217	29	20±23	19±16	0.94	0.83
All	45	21±23	22±20	0.31	0.68
All PW	45	21±22	22±20	0.31	0.68

4. Discussion and Conclusion

4.1 Review of our 1996-97 EMF-tumour studies

4.1.1 Tumour-growth-promotion of RG2 and N32 implanted brain tumours in Fischer rats.
Based on the insufficient clarity and consistency regarding a possible association between mobile phone use and an increased risk of tumours in the brain we have re-evaluated the results of our 1996 tumour-growth-promotion study in rats (Salford, L. G., et al. 1997). The following modulation frequencies of 915 MHz microwaves have been used in our study:
- 16 Hz was chosen due to the reported biological effects of microwaves modulated

with this frequency (Bawin, et al. 1978; Byus, et al. 1988).

- 8 Hz and 217 Hz was chosen due to GSM communication system modulation.
- 50 Hz has been proposed as wireless communication system modulation frequency (DUX) and is also the electrical power frequency in Europe.
- 4 Hz was chosen as a harmonic to the 16 and 8 Hz frequencies.

In our neuro-oncological research activity, we have since long used the RG2 tumour cell line, which causes rat glioma (Aas , et al. 1995). This kind of tumour is very similar to the human malignant astrocytoma, which grows very fast. If in humans no treatment is given, patients with this grim diagnosis survive only for about half a year in average, and given all available therapy survival is prolonged to about 15 months in average.

The human malignant astrocytoma sends migrating tumour cells far out in the normal brain, where they hide behind the blood-brain barrier (BBB). Also in our RG2 model, small clusters of migrating cells, separated from the main tumour, are found in the normal surrounding rat brain. The RG2 model mimics the human malignant astrocytoma also by having at least partially incompetent BBB (Groothuis, et al. 1982). It also produces an oedema surrounding the tumour.

In the re-evaluation of the results of our 1996 tumour-growth-promotion study we studied the tumour-volume "Observed Ratio" in the various groups of whole-body SAR values (W.kg^{-1}). The results of the tumour-volume "Observed Ratio" of exposed versus control rats are displayed in **Figure 2** for different SAR values from the various groups according to **Table 1**. Although the individual values are not significantly different from 1, it is interesting to note that the shape of the curve has the same "bath-tube" pattern as was found in the study of the albumin leakage through the blood-brain barrier (Persson, B.R.R., et al. 2012; Persson, B. R. R., et al. 1997).

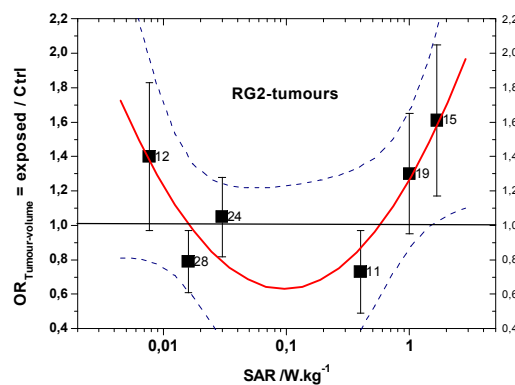**Figure 2**.   Observed ratio of tumour volume of exposed rats with implanted RG2-tumours to unexposed control rats with the same tumour implanted are displayed for different SAR values. The experimental data were fitted to a 2nd degree polynomial. The results of a Wilcoxon signed rank statistical test on p=0.05 level do indicate a 100% agreement between the experimental and fitted values.

In collaboration with the Department of Tumour immunology, Lund University, we developed another rat glioma cell line called "N32" induced by *N-ethyl-N-nitrosourea.* When these cells are inoculated in the rat brain they produce glioma tumours similar to the human malignant astrocytoma (Siesjö, et al. 1993). The N32 cells produce tumours with only half the growth rate of the RG2 cells, and would provide a less aggressive model appropriate for continued experiments. The N32 cell line was inoculated in 45 pair of rats of which the one mate was exposed to EMF, (frequencies 8, 50 and 217 Hz studied). The results of the N32 tumour volume "Observed Ratio" were calculated for various SAR valued and the results were plotted together with corresponding result for the RG2 tumours. The plot thus obtained is displayed in **Figure 3** shows the similar "bath-tube" patterns for both N32 and the RG2 tumours.
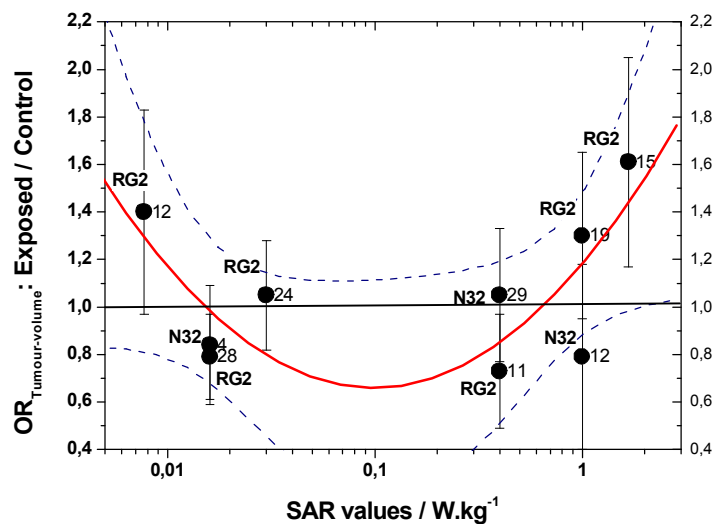

Figure 3   Observed ratio of tumour volume of exposed rats with implanted RG2 - or N32 tumours - to their unexposed control rats with tumours implanted are displayed for different SAR values. The experimental data were fitted to a 2nd degree polynomial (R^2=0.47).

The results of a Wilcoxon signed rank statistical test of the 9 experimentally estimated OR-values versus the values estimated by the fitted curve is given in Table 5.

Table 5. Wilcoxon signed rank test

Variable	Minimum	Maximum	Mean	Std. deviation	p-value (Two-tailed)	alpha
OR_{fit}	0,820	1,410	1,059	0,224	0,77	0,05
OR_{exp}	0,730	1,610	1,062	0,312		

The interpretation of the test:

   H_0 hypothesis: The distribution of the two samples is not significantly different.

   H_a hypothesis: The distributions of the two samples are significantly different.

As the computed p-value is greater than the significance level α=0,05, one should accept the null hypothesis H_0 that distributions of the experimental and fitted samples are not significantly different and the risk to reject the null hypothesis H_0 while it is true, is 77%.

   Most other animal studies for estimating the risk of cancer from exposure for wireless communication, however, are mostly applying SAR values higher than 0.1 W/kg. Thus the "bath-tube" SAR/response-pattern found in our study does indicate that most other *in vivo* animal studies are applying too high SAR values to be comparable with human epidemiological studies. The total exposure time in our study was 80 h which at SAR value of 0.1 W/kg corresponds to a ***Cumulative Specific Absorbed Energy***, of about 30 kJ/kg which is in the same order of magnitude as in epidemiological studies where the subjects are mostly exposed to a ***Cumulative Specific Absorbed Energy*** < 200 kJ/kg (Cardis, E., et al. 2011b). This might explain the discrepancy between the conclusions made from animal studies with very high ***Cumulative Specific Absorbed Energy*** and some human epidemiological studies (Cardis, E., et al. 2011a; Cardis, Elisabeth & Sadetzki 2011; I.A.R.C. 2013; Repacholi, et al. 2012; Who 2011).

   In our study there is a large spread of tumour sizes from animal to animal and thus the mean values for tumour sizes show large standard deviations. We ascribe this to variations in number of cells that are sucked into the Hamilton syringe and then reach the target point in the caudate nucleus during the stereotaxic inoculation. The biological condition of the cells may as well differ from day to day, and also depend upon when in their division cycle they are harvested. There may also exist minor differences in the rat´s response to inoculated tumour cells, although the Fischer 344 rats used in our study are inbred since several years. This underlines the importance of matching every treated animal with its own control, which gets simultaneous inoculation of cells in identical amounts.

   The control of body temperature during EMF exposures is important for the exclusion of thermal effects. We found no increase in temperature for SAR values up to 1.7 W/kg. In separate experiments, however, we have seen thermal exhaustion effects at power levels five times higher than the maximum levels used in the present study (unpublished results).

   All our results with two different tumour cell lines implanted in rat brains and exposed during around 80 hours to continuous wave and differently modulated microwaves do not indicate any overall significant increased tumour growth. The daily exposure time to EMF is long as compared to even what the most devoted mobile telephone user may experience (Cardis, E., et al. 2011b). Even if the more slow-growing of the two tumour-models shows no promoting effect of exposure , it should be noted that both the two models give aggressive tumours similar to the human most aggressive malignant astrocytoma. Other forms of human brain tumours grow slower. The anaplastic astrocytoma and the even less malignant astrocytoma may grow for several years up to decades in the human brain before they give symptoms leading to diagnosis (Salford, L. G. &

Brun 1986).

Although the large uncertainty in the individual, measured tumour volumes, the averages of the tumour volume "Observed Ratio" calculated for various groups of SAR values for both the RG2 and N32 tumours, show a significant "bath-tube" shaped response patterns. The negative inclination for tumour volume "Observed Ratio" at SAR<0.03 Wkg^{-1} is significantly different (p<0.005) from the positive inclination at SAR>0.03 Wkg^{-1}.   This type of nonlinear SAR dependence might explain the discrepancy between the conclusions made from *in vivo* animal studies performed at moderate SAR levels and human epidemiological studies in which the subjects often are exposed to lower average SAR levels. Taking the results of all animals in consideration, however, we also found **no significant difference** between the growth of inoculated rat brain tumours in 154 animals exposed to 915 MHz microwaves and their 154 unexposed matched controls. Thus it is important to consider large dispersion of SAR values in experimental animal studies for investigating the   biological effects of microwaves.

4.1.2 Spontaneously developing tumours in GFAP-mice

The rat study presented above was conducted to evaluate the possible promoter effect of EMF in a rat brain tumour model. This study has no bearing on the initiation of cancer development as the tumour cells are inoculated into the animals. But we have also performed another study with GFAP-mice which spontaneously develop tumours in several organs including the CNS. The GFAP-mice were exposed in the TEM Cells for about 8 hours a day from November16 1995 to July 9 1996. The total time of exposure was 1143 hours (47.62 days).

As shown in Table 6 we found tumours in all 6 unexposed controls of male GFAP-mice and in 4 out of 6 exposed. We found, however, no tumours in female controls and in the female group of exposed GFAP-mice we found only 1 tumour. Thus microwave exposure revealed no significant effect on tumour initiating or evolution in the GFAP-mice after exposure of those mice to EMF with a total exposure time of 1143 hours (Salford, L.G., et al. 2012).

Table 6
Tumour evolution in GFAP-mice, with and without Microwave exposure. P-values from Fisher test for exposed versus control (Salford, L.G., et al. 2012).

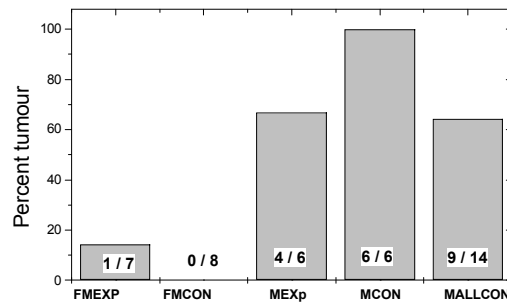|		Tumour	No Tumour	Sum	p-1 tail	p-2 tail
FM	Exposed	1	6	7	0.11	0.11
	Control	0	8	8		
M	Exposed	4	2	6	0.23	0.45
	Control	6	0	6		
	Cage Control	3	5	8		
	All Control	9	5	14	0.66	1.0

Figure 4

The frequency of observed tumour from clinical and histopathological observations (Salford, L.G., et al. 2012).

4.2   Animal EMF-tumour studies by other research groups

The effects of 836 MHz microwaves on spontaneous tumour incidence of central nervous system (CNS) were studied by Adey around the millennium shift (1999/2000) (Adey, W. R., et al. 2000; Adey, W. R., et al. 1999). He also investigated tumours in the offspring of pregnant rats and also modified incidence of primary CNS tumours in rats treated with a single dose of *N-ethyl-N-nitrosourea (ENU)* *in utero*. No signs of increased incidence was found of either spontaneous or *ENU* induced primary tumours of the CNS, associated with lifetime exposure of rats to 836 MHz microwave fields simulating EMF characteristics of hand-held mobile telephone transmissions at the head of the user (Adey, W. R., et al. 2000; Adey, W. R., et al. 1999).

Zook, B. C. and S. J. Simmens (2001) studied 900 Sprague-Dawley rats irradiated with 860 MHz frequency continuous-wave (CW) or a pulsed-wave (P) EMF at SAR values of 1.0 W.kg^{-1} for 6 hours per day for 5 days every week from 2 up to 24 months of age. No statistically significant effect on the number of tumours, volume, location, multiplicity, histological type, malignancy or fatality of brain tumours was found neither in the rats exposed to pulsed-wave (P) or continuous-wave (CW) compared to unexposed controls (Zook & Simmens 2001). They continued the study by investigating the promoting effect of the pulsed RF signal on latency and other characteristics of neuro-genic tumours in the progeny of pregnant rats given *ENU* at whole-body concentrations 6.25 or 10 mg/kg. A study of the tumour characteristics in 1283 tumours of the 1080 offspring disclosed no evidence that exposure to the pulsed RF signal affected the incidence, malignancy, volume, multiplicity, latency or fatality associated with any kind of neuro-genic tumour (Zook & Simmens 2006).

La Regina et al. (2003) investigated the effect of chronic exposure to 835.62 MHz FDMA or 847.74 MHz CDMA radiofrequency radiation on the incidence of spontaneous tumours in rats (La Regina, et al. 2003). Rats were irradiated 4 h per day, 5 days per week during more than 2 years. The nominal time-averaged brain SAR was 1.3 +/- 0.5 W.kg^{-1} (mean SD). The brain and all other major organs were evaluated grossly and histologically. There were no significant differences among final body weights or survival days for either males or females in any group. The number of tumours, types of tumour and incidence of hyperplasia for each organ were recorded. No significant differences were found on the incidence of spontaneous tumours in F344 rats between treated and sham-exposed animals for any tumour in any organ (La Regina, et al. 2003).

Tillman (2010) studied the effects on tumour susceptibility in mice exposed to a UMTS (universal mobile telecommunications system) test signal for up to 24 months, commencing with embryo-foetal exposure of   0, 4.8, and 48 W.m^{-2}. The low-dose group (4.8 W.m^{-2}) was subjected to additional prenatal *ENU* treatment (40 mg/kg body weight). In the high-level UMTS exposure (48 W.m^{-2}), the sham exposure, and the cage control groups showed comparable tumour incidences in the protocol organs. But in the ENU-treated group UMTS-exposed at 4.8 W.m^{-2} an enhanced lung tumour rate and an increased incidence of lung carcinomas was recorded as compared to the unexposed controls treated with ENU only. But no incidence of brain tumour or brain-tumour promotion was found (Tillmann, et al. 2010).

Anderson et al. (2004) performed a 2-year chronic bioassay study to determine whether long-term exposure to a 1.6 GHz radiofrequency (RF) field would affect the incidence of cancer in Fischer 344 rats (Anderson, et al. 2004). The 1.6 GHz RF was modulated with an Iridium signal (frame length of 90 ms, with 11 data slots per frame) used in a system of satellite phones and had the additional advantage of being in a frequency range approximately midway between the major cellular telephone frequencies (0.9 - 2,4 GHz) in current use (Bissell & Chapman 1992). Two different exposure systems were used during the course of the study: One system was designed to simulate a far-field exposure condition during the last 4 days of pregnancy through weaning of the pups at 23 ± 2 days old. Another system was designed for near-field, principally head-only exposure system used from day 35 (of age) through 2 years. The basic study design included treatment of pregnant rats and their 700 litters with 1.6 GHz far-field exposures of 0 and 0.16 W/kg until weaning. Perinatal exposed female and male offspring were then selected for exposure to 1.6 MHz near fields of 0, 0.16 or 1.6 W.kg^{-1}. Whole-body far-field exposures were initiated at 19 days of gestation and continued at 2 h/day, 7 days/week for dams and pups after parturition until weaning (similar to 23 days old).

- Exposed with SAR 1.6 W/kg (90 males and 90 females)
- Exposed with 0.16 W/kg   (90 males and 90 females)
- Near-field sham controls   (90 males and 90 females)
- Shelf controls   (80 males and 80 females)

Confining, head-first, near-field exposures of 2 h/day, 5 days/week were initiated when the offspring were 1 days old and continued until the rats were 2 years old. No statistically significant

differences were observed among treatment groups for number of live pups/litter, survival index, and weaning weights. No differences in clinical signs or neoplastic lesions were found among the treatment groups. No evidence of brain tumour incidence promotion was found in this study at 1.6 GHz which is in agreement with other long-term bioassay studies investigated radiofrequency field exposures of   860 or 836 MHz (Adey, W. R., et al. 2000; Adey, W. R., et al. 1999; Zook & Simmens 2001).

Shirai et al. (2005) investigated the effect of a 2 year long exposure to a 1439 MHz electromagnetic field on promotion of *(ENU)* induced central nervous system tumours in 500 pups of F344 rats (Shirai, T., et al. 2005).

- Group 1, untreated control;
- Group 2, *ENU* alone;
- Group 3, *ENU* (sham exposure)
- Group 4, *ENU* + EMF (SAR 0.67 W/kg)
- Group 5, *ENU* + EMF (SAR 2.0 W/kg)

Japanese standard cellular system was used for the exposure of the rat head starting from 5 weeks of age, 90 min a day, 5 days a week, for 104 weeks. There were no inter-group differences in body weights, food consumption, and survival rates. No increase in the incidences or numbers per group of brain and/or spinal cord tumours, either in the males or females, was detected in the EMF exposed groups. In addition, no clear changes in tumour types were evident. Thus, under the present experimental conditions, 1439 MHz EMF exposure to the heads of rats for a 2 year period was not demonstrated to accelerate or affect ENU initiated brain *tumori-genesis*. But the incidences of pituitary tumours were suppressed by EMF exposure (Shirai, T., et al. 2005).

Therefore Shirai et al. (2007) performed, another study designed for long-term investigation of effects of chronic exposure to 1.95-GHz W-CDMA signals for IMT-2000 cellular system on development of ENU-induced CNS tumours in F344 rats. A total of 100 pregnant F344 rats were given a single administration of *ENU* on gestational day 18. Those rats delivered a total of 500 pups which were divided into five groups, each composed of 50 males and 50 females analogous to the previous study. In either of the groups of males or females pups exposed to 1.95-GHz W-CDMA signals no significant increase in incidences or numbers of brain tumours in was detected after 104 weeks with 5 days a week EMF-exposure (Shirai, Tomoyuki, et al. 2007).

Heikkinen et al. (2006) investigated the effects of radiofrequency radiation on MX (*3-chloro-4-(dichlormethyl)-5-hydroxy-2(5H)-furanone)* -induced *tumori-genesis* given in drinking water to female Wistar rats (Heikkinen, et al. 2006; Heikkinen, et al. 2007). Female Wistar rats aged 7 weeks at the beginning of the experiments were randomly divided into following groups of in total 72 animals:

- a cage-control group
- a sham group of only MX exposed with a daily average dose of 1.7 mg MX/kg body weight for 104 weeks.
- a sham group of only RF-radiation exposed.

http://www2.msf.lu.se/b-persson/

- a group of both MX and exposed to 900 MHz pulsed RF radiation 2 h per day, 5 days per week for 104 weeks at nominal whole-body average SARs of 0.3 W/kg.
- a group of both MX and exposed to 900 MHz pulsed RF radiation 2 h per day, 5 days per week for 104 weeks at nominal whole-body average SARs of 0.9 W/kg.

After complete histopathology of the rats of the three MX-exposed groups, a statistically significant increase of vascular tumours of the mesenteric lymph nodes in the high-RF-radiation group compared to the sham-RF-radiation group was found. Statistically significant non-neoplastic findings commonly seen in aged rats were observed in the lacrimal glands of the eyes, lungs, liver and skin. But for those findings the overall results of the study did not support co-carcinogenic effects of low-level long-term RF-radiation exposure in rats (Heikkinen, et al. 2006; Heikkinen, et al. 2007).

Saran et al. (2007) investigated the effects of GSM modulated 900 MHz microwave exposure to newborn *Patched1 heterozygous mice* of the highly tumour-susceptible mouse strain Ptcl(+/-). Two hundred Ptcl(+/-) mice and their wild-type siblings were enrolled in the study. Newborn mice were exposed to GSM modulated 900 MHz radiofrequency radiation (average SAR: 0.4 W/kg for 5 days, 0.5 h twice a day) or were sham exposed. They found that EMF exposure with GSM modulated 900 MHz microwaves did not affect the length of survival of the mice. No effects attributable to EMF exposure were observed on the incidence and histology of Ptc1-associated cerebellum tumours. Moreover, they found no evidence of proliferative or promotional effects in the skin from neonatal EMF exposure. Furthermore, they detected no difference in Ptc1 - associated *rhabdomyosarcomas* between sham-exposed and EMF exposed mice. Thus, under the experimental conditions tested, there was no evidence of life shortening or *tumori-genic* effects of neonatal exposure to GSM modulated 900 MHz RF radiation in a highly tumour-susceptible mouse model (Saran, et al. 2007). Their results are in agreement with our study of a similar model with GSM 900 MHz exposure of Transgenic GFAP-Mice (Salford, L.G., et al. 2012).

Tillman and collaborators (2007) studied a total of 1170 B6C3F1, mice exposed to radio-frequency-radiation at 902 MHz (GSM) and 1747 MHz (DCS) (Tillmann, et al. 2007). The restrained mice were exposed for 2 h per day, 5 days per week over a period of 2 years, to three different whole-body averaged specific absorption rate (SAR) levels of 0.4, 1.3, 4.0 W/kg body weight (SAR), or were sham exposed. Regarding the organ-related tumour incidence, pair-wise Fisher's test did not show any significant increase in the incidence of any particular tumour type in the RF exposed groups as compared to the sham exposed group (Tillmann, et al. 2007).

Smith et al. (2007) studied Combined chronic Toxicity/Carcinogenicity in 1170 Wistar Rat after 104 weeks (2 h/day and 5 days/week) exposed to GSM or DCS wireless communication signals at three nominal SARs of 0.44, 1.33 and 4.0 W/kg. Additional groups for each modulation were sham exposed, and there was also an unrestrained, unexposed (cage) control group. Fifteen male and 15 female rats per group were killed after 52 weeks. From the remaining 50 male and 50 female rats per group, surviving animals were killed after 104 weeks. There was no adverse response to the wireless communication signals. In particular, there were no significant differences

between the rats exposed to wireless communication signals and rats that were sham exposed for the following endpoints; (Smith, et al. 2007).

- incidence of primary neoplasms,
- number of rats with more than one primary neoplasm,
- multiplicity and latency of neoplasms,
- number of rats with metastases,
- number of benign and malignant neoplasms

4.3 Epidemiological studies

During the last two decades of the past millennium some epidemiological studies have given weak evidence for a correlation between exposure to EMF and increased incidence of leukaemia and tumours of the central nervous system (CNS) among people living close to high power electrical lines or working in electrical occupations (Szmigielski 1996; Verkasalo, et al. 1996). If such a correlation exists, it can be caused either by initiation of cancer development or the promotion of a cancer initiated by other reasons. Some recent results of principal human epidemiology studies also suggest an increased risk of glioma, at the highest exposure levels, for ipsilateral exposures (Cardis, E., et al. 2010). Based on available epidemiological evidence the Health Council of the Netherlands, recently prepared a meta-analysis taking into account the quality of the different studies and their strengths and weaknesses. The final conclusion from their analysis is, however, that there is no clear and consistent evidence for an increased risk of tumours in the brain and other regions in the head in association with up to approximately 13 years use of a mobile telephone (Van Gool 2013). For longer term use, however, for which no data are available, such risk cannot be excluded at present. In a recent study of 1,251 patients with a malignant brain tumour, decreased survival of glioma cases with long-term and high cumulative use of wireless phones was found (Hardell & Carlberg 2013).

5. Conclusion

For both spontaneous brain tumours and tumours promoted by chemical carcinogens, we found no evidence of a significantly increased risk of EMF exposure from wireless communication. Thus there is an agreement between other animal studies and ours that there is no statistical significant relationship between EMF exposure from wireless communication and brain tumour promotion in rodents (Repacholi, et al. 2012; Salford, L. G., et al. 1997; Salford, L.G., et al. 2012; Van Gool 2013).

We found, however, in our rat study a "bath-tube" shape of response pattern of tumour volume "Observed Ratio" versus SAR (W/kg) as well as cumulative specific absorbed energy, SAE (Joule/kg), This non-linear relation between OR and SAR, might explain the discrepancy between the conclusions made from animal studies and some human epidemiological studies

(Cardis, E., et al. 2011a; Cardis, Elisabeth & Sadetzki 2011; I.A.R.C. 2013; Repacholi, et al. 2012; Van Gool 2013; WHO 2011). Thus it is important for future *in vivo* animal studies of tumour promotion also to consider exposure levels below SAR 0.1 W/ kg or SAE < 30 kJ/kg.

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