1990		magnetic field		Sprague Dawley rats	acid (DTPA)	procedure
Prato et al. 1994	23 min x 2	1.5 T static magnetic field	Yes	50 rats		Standard MRI procedure
Garber et al. 1989		0.3-0.5 T static magnetic field	Yes	Rats	Mannitol	Standard MRI procedure
Adzamli et al. 1989			No			Standard MRI procedure
ELF exposure						
Öztas et al. 2004	50 8 hours daily for 21 days	0.005T	Yes	34 Wistar rats	Evans-blue	BBB disruption in diabetic rats, but not in normoglycemic rats

In an attempt to repeat the findings of Oscar and Hawkins (26), Preston et al. (28) found no increase in the uptake of 14C-mannitol in anaesthetised rats after 2450MHz CW exposure for 30 min at power densities of 0.1 to 30mW/cm^2. Preston et al. further concluded that the increased BBB permeability, which had been observed by Oscar and Hawkins (26) in cerebellum and medulla, possibly had been misinterpreted and was not due to the EMF exposure. Rather, changes in blood flow and water influx or egress were supposed to be responsible for the BBB permeability in these caudal parts of the brain. Also, further attempts, made by Merritt et al. (1978) (29), to replicate the findings of Oscar and Hawkins from 1977, resulted in the conclusion that no repetition of the initial findings could be made. Merritt et al. (29) tried to replicate also the findings of Frey et al. (25), but reported that no changes were seen.

However, Frey commented upon this in an article in 1998, where he pointed out that, in fact, statistical analysis by the editor and reviewer of the data from the study by Merritt et al. provided a confirmation of the findings of Frey et al. (25) (30).

No alteration of BBB permeation of 14C-sucrose and 3H-inulin was found by Ward et al. (31)after exposure of anaesthetised rats to CW at 2450MHz for 30 min at power densities of 0, 10, 20, or 30 mW/cm^2 after correction for thermal effects. Similarly, Ward and Ali (32) observed no permeation after 1.7GHz exposure at SAR of 0.1 W/kg, using the same exposure duration and injected tracers as Ward et al. (31). Absence of EMF induced BBB permeability was also reported by Gruenau et al. (33), after injection of 14C-sucrose in conscious rats and exposure 30 min pulsed energy (2.8GHz at 0, 1, 5, 10, or 15mW/cm^2) or continuous wave (2.8 GHz, 0, 10, or 40 mW/cm^2).

Proof of EMF-induced BBB permeability was put forward by Albert and Kerns (34), who exposed un-anaesthetised Chinese hamsters to 2,450MHz CWs for 2 h at SARs of 2.5 W/kg. In one-third of the exposed animals there was an increased permeability of the BBB to horseradish peroxidase (HRP) and the endothelial cells of these irradiated animals had a 2–3-fold higher number of pinocytotic vesicles with HRP than the sham animals. The mechanism of BBB permeability seemed to be reversible, since animals allowed to recover for 1 or 2 h after the EMF exposure had almost no HRP permeation. A total number of 80 animals were included in this study.

Temperature Dependence

In further studies, more attention was directed towards the effects of hyperthermia, resulting from exposure at high SAR-levels, on BBB permeability.

A study correlating changes of BBB permeability with the quantity of absorbed microwave energy by Lin and Lin (35), using Evans blue and sodium fluorescein as indicators of BBB permeation, showed that 20 min of 2,450MHz exposure of anaesthetised Wistar rats caused no alteration of BBB permeability even at SAR values of 80 W/kg. Notably, the same lack of alteration was observed also at lower SAR-values, down to 0.04 W/kg. In further studies by the same group (36), no permeation of Evans blue could be observed after exposure to 2,450MHzB RFs for 5–20 min when the SAR-values ranged from 0.04–200 W/kg. Not until a SAR-value of 240 W/kg, with ensuing rise in brain temperature to 43ºC, was applied, the BBB permeability increased. These observations of demonstrable increases of BBB permeability associated with intense, microwave-induced hyperthermia were supported by another study by the same group (37).

In a series of EMF exposures at 2,450MHz CW, Williams et al. (38-40) concluded that increase of BBB permeability might not be explained by microwave exposure, but rather temperature increases and technically derived artefacts such as increase of the cerebral blood volume and a reduction in renal excretion of the tracer. Significantly elevated levels of sodium fluorescein (38) were found only in the brains of conscious rats made considerably hyperthermic by exposure to ambient heat for 90 min or 2,450MHz CW microwave energy for 30 or 90 min, but this was at high SAR values, 13 W/kg—far beyond the ICNIRP limit of 2 W/kg (41) —and not comparable to the experiments performed by, among others, our group, as described below.

With more research into the area of EMF induced BBB permeability, it became evident that with high-intensity EMF exposure resulting in tissue heating, the BBB permeability is temperature dependent (42). Thus, the importance of differentiating between thermal and non-thermal effects on the integrity of the BBB was realized. This is the reason why studies with increases of BBB permeability due to exposure to SAR-values well above recommended

exposure levels (43-46) need to be considered from another point of view, as compared to those focusing on the non-thermal effects of EMFs.

Continued Studies—MRI and BBB Permeability

Following the increasing use of magnetic resonance imaging (MRI), the effects of MRI radiation upon BBB permeability were investigated more thoroughly. MRI entails the concurrent exposure of subjects to a high-intensity static field, a radiofrequency field, and time-varying magnetic field. Shivers et al. (47) observed that exposure to a short (23 min) standard (of those days) clinical MRI procedure at 0.15 Tesla (T) temporarily increased the permeability of the BBB to horseradish peroxidase (HRP) in anaesthetised rats. This was revealed by electron microscopy (EM), to be due to an amplified vesicle-mediated transport of HRP across the microvessel endothelium, to the ablumenal basal lamina and extracellular compartment of the brain parenchyma. This vesicle-mediated transport also included transendothelial channels. However, no passage of the tracer through disrupted interendothelial tight junctions was present.

During the next few years, more groups studied the effects of MRI exposure on the BBB permeability by injection of radioactive tracers into rats. One supported (48)while others contradicted (49, 50) the initial findings made by Shivers et al. (47). Garber et al. exposed rats to MRI procedures at 1.5, 0.5, and 0.3 T with RFs of 13, 21, and 64 MHz, respectively (48). Brain mannitol concentration was significantly increased at 0.3 T and 0.5 T but not at 1.5 T. No decrease in plasma mannitol concentration of MRI exposed animals was found and thus the authors concluded that effects of MRI associated energies on mannitol transport do not occur measurably in the body, and might be more specific to brain vasculature. Preston et al. (50) found no significant permeation of blood-borne 14C-sucrose into brain parenchyma in anesthetized rats subjected to 23 min of MRI at 4.7 T and RFs at 12.5 kHz. However, the authors pointed out that if the MRI effect was focal and excess tracer counts were found only in restricted sites, there could have been MRI induced extravasation of sucrose that was not detected, due to the preponderance of normal tissue counts. When Preston et al. (50) compared the lack of BBB leakage in their study to the MRI induced leakage which had been observed by Shivers et al. (47), they also concluded that certain characteristics of electric and

magnetic fields, which were present in the study by Shivers et al. but not in their own work, could have been critical to the observed effects.

In 1990, further studies by the Shivers-Prato group were presented (51) and the group could now quantitatively support its initial findings, in a series of 43 Sprague-Dawley rats. The BBB permeability to diethylenetriaminepentaacetic acid (DTPA) increased in rats after two sequential 23 min MRI exposures at 0.15 T. It was suggested that the increased BBB permeability could result from a time-varying magnetic field mediated stimulation of endocytosis. Also, the increased BBB permeability could be explained by exposure-induced increases of intracellular Ca^{2+} in the vascular endothelial cells. Since the Ca^{2+} is an intracellular mediator, increases of BBB permeability could possibly be initiated in this way. A few years later, in a series of 50 rats, the Shivers - Prato group also found that the BBB permeability in rats is also altered by exposure to MRI at 1.5T for 23 min in 2 subsequent exposure sessions (52).

Studies by the Lund Group

Two of us found these observations highly interesting:

       - the neurosurgeon (LGS) in the hope to utilize possible applications of EMF to make the blood-brain barrier (BBB) more penetrable to chemotherapy, in order to treat brain cancers more effectively. An intact BBB keeps out chemotherapy agents, allowing cancer cells to hide behind the BBB.

       - the radiophysicist (BRRP) interested in possible adverse effects of the MRI technique.

After a visit to Shivers' group in London Ontario in 1988, we started work in Lund in 1988, studying the effects of MRI on rat brain and we found, by the use of Evans Blue, the same increased permeability over BBB for albumin (53).

This work was continued by separating the constituents of the MRI field: RF, undulant magnetic field, and static magnetic field. Since RF turned out to be the most efficient component of the MRI, the following studies focused mainly on the RF effects. Striving for

investigating the actual real-life situation, endogenous substances, which naturally circulate in the vessels of the animals, were used. In line with this, albumin and also fibrinogen leakage over the BBB were followed after identification of albumin with rabbit antibodies (see Figure 2 and 3) and rabbit anti-human fibrinogen.
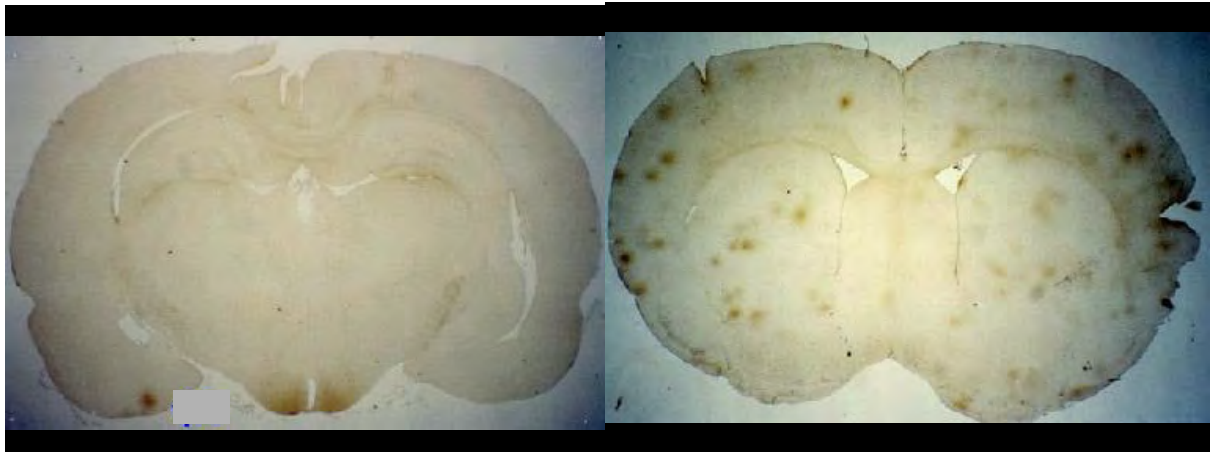

Figure 2. Albumin extravasation in rat brain (material from Persson et al. 1997)(54).
Left: control brain with albumin staining in hypothalamus, which serves as an inbuilt-control of the staining method, since the hypothalamus lacks BBB, and one occasional staining.
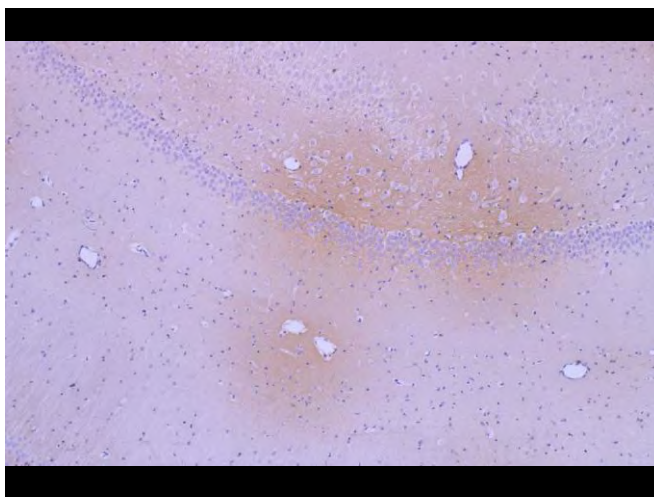Right: Brain of EMF exposed rat, with multiple albumin positive foci.

Figure 3. Albumin extravasation around vessels in the brain of an EMF exposed rat.

The work by Blackman et al. (55, 56) ~~made the ground~~ laid the groundwork for studies on the frequency modulation 16 Hz and its ~~harmonies~~ harmonics 4 and 8 Hz. A carrier wave of 915 MHz was used. At the suggestion of Östen Mäkitalo (Telia), a pioneer in mobile phone

development, who introduced 50 Hz (DUX) and 217 Hz (GSM) modulation in new digital wireless communication systems, we also included theses frequencies. This paralleled the first BBB study results that were published in 1992-1994 (57-59).

The result of our continued work, comprising more than 1000 animals, with exposure to both CWs and pulsed modulated waves, in the most cases lasting for 2 h, showed that there was a significant difference between the amount of albumin extravasation in the exposed animals as compared to the controls. In the exposed group 35–50% of the animals had a disrupted BBB as seen by the amount of albumin leakage, while the corresponding leakage in the sham exposed animals was only 17% (for results see Figure 4) (54).
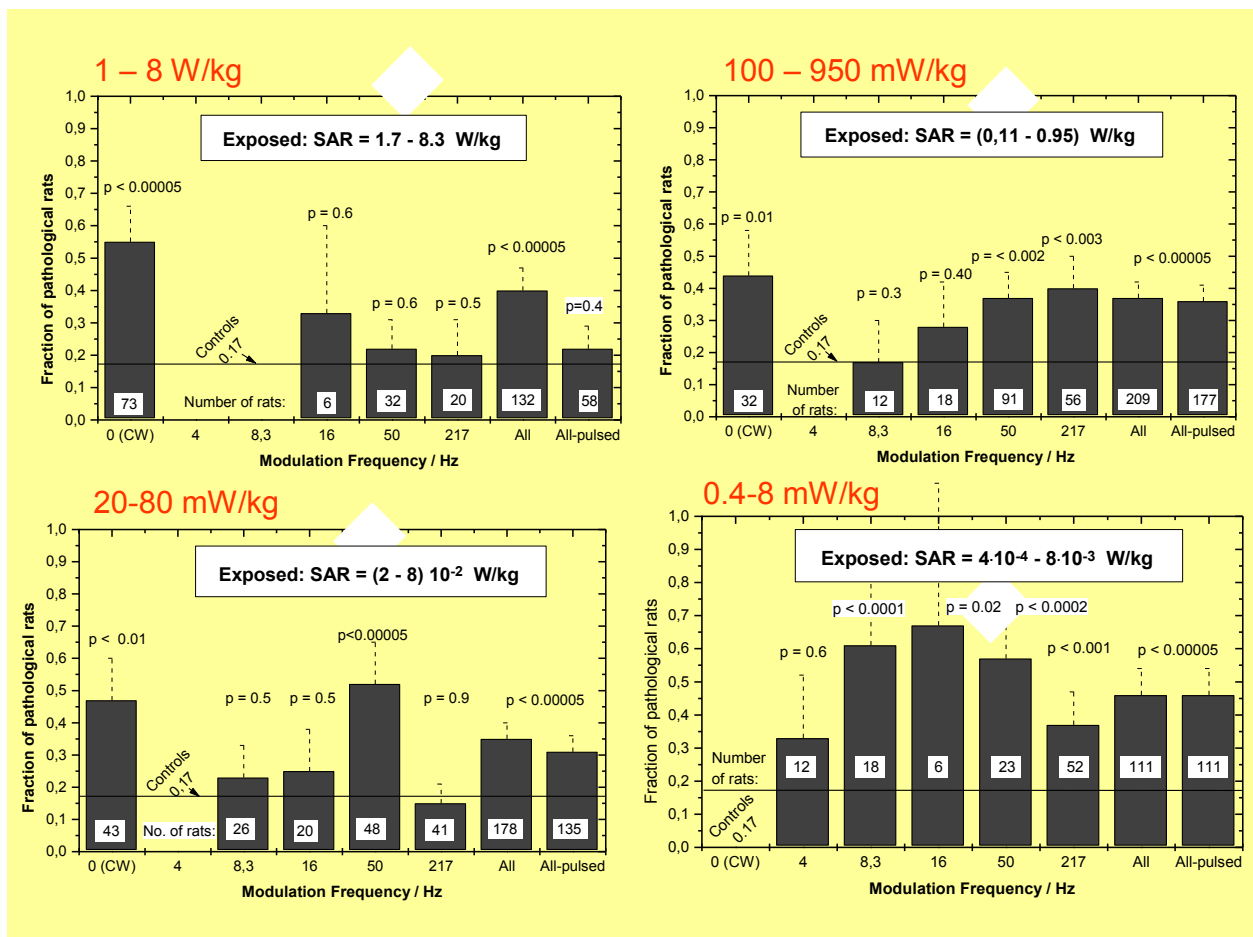

Figure 4. Albumin extravasation score as a result of EMF exposure (results from the study by Persson et al. (54)).

The fact that sham-exposed control animals also show some amount of albumin extravasation (see Figure 4), is most likely due to our very sensitive methods for immune histological examination. However, it is hard to explain the fact that although all animals in the 1997 series were inbred Fischer 344 rats, only every second animal, at the most, showed albumin leakage after EMF exposure. The question, what might protect the remaining 50% of the exposed animals from BBB disruption, is highly intriguing. It should be noted that in our large series, only in one single animal fibrinogen leakage has been observed (54).

Another conclusion from the 1997 study is that the number of pathological leakages in exposed animals is more frequent, and also more severe, per animal compared to the controls. This is an interesting observation as the prevailing opinion is that pulse modulated electromagnetic fields are more potent in causing biological effects.

In a statistical re-evaluation of our material published in 1997, where only exposed rats with a matched unexposed control rat are included, we found for the most interesting modulation frequency 217 Hz, i.e. that of GSM, that at SAR-values of 0.2 to 4 mW/kg 48 exposed rats had a significantly increased albumin leakage ($p < 0.001$) as compared their 48 matched controls. On the other hand, SAR-values of 25-50 mW/kg, gave no significant difference between 22 exposed rats vs their matched controls (Wilcoxon´s Rank Test, 2-sided p-value) (60).

In all our earlier studies we showed albumin extravasation immediately after exposure as described above. In later years we have performed a series of experiments where the animals were allowed to survive for 7 days (61), 14 days, 28 days (62) or 50 days (63) after one single 2-hour exposure to the radiation from a GSM mobile phone. All were exposed in TEM-cells to a 915 MHz carrier wave as described below. The peak power output from the GSM mobile phone fed into the TEM-cells was 1 mW, 10 mW, 100 mW and 1000 mW per cell respectively for the 7-14-28-days survival animals, resulting in average whole-body SAR of 0.12 mW/kg, 1.2 mW/kg, 12 mW/kg and 120 mW/kg for four different exposure groups SAR-values of 2, 20 and 200 mW/kg mW/kg for 2 hours for the 50-days survival animals.

Albumin extravasation over the BBB after GSM exposure seemed to be time-dependent, with significantly increased albumin in the brain parenchyma of the rats, which had survived for 7 and 14 days, but not for those surviving 28 days. After 50 days, albumin extravasation was

22

significantly increased again, with albumin-positive foci around the finer blood vessels in white and gray matter of the exposed animals.

In connection to the albumin passage over the BBB, albumin also spread in the surrounding brain tissue. A significantly increased uptake of albumin in the cytoplasm of neurons could be seen in the GSM exposed animals surviving 7 and 14 days after exposure, but not in those surviving 28 or 50 days.

Neuronal uptake

Extravasated albumin rapidly diffused down to, and beyond, concentrations possible to demonstrate accurately immunohistologically. However, the initial albumin leakage into the brain tissue (seen within hours in ~40% of exposed animals in our previous studies) most likely started a vicious circle of further BBB opening.

It has been postulated that albumin is the most likely neurotoxin in serum (64). Hassel et al. (65) have demonstrated that injection of albumin into the brain parenchyma of rats gives rise to neuronal damage. When 25 µl of rat albumin is infused into rat neostriatum, 10 and 30, but not 3 mg/ml albumin causes neuronal cell death and axonal severe damage. It also causes leakage of endogenous albumin in and around the area of neuronal damage. Albumin in the dose 10 mg/ml is approximately equivalent to 25% of the serum concentration.
It is less likely that the albumin leakage demonstrated in our experiments locally reaches such concentrations. However, we have seen that in the animals surviving 28 and 50 days after 2 hours of GSM exposure, there was a significantly increased incidence of neuronal damage as compared to the sham controls. In the 7-days and 14-days survival animals, on the other hand, no such increase of neuronal damage was seen.

In the 50-days post-exposure survival study, a 2 h exposure to GSM at SAR values 200, 20, and 2 mW/kg resulted in a significant (p = 0.002) neuronal damage in rat brains of the exposed animals as compared to the controls 50 days after the exposure occasion (Salford et al., 2003)(63). We have followed up this observation, as mentioned above, in a study where 96 animals were sacrificed 14 and 28 days respectively after an exposure for 2 h to GSM mobile phone electromagnetic fields at SAR values 0 (controls), 0.12, 1.2, 12 and 120 mW/kg. Significant neuronal damage is seen after 28 days and albumin leakage after 14. Our

findings may support the hypothesis that albumin leakage into the brain is the cause for the neuronal damage observed after 28 and 50 days (62).

The damaged neurons in the above mentioned studies took the shape of so-called dark neurons. Three main characteristics of the damaged dark neurons have been proposed (66): (i) irregular cellular outlines, (ii) increased chromatin density in the nucleus and cytoplasm and (iii) intensely and homogenously stained nucleus. The damaged dark neurons found in the 50 days-survival animals were investigated regarding signs of apoptotic markers, but we found no positive staining for Caspase-3, a marker for apoptosis (Bexell et al. unpublished results). However, the albumin leakage out in the neuropil in connection to EMF exposure might start other deleterious processes, leading to the formation of the dark neurons.

A group in Turkey performed similar experiments. However, also the presumed protective effects of the antioxidant Ginko biloba (Gb) were examined by Ilhan et al. (67). About 22 female Wistar rats were exposed to a 900 MHz electromagnetic GSM near-field signal for 1 h a day for 7 days. In the GSM only group, the pathological examination revealed scattered and grouped dark neurons in all locations, but especially in the cortex, hippocampus and basal ganglia, mixed in among normal neurons. A combined non-parametric test for the four groups revealed that the distributions of scores differed significantly between the control and the GSM only exposure group ($p < 0.01$).

Long-term study, including studies of memory and behaviour
In a recent long-term study from our laboratory, rats were exposed to GSM radiation 2 hours weekly during 55 weeks (two different exposure groups with 0.6 mW/kg and 60 mW/kg at the initiation of the exposure period). After this protracted exposure, behaviour and memory of the exposed animals were tested. Whereas the behaviour of the animals was not affected, the GSM exposed rats had significantly impaired episodic memory as compared to the sham controls (68). After the finalization of these tests, that is 5-7 weeks after the last exposure, the animals were sacrificed by perfusion fixation. Albumin extravasation, an indicator of BBB leakage, was increased in about 1 animal in each group of low GSM exposed, high GSM exposed, sham exposed and cage control rats. About 40 % of the animals had neuronal damage. GFAP staining, as an indicator of glial reaction, revealed positive results in 31-69 % of the animals for different groups and the aggregation product lipofuscin was increased in

44-71 % of the animals for different groups. With the Gallyas staining (aiming at cytoskeletal structures), no changes were seen. When comparing the results between the different groups, it turned out that there was no statistically significant difference for any of these parameters due to GSM exposure (69). When comparing these findings to those from animals which had been exposed only once for 2 hours, it seems likely that during the 55 weeks of repeated exposure, albumin leakage at an initial stage of the experimental period might have been absorbed after some time, and that at a certain, but unknown, time point during this protracted, more than 1 year long-exposure period, some adaptation process might have been activated. However, this could not compensate for cognitive alterations, demonstrated by the episodic memory tests.

TEM-cells

In the majority of our studies, EMF exposure of the animals has been performed in transverse electromagnetic transmission line chambers (TEM-cells, see Figure 5) (53, 54, 59, 61-63, 68-71). These TEM-cells are known to generate uniform electromagnetic fields for standard measurements. Each TEM-cell has two compartments, one above and one below the center septum. Thus, two animals can be exposed at a time. The animals are un-anaesthetized during the whole exposure. Since they can move and turn in the TEM-cells as they like, the component of stress-induced immobilization (described by Stagg et al. (72)) is effectively minimized. Through our studies, we have concluded that the amount of albumin leakage is neither affected by the sex of the animals, nor their placement in the upper or lower compartments of the TEM-cells.
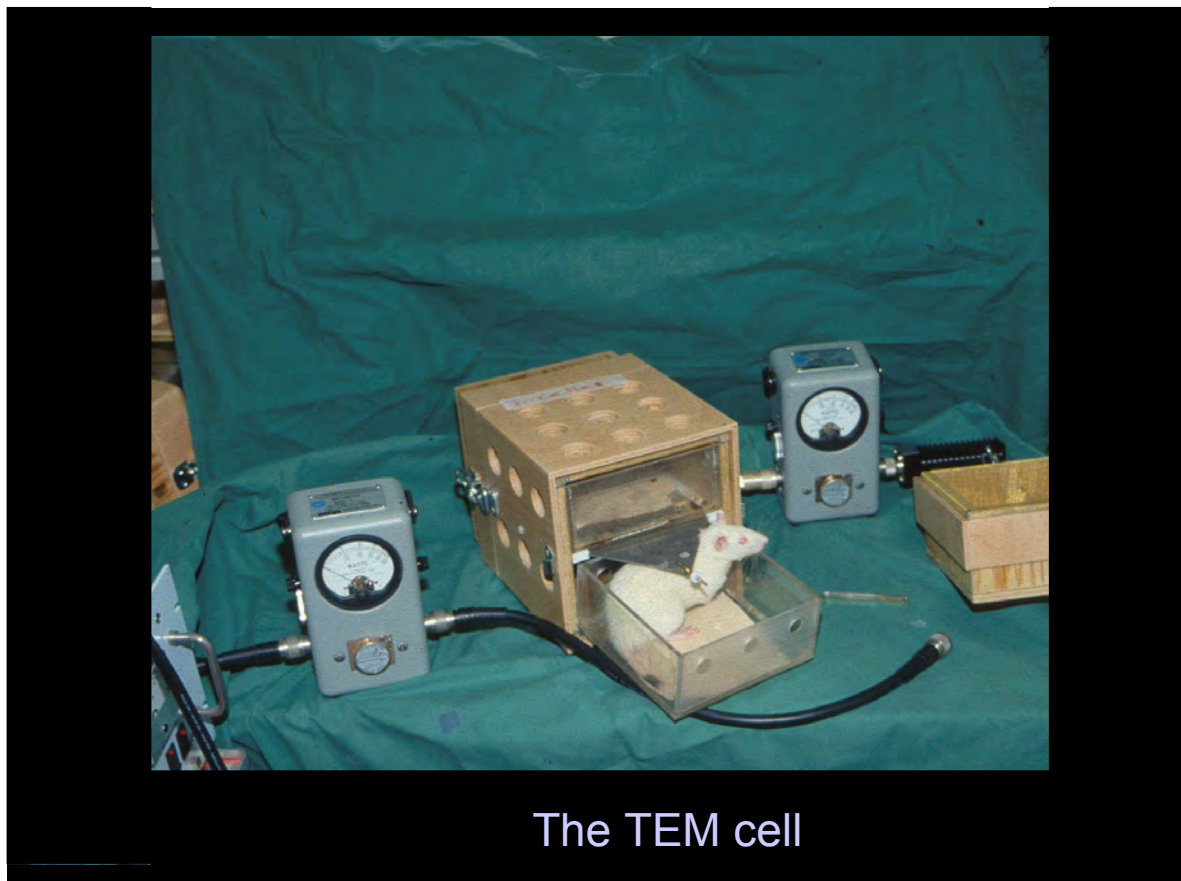
The TEM cell

Figure 5. TEM-cells for EMF exposure.

GSM-1800 modulated and CW microwaves in an anechoic chamber

In Lund we have also utilized an anechoic chamber for studies on microwaves from a real GSM-1800 mobile telephone, which were amplified and transferred to a dipole antenna in the anechoic chamber. The output power was varied to study the effect of various SAR values. In a series of 65 rats exposed for 2 h with 1800-GSM at SAR: 0.027 mW/kg, and 12 rats exposed for 2 h with continuous wave, we found significantly increased albumin leakage (see figure 6) as compared to 103 control rats ($p < 0,03$ and $p < 0,02$, respectively). (Unpublished results).
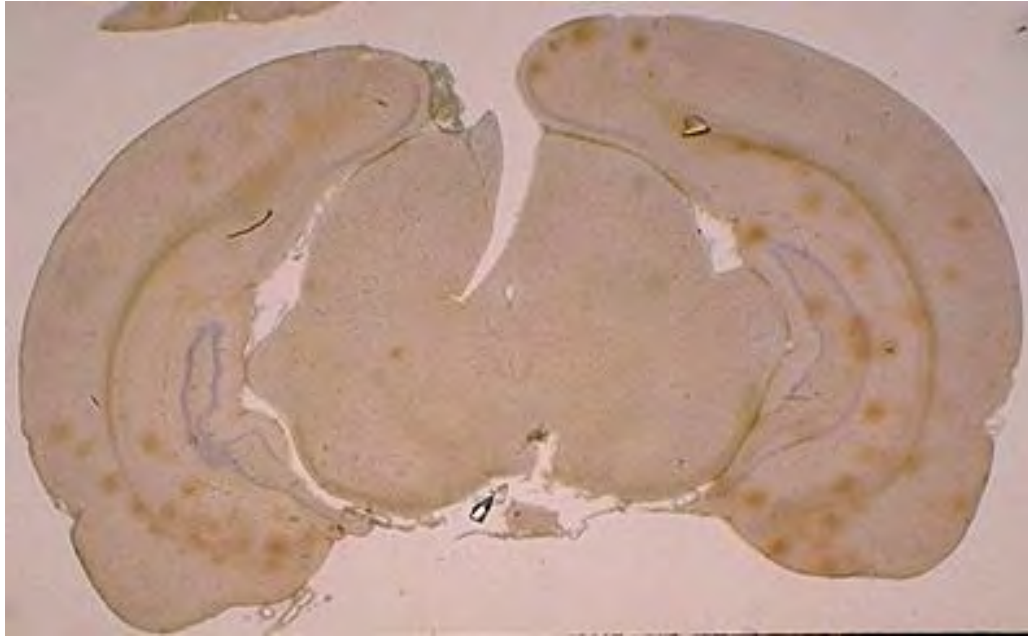
Figure 6.

Pathological leakage around vessels demonstrated by immunostaining against albumin.

Fischer 344 rat exposed for 2 h with 1800-GSM at SAR: 0.027 mW/kg

Other Studies on BBB Permeability, Focusing on the Effects of RF EMFs of the Type Emitted by Mobile Phones

With the increasing use of mobile phones, much attention has been directed towards the possible effects on BBB permeability, after exposure to the type of RF EMFs emitted by the different sorts of mobile phones.

Repetitions of our initial findings of albumin leakage have been made by Fritze et al. (73), with 900 MHz exposure of rats for 4 h at brain power densities ranging from 0.3–7.5 W/kg. Albumin extravasation into the brain tissue was seen, with significant difference between controls and rats exposed reported for 7.5 W/kg, which is a thermal level. However, Fisher exact probability test (two-tailed) performed on the reported results, reveals significant ($p <$ 0.01, Fisher exact probability test) difference for the subthermal level group (SAR 0.3 W/kg plus 1.3 W/kg, compared to sham exposed and cage control animals) where in total 10 out of 20 animals showed one or more extravasations direct after exposure (Salford et al. (20)).

27

Another group, working in Bordeaux, and led by Prof Pierre Aubineau, has also demonstrated evidence of albumin leakage in rats exposed for 2 h to 900 MHz at non thermal SAR-values, using fluorescein-labeled proteins. The results were presented at two meetings by Töre et al. (74, 75). The findings are very similar to those of our group, described above.

At the BEMS meeting in 2002 in Quebec City in Canada, the Aubineau-Töre group presented results from exposure GSM-900 EMFs at SAR values of 0.12, 0.5, and 2.0 W/kg. Seventy Sprague-Dawley rats were included in the study. In addition to normal sham and normal GSM exposed rats, also rats subjected to chronic dura mater neurogenic inflammation, induced by bilateral sympathetic superior cervical ganglionectomy, were included. Arterial blood pressure was measured during the exposure, and Töre et al. (74, 75) concluded that the pressure variations (100–130mm Hg) were well below those limits, which are considered to be compatible with an opening of the BBB of rats. In order to induce opening of the BBB in rats, arterial blood pressure needs to reach values of 170 mmHg, according to Töre et al. (74, 75). At SAR of 2 W/kg a marked BBB permeabilization was observed, but also at the lower SAR-value of 0.5 W/kg, permeabilization, although somewhat more discrete, was present around intracranial blood vessels, both those of the meninges and of the brain parenchyma. Comparing the animals, which had been subjected to ganglionectomy, to the other animals, Töre et al. made an interesting observation: as expected, albumin extravasation was more prominent in the sympathectomised sham-exposed rats as compared to normal exposed rats. This was due to the fact that the sympathectomised rats were in a chronic inflammation-prone state with hyper-development of pro-inflammatory structures, such as the parasympathetic and sensory inputs as well as mast cells, and changes in the structure of the blood vessels. Such an inflammation-prone state has a well-known effect on the BBB leakage. However, when comparing sham-exposed sympathectomised rats to GSM-exposed sympathectomised rats, a remarkable increase in albumin leakage was present in the GSM exposed sympathectomised rats compared to the sham rats. In the GSM-exposed sympathectomised rats, both brain areas and the dura mater showed levels of albumin leakage resembling those observed in positive controls after osmotic shock. Indeed, more attention should be paid to this finding, since it implicates that the sensitivity to EMF-induced BBB permeability depends not only on power densities and exposure modulations, but also on the initial state of health of the exposed subject.

In rats, uptake of a systemically administered rhodamine-ferritin complex through the BBB also has been observed, after exposure to pulsed 2.45GHz EMFs at average power densities of

2 W/kg by Neubauer et al. (76). The authors observed that the magnitude of BBB permeability depended on power density and duration of exposure. Exposure to a lower power density (1 W/kg) and shorter duration of the exposure (15 min) did not alter the BBB permeability, as compared to higher power densities (SAR 2 W/kg) and longer duration of exposure (30–120 min). The microtubules seemed to play a vital role in the observed BBB permeability, since treatment with colchicine, which inhibits microtubular function, resulted in near-complete blockade of rhodamine-ferritin uptake. The mechanism underlying the observed leakage was presumed to be correlated to pinocytotic-like transport.

In other studies, no effect of EMF exposure has been observed on the BBB integrity. With exposure to 1,439MHz EMFs, 1 h daily during 2 or 4 weeks (average whole-body energy doses of 0.25 W/kg) no extravazation of serum albumin trough the BBB was observed in a series of 36 animals by Tsurita et al.(77). However, in this small material only 12 animals in total were EMF exposed (6 rats exposed for 2 weeks and 6 rats exposed for 4 weeks). Also, lack of interference with the BBB function of rats was found after 1,439MHz exposure for 90 min/d for 1–2 weeks at average brain power densities of either 2 or 6W/kg by Kuribayashi et al.(78). A total number of 40 animals were included in the study.

Finnie et al. (79) came to the conclusion that no increase in albumin leakage over the BBB resulted from EMF exposure in a series of 60 mice. With whole body exposure of mice to GSM-900 EMFs for 1 h at a SAR of 4 W/kg or sham exposure, no difference in albumin extravazation was observed between the different groups. Also, free-moving cage controls were included in the study, and interestingly, there was no significant difference between these non-restrained mice as compared to the sham and EMF-exposed animals. Thus, the authors concluded that there were no stress-related exposure module confinement effects on the BBB permeability.

Finnie et al. (80) continued to investigate more long-lasting exposure effects. In a series of experiments, a total of 207 mice were exposed 60 min daily, 5 days per week for 104 weeks at average whole body SARs of 0.25, 1.0, 2.0, and 4.0W/kg. This led to a minor disruption of the BBB, as seen by the use of endogenous albumin as a vascular tracer. However, it should be added that the authors performed no statistical analyses to evaluate the albumin leakage through the small vessels in the brain. In an answer to correspondence in the same journal (81), the authors presented the original data from the long-term study in one table, from which

one can conclude that non-leptomeningeal albumin leaking vessels were seen in few sham-exposed animals, and in one-third of the animals in the 0.25 W/kg group and to a lesser extent in the higher SAR groups.

The fact that some research groups observe albumin leakage/transport over the BBB after EMF exposure and others do not, has led to a rather intense debate between the researchers but also in society, which is puzzled by the divergent findings. A major concentration of the involved research groups took place at Schloss Reisensburg in Germany in 2003, where the technical approaches in the studies of BBB effects were discussed. Two world-renowned researchers in the BBB field, Dr. David Begley of Kings College, London, and Prof. Olaf Poulsen of Copenhagen, Denmark, chaired the FGF/COST 281 Reisensburg, November 2–6 meeting. They made the final statement as a summary of the meeting: ''It seems clear that RF fields can have some effects on tissues''. The statement was made to a large extent on the basis of the concordant findings of the Bordeaux group, represented by Prof. Aubineau, and the Lund group, represented by Prof. Salford and Prof. Persson.

The histopathological examinations of the brains are not uncomplicated. Some laboratories that have tried to replicate our studies have not been able to demonstrate the albumin leakage. We have recently had problems with the albumin staining due to change of suppliers of avidin, biotin, serum and antibodies. The lateral hypothalamic nuclei in the immediate vicinity of the third ventricle are well known for their normally insufficient BBB. This has served as an inbuilt control of adequate albumin staining in all our experiments since 1990. In our study on combined effects of RF- and ELF-EMF, for the first time, we could not demonstrate albumin extravasation in basal hypothalamus. Not until our third attempt with new staining material, we got our positive control and could also demonstrate albumin leakage in the exposed brains (61).

The biological effects of RF exposure depend on many parameters, such as mean power level and the time variations of the power (82) and whether in vivo or in vitro experiments are performed. In the in vivo situation, different kinds of animals, and also the same kind of animals but of different breeds, might react differently. It might not necessarily be the strongest RF fields that give rise to the most obvious biological effects (54, 63). In many cases, the weak and precisely tuned EMFs have the most important biological function; two examples of this are cellular communication and protein folding. It seems quite likely that in

different experimental set-ups, and in different living organisms, the signal has to be tuned to different properties in order to cause any effect. This could perhaps in some part explain why, in some cases, there are quite obvious effects of RF exposure, whereas in others, no such effects can be seen.

Other Studies on BBB permeability and neuronal damage

As has been mentioned above (p. 26) Ilhan et al. (67), in 2004 reported neuronal damage in female Wistar rats, which had been exposed to a 900 MHz electromagnetic GSM near-field signal for 1 h. a day for 7 days. They found scattered and grouped dark neurons in the cortex, hippocampus and basal ganglia, mixed in among normal neurons. A combined non-parametric test for the four groups revealed that the distributions of scores differed significantly between the control and the GSM only exposure group ($p < 0.01$).

Later, Masuda et al. (83) tried to replicate the findings by our group of albumin extravasation and dark neurons. F344 rats (n=64) were exposed to 915 MHz signals for 2 hours (SAR of 0, 0.02, 0.2 and 2 W/kg), and albumin extravasation and dark neurons were investigated 14 and 50 days after the exposure. No albumin extravasation was seen, neither in control or exposed rats, and no difference in the occurrence of dark neurons could be found due to EMF exposure. An interesting difference as compared to the studies by Salford et al. mentioned above, was that animals, after perfusion fixation, were left in a 4°C storage for 18 hours before the brains were removed. The question is whether this might have led to dilution of the very sensitive albumin extravasation, which is often more pronounced in the circumventrical organs as compared to the brain extravasates (personal communications with our neuropathologist Arne Brun). This might explain the fact, that no albumin extravasation could be seen in neither the cage control animals, the shams or the GSM exposed animals.

Another study by Mason and his group at Brooks Airforce Resarch Laboratory, San Antonio, also tried to confirm our findings of albumin extravastion by using the same type of TEM-cells for EMF Exposure (84), although the exposure parameters where somewhat different with only 30-min exposure, including only male rats of the Fischer 344 CD-VAF strain and utilizing only the upper compartment of the TEM cells. Exposure was at whole-body SAR values of 0.002 to 20 W/kg. Regarding extracellular albumin accumulation, the results were

not formally analyzed, as motivated by too low scores of albumin. Regarding intracellular albumin uptake, no significant difference between the different groups was reported. However, as presented in the paper by McQuade et al.(84), at the lowest SAR of 1.8 mW/kg at 16 Hz, of 33 exposed rats, 11 had 2 or 3 positivities (33% of the animals) and 22 had none or 1 positivity. In the sham animals, 18% were positive and among the cage controls only 12%. These results are reminiscent of prior work by the Lund group reporting that 17% of the sham animals had some albumin leakage, while only at the most 50% of the identical and equally handled, but RF exposed animals displayed albumin extravasation (60).

In a third study aiming to replicate the Lund findings of dark neurons, a group in Bordeaux (85) exposed 14 weeks old Fischer 344 rats (which, however, were restrained in a rocket-type exposure setup), to the GSM-900 signal for 2 h at various brain-averaged SARs (0, 0.14 and 2.0 W/kg). Eight rats were included in each of these groups.
Albumin leakage and neuronal degeneration was evaluated 14 and 50 days after exposure.
It was reported that no statistically significant albumin leakage was observed and that neuronal degeneration assessed using cresyl-violet or the more specific marker Fluoro-Jade B, was not significantly different among the tested groups. Here we want to point out that the Bordeaux group makes a major deviation from the way we have evaluated the occurrence of dark neurons in the tissue slices. While we counted the overall number of dark neurons, de Gannes et al. (85) chose to subdivide the slices into 12 different small regions, which were compared individually to each other (fig 3 in the publication). This gave the effect that a clear overall difference in number of observed dark neurons between animals 50 days after exposure to 2 W/kg for two hours versus sham exposed, disappeared in the statistics. On the contrary, if all the numerical values for the bars representing the scored dark neurons observed in each brain zone and region 50 days after exposure to 2 W/kg are compared to all those of the sham animals, a highly significant difference (Kruskall-Wallis) between animals exposed to 2 W/kg and sham is demonstrated (Mann-Whitney) p = 0.003! This is in concordance with the Lund experience!

Indirect studies and studies on the blood cerebrospinal fluid barrier

The integrity of the BBB has also been investigated indirectly. Cosquer et al. (86) treated rats with the muscarinic antagonist scopolamine methylbromide, which is known to induce

memory impairments, followed by EMF exposure at 2.45GHz for 45 min at average whole body SARs of 2W/kg. Opening of the BBB after EMF exposure was hypothesised to affect the performance in a radial arm maze. However, no such alterations were observed and the authors concluded that no BBB opening seemed to have occurred. In agreement with this, no albumin extravasation was noticed.

Ushiyama et al. (87) investigated the effects on the blood cerebrospinal fluid barrier after RF-EMF exposure. With a microperfusion method, cerebrospinal fluid from rat brain was collected in vivo. Fluorescent intensity of FITC-albumin in perfusate was measured. Rats exposed to 1.5GHz RFs during 30 min at SAR-values of 0.5, 2.0, 9.5W/kg for adult rats and 0.6, 2.2, 10.4W/kg for juvenile rats, respectively, were compared to sham-exposed controls. Under these conditions, no increase in FITC-albumin was seen in the cerebrospinal fluid of exposed rats as compared to sham exposed controls. It was concluded that no effect on the function of the blood cerebrospinal fluid barrier was seen.

In a recent study, the permeability of the human BBB after mobile phone exposure was assessed measuring blood levels of S100B and transthyretin in human volunteers by Söderqvist et al. (88). S100B is a calcium-binding protein, and it has been shown to be increased in serum after damage to the BBB. Transthyretin, also known as pre-albumin, is synthesised both in the liver and the choroid plexus. 30 min of GSM-900-like exposure at SAR-values of 1 W/kg was used. No difference was seen regarding S100, but transthyretin was increased 60 min after the termination of exposure as compared to the control situation. The concentrations of S100B and transthyretin were also analysed 30 min prior to provocation and after 30 min rest, showing a decrease after 30 min rest, which was suggested, might be due to less stress after the 30 min rest. Thus, it is interesting that despite this decline, which might be due to relaxation, still an increase in thransthyretin could be measured 30 min after exposure. It was also put forward, that it could not be excluded that the thransthyretin rise might be a compensation to the previous decrease, and that new studies including more participants and also a sham group would be needed.

We have in the past investigated whether MW exposure, CW and at different SAR levels might enhance S-100 protein levels in the blood of a large proportion of our rats. We could conclude that no significant differences were seen (see Figure 7 below) (to be published).
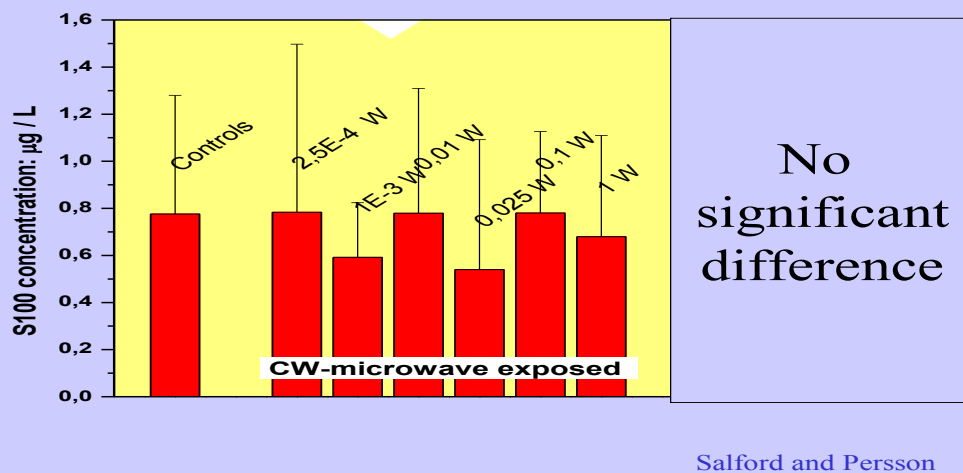
Figure 7. S-100 in the blood of rats after EMF exposure (to be published in Acta Scientiarum Lundensia).

In another study, by Sirav and Seyhan (89), exposure to CW EMFs at 900 and 1,800 MHz for 20 min, increased the BBB permeability of male but not female rats. Evans blue dye, which binds to serum albumin after injection, was used to quantitatively measure BBB permeability. A strength of this study, was the ability to objectively quantify the Evans blue uptake in the brain. The finding that only male, and not female rats, are affected, is however not fully addressed.

In Vitro Models

In recent years, there has been an increasing use of in vitro models in the search for BBB effects of EMF exposure. In vitro models of the BBB have been studied, as by Schirmacher et al. (90), with co-cultures consisting of rat astrocytes and porcine brain capillary cells. Exposure to GSM-1800 for 4 d with average SAR of 0.3 W/kg increased the permeability of 14C-sucrose significantly compared to unexposed samples in the studied BBB model. These findings were not repeated in experiments performed later by the same group, after modifications of their in vitro BBB model (91). The modified BBB model had a higher general tightness. It was speculated that at a higher original BBB permeability, which was

present in the first study by Schirmacher et al. (90), the cultures were more susceptible to the RF EMFs. Using porcine brain microvascular-endothelial cell cultures as an in vitro model of the BBB, no effects on barrier-tightness, transport behavior, and integrity of tight junction proteins were observed-after exposure to UMTS EMFs at 1.966 GHz for 1–3 d at different field strengths at 3.4–34 V/m, generating a maximum SAR of 1.8 W/kg (92).

In the search after the mechanism underlying non thermal EMF effects, Leszczynski et al. (93) observed human endothelial cells, with the interesting finding that GSM-900 exposure for 1 h with SAR-values of 2 W/kg resulted in changes in the phosphorylation status of many proteins. Among the affected pathways, the hsp27/p38MAPK stress response pathway was found, with a transient phosphorylation of hsp27 as a result of the mobile phone exposure. This generated the hypothesis that the mobile-phone induced hsp27-activation might stabilize stress fibers and in this way cause an increase in the BBB permeability. Furthermore, it was also suggested that several brain-damaging factors might all contribute to the mobile phone-induced effects observed in the brain and other structures as well.

Further perspectives of the importance of the BBB including the human situation

BBB in the Context of Alzheimer's Disease and the findings by the Zlokovic Group

The BBB, as mentioned previously, is of essential role for maintaining an accurate brain function. As described by Zlokovic (94), in a review regarding BBB in correlation to neurodegenerative disorders, BBB breakdown can be due to tight junction disruption, alterations of angiogenesis or vessel regression, hypoperfusion, inflammatory response and alterations of the transport of molecules across the BBB (94). Further, as Zlokovic hypothesises, this might contribute to neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease, multiple sclerosis and amyotrophic lateral sclerosis.

In the review by Zlokovic (94), a neurovascular disease pathway is presented, regarding possible genesis of AD, where it is suggested that changes in vascular genes and receptors in brain capillaries and small arteries might disrupt BBB functions, leading to an accumulation

of amyloid beta (Aβ), a neuroinflammatory response and BBB breakdown and further on accumulation of Aβ, loss of the BBB to clear Aβ (due to affected synaptic transmission, neuronal injury and recruitment of microglia) and secretion of proinflammatory cytokines. Ultimately, this is suggested to lead to disappearance of the capillary unit, increasing Aβ deposits and synaptic and neuronal loss (94).

This observation might explain how vascular disease contributes to Alzheimer's disease (AD) risk; the heterogeneity of AD; and supports the idea that exclusively focusing on amyloid is likely to be disappointing.

Neuronal injury resulting from vascular defects that are not related to amyloid-beta but is related to damage results from a breakdown of the blood-brain barrier and a reduction in blood flow (94). Although Amyloid beta definitely has an important role in Alzheimer's disease it's very important to investigate other leads, perhaps where amyloid-beta isn't as centrally involved.

Human apolipoprotein E has three isoforms: APOE2, APOE3 and APOE4. APOE4 is a major genetic risk factor for Alzheimer's disease and is associated with Down's syndrome dementia and poor neurological outcome after traumatic brain injury and haemorrhage. Neurovascular dysfunction is present in normal APOE4 carriers and individuals with APOE4-associated disorders. In mice, lack of APOE leads to blood-brain barrier (BBB) breakdown, whereas APOE4 increases BBB susceptibility to injury. How APOE genotype affects brain microcirculation remains elusive. Using different APOE transgenic mice, including mice with ablation and/or inhibition of cyclophilin A (CypA), it has been shown show that expression of APOE4 and lack of murine APOE, but not APOE2 and APOE3, leads to BBB breakdown by activating a proinflammatory CypA-nuclear factor-kappa B-matrix-metalloproteinase-9 pathway in pericytes. These findings suggest that CypA is a key target for treating APOE4-mediated neurovascular injury and the resulting neuronal dysfunction and degeneration. The data reviewed above support an essential role of neurovascular and BBB mechanisms in contributing to both, onset and progression of AD (95, 96).

BBB in the context of Alzheimer's Disease – Importance of EMF Exposure

In this context, the findings of Arendash et al., that long-term EMF reduced brain Aβ deposition through Aβ anti-aggregation actions in AD mice, are highly interesting (97). It was also found, by Mori and Arendash et al., that long-term exposure to high frequency EMF treatment prevented cognitive impairment in AD transgenic (Tg) mice and improved memory in normal mice and that an increase in neuronal activity could be observed in the EMF exposed groups (98). Furthermore, it was found by the group that EMF treatment enhances brain mitochondrial functions in AD Tg as well as normal mice and that no increase in brain temperature could be found in connection to the EMF exposure (99). An interesting aspect in this context, is the role of mitochondria for many cellular functions, including reactive oxygen species generation, apoptosis, and $Ca2^+$ homeostasis as was mentioned by Dragicevic et al. and reviewed by Nicholls (99, 100).

In the first mentioned study by Arendash et al. (97), mice were EMF exposed with start at young age or at adult age. In the young-age group, 24 mice were divided into 4 subgroups: n=6 were Tg controls, n=6 were Tg animals treated with EMF, n=6 were non-transgenic (NT) controls and n=6 were NT animals treated with EMF. 2.5, 4-5 and 6-7 months after daily GSM-900 EMF exposure (two 1-hour sessions daily, at SAR 0.25 W/kg), the animals were evaluated by cognitive tests. At the end of the study, Aβ in the brains was evaluated by immunohistochemistry. No effect on cognitive functions was observed after 2 months of exposure. However, for the Tg+EMF mice with start of EMF exposure at young age, the cognitive function was maintained after 6-7 months of exposure, while it deteriorated in the Tg group. In a final task for NT mice after 7 months of EMF, the EMF actually improved the mnemonic function. In the adult-age group, Tg animals had impaired cognitive functions at the age of 4 months. 28 Tg and NT mice were included. After long-term EMF exposure (2, 5 and 8 months) the memory was tested. While 2 months of EMF exposure had no effect, 5 months of exposure had positive effects only on NT mice, and 8 months of exposure had beneficial effects for the Tg mice, with better results in the Tg+EMF group as compared to the Tg controls. Also the NT+EMF mice had an improved function as compared to NT controls after 8 months. Staining for Aβ revealed lower values on both hippocampus and the entorhinal cortex in the Tg+EMF group as compared to the Tg control group. Hippocampal

tissue from Tg mice were then exposed to EMF for 4 days, after which it was shown that the Aβ amount had decreased as compared to non-exposed control tissue. It was also reported that a ±1° temperature increase was observed in EMF exposed animals during exposure, but not in between exposure sessions (97).

In the study by Mori and Arendash (98), n=6 mice were Tg controls, carrying the mutant APPK670N , n=10 mice were Tg treated with EMF, n=4 mice were NT controls and n=5 mice were NT treated with EMF. EMF exposed animals were placed in a Faraday cage, receiving two 2-hour periods of EMF treatment at GSM-900 frequencies, pulse modulated at SAR 0.25-1.05 W/kg. The neuronal expression of c-Fos was taken as an indicator of neuronal activity. With immunohistochemistry, it was found that c-Fos was increased in both the NT+EMF group, as well as in the Tg+EMF group in the entorhinal cortex. However, only this one brain region was analyzed, since c-Fos expression was too low in other regions, which the authors hypothesised might be due to that c-Fos in an early response gene, and that at a certain time after stimulation, when the animals were sacrificed, the expression had already declined in other regions, such as hippocampus. In a cognitive test (Y-maze), it was found that EMF improved the performance in both NT and Tg group as compared to untreated controls. It should also be noted, that despite the very interesting findings, the number of included animals is quite small (98).

EMF and ^{18}FDG Uptake – Recent Studies

The question whether EMF exposure from mobile phones has neuronal effects in the human situation was recently addressed by an American research group led by Volkow et al., conducting a PET study on ^{18}F-fluorodeoxyglucose (^{18}FDG) uptake (101). Though PET-studies on humans in correlation to EMF exposure have also been previously made, the purpose of this study was to extend the study material and use the more direct measure of brain glucose metabolism by the uptake of ^{18}FDG instead of the previously used CBF (cerebral blood flow) measure, which might be a more indirect sign of neuronal activity and also reflect short-term alterations (60s) as compared to the more long-lasting ones observed with ^{18}FDG (suggested to be in the range of 30 min). ^{18}FDG is actively transported across the BBB into the cells, where it is phosphorylated, and is, among others, used as a prognostic value for following low-grade brain tumours, where an increased uptake in previously low-

grade tumours is an indicator of anaplastic transformation (for review into the topic of [18]FDG and brain tumours (102).
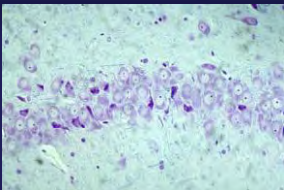
(space)

In the study by Volkow et al. (101), in total, 47 persons were involved, and effects upon brain glucose metabolism of EMF exposure were evaluated using PET with injection of [18]FDG. PET scans were performed both with and without EMF exposure (50 min of GSM-900 with maximum SAR of 0.901 W/kg), and the participants were blinded to the exposure situation. Whereas whole-brain metabolism was not affected, there were regional differences, in the right orbitofrontal cortex and the lower part of the right superior temporal gyrus (that is, the same side as the mobile phone was placed at) with increased metabolism in the exposure situation of about 7% as compared to control. There was a positive correlation between the strength of the E-field from the phones and the brain activation. Interestingly, it was hypothesized that RF-EMF exposure might increase the excitability of brain neurons.

Following the study by Volkow et al. (101), Kwon et al. (103) also investigated effects of GSM-900 exposure upon brain [18]FDG uptake. Thirteen persons were exposed to GSM-900 for 30 minutes to the right side of the head, and all subjects were also sham-exposed, and blinded to the exposure situation (SAR-values of maximum 0.74 W/kg in the head and 0.23 W/kg in the brain tissue). Contrary to the findings of Volkow et al. (101), the study by Kwon et al. (103) demonstrated a decrease in brain [18]FDG uptake after GSM-900 exposure, with decreased uptake values in the temporoparietal junction. A volume-of-interest analysis focused upon the right temporal lobe, showed a decreased [18]FDG uptake in the anterior inferior temporal cortex. No effects on task performance were found, and no correlation between temperature or [18]FDG uptake (a temperature increase of <0.21°C was found on the skin on the exposed side of the head) (103).

In the animal situation, Frilot et al. investigated the effect of ELF magnetic field exposure (2.5 G at 60 Hz) upon [18]FDG uptake in rats, comparing uptake with and without EMF exposure. An increased glucose uptake was found in the hindbrain when the field was orthogonally to the sagittal plane, but not when the angle varied randomly between the field and sagittal plane. These effects were hypothesized to be coupled to induction of electric field on the gate of ion channels (104).

Possible connection between BBB leakage and nerve cell injury

It has been suggested that BBB leakage is the major reason for nerve cell injury, such as that seen in dark neurons in stroke-prone spontaneously hypertensive rats (105). Much speaks in favour of this possibility. The parallel findings in the Lund material of neuronal uptake of albumin and dark neurons may support the hypothesis that albumin leakage into the brain is the cause for the neuronal damage observed after 28 and 50 d. It should, however, be pointed out that the connection is not yet proven (Figure 8).

Exposed vs sham	7d	14 d	28 d	50 d
Albumin foci	0.04	0.02	ns	0.04
Neuronal albumin	0.02	0.005	ns	ns
Dark neurons	ns	ns	0.01	0.001

© Salford et al

Figure 8. Results from the Lund group (61-63)

Also, other unwanted and toxic molecules in the blood may leak into the brain tissue in parallel with the albumin, and concentrate in and damage the neurons and glial cells of the brain. In favour of a causal connection between albumin and neuronal damage is a series of experiments performed in rats by another group at Lund University; albumin leaks into the brain and neuronal degeneration is seen in areas with BBB disruption in several circumstances: after intracarotid infusion of hyperosmolar solutions in rats (106) in the stroke

prone hypertensive rat (105); and in acute hypertension by aortic compression in rats (22). Furthermore, it has been shown in other laboratories that epileptic seizures cause extravasation of plasma into brain parenchyma (21), and in the clinical situation the cerebellar Purkinje cells are heavily exposed to plasma constituents and degenerate in epileptic patients . There are indications that an already disrupted BBB is more sensitive to the RF fields than an intact BBB (74, 91). It has been stated by other researchers that albumin is the most likely neurotoxin in serum (64). It has been demonstrated that injection of albumin into the brain parenchyma of rats gives rise to neuronal damage. When 25 micro-litres of rat albumin is infused into rat neostriatum, 10 and 30, but not 3 mg/ ml albumin causes neuronal cell death and axonal severe damage (65). It also causes leakage of endogenous albumin in and around the area of neuronal damage. However, it is still unclear whether the albumin leakage demonstrated in our experiments locally reaches such concentrations.

Possible mechanisms

Microarray analysis of the expression of all the rats' genes in cortex and hippocampus, after exposure to GSM RFs or sham exposure for 6 h, has shown interesting differences between exposed animals and controls as described by Nittby et al. (107). Genes of interest for membrane transport show highly significant differences. This may be of importance in conjunction with our earlier findings of albumin leakage into neurons around capillaries in exposed animals. It can be noted here that among the significantly altered genes from these evaluations, two variants of the gene RGS4 are up-regulated in hippocampal tissue from exposed rats as compared to the sham-exposed rats (unpublished results). RGS is a regulator of G protein signalling, and it has been proposed that RGS4 might regulate BBB permeability in mammals, in a way corresponding to the role of its Loco homolog G protein coupled receptor (GPCR) in developing and maintaining the BBB permeability of Drosophila (7).

It has also been suggested in other connections that manifestations of BBB disruption might also be mediated by the formation of free radicals, such as O_2^-, H_2O_2, and hydroxyl radical, which are supposed to oxidize cell membrane lipids by virtue of the high concentration of polyunsaturated fatty acids in these membrane constituents (108). As an example of this, it was reported by Chan et al.(109), that treatment of the brain of rats with a free-radical

generating system resulted in lipid-peroxidation, and an increased permeation of Evans blue due to barrier breakdown.

Recently, a detailed molecular mechanism, by means of which mobile phone radiation might exert its effects, has been proposed (110). By using Rat1 and HeLa cells, it was shown that EMF exposure resulted in rapid activation of ERK/ MAPKs (mitogen-activated protein kinase). The activation of these ERKs was mediated by reactive oxygen species (ROS), resulting in a signalling cascade ultimately affecting transcription, by the central key role of ERKs in signalling pathways.

In the continued search for the mechanisms behind EMF mediated effects, their interaction with calcium-45 transport in bio-membranes has been studied (111) and $Ca2^+$-efflux over plasma membranes has been observed in plasma vesicles from spinach exposed to ELF magnetic fields (112). With this model, quantum mechanical theoretical models for the interaction between magnetic fields and biological systems are tested. The model proposed by Blanchard and Blackman (113), in which it is assumed that biologically active ions can be bound to a channel protein and in this way alter the opening state of that channel, could in this way be quantitatively confirmed. Thus, the membrane is one site of interaction between the magnetic fields and the cell, and more specifically, the $Ca2^+$-channels, are one of the targets. More recently, new models for the interaction between magnetic fields and hydrogen nuclei also have been proposed.

EMF-induced $Ca2^+$-efflux over plasma membranes, understandably, can have many different effects on the target cells. Some agents that increase the BBB permeability act through a contractile mechanism that widens the intercellular junctions of the capillary endothelium. An increase of free $Ca2^+$ should mediate these changes, thereby resulting in measurable alterations of intracellular $Ca2^+$-levels in brain capillary cells after exposure to BBB-disrupting agents (108).

Another hypothesis is that EMF-induced intracellular $Ca2^+$-alterations might affect Ets genes, which are transcription factors expressed in different tissues (114). In this context, we could add that in our gene expression material from GSM-exposed rats vs., sham-exposed rats, one Ets variant gene is actually significantly up-regulated in hippocampus and one Ets1 gene is significantly up-regulated in cortex of the exposed animals.

EMF induced BBB permeability – with the aim of medical use

In the attempt to further try to understand the underlying mechanisms of the RF effects, we recently undertook a study upon snail nociception, with 1-hour GSM-1800 exposure of the land snail *H. pomatia*. This revealed, that the exposure induced analgesia in the snail model, with a significantly increased latency of reaction when placed on a hot plate, as compared to when only sham exposed. The vast knowledge about the physiology of the snail, its neurotransmission systems and it simplicity as compared the mammals may provide a tool for successful continued search for the mechanisms behind the effects of the GSM EMF upon biology (115).

In a recent study by Kuo et al (116), it was described how EMFs might be utilized to facilitate transport across the BBB. In an *in vitro* model, human micro-vascular endothelial cells were co-cultured with human astrocytes. Effects of EMF upon P-glycoprotein (P-gp) and multi-drug resistance -associated proteins (MRP) were tested in connection to treatment with anti-retroviral drugs, where the MRPs and P-gp are known to play an important role in multidrug resistance, which is encountered in carcinomas and therapies for acquired immune-deficiency (Kuo et al. 2012). With increasing EMF frequencies up to 900 MHz (both 715MHz and 900 MHz), the endocytotic uptake of calcein was increased (5mW, square wave with amplitude modulation at 20 MHz for 4 hours). Treatment with EMF could also inhibit expression of MRP and P-gp after treatment with anti-retroviral drugs, indicating that it might be useful in order to deliver antiretroviral proteins into the brain, by decreasing the efflux of the drugs due to the MRPs and P-gl.

Kuo et al. (117) also showed that EMF exposure (915 MHz EMFs at 5 mW with 20 MHz amplitude modulation for 4 hours) in combination with cationic solid lipid nanoparticles (CSLNs) could increase the transport of the antiretroviral drug Saquinavir 22-fold across human brain-microvascular endothelial cells (as compared to a 17-fold increase when only CSLNs were used).

43

Conclusions

In this review, we have reported the results of our group's research during the last 24 years, and the results of similar, but seldom identical, experiments of several other groups around the world. When summing up what we have described here, we are convinced that RF electromagnetic fields have effects upon biology, and we believe that it is more probable than unlikely, that non-thermal electromagnetic fields from mobile phones and base stations do have effects also upon the human brain. However, in this context, it is also important to point out, that the studies from our laboratory, as well as most studies presented above and available in literature, have been performed using animals and not humans. Thus no definitive conclusions can be drawn regarding effects of mobile phone use upon the human BBB.

However, studies in humans utilizing radiopharmaceuticals have been performed by Volkow et al. (101) upon brain glucose metabolism, and as was described by Saha et al. (118) already in 1994, studies with PET or SPECT and radiopharmaceuticals are used in brain imaging.

Further, a tool to directly study the human BBB has recently been described (119). It is based upon a non-radioactive methodology for *in vivo* non-invasive, real-time imaging of BBB permeability for conventional drugs, using nitroxyl radicals as spin-labels and MRI. In this connection, it should be mentioned though, that MRI has the drawback of possibly itself influence upon the results.

Based upon what has been presented here, we feel that the WHO IARC classification of RFR at the level 2B is adequate at present.

The question whether existing FCC/IEE and/or ICNIRP public safety limits and reference levels are adequate to protect the public is not easily answered. The reported studies on EMF induced BBB disruption have shown partially contradictory results from different laboratories. However, the fact that an abundance of studies do show effects is an important warning. This is true even if it can be summarized that the effects most often are weak and are seen in about 40% of the exposed animals.

However, we have stressed the following opinion in several publications during the past years: - *"The intense use of mobile phones, not least by youngsters, is a serious memento. A neuronal damage may not have immediately demonstrable consequences, even if repeated. It may, however, in the long run, result in reduced brain reserve capacity that might be unveiled by other later neuronal disease or even the wear and tear of ageing. We can not exclude that after some decades of (often), daily use, a whole generation of users, may suffer negative effects such as autoimmune and neuro-degenerative diseases maybe already in their middle age"*.

One remarkable observation, which we have made in our studies throughout the years, is that exposure with whole-body average power densities below 10 mW/kg gives rise to a more pronounced albumin leakage than higher power densities, all at non-thermal levels. These very low SAR-values, such as 1 mW/kg, exist at a distance of more than one meter away from the mobile phone antenna and at a distance of about 150–200 m from a base station. Further, when a mobile phone operating at 915 MHz (and its antenna) is held 1.4 cm from the human head, the very low SAR levels of 10 mW/kg exist in deep-lying parts of the human brain such as the basal ganglia, and the power density of 1 mW/kg and less is absorbed in thalamus bilaterally.

With this information as a background, it is difficult to recommend safety limits as the function of existing mobile systems might not allow for limits that produce SAR levels below 1 or 0,1 mW/kg in the human brain, which are reported to cause a pathological leakage of the BBB and to neuronal damage.

Demonstrated effects on the BBB, as well as a series of other effects upon biology (120) have given rise to scientific concern and to public anxiety. It is up to the society and our politicians and also the providers of the radiofrequency-emitting technologies to support continued research in order to understand the nature of the effects, thereby neutralizing or at least reducing them.   Also, it should be kept in mind that proven effects on biology also means that positive potentials might be revealed. This might be useful in medical applications, for example a controlled opening of the BBB would enable previously excluded pharmaceuticals to reach their targets within the brain tissue.

Acknowledgements

References

1.   J.J. Carlsson SD, Hilgers LS. Blood barriers of the insect. Annu Rev Entomol 45:(2000).
2.   N.J. Abbottand Y. Pichon. The glial blood-brain barrier of crustacea and cephalopods: a review. Journal de physiologie. 82:304-313 (1987).
3.   T. Gotowand P.H. Hashimoto. Plasma membrane organization of astrocytes in elasmobranchs with special reference to the brain barrier system. Journal of neurocytology. 13:727-742 (1984).
4.   M. Reinecke. The glial cells of the cerebral ganglia of Helix pomatia L. (Gastropoda, Pulmonata). II. Uptake of ferritin and 3H-glutamate. Cell and tissue research. 169:361-382 (1976).
5.   H.M. Dziegielewska KM, Jones SE, Reader M, Saunders NR. . Proteins in cerebrospinal fluid and plasma of postnatal Monodelphis domestica (grey shorttailed opossum). Comp Biochem Physiol. 92B:569-576 (1989).
6.   J.A. Alberts B. Cell junctions, cell adhesion and the extracellular matrix. In G. S (ed.), Molecular Biology of the Cell, Garland Publishing, New york, 2002.
7.   R. Danemanand B.A. Barres. The blood-brain barrier--lessons from moody flies. Cell. 123:9-12 (2005).
8.   R.N. Frank, S. Dutta, and M.A. Mancini. Pericyte coverage is greater in the retinal than in the cerebral capillaries of the rat. Investigative ophthalmology & visual science. 28:1086-1091 (1987).
9.   W.E. Thomas. Brain macrophages: on the role of pericytes and perivascular cells. Brain research Brain research reviews. 31:42-57 (1999).
10. W.H. Oldendorf, M.E. Cornford, and W.J. Brown. The large apparent work capability of the blood-brain barrier: a study of the mitochondrial content of capillary endothelial cells in brain and other tissues of the rat. Annals of neurology. 1:409-417 (1977).
11. J.F. Ghersi-Egea, A. Minn, and G. Siest. A new aspect of the protective functions of the blood-brain barrier: activities of four drug-metabolizing enzymes in isolated rat brain microvessels. Life sciences. 42:2515-2523 (1988).
12. A.L. Betz, J.A. Firth, and G.W. Goldstein. Polarity of the blood-brain barrier: distribution of enzymes between the luminal and antiluminal membranes of brain capillary endothelial cells. Brain research. 192:17-28 (1980).
13. N.A. Oberheim, X. Wang, S. Goldman, and M. Nedergaard. Astrocytic complexity distinguishes the human brain. Trends in neurosciences. 29:547-553 (2006).
14. N.A. Oberheim, T. Takano, X. Han, W. He, J.H. Lin, F. Wang, Q. Xu, J.D. Wyatt, W. Pilcher, J.G. Ojemann, B.R. Ransom, S.A. Goldman, and M. Nedergaard. Uniquely hominid features

of adult human astrocytes. The Journal of neuroscience : the official journal of the Society for Neuroscience. 29:3276-3287 (2009).

15. W.A. Banks, V.B. Kumar, M.W. Franko, J.W. Bess, Jr., and L.O. Arthur. Evidence that the species barrier of human immunodeficiency virus-1 does not extend to uptake by the blood--brain barrier: comparison of mouse and human brain microvessels. Life sciences. 77:2361-2368 (2005).

16. P. Hsiao, L. Sasongko, J.M. Link, D.A. Mankoff, M. Muzi, A.C. Collier, and J.D. Unadkat. Verapamil P-glycoprotein transport across the rat blood-brain barrier: cyclosporine, a concentration inhibition analysis, and comparison with human data. The Journal of pharmacology and experimental therapeutics. 317:704-710 (2006).

17. D.Z. Gerhart, R.L. Leino, N.D. Borson, W.E. Taylor, K.M. Gronlund, A.L. McCall, and L.R. Drewes. Localization of glucose transporter GLUT 3 in brain: comparison of rodent and dog using species-specific carboxyl-terminal antisera. Neuroscience. 66:237-246 (1995).

18. W. Oldendorf. Permeability of the BBB. In T. D (ed.), The Nervous System, Raven Press, New York, 1975, pp. 229–289.

19. S. Rapoport. BBB in Physiology and Medicine, Raven Press, New York, 176.

20. L.G. Salford, Persson, B., Malmgren, L., & Brun. Téléphonie Mobile et Barrière Sang-Cerveau. In P. Marco (ed.), Téléphonie mobile—effects potentiels sur la santé des ondes électromagnétiques de haute fréquence, Emburg, Belgium, 2001, pp. 141–152.

21. A. Mihalyand B. Bozoky. Immunohistochemical localization of extravasated serum albumin in the hippocampus of human subjects with partial and generalized epilepsies and epileptiform convulsions. Acta neuropathologica. 65:25-34 (1984).

22. T.E. Sokrab, B.B. Johansson, H. Kalimo, and Y. Olsson. A transient hypertensive opening of the blood-brain barrier can lead to brain damage. Extravasation of serum proteins and cellular changes in rats subjected to aortic compression. Acta neuropathologica. 75:557-565 (1988).

23. S.R. Parathath, S. Parathath, and S.E. Tsirka. Nitric oxide mediates neurodegeneration and breakdown of the blood-brain barrier in tPA-dependent excitotoxic injury in mice. Journal of cell science. 119:339-349 (2006).

24. H. Nittby, G. Grafstrom, J.L. Eberhardt, L. Malmgren, A. Brun, B.R. Persson, and L.G. Salford. Radiofrequency and extremely low-frequency electromagnetic field effects on the blood-brain barrier. Electromagnetic biology and medicine. 27:103-126 (2008).

25. A.H. Frey, S.R. Feld, and B. Frey. Neural function and behavior: defining the relationship. Annals of the New York Academy of Sciences. 247:433-439 (1975).

26. K.J. Oscarand T.D. Hawkins. Microwave alteration of the blood-brain barrier system of rats. Brain research. 126:281-293 (1977).

27. K.J. Oscar, S.P. Gruenau, M.T. Folker, and S.I. Rapoport. Local cerebral blood flow after microwave exposure. Brain research. 204:220-225 (1981).

28. E. Preston, E.J. Vavasour, and H.M. Assenheim. Permeability of the blood-brain barrier to mannitol in the rat following 2450 MHz microwave irradiation. Brain research. 174:109-117 (1979).

29. J.H. Merritt, A.F. Chamness, and S.J. Allen. Studies on blood-brain barrier permeability after microwave-radiation. Radiation and environmental biophysics. 15:367-377 (1978).

30. A.H. Frey. Headaches from cellular telephones: are they real and what are the implications? Environmental health perspectives. 106:101-103 (1998).

31. T.R. Ward, J.A. Elder, M.D. Long, and D. Svendsgaard. Measurement of blood-brain barrier permeation in rats during exposure to 2450-MHz microwaves. Bioelectromagnetics. 3:371-383 (1982).

32. T.R. Wardand J.S. Ali. Blood-brain barrier permeation in the rat during exposure to low-power 1.7-GHz microwave radiation. Bioelectromagnetics. 6:131-143 (1985).

33. S.P. Gruenau, K.J. Oscar, M.T. Folker, and S.I. Rapoport. Absence of microwave effect on blood-brain barrier permeability to [14C]sucrose in the conscious rat. Experimental neurology. 75:299-307 (1982).

34. E.N. Albertand J.M. Kerns. Reversible microwave effects on the blood-brain barrier. Brain research. 230:153-164 (1981).

35. J.C. Linand M.F. Lin. Studies on microwave and blood-brain barrier interaction. Bioelectromagnetics. 1:313-323 (1980).

36. J.C. Linand M.F. Lin. Microwave hyperthermia-induced blood-brain barrier alterations. Radiation research. 89:77-87 (1982).

37. H. Goldman, J.C. Lin, S. Murphy, and M.F. Lin. Cerebrovascular permeability to 86Rb in the rat after exposure to pulsed microwaves. Bioelectromagnetics. 5:323-330 (1984).

38. W.M. Williams, W. Hoss, M. Formaniak, and S.M. Michaelson. Effect of 2450 MHz microwave energy on the blood-brain barrier to hydrophilic molecules. A. Effect on the permeability to sodium fluorescein. Brain research. 319:165-170 (1984).

39. W.M. Williams, M. Del Cerro, and S.M. Michaelson. Effect of 2450 MHz microwave energy on the blood-brain barrier to hydrophilic molecules. B. Effect on the permeability to HRP. Brain research. 319:171-181 (1984).

40. W.M. Williams, J. Platner, and S.M. Michaelson. Effect of 2450 MHz microwave energy on the blood-brain barrier to hydrophilic molecules. C. Effect on the permeability to [14C]sucrose. Brain research. 319:183-190 (1984).

41. ICNIRP. Guidelines for limiting exposure to time-varying electric, magnetic and electromagnetic fields Health Physiology:494–522 (1998).

42. W.M. Williams, S.T. Lu, M. Del Cerro, and S.M. Michaelson. Effect of 2450 MHz microwave energy on the blood-brain barrier to hydrophilic molecules. D. Brain temperature and blood-brain barrier permeability to hydrophilic tracers. Brain research. 319:191-212 (1984).

43. R.M. Quock, J.M. Fujimoto, T.K. Ishii, and D.G. Lange. Microwave facilitation of methylatropine antagonism of central cholinomimetic drug effects. Radiation research. 105:328-340 (1986).

44. R.M. Quock, F.J. Kouchich, T.K. Ishii, and D.G. Lange. Microwave facilitation of domperidone antagonism of apomorphine-induced stereotypic climbing in mice. Bioelectromagnetics. 8:45-55 (1987).

45. E. Moriyama, M. Salcman, and R.D. Broadwell. Blood-brain barrier alteration after microwave-induced hyperthermia is purely a thermal effect: I. Temperature and power measurements. Surgical neurology. 35:177-182 (1991).

46. M. Nakagawa, K. Matsumoto, H. Higashi, T. Furuta, and T. Ohmoto. Acute effects of interstitial hyperthermia on normal monkey brain--magnetic resonance imaging appearance and effects on blood-brain barrier. Neurologia medico-chirurgica. 34:668-675 (1994).

47. R.R. Shivers, M. Kavaliers, G.C. Teskey, F.S. Prato, and R.M. Pelletier. Magnetic resonance imaging temporarily alters blood-brain barrier permeability in the rat. Neuroscience letters. 76:25-31 (1987).

48. H.J. Garber, W.H. Oldendorf, L.D. Braun, and R.B. Lufkin. MRI gradient fields increase brain mannitol space. Magnetic resonance imaging. 7:605-610 (1989).

49. I.K. Adzamli, Jolesz, E. A., Blau, M. An assessment of BBB integrity under MRI conditions: brain uptake of radiolabelled Gd-DTPA and In-DTPA-IgG. J Nucl Med:839-840 (1989).

50. E. Preston, Buffler, K., Haas, N. Does magnetic resonance imaging compromise integrity of the BBB? Neuroscience letters:46–50 (1989).

51. F.S. Prato, J.R. Frappier, R.R. Shivers, M. Kavaliers, P. Zabel, D. Drost, and T.Y. Lee. Magnetic resonance imaging increases the blood-brain barrier permeability to 153-gadolinium diethylenetriaminepentaacetic acid in rats. Brain research. 523:301-304 (1990).

52. F.S. Prato, J.M. Wills, J. Roger, H. Frappier, D.J. Drost, T.Y. Lee, R.R. Shivers, and P. Zabel. Blood-brain barrier permeability in rats is altered by exposure to magnetic fields associated with magnetic resonance imaging at 1.5 T. Microscopy research and technique. 27:528-534 (1994).

53. B.A. Salford LG, Eberhardt J, Malmgren L, Persson BRR. Electromagnetic field-induced permeability of the blood-brain barrier shown by immunohistochemical methods. Interaction mechanism of low-level electromagnetic fields, living systems, Oxford University Press, Oxford, 1992, pp. 251–258.

54. B.R.R. Persson, Salford, L. G., Brun, A. BBB permeability in rats exposed to electromagnetic fields used in wireless communication. Wireless Networks:455–461 (1997).

55. C.F. Blackman, S.G. Benane, J.R. Rabinowitz, D.E. House, and W.T. Joines. A role for the magnetic field in the radiation-induced efflux of calcium ions from brain tissue in vitro. Bioelectromagnetics. 6:327-337 (1985).

56. C.F. Blackman, L.S. Kinney, D.E. House, and W.T. Joines. Multiple power-density windows and their possible origin. Bioelectromagnetics. 10:115-128 (1989).

57. L.G. Salford, A. Brun, J. Eberhardt, L. Malmgren, and B. Persson. Electromagnetic field-induced permeability of the blood-brain barrier shown by immunohistochemical methods. In B. Nordénand C. Ramel (eds.), Interaction Mechanism of Low-Level Electromagnetic Fields in Living Systems, Oxford University Press, Oxford, 1992, pp. 251-258.

58. L.G. Salford, A. Brun, J.L. Eberhardt, and B.R.R. Persson. Permeability of the blood-brain-barrier induced by 915 MHz electromagnetic-radiation, continuous wave and modulated at 8, 16, 50 and 200 Hz. Bioelectrochemistry and Bioenergetics. 30:293-301 (1993).

59. L.G. Salford, A. Brun, K. Sturesson, J.L. Eberhardt, and B.R. Persson. Permeability of the blood-brain barrier induced by 915 MHz electromagnetic radiation, continuous wave and modulated at 8, 16, 50, and 200 Hz. Microscopy research and technique. 27:535-542 (1994).

60. N.H. Salford LG, Brun A, Grafström G, Eberhardt J, Malmgren L, Persson BRR. Non-thermal effects of EMF upon the mammalian brain: the Lund experience. Environmentalist:493–500 (2007).

61. H. Nittby, A. Brun, J. Eberhardt, L. Malmgren, B.R. Persson, and L.G. Salford. Increased blood-brain barrier permeability in mammalian brain 7 days after exposure to the radiation from a GSM-900 mobile phone. Pathophysiology. 16:103-112 (2009).

62. J.L. Eberhardt, B.R. Persson, A.E. Brun, L.G. Salford, and L.O. Malmgren. Blood-brain barrier permeability and nerve cell damage in rat brain 14 and 28 days after exposure to microwaves from GSM mobile phones. Electromagnetic biology and medicine. 27:215-229 (2008).

63. L.G. Salford, A.E. Brun, J.L. Eberhardt, L. Malmgren, and B.R. Persson. Nerve cell damage in mammalian brain after exposure to microwaves from GSM mobile phones. Environmental health perspectives. 111:881-883; discussion A408 (2003).

64. S. Eimerland M. Schramm. Acute glutamate toxicity and its potentiation by serum albumin are determined by the Ca2+ concentration. Neuroscience letters. 130:125-127 (1991).

65. B. Hassel, E.G. Iversen, and F. Fonnum. Neurotoxicity of albumin in vivo. Neuroscience letters. 167:29-32 (1994).

66. T. Sugimoto, G.J. Bennett, and K.C. Kajander. Transsynaptic degeneration in the superficial dorsal horn after sciatic nerve injury: effects of a chronic constriction injury, transection, and strychnine. Pain. 42:205-213 (1990).

67. A. Ilhan, A. Gurel, F. Armutcu, S. Kamisli, M. Iraz, O. Akyol, and S. Ozen. Ginkgo biloba prevents mobile phone-induced oxidative stress in rat brain. Clinica chimica acta; international journal of clinical chemistry. 340:153-162 (2004).

68. H. Nittby, G. Grafstrom, D.P. Tian, L. Malmgren, A. Brun, B.R. Persson, L.G. Salford, and J. Eberhardt. Cognitive impairment in rats after long-term exposure to GSM-900 mobile phone radiation. Bioelectromagnetics. 29:219-232 (2008).

69. G. Grafstrom, H. Nittby, A. Brun, L. Malmgren, B.R.R. Persson, L.G. Salford, and J. Eberhardt. Histopathological examinations of rat brains after long-term exposure to GSM-900 mobile phone radiation. Brain Research Bulletin. 77:257-263 (2008).

70. J. Van Hese, Martens, L., De Zutter, D., De Wagter, C., Malmgren, L., Persson, B. R. R, & Salford, L. G. Simulations of the effect of inhomogenities in TEM transmission cells using the FDTD-method. IEEE Transactions on Electromagnetic Compatibility:292–298 (1991).

71. L. Martens, van Hese, J. Electromagnetic field calculations used for exposure experiments on small animals in TEM-cells. Bioelectrochem Bioenerget:73–81 (1993).

72. R.B. Stagg, Havel, L. H. III, Pastorian, K., Cain, C., Adey, W. R., & Byus, C. V. Effect of immobilization and concurrent exposure to a pulse-modulated microwave field on core body temperature, plasma ACTH and corticosteroid, and brain ornithine decarboxylase, Fos and Jun mRNA. Radiation research:584–592 (2001).

73. K. Fritze, C. Sommer, B. Schmitz, G. Mies, K.A. Hossmann, M. Kiessling, and C. Wiessner. Effect of global system for mobile communication (GSM) microwave exposure on blood-brain barrier permeability in rat. Acta neuropathologica. 94:465-470 (1997).

74. F. Töre, Dulou, P. E., Haro, E., Veyret, B., & Aubineau, P. Two-hour exposure to 2-W/kg, 900-MHZ GSM microwaves induces plasma protein extravasation in rat brain and dura mater., *Proceedings of the 5th International congress of the EBEA*, Helsinki, Finland, 2001, pp. 43-45.

75. F. Töre, Dulou, P. E., Haro, E., Veyret, B., & Aubineau, P. Effect of 2 h GSM-900 microwave exposures at 2.0, 0.5 and 0.12 W/kg on plasma protein extravasation in rat brain and dura mater., *Proceedings of the 24th annual meeting of the BEMS*2002, pp. 61-62.

76. C. Neubauer, A.M. Phelan, H. Kues, and D.G. Lange. Microwave irradiation of rats at 2.45 GHz activates pinocytotic-like uptake of tracer by capillary endothelial cells of cerebral cortex. Bioelectromagnetics. 11:261-268 (1990).

77. G. Tsurita, H. Nagawa, S. Ueno, S. Watanabe, and M. Taki. Biological and morphological effects on the brain after exposure of rats to a 1439 MHz TDMA field. Bioelectromagnetics. 21:364-371 (2000).

78. M. Kuribayashi, J. Wang, O. Fujiwara, Y. Doi, K. Nabae, S. Tamano, T. Ogiso, M. Asamoto, and T. Shirai. Lack of effects of 1439 MHz electromagnetic near field exposure on the blood-brain barrier in immature and young rats. Bioelectromagnetics. 26:578-588 (2005).

79. J.W. Finnie, P.C. Blumbergs, J. Manavis, T.D. Utteridge, V. Gebski, J.G. Swift, B. Vernon-Roberts, and T.R. Kuchel. Effect of global system for mobile communication (gsm)-like radiofrequency fields on vascular permeability in mouse brain. Pathology. 33:338-340 (2001).

80. J.W. Finnie, P.C. Blumbergs, J. Manavis, T.D. Utteridge, V. Gebski, R.A. Davies, B. Vernon-Roberts, and T.R. Kuchel. Effect of long-term mobile communication microwave exposure on vascular permeability in mouse brain. Pathology. 34:344-347 (2002).

81. J.W. Finnieand P.C. Blumbergs. Mobile telephones and brain vascular leakage. Pathology. 36:96-97 (2004).

82. J. Bach Andersen, Mogensen, P., Pedersen, G. F. . Possible exposures from future mobile communication systems. Review of Radio Science 1999–2002, Wiley- Interscience, New York, 2002, pp. 935–941.

83. H. Masuda, A. Ushiyama, M. Takahashi, J. Wang, O. Fujiwara, T. Hikage, T. Nojima, K. Fujita, M. Kudo, and C. Ohkubo. Effects of 915 MHz electromagnetic-field radiation in TEM cell on the blood-brain barrier and neurons in the rat brain. Radiation research. 172:66-73 (2009).

84. J.M. McQuade, J.H. Merritt, S.A. Miller, T. Scholin, M.C. Cook, A. Salazar, O.B. Rahimi, M.R. Murphy, and P.A. Mason. Radiofrequency-radiation exposure does not induce detectable leakage of albumin across the blood-brain barrier. Radiation research. 171:615-621 (2009).

85. F.P. de Gannes, B. Billaudel, M. Taxile, E. Haro, G. Ruffie, P. Leveque, B. Veyret, and I. Lagroye. Effects of head-only exposure of rats to GSM-900 on blood-brain barrier permeability and neuronal degeneration. Radiation research. 172:359-367 (2009).

86. B. Cosquer, A.P. Vasconcelos, J. Frohlich, and J.C. Cassel. Blood-brain barrier and electromagnetic fields: effects of scopolamine methylbromide on working memory after whole-body exposure to 2.45 GHz microwaves in rats. Behavioural brain research. 161:229-237 (2005).

87. A. Ushiyama, Masuda, H, Hirota S, Wake K, Kawai H, Watanabe S, Taki M, Ohkubo C. Biological effect on blood cerebrospinal fluid barrier due to radio frequency electromagnetic fileds exposure of the rat brain in vivo. Environmentalist 489–492 (2007).

88. F. Soderqvist, M. Carlberg, K. Hansson Mild, and L. Hardell. Exposure to an 890-MHz mobile phone-like signal and serum levels of S100B and transthyretin in volunteers. Toxicology letters. 189:63-66 (2009).

89. S.N. Sirav B. Blood–brain barrier disruption by continuous-wave radio frequency radiation. Electromagnetic biology and medicine:215–222 (2009).

90. A. Schirmacher, S. Winters, S. Fischer, J. Goeke, H.J. Galla, U. Kullnick, E.B. Ringelstein, and F. Stogbauer. Electromagnetic fields (1.8 GHz) increase the permeability to sucrose of the blood-brain barrier in vitro. Bioelectromagnetics. 21:338-345 (2000).

91. H. Franke, E.B. Ringelstein, and F. Stogbauer. Electromagnetic fields (GSM 1800) do not alter blood-brain barrier permeability to sucrose in models in vitro with high barrier tightness. Bioelectromagnetics. 26:529-535 (2005).

92. H. Franke, J. Streckert, A. Bitz, J. Goeke, V. Hansen, E.B. Ringelstein, H. Nattkamper, H.J. Galla, and F. Stogbauer. Effects of Universal Mobile Telecommunications System (UMTS) electromagnetic fields on the blood-brain barrier in vitro. Radiation research. 164:258-269 (2005).

93. D. Leszczynski, S. Joenvaara, J. Reivinen, and R. Kuokka. Non-thermal activation of the hsp27/p38MAPK stress pathway by mobile phone radiation in human endothelial cells: molecular mechanism for cancer- and blood-brain barrier-related effects. Differentiation; research in biological diversity. 70:120-129 (2002).

94. B.V. Zlokovic. The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron. 57:178-201 (2008).

95. R.D. Bell, E.A. Winkler, I. Singh, A.P. Sagare, R. Deane, Z. Wu, D.M. Holtzman, C. Betsholtz, A. Armulik, J. Sallstrom, B.C. Berk, and B.V. Zlokovic. Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. Nature. 485:512-516 (2012).

96. R.D. Belland B.V. Zlokovic. Neurovascular mechanisms and blood-brain barrier disorder in Alzheimer's disease. Acta Neuropathol. 118:103-113 (2009).

97. G.W. Arendash, J. Sanchez-Ramos, T. Mori, M. Mamcarz, X. Lin, M. Runfeldt, L. Wang, G. Zhang, V. Sava, J. Tan, and C. Cao. Electromagnetic Field Treatment Protects Against and Reverses Cognitive Impairment in Alzheimer's Disease Mice. J Alzheimers Dis. 19:191-210 (2010).

98. T. Moriand G.W. Arendash. Long-Term Electromagnetic FieldTreatment Increases Brain Neuronal Activity: Linkage to Cognitive Benefit andTherapeutic Implications for Alzheimer's Disease. J Alzheimers Dis. 1:102 (2011).

99. N. Dragicevic, P.C. Bradshaw, M. Mamcarz, X. Lin, L. Wang, C. Cao, and G.W. Arendash. Long-term electromagnetic field treatment enhances brain mitochondrial function of both Alzheimer's transgenic mice and normal mice: a mechanism for electromagnetic field-induced cognitive benefit? . Neuroscience. 185:135-149 (2011).

100. D. Nicholls. Mitochondrial Bioenergetics, Aging, and Aging-Related Disease. Sci Aging Knowl Environ. 2002:12 (2002).

101. N.D. Volkow, D. Tomasi, G.-J. Wang, P. Vaska, J.S. Fowler, F. Telang, D. Alexoff, J. Logan, and C. Wong. Effects of Cell Phone Radiofrequency Signal Exposure on Brain Glucose Metabolism. Jama-Journal of the American Medical Association. 305:808-813 (2011).

102. W. Chen. Clinical applications of PET in brain tumors. Journal of Nuclear Medicine. 48:1468-1481 (2007).

103. M.S. Kwon, V. Vorobyev, S. Kannala, M. Laine, J.O. Rinne, T. Toivonen, J. Johansson, M. Teras, H. Lindholm, T. Alanko, and H. Hamalainen. GSM mobile phone radiation suppresses brain glucose metabolism. Journal of Cerebral Blood Flow and Metabolism. 31:2293-2301 (2011).

104. C. Frilot, II, S. Carrubba, and A.A. Marino. Transient and Steady-State Magnetic Fields Induce Increased Fluorodeoxyglucose Uptake in the Rat Hindbrain. Synapse. 65:617-623 (2011).

105. K. Fredriksson, H. Kalimo, C. Nordborg, B.B. Johansson, and Y. Olsson. Nerve cell injury in the brain of stroke-prone spontaneously hypertensive rats. Acta neuropathologica. 76:227-237 (1988).

106. T.S. Salahuddin, H. Kalimo, B.B. Johansson, and Y. Olsson. Observations on exsudation of fibronectin, fibrinogen and albumin in the brain after carotid infusion of hyperosmolar solutions. An immunohistochemical study in the rat indicating longlasting changes in the brain microenvironment and multifocal nerve cell injuries. Acta neuropathologica. 76:1-10 (1988).

107. W.B. Nittby H, Krogh M, Grafström G, Berlin H, Rehn G, Eberhardt JL, Malmgren L, Persson BRR, Salford LG. Exposure to radiation from global system for mobile communications at 1, 800 MHz significantly changes gene expression in rat hippocampus and cortex. Environmentalist:458–465 (2008).

108. H. Davson, Segal, M. B. Breakdown of the barriers and cerebral edema. In D. H (ed.), Physiology if the CSF and the BBBs, CRC Press, Inc1996, pp. 538–539.

109. P.H. Chan, R.A. Fishman, J. Caronna, J.W. Schmidley, G. Prioleau, and J. Lee. Induction of brain edema following intracerebral injection of arachidonic acid. Annals of neurology. 13:625-632 (1983).

110. J. Friedman, S. Kraus, Y. Hauptman, Y. Schiff, and R. Seger. Mechanism of short-term ERK activation by electromagnetic fields at mobile phone frequencies. The Biochemical journal. 405:559-568 (2007).

111. B.R.R. Persson, Salford, L. G. EMF interaction with calcium-45 transport in biomembranes. Nanobiology. 3:483–490 (1992).

112. C.L. Baureus Koch, M. Sommarin, B.R. Persson, L.G. Salford, and J.L. Eberhardt. Interaction between weak low frequency magnetic fields and cell membranes. Bioelectromagnetics. 24:395-402 (2003).

113. J.P. Blanchardand C.F. Blackman. Clarification and application of an ion parametric resonance model for magnetic field interactions with biological systems. Bioelectromagnetics. 15:217-238 (1994).

114. V. Romano-Spicaand N. Mucci. Biological effects of EMF exposure on Ets genes. Radiatsionnaia biologiia, radioecologiia / Rossiiskaia akademiia nauk. 43:528-530 (2003).

115. H. Nittby, M.K. Moghadam, W. Sun, L. Malmgren, J. Eberhardt, B.R. Persson, and L.G. Salford. Analgetic effects of non-thermal GSM-1900 radiofrequency electromagnetic fields in the land snail Helix pomatia. International Journal of Radiation Biology. 88:245-252 (2012).

116. L.C.-H. Kuo Y-C. Expression of P-glycoprotein and multidrug resistance-associated protein on human brain-microvascular endothelial cells with electromagnetic stimulation. Colloids and Surfaces B: Biointerfaces 57– 62 (2012).

117. Y.C. Kuoand H.H. Chen. Effect of electromagnetic field on endocytosis of cationic solid lipid nanoparticles by human brain-microvascular endothelial cells. Journal of drug targeting. 18:447-456 (2010).

118. Saha, G. B., W. J. Macintyre, et al. (1994). "RADIOPHARMACEUTICALS FOR BRAIN IMAGING." Seminars in Nuclear Medicine **24**(4): 324-349.

119. Zhelev Z, Bakalova R, Aoki I, *et al*. Nitroxyl radicals as low toxic spin-labels for non-invasive magnetic resonance imaging of blood–brain barrier permeability for conventional therapeutics. Chem Commun 2009; 1: 53-5

120. G.J. Hyland. Physics and biology of mobile telephony. Lancet. 356:1833-1836 (2000).

Evidence For Brain Tumors And Acoustic Neuromas

Lennart Hardell, MD, PhD, Professor

Department of Oncology, Örebro University Hospital, Sweden

Kjell Hansson Mild, PhD, Professor

Department of Radiation Physics, Umeå University, Sweden

Michael Kundi, PhD, med.habil, Professor

Institute of Environmental Health, Center for Public Health,

Medical University of Vienna, Austria

Table of Contents

I. Introduction

During the recent decade potential health risks from microwave exposure during use of wireless phones has been discussed both in scientific settings but also by the layman. Especially the use of mobile phones has been of concern, to less extent use of cordless desktop phones (digital enhanced cordless telephone; DECT). The Nordic countries were among the first in the world to widely adopt use of such devices, probably due to the mobile phone companies like Ericsson in Sweden and Nokia in Finland.

These countries may be taken as models for the introduction of this new technology on the market. Thus, the analogue mobile phone system (Nordic Mobile Telephony, NMT) using 450 MHz started to operate in Sweden in 1981. First, it was used in cars with external antenna but from 1984 mobile (portable!) phones existed. This system is still used in Sweden but only to a minor extent. The 900 MHz NMT system operated in Sweden between 1986-2000. The GSM phone (Global System for Mobile communication) started in 1991 and is the most used phone type today, although the 3G phone (third generation mobile phone, UMTS) is increasingly used now.

The risk of brain tumors has been of special concern since the brain is the organ mainly exposed during such phone calls. Most studies on this topic have been of the case-control design and no results exist from prospective cohort studies. However, the results have been hampered by too short tumor-induction period in most studies or with limited number of long-term users, i.e. $\geq$ 10 years latency time. As to carcinogenesis short latency period is of limited value to predict long-term health risks. Usually a latency period of at least 10 years is needed for more firm conclusions. It should noted that for several carcinogens longer latency periods are often

required, such as smoking and lung cancer, asbestos and lung cancer, dioxins and certain cancer types etc.

By now a number of studies exist that give results for brain tumour risk and use of mobile phones for subjects with latency period ≥ 10 years. Most of these results are based on low numbers but nevertheless may together give a pattern of increased risk. In this review we discuss all studies on this topic that have been published so far. Moreover, we present a meta-analysis of results from studies with at least 10 years latency period. Only the Hardell group in Sweden has published results also for use of cordless phones. Recently the same group published an overview of long-term use of cellular phones and the risk for brain tumors, especially with use for 10 years or more (Hardell et al 2007). In the following a brief summary is given of these results with the addition of two more study published after that review (Klaeboe et al 2007, Schlehofer et al 2007). For further details see Hardell et al (2007).

II. Materials and Methods

The Pub Med database (**www.ncbi.nlm.nih.gov**) was used for an up-dated search of published studies in this area using mobile/cellular/cordless telephone and brain tumour/neoplasm/acoustic neuroma/meningioma/glioma as searching terms. Personal knowledge of published studies was also used in order to get as comprehensive review as possible. Regarding several publication of the same study the most recent one with relevant data was used. We identified 20 studies to be included. Two were cohort studies (one study analysed twice) and 18 were case-control studies. No mortality studies were included. Three studies came from USA, four from Denmark, one from Finland, five from Sweden, two from Germany, one from the UK, one from Japan, one from Norway and two from study groups partly overlapping previously mentioned studies.

4

III. Results

A. The first Swedish studies

The first study by Hardell et al (1999, 2001) included cases and controls collected during 1994-96 in Sweden. Only living cases were included. Two controls were selected to each case from the Population Registry. The questionnaire was answered by 217 (93 %) cases and 439 (94 %) controls. Overall no association between mobile phone use and brain tumours was found, but when analysing ipsilateral phone use a somewhat increased risk was seen especially for tumours in the temporal, occipital or temporoparietal lobe yielding odds ratio (OR) = 2.4, 95 % confidence interval (CI) = 0.97-6.1 (Hardell et al 2001).

Hardell et al (2006a) made a pooled analysis for benign brain tumours from their two case-control studies. Cases were reported from Cancer Registries and controls were population based. The questionnaire was answered by 1,254 (88 %) cases and 2,162 (89 %) controls. Also use of cordless desktop phones was assessed. Use of cellular phones gave for acoustic neuroma OR = 1.7, 95 % CI 1.2-2.3 increasing to OR = 2.9, 95 % CI = 1.6-5.5 with > 10 year latency period. The corresponding results for cordless phones were OR = 1.5, 95 % CI = 1.04-2.0, and OR = 1.0, 95 % CI 0.3-2.9, respectively. Regarding meningioma cellular phones gave OR = 1.1, 95 % CI = 0.9-1.3, and cordless OR = 1.1, 95 % CI = 0.9-1.4. Using > 10 year latency period ORs increased, for cellular telephones OR = 1.5, 95 % CI = 0.98-2.4, and for cordless phones OR = 1.6, 95 % CI = 0.9-2.8.

The pooled analyses of the two case control studies of malignant brain tumours by Hardell et al (2006b) included 905 (90%) cases and the same control group as for benign tumours was used,

2,162 (89 %) subjects. Overall for low-grade astrocytoma cellular phones gave OR= 1.4, 95 %

CI = 0.9-2.3 and cordless phones OR = 1.4, 95 % CI = 0.9-3.4. The corresponding results for

high-grade astrocytoma were OR = 1.4, 95 % CI = 1.1-1.8, and OR = 1.5, 95 % CI = 1.1-1.9,

respectively. Using > 10 year latency period gave for low-grade astrocytoma and use of cellular

phones OR = 1.5, 95 % CI = 0.6-3.8 (ipsilateral OR = 1.2, 95 % CI = 0.5-5.8), and for cordless

phones OR = 1.6, 95 % CI = 0.5-4.6 (ipsilateral OR = 3.2, 95 % CI = 0.6-16). For high-grade

astrocytoma in the same latency period cellular phones gave OR = 3.1, 95 % CI = 2.0-4.6

(ipsilateral OR = 5.4, 95 % CI = 3.0-9.6), and cordless phones OR = 2.2, 95 % CI = 1.3-3.9

(ipsilateral OR = 4.7, 95 % CI = 1.8-13).

B. Studies from USA

Muscat et al (2000) studied patients with malignant brain tumours from five different hospitals

in USA. Controls were hospital patients. Data from 469 (82 %) cases and 422 (90 %) controls

were available. Overall no association was found, OR for handheld cellular phones was 0.9, 95

% CI = 0.6-1.2, but the mean duration of use was short, only 2.8 years for cases and 2.7 years

for controls. For neuroepithelioma OR = 2.1, 95 % CI = 0.9-4.7, was reported. The study is

inconclusive since no data were available on long-term users (≥ 10 years latency period). Some

support of an association was obtained since of 41 evaluable tumours, 26 occurred at the side of

the head mostly used during calls and 15 on the contralateral side.

Also the study by Inskip et al (2001) from USA had few long-term users of mobile phones, only

11 cases with glioma, 6 with meningioma and 5 with acoustic neuroma with ≥ 5 years regular

use. No subjects had ≥ 10 years use. The study comprised 489 (92 %) hospital cases with

malignant brain tumours, 197 with meningioma and 96 with acoustic neuroma, and 799 (86 %)

hospital-based controls. Overall no significant associations were found. Regarding different

types of glioma OR = 1.8, 95 % CI = 0.7-5.1 was found for anaplastic astrocytoma. Duration of use ≥ 5 years gave for acoustic neuroma OR increased to 1.9, 95 % CI = 0.6-5.9.

In another study by Muscat et al (2002) presented results from a hospital based case-control study on acoustic neuroma on 90 (100 %) patients and 86 (100 %) controls. Cell phone use 1-2 years gave OR = 0.5, 95 % CI = 0.2-1.3 (n=7 cases), increasing to OR = 1.7, 95 % CI = 0.5-5.1 (n=11 cases), in the group with 3-6 years use. Average use among cases was 4.1 years and among controls 2.2 years.

C. Danish cohort study

A population based cohort study in Denmark of mobile phone users during 1982 to 1995 included over 700,000 users (Johansen et al 2001). About 200,000 individuals were excluded since they had company paid mobile phones. Of digital (GSM) subscribers only nine cases had used the phone for ≥ 3 years duration yielding standardised incidence ratio (SIR) of 1.2, 95 % CI = 0.6-2.3. No subjects with 10-year use were reported.

This cohort study was updated with follow-up through 2002 for cancer incidence (Schüz et al 2006). There was no truly unexposed group for comparison since a large part of the population uses wireless phones. Moreover the excluded company subscribers (> 200 000 or 32 %) were apparently included in the reference population. There was also a very skewed sex distribution with 85 % men and only 15 % women in the cohort. SIR was significantly decreased to 0.95, 95 % CI = 0.9-0.97 for all cancers indicating a "healthy worker" effect in the study. In the group with ≥ 10 years since first subscription significantly decreased SIR of 0.7, 95 % CI = 0.4-0.95 was found for brain and nervous system tumours indicating methodological problems in the study. No latency data were given or laterality of phone use in relation to tumour localisation in

the brain. This study was uninformative regarding long-term health effects from mobile phone use.

D. Finnish study

Auvinen et al (2002) did a register based case-control study on brain and salivary gland tumors in Finland. All cases aged 20-69 years diagnosed in 1996 were included; 398 brain tumour cases and 34 salivary gland tumour cases. The duration of use was short, for analogue users 2-3 years and for digital less than one year. No association was found for salivary gland tumours. For glioma OR = 2.1, 95 % CI = 1.3-3.4 was calculated for use of analogue phones, but no association was found for digital mobile phones. When duration of use of analogue phones was used as a continuous variable an increased risk was found for glioma with OR = 1.2, 95 % CI = 1.1-1.5 per year of use.

E. The Interphone studies

1. Acoustic neuroma

The Swedish part of the Interphone study on acoustic neuroma included exposure data from 148 (93 %) cases and 604 (72 %) population based controls (Lönn et al 2004). Use of digital phones with time ≥ 5 years since first use gave OR = 1.2, 95 % CI = 0.7-2.1. No subjects were reported with use of a digital phone ≥ 10 years. An association was found for use of analogue phones yielding for ≥ 10 years latency period OR = 1.8, 95 % CI = 0.8-4.3 increasing to OR = 3.9, 95 % CI = 1.6-9.5 for ipsilateral use.

In Denmark the Interphone study included 106 (82 %) interviewed cases with acoustic neuroma and 212 (64 %) population-based controls (Christensen et al 2004). Significantly larger tumours were found among cellular phone users, 1.66 cm^3 compared with 1.39 cm^3 among non-users, *p* =

0.03. However OR was not significantly increased but only two cases had use a mobile phone regularly ≥ 10 years.

Schoemaker et al (2005) presented results for acoustic neuroma as part of the Interphone study performed in 6 different regions in the Nordic countries and UK, as previously partly reported (Lönn et al 2004; Christensen et al 2004). The results were based on 678 (82 %) cases and 3,553 (42 %) controls. Lifetime use of mobile phone for ≥ 10 years gave for ipsilateral acoustic neuroma OR = 1.8, 95 % CI = 1.1-3.1, and for contralateral OR = 0.9, 95 % CI = 0.5-1.8.

The study from Japan by Takebayashi et al (2006) included 101 (84 %) acoustic neuroma cases aged 30-69 years and diagnosed during 2000-2004. Using random digit dialling 339 (52 %) controls were interview. No association was found, OR = 0.7, 95% CI = 0.4 – 1.2. No exposure related increase in the risk of acoustic neuroma was observed when the cumulative length of use (<4 years, 4-8 years, >8 years) or cumulative call time (<300 hours, 300-900 hours, >900 hours) was used as an exposure index. The OR was 1.1, 95% CI = 0.6 - 2.1, when the reference date was set to five years before the diagnosis. Further, laterality of mobile phone use was not associated with tumours. No cases with ≥ 10 years latency period were reported.

Use of mobile phones and risk of acoustic neuroma were published from Norway as part of the Interphone study (Klaeboe et al 2007). It included 45 (68 %) acoustic neuroma cases and 358 (69 %) controls. A decreased risk was found with OR = 0.5, 95 % CI = 0.2-1.0. Using different criteria such as duration of regular use, time since first regular use, cumulative use etc 22 additional ORs and CIs were calculated. Time since first regular use for < 6 years gave OR =

1.0, 95 % CI = 0.2-5.7. All 21 other ORs were < 1.0 indicating systematic bias in the study. No case had a latency period of 10 years.

Schlehofer et al (2007) reported results from the German part of the Interphone study on sporadic acoustic neuroma. The study was performed during October 2000 and October 2003. Four study areas were included and cases were aged 30-59 years, but from October 1, 2001 extended to include the age group 60-69 years. They were recruited from hospitals and included 97 (89 %) cases, however, three with trigeminus neuroma. Controls were randomly selected from population registries and in total 202 (55 %) agreed to participate. No association was found for regular mobile phone use, OR = 0.7, 95 % CI = 0.4-1.2. Most ORs were < 1.0 and a decreasing trend of the risk was found for time since first regular use, lifetime number of use and duration of calls. No case had a latency period > 10 years. However, increased OR was found for highly exposed in "specified occupational exposure" yielding OR = 1.5, 95 % CI =0.5-4.2.

E. The Interphone studies

2. Glioma, meningioma

Lönn et al (2005) also studied glioma and meningioma. Data were obtained for 371 (74 %) glioma and 273 (85 %) meningioma cases. The control group consisted of 674 (71 %) subjects. No association was found although time since first regular phone use for ≥ 10 years gave for ipsilateral glioma OR = 1.6, 95 % CI = 0.8-3.4 and for contralateral glioma OR = 0.7, 95 % CI = 0.3-1.5.

For ipsilateral meningioma OR = 1.3, 95 % CI = 0.5-3.9 was calculated and for contralateral OR = 0.5, 95 % CI = 0.1-1.7 using 10 ≥ years latency period.

The Danish part of the Interphone study on brain tumours (Christensen et al, 2005) included 252 (71 %) persons with glioma, 175 (74 %) with meningioma and 822 (64 %) controls. For meningioma OR = 0.8, 95 % CI = 0.5-1.3 was calculated and for low-grade glioma OR = 1.1, 95 % CI = 0.6-2.0, and for high-grade glioma OR = 0.6, 95 % CI = 0.4-0.9 were found. Use for $\geq$ 10 years yielded for meningioma OR = 1.0, 95 % CI = 0.3-3.2, low-grade glioma OR = 1.6, 95 % CI = 0.4-6.1 and for high-grade glioma OR = 0.5, 95 % CI = 0.2-1.3. Regarding high-grade glioma 17 ORs were presented and all showed OR < 1.0.

Results from England were based on 966 (51 %) glioma cases and 1,716 (45 %) controls (Hepworth et al 2006). Cases were ascertained from multiple sources including hospital departments and cancer registries. The controls were randomly selected from general practioners' lists. Regular phone use gave OR = 0.9, 95 % CI = 0.8-1.1, increasing to OR = 1.2, 95 % CI = 1.02-1.5 for ipsilateral use but OR = 0.8, 95 % CI = 0.6-0.9 for contralateral use. Ipsilateral use for $\geq$ 10 years produced OR = 1.6, 95 % CI = 0.9-2.8, and contralateral OR = 0.8, 95 % CI = 0.4-1.4.

Schüz et al (2006) carried out a population-based case-control study in three regions of Germany, with incident cases of glioma and meningioma aged 30-69 years during 2000-2003. Controls were randomly drawn from population registries. In total, 366 (80 %) glioma cases, 381 (88 %) meningioma cases, and 1,494 (61 %) controls were interviewed. For glioma OR = 1.0, 95% CI = 0.7 - 1.3 and for meningioma OR = 0.8, 95% CI = 0.6 - 1.1 were obtained. However, among persons who had used cellular phones for $\geq$ 10 years increased risk was found for glioma; OR = 2.2, 95% CI = 0.9 - 5.1 but not for meningioma; OR = 1.1, 95% CI = 0.4 – 3.4. Among women they found OR = 2.0, 95 % CI = 1.1-3.5 for high-grade glioma for "regular" cell-phone use.

Summary results for mobile phone use and risk of glioma in Denmark, and parts of Finland, Norway, Sweden and United Kingdom have been published (Lahkola et al 2007). Of the included Interphone studies results had already been published from Sweden (Lönn et al 2005), Denmark (Christensen et al 2005) and UK (Hepworth et al 2006). The results were based on 2,530 eligible cases but only 1,521 (60%) participated. Regular mobile phone use gave OR = 0.8, 95 % CI = 0.7-0.9, but cumulative hours of use yielded OR = 1.006, 95 % CI = 1.002-1.010 per 100 hours. Ipsilateral mobile phone use for $\geq$ 10 years gave OR = 1.4, 95 % CI = 1.01-1.9, *p* trend = 0.04 and contralateral use OR = 1.0, 95 % CI = 0.7-1.4.

Use of mobile phones and risk of glioma and meningioma were published from Norway as part of the Interphone study (Klaeboe et al 2007). It included 289 (71 %) glioma cases, 207 (69 %) meningioma cases and 358 (69 %) controls. Significantly decreased OR = 0.6, 95 % CI = 0.4-0.9 was found for glioma and decreased OR = 0.8, 95 % CI = 0.5-1.1 for meningioma. For glioma 22 additional ORs were calculated using different exposure criteria as discussed above and all calculations yielded OR < 1.0, seven significantly so. Also for meningioma most ORs were < 1.0. Again these results indicate systematic bias in the study.

F. Meta-analysis

A meta-analysis of the risk for acoustic neuroma, glioma and meningioma was performed for mobile phone use with a latency period of 10 years or more (Hardell et al 2007). For acoustic neuroma studies by Lönn et al (2004), Christensen et al (2004) Schoemaker et al (2005) and Hardell et al (2006a) were included, all giving results for at least 10 years latency period or

more. Overall OR = 1.3, 95 % CI = 0.6-2.8 was obtained increasing to OR = 2.4, 95 % CI = 1.1-5.3 for ipsilateral mobile phone use (Lönn et al 2004, Schoemaker et al 2005, Hardell et al 2006). For glioma OR = 1.2, 95 % CI = 0.8-1.9 was calculated (Lönn et al 2005, Christensen et al 2005, Hepworth et al 2006, Schüz et al 2006, Hardell et al 2006b, Lahkola et al 2007). Ipsilateral use yielded OR = 2.0, 95 % CI = 1.2-3.4 (Lönn et al 2005, Hepworth et al 2006, Hardell et al 2006b, Lahkola et al 2007). In total OR = 1.3, 95 % CI = 0.9-1.8 was found for meningioma (Lönn et al 2005, Christensen et al 2005, Schüz et al 2006, Hardell et al 2006a) increasing to OR = 1.7, 95 % CI = 0.99-3.1 for ipsilateral use (Lönn et al 2005, Hardell et al 2006b).

IV. Discussion

This review included 20 studies, two cohort studies and 18 case-control studies. We recently made a review on this topic and more details can be found in that publication (Hardell et al 2007). Only two studies have been published since then. Both were on acoustic neuroma (Klaeboe et al 2007, Schlehofer et al 2007). They were small with no cases with a latency period of at least 10 years. Furthermore, most ORs were < 1.0 indicating serious methodological problems in the studies.

So far most studies have had no or limited information on long-term users. No other studies than from the Hardell group has published results for use of cordless phones (Hardell et al 2006a,b). As we have discussed in our publications it is pertinent to include also such use in this type of studies. Cordless phones are an important source of exposure to microwaves and they are usually used for a longer time period on daily basis as compared with mobile phones. Thus, to exclude such use seems to underestimate the risk for brain tumors from use of wireless phones.

It should be noted that the Hardell group has included also use of cordless phones, and thus in the exposure assessment the "unexposed" cases and controls have not been exposed to either cordless or cellular phones. This is in contrast to the Interphone study where the "unexposed" may have been exposed to cordless phones of unknown amount.

Of the 18 case-control studies 11 gave results for ≥ 10 years use or latency period. However, most of the results were based on low numbers. Thus, it is necessary to get an overview if there is a consistent pattern of increased risk with longer latency period and to make a formal meta-analysis of these findings. Since brain tumours are a heterogenic group of tumours it is reasonable to separate the results for malignant and benign tumours, as has been done in the various studies.

The Danish cohort study (Johansen et al, 2001) is not very informative due to limits in study design, analysis and follow-up. Schüz et al. (2006) reported an update of this previous study on mobile phone subscribers in Denmark. Since this report has gained substantial media coverage as "proof" of no brain tumor risk from mobile phone use we will discuss the shortcomings of the study in more detail in the following.

The cohort was established for persons that some time during 1982–1995 were registered cellular telephone users and has now been followed against the Danish Cancer Registry until 2002, seven years more than in the previous study. Previously (Johansen et al, 2001) 9 persons with brain tumors had used GSM phones for > 3 years, and OR =1.2 was reported. Now, data were not provided for type of phone or years of use. Rather the calculation of latency was based on first year of registration.

During early 1980s almost all cellular telephones were used in cars with external antennae. These subjects were unexposed to electromagnetic fields (EMF). No information regarding such use is provided, and one may assume that such participants are now included as exposed although they were not. Over 200 000 (32 %) company subscribers were excluded from the cohort. These are the heaviest users and are billed 4.5 times more than the layman in Sweden. They started use the earliest, but were included in the "non-user" group, i.e., the general Danish population.

SIR among cellular telephone users was 1.21 for temporal glioma (Schüz et al 2006), a region most exposed to EMF, based on 54 persons and not on phone type or time of first use (latency period). No information regarding the ear used and correlation with tumor site was given. The expected numbers were based on the general population. Because a large part of the population uses mobile phones and/or cordless phones, and the latter use was not assessed at all in the study, there is no truly unexposed group for comparison. Risk of cancer was underestimated, e.g., in the group with first use $\geq$ 10 years, the associated risk for brain tumors was low (SIR =0.7, 95 % CI = 0.4- 0.95). Relying on private cellular network subscription as measure of mobile phone use has been questioned (Ahlbom et al 2004, Funch et al 1996).

There seems to be a "healthy worker" effect in the study because of the decreased overall cancer risk (SIR= 0.9, 95 % CI = 0.9-0.95). Of the subscribers 85 % were men and 15 % women. Certainly early mobile phone users are not socioeconomically representative of the whole Danish population, used for comparison. The cohort only included people > 18 years of age. We reported (Hardell et al 2004, 2006a,b) that cellular telephone use beginning before age 20 is associated with a higher risk of brain tumours than use starting after age 20.

The authors do not acknowledge the contribution by the telecom industry as cited in the first publication (Johansen et al 2001), i.e., TelemarkDanmarkMobil and Sonofom. Two of the authors are affiliated with the private International Epidemiology Institute, Rockville, MD, USA, which has contributed financially to the study. Where the International Epidemiology Institute gets its money from is not declared. In the application to the Danish National Mobile Phone Program, which funded part of the study, no mention of the involvement or payment of these two consultants was made, a fact that is now being set under question.

Regarding the case-control studies there seems to be a consistent pattern of an increased risk for acoustic neuroma using a 10-year latency period and considering ipsilateral exposure. It might be a "signal" tumour type for increased brain tumour risk from microwave exposure, since it is located in an anatomical area with high exposure during calls with cellular or cordless phones (Hardell et al, 2003). Christensen et al (2004) found no association using a ≥ 10 year latency period, but the result was based on only 2 cases. Interestingly, the tumours were significantly larger in the total group of regular mobile phone users.

In our study we found an increased risk also with shorter latency period than 10 years (Hardell et al 2006a). However, it is not known at what stage in the carcinogenesis microwaves act. An effect might exist at different stages both of promoter and initiator type. We conclude that the results on acoustic neuroma are consistent with an association with use of cellular phones using a latency period of ≥ 10 years.

Regarding meningioma no consistent pattern of an association was found, although ipsilateral exposure in the ≥ 10 years latency group increased the risk in the meta-analysis. For a definite

conclusion longer follow-up studies are needed. We conclude that the results are not consistent with an association between use of mobile phones and meningioma.

Malignant brain tumours have been studied in 8 case-control studies. One study was register based and showed an increased risk associated with analogue phone use although the latency period seemed to be short (Auvinen et al 2002). The risk of glioma increased significantly per year of use. Five studies gave results for use of cell phone for 10 years or more. The pattern of an association was consistent in the different studies, except for the Danish study by Christensen et al (2005). In that study all 17 odds ratios for high-grade glioma were < 1.0 indicating systematic bias in assessment of exposure.

Our meta-analysis showed a significantly increased risk for ipsilateral use. We conclude that using ≥ 10 years latency period gives a consistent pattern of an association between use of mobile phones and glioma.

Regarding the Interphone studies the German part (Schüz et al 2006) was commented on by Morgan (2006) and these comments may also apply to the other Interphone studies. Morgan noted that the definition of a "regular" cell-phone user was so minimal that almost all "regular" cell-phone users would not be expected to be at risk, even if cell-phone use was found to create very high risks of glioma and meningioma. As for longer periods of "regular" cell-phone use, Schüz et al (2006) reported that only 14 percent of the glioma cases and 6 percent of the meningioma cases had used a cell phone for 5 years or more. For 10 years or more, the percentages were 3 percent and 1 percent, respectively. The authors replied that even long-term users in the study had barely more than 10 years of regular use and, in the beginning, were not heavy users; hence, they could not draw conclusions on heavy long-term use.

Methodological issues in the Interphone studies have been also discussed by Vrijhed et al (2006a,b). It was concluded that actual use of mobile phones was underestimated in light users and overestimated in heavy users. Random recall bias could lead to large underestimation in the risk of brain tumours associated with mobile phone use. According to the authors there was a selection bias in the Interphone study resulting in under selection of unexposed controls with decreasing risk at low to moderate exposure levels. Some of the Interphone studies had a low response rate, especially among controls giving potential selection bias.

A formal meta-analysis on mobile phone use and intracranial tumors was performed by Lahkola et al (2006). No data were given for ≥ 10 year latency period. Overall the risk increased for ipsilateral tumors, OR = 1.3, 95 % CI = 0.99-1.9 whereas no increased risk was found for contralateral tumors, OR = 1.0, 95 % CI = 0.8-1.4.

V. Conclusions

In summary we conclude that our review yielded a consistent pattern of an increased risk for acoustic neuroma and glioma after ≥ 10 years mobile phone use. We conclude that current standard for exposure to microwaves during mobile phone use is not safe for long-term brain tumor risk and needs to be revised.

VI. References

Ahlbom A, Green A, Kheifets L, Savitz D, Swerdlow A. 2004. Epidemiology of health effects of radiofrequency exposure. ICNIRP (International Commission for Non-ionizing Radiation Protection) Standing Committee on Epidemiology. *Environ Health Perspect* 112:1741-1754.

Auvinen A, Hietanen M, Luukonen R, Koskela RS. 2002. Brain tumors and salivary gland cancers among cellular telephone users. *Epidemiology* 13:356-359.

Christensen HC, Schüz J, Kosteljanetz M, Poulsen HS, Thomsen J, Johansen C.2004. Cellular telephone use and risk of acoustic neuroma. *Am J Epidemiol* 159:277-283.

Christensen HC, Schüz J, Kosteljanetz M, *et al.* 2005. Cellular telephones and risk for brain tumors. A population-based, incident case-control study. *Neurology* 64:1189-1195.

Funch DP, Rothman KJ, Loughlin JE, Dreyer NA. 1996. Utility of telephone company records for epidemiologic studies of cellular telephones. *Epidemiology* 7:299-302.

Hardell L, Näsman Å, Påhlson A, Hallquist A, Hansson Mild K. 1999. Use of cellular telephones and the risk for brain tumours:A case-control study. *Int J Oncol* 15:113-116.

Hardell L, Hansson Mild K, Påhlson A, Hallquist A.2001. Ionizing radiation, cellular telephones and the risk for brain tumours. *Eur J Cancer Prev* 10:523-529.

Hardell L, Hansson Mild K, Sandström M. 2003. Vestibular schwannoma, tinnitus and mobile telephones. *Neuroepidemiology* 22:124-129.

Hardell L, Hansson Mild K, Carlberg M, Hallquist A. 2004. Cellular and cordless telephones and the association with brain tumours in different age groups. *Arch Environ Health* 59(3): 132-137.

Hardell L, Carlberg M, Hansson Mild K. Pooled analyis of two case-control studies on the use of cellular and cordless telephones and the risk of benign tumours diagnosed during 1997-2003. *Int J Oncol* 28:509-518.

Hardell L, Hansson Mild K, Carlberg M.2006a. Pooled analysis of two case-control studies on use of cellular and cordless telephones and the risk for malignant brain tumours diagnosed in 1997-2003. *Int Arch Occup Environ Health* 2006b, 79:630-639.

Hardell L, Carlberg M, Söderqvist F, Hansson Mild K, Morgan LL. Long-term use of cellular phones and brain tumours: increased risk associated with use for ≥ 10 years. *Occup Environ Med* 2007;64:626-632, doi:10.1136/oem.2006.029751.

Hepworth SJ, Schoemaker MJ, Muir KR, Swerdlow AJ, van Tongeren MJ, McKinney PA. 2006. Mobile phone use and risk of glioma in adults: case-control study. *BMJ*15;332(7546):883-887. Epub 2006 Jan 20.

Inskip PD, Tarone RE, Hatch EE, *et al*. Cellular-telephone use and brain tumors. 2001. *New Engl J Med* 344:79-86.

Johansen C, Boice JD Jr, McLaughlin JK, Olsen JH 2001. Cellular telephones and cancer – a nationwide cohort study in Denmark. *J Natl Cancer Inst* 93:203-207.

Klaeboe L, Blaasaas KG, Tynes T. 2007. Use of mobile phones in Norway and risk of intracranial tumours. *Eur J Cancer Prev* 16:158-164.

Lahkola A, Tokola K, Auvinen A. 2006. Meta-analysis of mobile phone use and intracranial tumors. *Scand J Work Environ Health* 32(3):171-177.

Lahkola A, Auvinen A, Raitanen J, *et al*. 2007. Mobile phone use and risk of glioma in 5 North European countries. *Int J Cancer* 120:1769-1775.

Lönn S, Ahlbom A, Hall P, Feychting M. 2004. Mobile phone use and the risk of acoustic neuroma. *Epidemiology* 15: 653-659.

Lönn S, Ahlbom A, Hall P, Feychting M 2005. Swedish Interphone Study Group.Long-term mobile phone use and brain tumor risk. *Am J Epidemiol* 161:526-535.

Muscat JE, Malkin MG, Thompson S, *et al*. 2000. Handheld cellular telephone use and risk of brain cancer. *JAMA* 284:3001-3007.

Muscat JE, Malkin MG, Shore RE, *et al*. 2002. Handheld cellular telephones and risk of acoustic neuroma *Neurology* 58:1304-1306

Schoemaker MJ, Swerdlow AJ, Ahlbom A, *et al*. 2005. Mobile phone use and risk of acoustic neuroma: results of the Interphone case-control study in five North European countries. *Br J Cancer* doi: 10.1038/sj.bjc.6602764.

Schüz J, Böhler E, Berg G, Schlehofer B, *et al.* 2006. Cellular phones, cordless phones, and the risks of glioma and meningioma (Interphone Study Group, Germany). *Am J Epidemiology* 163(6):512-520. Epub 2006 Jan 27. Comment by Morgan in: *Am J Epidemiol* 2006;164:294-295. Author reply 295.

Schüz J, Jacobsen R, Olsen JH, *et al.* 2006. Cellular telephone use and cancer risks: An update of a nationwide Danish cohort. *J Natl Cancer Inst* 98:1707-1713.

Schlehofer B, Schlafer K, Blettner M, *et al.* 2007. Environmental risk factors for sporadic acoustic neuroma (Interphone Study Group, Germany). *Eur J Cancer* doi:10.1016/j.ejca.2007.05.008.

Takebayashi T, Akiba S, Kikuchi Y, *et al*. 2006. Mobile phone use and acoustic neuroma risk in Japan. *Occup Environ Med* 63:802-807.

Vrijheid M, Cardis E, Armstrong BK, *et a*l. 2006a. Validation of short term recall of mobile phone use for the Interphone study. *Occup Environ Med* 63:237-243.

Vrijheid M, Deltour I, Krewski D, Sanchez M, Cardis E. 2006b. The effects of recall errors and selection bias in epidemiologic studies of mobile phone use and cancer risk. *J Expo Sci Environ Epidemiol* doi:10.1038/sj.jes.7500509.

Table. Summary of 20 studies on the use of cellular telephones and brain tumour risk. For further details, see Hardell et al (2007). Odds ratio (OR), 95 % confidence interval (CI) and standardised incidence ratio (SIR) are given.

Study	Years Study Type	Age	Tumour type	No. of Cases	Odds ratio, 95 % confidence interval	Comments
Hardell et al 1999, 2001 Sweden	1994-1996 Case-control	20-80 years	Brain tumours	217	OR 1.0 (0.7-1.4)	Analogue and digital cell phone use
				34	OR 1.1 (0.6-1.8)	Ipsilateral
				16	OR 1.2 (0.6-2.6)	> 10 year latency, analogue cell phone
Muscat et al 2000 USA	1994-1998 Case-control	18-80 years	Brain tumours	17	OR 0.7 (0.4-1.4)	Mean duration of use, 2.8 years
			Neuorepithelioma	35	OR 2.1 (0.9-4.7)	
Johansen et al 2001 Denmark	1982-1995 Cohort	0 to > 65 years	Brain tumours	20	SIR 1.3 (0.8-2.1)	Analogue and digital cell phone use
				9	SIR 1.2 (0.6-2.3)	≥ 3 years duration of digital subscription
Inskip et al 2001 USA	1994-1998 Case-control	≥ 18 years	Acoustic neuroma	5	OR 1.9 (0.6-5.9)	≥ 5 years of cell phone use
			Glioma	11	OR 0.6 (0.3-1.3)	
			Meningioma	6	OR 0.9 (0.3-2.7)	
Muscat et al 2002 USA	1997-1999 Case-control	≥ 18 years	Acoustic neuroma	11	OR 1.7 (0.5-5.1)	3-6 years of cell phone use
Auvinen et al 2002 Finland	1996 Case-control, register based	20-69 years	Glioma	119	OR 1.5 (1.0-2.4)	Analogue and digital cell phone "ever" use
				40	OR 2.1 (1.3-3.4)	Analogue cell phone "ever" used
				11	OR 2.4 (1.2-5.1)	Analogue cell phone use 1-2 years
				11	OR 2.0 (1.0-4.1)	Analogue cell phone use, >2 years
Lönn et al 2004 Sweden Interphone	1999-2002 Case-control	20-69 years	Acoustic neuroma	12	OR 1.8 (0.8-4.3)	≥10 years of cell phone use, result for either side of head
				12	OR 3.9 (1.6-9.5)	≥10 years of cell phone use on same side of head as tumour

Study	Years Study Type	Age	Tumour type	No. of Cases	Odds ratio, 95 % confidence interval	Comments
Christensen et al 2004 Denmark Interphone	2000-2002 Case-control	20-69 years	Acoustic neuroma	45	OR 0.9 (0.5-1.6)	Regular use
				2	OR 0.2 (0.04-1.1)	$\geq$ 10 years cell phone use on same side of head as tumour. Significantly larger tumours among cellular phone users 1.66 cm^3 *versus* 1.39 cm^3, p=0.03.
Lönn et al 2005 Sweden Interphone	2000-2002 Case-control	20-69 years	Glioma	214	OR 0.8 (0.6-1.0)	Regular use
				15	OR 1.6 (0.8-3.4)	$\geq$10 years since first "regular" cell phone use on same side of head as tumour
				11	OR 0.7 (0.3-1.5)	$\geq$10 years since first "regular" cell phone use on opposite side of head as tumour.
			Meningioma	118	OR 0.7 (0.5-0.9)	Regular use
				5	OR 1.3 (0.5-3.9)	$\geq$10 years since first "regular" cell phone use on same side of head as tumour
				3	OR 0.5 (0.1-1.7)	$\geq$10 years since first "regular" cell phone use on opposite side of head as tumour.

Study	Years Study Type	Age	Tumour type	No. of Cases	Odds ratio, 95 % confidence interval	Comments
Schoemaker et al 2005 Denmark, Finland, Sweden, Norway, Scotland, England, Interphone	1999-2004 Case-control	18-69 years (variable)	Acoustic neuroma	360	OR 0.9 (0.7-1.1)	Regular use
				23	OR **1.8** (1.1-3.1)	≥ 10 lifetime years of cell phone use on same side of head as tumour
				12	OR 0.9 (0.5-1.8)	≥ 10 lifetime years of cell phone use on opposite side of head as tumour
Christensen et al 2005 Denmark Interphone	2000-2002 Case-control	20-69 years	Low-grade glioma	47	OR 1.1 (0.6-2.0)	Regular use
				9	OR 1.6 (0.4-6.1)	≥10 years since first regular use of cell phone
			High-grade glioma	59	OR 0.6 (0.4-0.9)	Regular use
				8	OR 0.5 (0.2-1.3)	≥10 years since first regular use of cell phone\n\n17 odds ratios for high-grade glioma, all < 1.0, indicates systematic bias
			Meningioma	67	OR 0.8 (0.5-1.3)	Regular use
				6	OR 1.0 (0.3-3.2)	≥10 years since first regular use of cell phone
Hepworth et al 2006 UK Interphone	2000-2004 Case-control	18-69 years	Glioma	508	OR 0.9 (0.8-1.1)	Regular use
				NA	OR 1.6 (0.9-2.8)	≥10 years of cell phone use on same side of head as tumour.
				NA	OR 0.8 (0.4-1.4)	>10 years of cell phone use on opposite side of head as tumour.

Study	Years Study Type	Age	Tumour type	No. of Cases	Odds ratio, 95 % confidence interval	Comments

Study	Years Study Type	Age	Tumour type	No. of Cases	Odds ratio, 95 % confidence interval	Comments
Schüz et al 2006 Germany Interphone	2000-2003 Case-control	30-59 years	Glioma	138	OR 1.0 (0.7-1.3)	Regular use
				12	OR 2.2 (0.9-5.1)	≥ 10 years since first regular use of cell phone
				30	OR **2.0** (1.1-3.5)	Female regular use of cell phone
			Meningioma	104	OR 0.8 (0.6-1.1)	Regular use
				5	OR 1.1 (0.4-3.4)	≥ 10 years since first regular use of cell phone

Hardell et al 2006a Sweden	1997-2003 Case-control	20-80 years	Acoustic neuroma	130	OR **1.7** (1.2-2.3)	> 1 year latency of cell phone use
				20	OR **2.9** (1.6-5.5)	> 10 years latency of cell phone use
				10	OR **3.5** (1.5-7.8)	> 10 years of ipsilateral cell phone use
				4	OR 1.0 (0.3-2.9)	> 10 years latency of cordless phone use
				3	OR 3.1 (0.8-12)	> 10 years latency of ipsilateral cordless phone use
			Meningioma	347	OR 1.1 (0.9-1.3)	> 1 year latency of cell phone use
				38	OR 1.5 (0.98-2.4)	> 10 years latency of cell phone use
				15	OR 2.0 (0.98-3.9)	> 10 years latency of ipsilateral cell phone use
				23	OR 1.6 (0.9-2.8)	> 10 years latency of cordless phone use
				9	OR **3.2** (1.2-8.4)	> 10 years latency of ipsilateral cordless phone use
Hardell et al 2006b Sweden	1997-2003 Case-control	20-80 years	Glioma, high-grade	281	OR **1.4** (1.1-1.8)	> 1 year latency of cell phone use
				71	OR **3.1** (2.0-4.6)	> 10 years latency of cell phone use
				39	OR **5.4** (3.0-9.6)	> 10 years latency of ipsilateral cell phone use
				23	OR **2.2** (1.3-3.9)	> 10 years of cordless phone use
				10	OR **4.7** (1.8-13)	> 10 years latency of ipsilateral cordless phone use
			Glioma, low-grade	65	OR 1.4 (0.9-2.3)	> 1 year latency of cell phone use
				7	OR 1.5 (0.6-3.8)	> 10 years latency of cell phone use
				2	OR 1.2 (0.3-5.8)	> 10 years latency of ipsilateral cell phone use
				5	OR 1.6 (0.5-4.6)	> 10 years latency of cordless phone use
				3	OR 3.2 (0.6-16)	> 10 years latency of ipsilateral cordless phone use

Study	Years Study Type	Age	Tumour type	No. of Cases	Odds ratio, 95 % confidence interval	Comments
Takebayashi et al 2006 Tokyo Interphone	2000-2004 Case-control	30-69 years	Acoustic neuroma	51	OR 0.7 (0.4-1.2)	Regular use
				4	OR 0.8 (0.2-2.7)	Length of use > 8 years
				20	OR 0.9 (0.5-1.6)	Ipsilateral use
Schüz et al 2006 Denmark	1982-2002 Cohort	>18 years	Glioma	257	SIR 1.0 (0.9-1.1)	420 095 telephone subscribers
			Meningioma	68	SIR 0.9 (0.7-1.1)	
			Nerve sheat tumors	32	SIR 0.7 (0.5-1.0)	
			Brain and nervous system	28	SIR 0.7 (0.4-0.95)	Latency $\geq$ 10 years
Lahkola et al 2007 Denmark, Norway, Finland, Sweden, UK Interphone	September 2000-February 2004 (differed between countries) Case-control	20-69 years (Nordic countries), 18-59 years (UK)	Glioma	867	OR 0.8 (0.7-0.9)	Regular use
				77	OR 1.4 (1.01-1.9)	Ipsilateral mobile phone use, $\geq$ 10 years since first use, *p* for trend = 0.04
Klaeboe et al 2007 Norway Interphone	2001-2002 Case-control	19-69 years	Glioma	161	OR 0.6 (0.4-0.9)	Regular use
			Meningioma	111	OR 0.8 (0.5-1.1)	
Schlehofer et al 2007 Germany Interphone	2000-2003 Case-control	30-69 years	Acoustic neuroma	29	OR 0.7 (0.4-1.2)	Regular use

Evidence for Brain Tumors

(EPIDMIOLOGICAL)

Michael Kundi, PhD med. habil., Professor

Institute of Environmental Health, Center for Public Health,

Medical University of Vienna, Austria

Prepared for the BioInitiative Working Group

July 2007

TABLE OF CONTENTS

I.   INTRODUCTION

Primary central nervous system (CNS) tumors are a heterogeneous group of benign and malignant neoplasms localized in the brain, the spinal cord and their coverings. They differ in histological type, tissue of origin, anatomic site, growth pattern, age distribution, sex ratio, clinical appearance and many other features including molecular neuropathological markers. These features are not independent but little is known about the etiology of these tumors and the reason for the observed epidemiological patterns. The rapidly developing field of molecular neuropathology may provide clues to solve these problems in the future.

Brain tumors, accounting for the majority of CNS tumors, are rare. Annually about 36,000 36000 new cases are diagnosed in the US and about 180,000 180000 world-wide. The age distribution has two peaks: incidence is about 35 cases per million per year below 10 years of age (which is mainly due to tumors originating from mesodermal and embryonic tissues, medulloblastoma and astrocytoma of the juvenile pilocytic type), and after age 15 there is a steady increase of incidence with increasing age reaching its second peak of about 200 cases per million per year at an age around 75 years. The burden of CNS cancers is distinctly higher in children making up around 20% of all childhood malignancies, while in adults less than 2% of all cancers are primary brain cancers.

There are some rare cases of inherited cancer syndromes (e.g. von Hippel-Lindau disease, Li-Fraumeni syndrome) that are related to brain tumor risk, accounting for a small fraction of cases. Except for therapeutic x-rays no environmental or lifestyle life-stile factor has unequivocally been established as risk factor for brain tumors. Non-whites Non whites seem to have lower risk, and incidence tends to be higher with increasing socio-economic status. However, because of the rather advanced age of 75 of peak incidence, such differences may partly be due to differences in life-expectancy. During the last decades some types of brain tumors show a steady increase of a few percent per year, which might to some extent be related to the introduction of computed tomography and other high-resolution neuroimaging methods.

Since the report of Wertheimer and Leeper in 1979 of an increased incidence of brain tumors in children living in homes with an expected higher exposure to power-frequency electric and

magnetic fields, exposure to electromagnetic fields have become an area of interest in the study of factors affecting brain tumor risk.

This review focuses on the radio frequency (RF) part of the electromagnetic spectrum (3 kHz to 300 GHz). However, because the epidemiology of mobile phone use is covered in another section, it will be restricted to RF exposure conditions other than microwaves from mobile phone use. Exposure to ELF magnetic fields and childhood brain tumors is covered in the chapter about childhood cancers.

II.   Material and Methods

Published articles of relevant studies restricted to the last 20 years were obtained by searching PubMed using the following terms:

("radio frequency" OR electromagnetic* OR microwaves) AND ("brain cancer" OR brain tumor* OR "CNS cancer" OR CNS tumor* OR glioma* OR meningioma* OR neuroma*) NOT ("power frequency" OR "low frequency") AND epidemiology

The search resulted in 101 hits. After removing reviews and animal or in vitro studies as well as studies of mobile phone use, 8 articles remained. A hand search in review papers (Krewski et al. 2001; Elwood 2003; Ahlbom et al. 2004; Kundi et al. 2004) and reference lists of the articles found in PubMed revealed another 7 papers; hence the final body of evidence consists of 15 studies of exposure to various types of RF fields.

Of the 15 studies 8 were cohort studies, 3 case-control studies and 4 of an ecological type. The majority (11) were occupational studies, two studies investigated children, and one ecological study investigated adults and one study both, adults and children.

III.   Epidemiological studies of RF fields and brain tumors

Table 1 gives an overview of the 15 studies obtained by the literature search with respect to study type, assessment of exposure and outcome, confounders considered and matching variables used, number of cases included and selection method of study participants. Results are summarized in Table 2.

In the following paragraphs each study is briefly discussed with respect to its strengths and weaknesses.

A. Thomas et al. 1987

This case-control study included 435 deaths from brain or CNS tumors and 386 deaths from other causes as controls. Only adult males were included. Basis of data collection on occupational history were interview with next-of-kin. Two methods of classification were used: one method assigned subjects to one of three categories (never exposed to RF/ever exposed to RF in an electrical or electronics job/ever exposed to RF but not in an electrical or electronics job), the other method consisted in a classification of each job by an industrial hygienist hyginiest for presumed exposure to RF, soldering fumes, and lead. Both methods revealed significantly increased brain tumor risks of presumed occupational exposure to RF fields. This increase was due to an association in electronics and electrical jobs with astrocytic tumors as the predominant outcome associated with employment in these categories. In addition a significant increase of brain tumor risk was found for increasing duration of exposure.

Although relying on information of next-of-kin could be a source of misclassification, one strength of this study is it's its relying on occupational history only that could be assumed to be more accurate than recall of exposure to various agents. The two methods of classification led to almost the same results, which lends support to the hypothesis that indeed exposure in electrical and electronics jobs is associated with an increased brain tumor risk. Due to the strong relationship between RF exposure and exposure to lead, solvents or soldering fumes in these jobs, it is not possible to separate effects of these exposures. However, analysis of exposure to lead did not show a consistent relationship with brain tumor risk, indicating that it may not confound the relationship to RF exposure.

Because this study is of dead cases only it is likely over-representing high grade brain tumors that may not all be associated with exposure which leads to an effect dilution. Exposure misclassification, if it is non-differential in cases and controls, also tends to reduce effect estimates.

A weakness of this study is obviously its lack of an exposure indicator other than the occupational category. While there is no doubt that in these jobs some exposure to RF fields occur quite regularly, specific characteristics including frequency ranges, modulation, intensity, duration and distance from the source vary considerably. Overall the study (as well as two earlier ones outside the search window: Lin et al. 1985 and Milham 1985) are sufficient to formulate a research hypothesis that can be tested in appropriately designed subsequent investigations. Unfortunately such studies have never been conducted.

B. Milham 1988

In this cohort study of 67,829 amateur radio operators holding a license within 1/1979 to 6/1984 in Washington and California 29 brain tumor deaths occurred during the follow up period with 21 expected.

It should be noted that there was a substantial and statistically significant lower number of overall deaths of less than three quarters of deaths expected from country mortality rates. This could be due to both a 'healthy-worker' effect as well as an effect of socio-economic status. In lieu of computing standardized mortality ratios (SMR) it may be instructive to look at the proportional mortality rates in the reference population and the amateur radio operators: 0.6% of all deaths are expected to be due to brain tumors in the reference population while in amateur radio operators twice as many occurred (1.2%). Whether or not this is an indication of an increased brain tumor risk due to RF exposure is difficult to assess. First of all this study is a register only investigation and no information on intensity, frequency and duration of engagement in amateur radio operations are available. In a later analysis the author reported about results using a proxy of intensity and duration of exposure: the license class. In this analysis indications of an increase of risk with increasing license class were obtained.
This study could and should have started off a thorough follow up of amateur radio operators and nested case-control studies to address the problem of potential confounders and to narrow down the conditions that may be responsible for the increased mortality from some cancers. It is another loose end that leaves us without a clear message.

Although no risk factor for brain cancer except therapeutic ionizing radiation is known, there are some indications that risk increases with social class. The reason for this association is unknown but life-style factors may play a role as well as concomitant causes of death that

could lead to a spurious reduction of risk in lower class populations because brain tumors have their peak close to life-expectancy.

C. Selvin et al. 1992

The objective of this investigation was not primarily to study the relationship between RF exposure and childhood cancer but to address the general problem of how to assess disease incidence or mortality in relation to a point source. As the point source the Sutro Tower in San Francisco, the only microwaves emitting tower in this county, was chosen. A total of 35 brain tumor deaths occurred among 50,686 white individuals at risk aged less than 21 in the years 1973-88 in an area of approximately 6 km around the tower. The exact location of residence could not be obtained; therefore each case was located in the center of the census tract. Different methods of analysis were applied to assess a potential relationship between distance from the tower and brain tumor risk. Relative risk for brain tumors for a distance less than 3.5 km from Sutro Tower compared to more than 3.5 km was 1.162 and not significant. The study explored different methodological procedures and has its merits from a methodological point of view. However, it starts from the wrong assumption: that distance to a point source is a valid proxy for intensity of exposure. Under ideal conditions of spherical symmetry of an emission this assumption holds, however, there are almost no real life situations where this assumption is sufficiently close to actual exposure levels. And it is definitely not true for the Sutro Tower. Radiations from the antennae are directed towards the horizon and the complex pattern of emission with main and side lobes results in a complex pattern of RF exposure at ground level. Furthermore, the area is topographically structured with hills and valleys such that areas of high exposure at the vertices are in close proximity to areas of low exposure at the shadowed side downhill.

Studying the relationship between a point source and disease is not only difficult due to the complex relationship between distance and exposure but also because of the fact that humans are not stable at a certain location. This is of greater importance for adults who may commute from and to work places and have generally a greater radius of activity as compared to children. Nevertheless, there is at least a high chance of one long-lasting stable location that is when people sleep in their beds. Therefore, studies in relation to a point source should attempt to assess exposure at the location of the bed. Because the objective of this study was not the

assessment of a potential brain tumor risk but the application of methods for the analysis of spatial data, no attempts were made to measure actual exposure.

D. Tynes et al. 1992

In this study information on occupations obtained for all Norwegians every 10 years was used to assess cancer incidence in relation to job titles. In 1960 37,945 male workers were identified that had jobs with possible exposure to EMFs and among these 3,017 with possible RF exposure. Overall 119 brain tumor cases were found in the cancer registry between 1961 and 1985. Of these cases 6 occurred in the subgroup of workers possibly exposed to RF fields. The overall expected number of brain tumor cases was 109 and 12 for the subgroup with possible RF exposure. Hence no increased brain tumor risk could be detected.

Despite the long follow-up period of 25 years with an accumulated number of 65,500 person-years the expected number of brain tumors diagnosed during that period is too low to detect a moderately elevated risk of 1.3 to 1.5.

As mentioned above, all studies solely relying on job titles lead to exposure misclassification and, therefore, to a dilution of risk. For dichotomous exposure variables (exposed/not exposed) and assuming a negligibly small proportion of exposed in the reference population standardized incidence ratios (SIR) are biased by a factor $(1+f*(SIR-1))/SIR$, if f denotes the fraction of true exposed and SIR is the true incidence ratio. Hence a true SIR of 2.0 is reduced to 1.5 if only 50% in the cohort are actually exposed. The observed SIR is further reduced if the assumption of a negligible fraction of exposed in the reference population is wrong. In this case the bias factor given above is further divided by $(1+g*(SIR-1))$, where g is the fraction of exposed in the general population.

While a cohort study that is based on registry data has the advantage of independence from recall errors and selection bias due to possible differential participation, it has the disadvantage that registry data are generally insufficient to provide reliable exposure indicators. While no association with brain tumors could be detected in this study it revealed an increased number of leukemia cases in occupations with possible RF exposure. This could

be due to the higher incidence of leukemia or to a stronger association or to different latency periods and various other reasons including chance.

E. Grayson 1996

In this case-control study nested within approx. 880,000 US Air Force personnel with at least one years of service during the study period of 1970-89 primary malignant brain tumor cases were ascertained by screening hospital discharge records. The study included only males and only as long as they were on Air Force records. From 246 cases detected 16 were dropped due to incomplete or ambiguous data. For each case four controls were randomly selected from the case's risk set matching it exactly on year of birth and race. Controls who were diagnosed with diseases that may be associated with EMF exposure (leukemia, breast cancer, malignant melanoma) were excluded from the risk set.

One strength of this study is the detailed job history filed for each cohort member that could be used for retrospective exposure assessment. Furthermore, Air Force files contained detailed data from personal dosímetry on ionizing radiation for the different posts and jobs. Classification of RF field exposure was based on a detailed job exposure matrix with over 1,950 entries, indexing 552 different job titles. One source of classification was recorded events of exposure to RF fields above 100 W/m2. By this method probable exposure was assigned if for a job such events were recorded in the past as well as for closely related jobs. Possible exposure was assigned for jobs that required operation of RF emitters but without recorded overexposure.

A further strength is the thorough consideration of possible confounders. Because of the possible relationship of brain tumor risk with socio-economic status (SES), military rank was used as a surrogate for SES and included in the analysis as well as ionizing radiation exposure that has previously been shown to increase brain tumor risk.

Exposure to RF fields was associated with a moderate but statistically significant increased risk of OR=1.39. Investigation of duration of exposure was compromised by an ambiguity introduced by the calculation of an exposure score as the product of exposure and months.

Nevertheless, for those ever exposed there were indications of an increasing risk with increasing exposure duration.

A weakness of this investigation is its incomplete follow-up of cohort members. This could have resulted in an underestimation of the true risk. Leaving the Air Force could have been more likely in those exposed to RF fields and developing a brain tumor. Some malignant brain tumors have early signs that could be incompatible with the Air Force job especially if involving operation of RF equipment (like seizures, severe headaches, somnolence, and absences). Because the study did not involve personal contact it is free of other selection biases.

F. Szmigielski 1996

In this military cohort study of cancer morbidity Polish military career personnel was assessed for occupational exposure to RF fields based on service records. The study covered 15 years (1971-85) including approx. 128,000 persons per year. Expected rates for 12 cancer types were calculated based on the age specific morbidity in those classified as unexposed.
For brain and nervous system tumors a significantly increased ratio of observed to expected (OER=1.91) was found. Other malignancies with significantly increased incidence in exposed were: esophageal and stomach cancers, colorectal cancers, melanoma, and leukemia/lymphoma.

One strength of this study is its substantial size with almost 2 million person-years of follow-up. Furthermore, accurate military records on job assignment and on exposure from military safety groups gives a unique opportunity to assess long-term exposure effects based on already filed data.

Some important data are missing because they were military classified information that could not be provided in the paper. This includes the exact number of cases of the different neoplasms. However, from the data presented an observed number of brain tumors of about 46 can be calculated.

The study has been criticized for an alleged bias because more information on risk factors was available for cancer cases. It is true that military medical boards collected data for cases such

as life style factors and exposure to possible carcinogens during service, however, at no stage this information entered the analysis. Therefore, this criticism is unfounded. Such information could have been utilized within a nested case-control study applying the same methods of assessment of risk factors for controls as has been done for cases. Because some findings, such as the increased risk for esophagus/stomach cancer, that are rarely reported in relation to RF exposure warrant further study, such a nested case-control approach is recommended. It could, albeit with some difficulties, even be successfully conducted retrospectively.

G. Hocking et al. 1996

In an ecological study cancer incidence and mortality in nine municipalities of northern Sydney during 1972-90 three of which surround three TV towers were assessed. Population size in the three municipalities located within a radius of approximately approx. 4 km around the TV towers amounts to 135,000 while population size in the six municipalities further away was 450,000. High-power transmission commenced in 1956, an additional 100 kW transmission started in 1965 and another 300 kV broadcast in 1980. Carrier frequencies varied between 63 and 533 MHz for TV broadcasting and was around 100 MHz for FM radio broadcast.

During the study period 740 primary malignant brain tumors were diagnosed in adults and 64 in children, 606 deaths due to brain cancer occurred in adults and 30 in children. While incidence of lymphatic leukemia was significantly higher in adults as well as in children inhabiting the three municipalities surrounding the transmission towers compared to the six districts further away, brain tumor incidence was not significantly elevated (RR=0.89 in adults and 1.10 in children).

As has been stated above, distance from a transmitter is a poor proxy for exposure. Some measurements done in the study area obtained levels much lower than those calculated from the emission power and antenna gain. Several factors are responsible for this effect: multiple reflections, attenuation by buildings and vegetation, ground undulations, non-coincidence of maxima for the different signals as well as complex radiation characteristics of the broadcast antennae.

The exact location of the residence of cases could not be provided which reduces the potential of the study to relate incidences to measurements or calculations of RF fields. Authors discussed some potential sources of bias such as migration and other exposures in the different regions. However, the most important disadvantage in such studies is that individual risk factors cannot be adjusted for. Both spurious positive as well as false negative results can be obtained by disregarding such individual variables.

H. Tynes et al. 1996

In a historical cohort study 2,619 Norwegian female radio and telegraph operators certified between 1920 and 1980 were followed from 1961 through 1991 for entries in the cancer registry. During this period a total of 140 cases of cancer occurred which are about 20% more than expected from the Norwegian population. Among these were 5 brain tumor cases closely matching the number expected.

An excess for breast cancer was found in this study that may be related to a combination of RF field exposure and night work. For other cancers including brain cancer numbers of cases were too low to address exposure risk.

In this very thoroughly conducted study including a nested case-control approach for breast cancer, measurements at historical transmitters on ships, comparison with women at other jobs on sea, brain tumors were not distinctly higher than expected from the reference population. However, because of the limited cohort size a moderately increased risk cannot be excluded.

I. Dolk et al. 1997a

This ecological small area study of cancer incidence 1974-86 near the Sutton Coldfield TV/radio transmitter at the northern edge of the city of Birmingham (England) was initiated by an unconfirmed report of a 'cluster' of leukemias and lymphomas. The transmitter came into service in 1949. Transmission at 1 megawatt (effective radiated power erp) began in 1964, at 3 MW in 1969, and at 4 MW in 1982. The tower has a height of 240 m with no big hills in the surrounding area. The study area was defined by a circle of 10 km radius centered at the transmitter. The population within this area was about 408,000. All cancers, excluding

non-melanoma skin cancer, were considered focusing on hematopoietic and lymphatic cancers, brain and nervous system cancers, eye cancer, and male breast cancer. Childhood cancers were restricted to all cancers and all leukemias.

In the study area a small but significant excess of all cancers was observed in adults. All leukemias and non-Hodgkin's lymphoma were particularly elevated and incidence within 2 to 4 km from the tower was about 30% higher than expected. Brain tumors were only analyzed for distances of within 2 km and the whole study area. Within 2 km an increased OER of 1.29 for all brain tumors and 1.31 for malignant brain tumors was calculated based on 17 and 12 cases, respectively.

Also this investigation suffers from using distance from the tower as proxy for intensity of exposure. The wrong assumption that exposure decreases with increasing distance invalidates the statistical trend test applied. Measurements conducted in the study area revealed the poor relationship with distance but without consequences on the evaluation of the data. Overall the study is consistent with a moderately increased risk of hematopoietic and lymphatic cancers as well as some other cancers including brain cancer in the vicinity of high-power transmitters that, if related to RF fields, must be substantially higher for actual exposure.

The Sutton Coldfield study was later continued (Cooper & Saunders 2001) to cover the period 1987-94. The study revealed, compared to the earlier period, an almost unchanged increase of leukemias and non-Hodgkin's lymphoma in adults and a slight increase in children.

J.   Dolk et al. 1997b

Because the Sutton Coldfield study was triggered by a cluster report and to provide independent test of hypotheses arising from that study, similar methods as applied in the previous study were used to study all high-power TV/radio transmitters ($\geq$ 500 kW ERP) in Great Britain. In adults leukemias, bladder cancer, and skin melanoma, and in children, leukemias and brain tumors were studied. The study period was 1974-86 for England and somewhat shorter in Wales and Scotland.

Although population density around transmitters was not always as high as in the case of the Sutton Coldfield tower, with an average population density of only about one third of that

around Sutton Coldfield tower within 2 km from the towers, in the most important range of 2 to 4 km from the transmitters, where in many cases the maximum of radiated RF at ground level is reached, population density was similar. The study of all high-power transmitters essentially corroborated the findings for adult leukemias with an increase of incidence between 10 and 50% in the distance band of 2 to 4 km from the transmitters for the different transmitter types. Most of these increased incidences were statistically significant.

For children only the incidence in the whole study area and within a distance of 2 km was calculated, which is unfortunate because the area close to the towers is sparsely populated and exposure is low. Number of brain tumors in children was slightly above expectation (244 observed and 231 expected).

In contrast to the interpretation by the authors, the study of all high power transmitters essentially replicated and supported the findings of an excess incidence of leukemias in relation to RF emission from TV/radio towers. Because the different heights and radiation characteristics of the transmitters result in different exposure patterns at ground level, the consistent increase in an area that is likely close to the maximum of exposure supports the hypothesis of an association.

K. Lagorio et al. 1997

A mortality study of a cohort of 481 female plastic-ware workers employed between 1962-92 in an Italian plant, 302 of which were engaged in the sealing department with exposure to RF fields, was reported by Lagorio et al. (1997). For RF-sealers 6,772 person-years of follow-up were accumulated and overall 9 deaths occurred, 6 of which were from malignant neoplasms (which are twice as many as expected from comparison with the local reference population). In the 31 years only one brain cancer occurred but only 0.1 were expected.

Although the small size of the cohort and the potential exposure to other agents except RF fields such as solvents and vinyl chloride prohibit far reaching conclusion, much more of such thorough follow-up studies of exposed cohorts are needed to accumulate a body of evidence that can provide a useful basis for analysis.

L. Finkelstein 1998

A preliminary study intended to form the basis for an assessment of cancer risks associated with handheld radar devices was conducted among a cohort of 20,601 male Ontario police officers. The retrospective follow up covered the period of 1964-95. By linkage with the cancer registry and mortality database 650 cases of cancer were detected.

Testicular cancer and melanoma showed an excess incidence while overall cancer incidence was reduces as expected from a working cohort. Overall 16 cases of primary malignant brain tumors occurred which are slightly less than expected.

The author had difficulties to build up a proper cohort because some departments refused to participate and others couldn't spare the time to provide lists of all officers employed during the target period. Furthermore, while cancer sites of primary interest showed actually an increased incidence calling for a nested case-control approach, this study was never conducted due to lack of interest and support of the authorities.

M. Morgan et al. 2000

In an occupational cohort study all US Motorola employees with at least 6 months cumulative employment and at least 1 day of employment in the period 1976-96 were included. A total of 195,775 workers contributing about 2,7 million person-years were available for the study. The cohort was compared to the SSA Master Mortality File and the National Death Index to obtain vital status. Death certificates were obtained by states' vital statistics offices and company records. Exposure was assessed by expert opinion. Four RF exposure groups were defined with increasing level of estimated RF exposure. Only about 5% of the total cohort was classified as highly exposed and more than 70% with only background exposure. Neither private nor occupational mobile phone use was included.

Overall 6,296 deaths occurred in the cohort in 21 years, which were only two thirds of deaths expected from mortality data of the four countries where most Motorola facilities are located. This reduction is too pronounced to be solely due to a healthy worker effect, other factors such as higher SES must have contributed, an interpretation supported by the substantial reduction of mortality from all life-style associated causes of death. Internal comparisons were done for mortality from brain cancer and hematopoietic and lymphatic cancers. Brain tumor mortality was slightly but insignificantly elevated in high and moderately high exposed workers as compared to those with no or low RF exposure.

15

This study of a huge cohort demonstrates the limitations of such a study design. The majority of the cohort (58%) consisted of retired or terminated workers that may or may not accumulate further RF exposure at other companies. Furthermore, it can be assumed that Motorola employees were among the first that used mobile phones at the workplace and privately. Neglecting mobile phone use may diminish the gradient of exposures between occupational groups studied. It would have been better to conduct nested case-control studies instead of using internal comparison that may be compromised by mobility bias, exposure misclassification and other sources of bias.

N. Groves et al. 2002

In this military cohort study of 40,581 men followed from the year of graduation (1950-1954) from Navy technical schools through 1997, known as the Korean War Veterans study, groups of sailors with imputed difference in likelihood and amount of exposure to radar waves were compared with respect to mortality. The original study, with a follow up through 1974, (Robinette et al. 1980) reported increased risks of cancer of the hematopoietic and lymphatic system, of the lung and digestive system for the high exposure group but was handicapped by the lack of information on date of birth of the cohort members. For the extended follow up study many missing birth dates were found in the Veterans Administration Master Index. Nevertheless, birth date remained unknown for over 8% of the cohort. Based on expert opinion low RF exposure was assigned to job classifications of radioman, radarman, and aviation electrician's mate, high exposure stratum included men with job classifications of electronics technician, aviation electronics technician, and fire control technician.

By matching against the Social Security Administration's Death Master File and the National Death Index 8,393 deceased subjects were identified through 1997. This number is substantially and significantly lower as expected from the male white US population. A healthy soldier effect may have been responsible for a lower mortality rate in the 1950ies but cannot explain the reduced mortality after 40 years. It has not been reported how long the cohort members stayed in service nor were life-style factors investigated; however, of more than 40% of the cohort no social security number could be obtained suggesting possible under-estimation of deaths.

Comparison of high- with low-exposure groups revealed significantly lower mortality from life-style associated causes of death (lung cancer, vascular diseases, diabetes mellitus, chronic obstructive pulmonary disease, liver cirrhosis) and significantly higher mortality from all leukemias and external causes of death. Increased mortality from leukemias was found in all high exposure groups but the most pronounced increase was observed in aviation electronics technicians. Brain cancer was less frequent in all high exposure groups compared to the low exposure category.

The long period of follow up of this large cohort with start of follow up almost at the same time (1950-54) and at a time when exposure commenced is a great advantage of this investigation. However, there are a number of shortcomings: follow up was possibly incomplete by unknown social security number of a substantial proportion of the cohort; almost half of all deaths in the first 20 years were from external causes which could have obscured an effect of exposure; duration and intensity of exposure is unknown as well as potential exposure after leaving the Navy; classification into low and high exposure groups may introduce substantial misclassification. In the earlier report, inspection of Navy records for a sample from the high exposure group revealed that 24% had no exposure to radar waves at all.

Concerning brain tumors, assuming an effect of radar exposure on growth rate, exposure during the Korean War and no exposure afterwards would be expected to result in only a slightly increased risk during a period of about 10 years after the war. Sailors were about 20 to 25 years at that time. The fraction with an already initiated brain tumor during this age range is estimated to be less than 3 in 100,000 per year. Increase of growth rate even if substantial cannot result in an effect observable in a cohort of that size. If radar exposure increases the likelihood of malignant transformation this could increase the incidence during a time window of 10 to 20 years after the exposure period. Results of the Israeli study of x-ray treated tinea capitis (Sadetzki et al. 2005) suggest an even longer latency, however, risk decreased with increasing age at first exposure to x-rays. In addition, for malignant brain tumors there is a less pronounced relationship to ionizing radiation, and a higher risk was observed for meningioma that were not investigated in the Korean War Veterans study. Taking the data on ionizing radiation as a guiding principle for brain tumor initiation, radar exposure of sailors during their twenties might result in an increase of brain tumor mortality of about 10 to 15%, i.e. a maximum of 8 additional cases among 20,000. Considering the

biases of the study such a low risk is easily obscured. Hence neither tumor promotion nor initiation may be detected in this study even if there is an increased risk. Because of the mentioned limitation to a certain time window with possibly increased incidence due to exposures during service in the Korean War, it would have been instructive to compute Kaplan-Meier estimates for cumulative brain tumor mortality.

N. Berg et al. 2006

In the German part of the Interphone study special attention was paid to occupational history and exposure to RF fields at workplaces. Incident meningioma (n=381, response rate 88%) and glioma cases (n=366, response rate 80%) aged 30-69 years were selected from four neurological clinics. Overall 1,535 (participation rate 63%) were randomly selected from population registries matched to the cases by sex, age, and region. Most cases were interviewed during their stay in hospitals, controls were interviewed at home. The interview contained several screening questions about occupations that are probably associated with RF exposure. If any of these screening questions were marked additional questions were asked about the job. Based on the literature and the evaluation by two industrial hygienists a classification into the following categories was performed: no RF exposure/not probably RF exposed/probably ER exposed/highly RF exposed. In total about 13% (299 cases and controls) were classified with at least possible RF exposure at the workplace. Analyses were adjusted for region, sex, age, SES, urban/rural residence, ionizing radiation exposure in the head/neck region. Mobile phone use was not considered as a confounder.

While overall RF exposure at workplaces showed no increased odds-ratios, high exposure and especially for durations of 10 years or more resulted in elevated risk estimates that were, however, not significant. This result was similar for meningioma (OR=1.55 for high exposure for 10 years or more) and glioma (OR=1.39).

The study tried to assess potential workplace exposure as precisely as possible in a personal interview, but still misclassification may have occurred especially in the probable and not probable categories while the high exposure group is likely to have had at least occasionally above average RF exposure. Odds ratios are in the range expected if exposure results in a substantial increase of growth rate. The small number of highly and long-term exposed cases (13 glioma and 6 meningioma) prohibit, however, far reaching conclusions.

IV. Evaluation of Evidence

Due to the varying endpoints, methods used and populations included and the small number of studies a formal meta-analysis is not possible. The following figure shows the results detailed in Table 2 in an easily comprehendible way.

Only few studies found clear indications of an association between RF exposure and brain tumors: one cohort study (Szmigielski 1996) and two case-control studies (Thomas et al. 1987, Grayson 1996). None of the ecological studies demonstrated a tendency for an increased risk in the vicinity of RF transmitters.

The discussion of the 15 published investigations revealed shortcomings in all studies. The greatest problem was encountered in the difficulties to reliably assess actual exposure. Even if we don't know the relevant aspect of the exposure, if any, that is responsible for an increased risk, the type, duration and amount of exposure must be determined in order to use the studies in derivations of exposure standards. None of the studies included a useful quantitative indicator of intensity of exposure and even duration of exposure was rarely addressed. Concerning type of exposure only quite crude and broad categories were used.
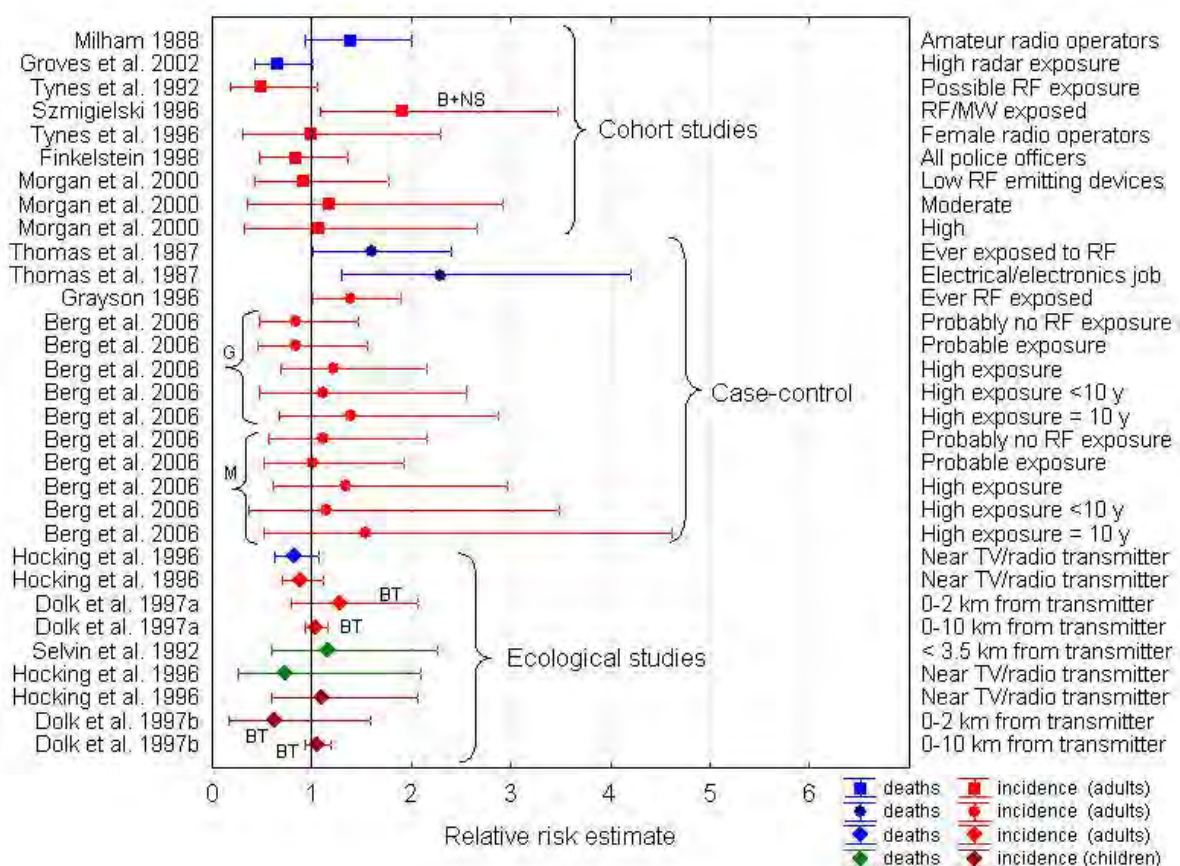
Fig. 1: Estimates of relative risk (and 95% confidence intervals) of various RF exposures with respect to brain tumors (B+NS...brain and nervous system tumors, BT...brain tumors, M...menigioma, G...glioma; all others primary malignant brain tumors)

In ecological studies, although for the studied population the exposure - despite considerable variations in time - is similar with respect to carrier frequency, modulation etc. it is quite different between various types of transmitters and hence results are not easily generalized. Considering the discussion of the different investigations and the fact that most biases encountered tend to dilute a potential risk, the compiled evidence from occupational cohorts is compatible with a moderately increased risk of RF exposure. Because of the lack of actual measurements but observing that exposure above guideline levels must have been a rare event a precautionary approach must result in a reduction of occupational exposure levels and organizational measures to avoid over-exposure. Although brain tumors are rare and the population attributable risk is low (assuming 13% of adults being occupationally exposed to RF fields as inferred from Berg et al. 2006, and assuming a relative risk of 1.3, about 4% of brain tumors can be attributed to RF exposure, i.e. 1,350 cases per years in the US).

V.   EVALUATION OF CANCER-RELATED ENDPOINTS (RF EXPOSURE)

A. Assessment of Epidemiological Evidence by IEEE (C95.1 Revision)

In their 2006 revision of the standard C95.1 IEEE has assessed the evidence from epidemiology for cancer related endpoints in chapter B.7.3. The assessment relies mainly on the reviews of Bergqvist (1997), Moulder et al. (1999) and Elwood (2003). These reviews and the IEEE overview share the same deficiencies. The main lines of argumentation would be impossible in any other field of environmental health and closely resemble the strategy used to dismiss a power frequency exposure/childhood leukemia association. In the following paragraphs the assessment by IEEE will be briefly discussed.

> *Cluster studies, such as the one performed in Sutton Coldfield in the U.K. in response to a cluster of leukemia and lymphoma in adults living close to an RF broadcasting transmitter (Dolk et al. [R624]), are inherently difficult to interpret because of the impossibility of assessing all of the effects that chance variation might have contributed to the cluster. In the initial Sutton Coldfield study, the authors correctly concluded that no causal association could be drawn between the presence of the cluster and RF exposure from broadcasting towers (Dolk et al. [R625]) (Cooper et al. [R760]). (IEEE C 95.1 – 2005, p.75)*

First of all the Sutton Coldfield study was no cluster study but an ecological investigation. It is true that it was initiated by an unconfirmed report of a cluster of leukemia and lymphoma in

the vicinity of a broadcasting transmitter but it proceeded independently of this initial report and used registry data on the population living within a radius of 10 km around the transmitter. The statement that such studies are "inherently difficult to interpret because of the impossibility of assessing all of the effects that chance variation might have contributed to the cluster" is ridiculous not only because the study is no cluster study but because it is impossible for any study to "assess all effects that chance variation might have contributed" to the endpoint under investigation. It is not mentioned that the study was supplemented by a larger investigation of another 20 high-power transmitters in Great Britain. The difficulties of interpreting ecological studies is related to the fact that potential confounders can only be related to a segment of the population but not to individuals and that in general duration and intensity of exposure are not known for individual members of the different strata. While evidence for an effect on brain tumor incidence from both studies (Dolk et al. 1997a, 1997b) is weak, there is consistent evidence for a relation to hematopoietic cancers. This evidence has been overlooked by the authors due their wrong assumption about the relation between proximity to the transmitter and exposure.

> *Inconsistent effects have been reported between residential proximity to other RF broadcast towers and adverse health endpoints (Bielski [R267]) (Maskarinec et al. [R579]) (Selvin and Merrill [R823]) (Michelozzi et al. [R858]) (Altpeter et al. [R977]) (Hallberg and Johansson [R995], [R996]) (Boscolo [R1012]), although many of these studies have significant flaws in their study design (making them difficult to interpret). (IEEE C 95.1 – 2005, p.75)*

Although it is not stated what these "inconsistent effects" might be, the statement is flawed in more than this respect. First of all the study by Bielski (1994) is an occupational investigation and not about residential proximity to RF broadcast towers, second three of these investigations (Selvin et al. 1992; Maskarinec et al. 1994; Michelozzi et al. 2002) included leukemia as an endpoint with indications of an increased incidence consistent with the studies from Great Britain (Dolk et al. 1997a, 1997b) and Australia (Hocking et al. 1996). Note that the study by Selvin et al. (1992), as stated previously, intended to compare different methods to assess the relationship between a point source and diseases and did erroneously assume a monotonous relationship between exposure and distance from a transmitter. Correcting this error there seems to be an increased probability of childhood leukemia in areas receiving the highest exposure from the Sutro tower. The other three investigations (Altpeter et al. 1995; Boscolo 2001; Hallberg & Johansson 2002) have nothing in common and hence cannot be inconsistent.

An increased incidence and mortality rate of childhood leukemia was reported in Australia with residential proximity to a specific RF broadcasting tower (Hocking et al. [R633]), although subsequent reanalysis of the data showed the results may have been influenced by other confounding variables within the study location (McKenzie et al. [R669]). (IEEE C 95.1 – 2005, p.75)

This is another example how carelessly and sloppy the evidence is dealt with by the IEEE committee. The study of Hocking et al. (1996) was not about "proximity to a specific RF broadcasting tower" but about an area where three broadcasting towers are located. While there is always the possibility of confounders influencing results of an epidemiologic investigation, the 'reanalysis' of McKenzie et al. (1998) is seriously flawed and cannot support the cited statement. Hocking et al. (1996) combined the districts near the broadcasting area and those further away based on homogeneity analyses, while McKenzie et al. (1998) omitted one area with high incidence (and highest exposure) based on inspection of data. Any statistical analysis subsequent to such data picking is useless.

While scattered reports of adverse health effects associated with occupational exposure to RF do exist (Demers et al. [R36]) (Kurt and Milham [R68]) (Pearce [R110]) (Speers et al. [R125]) (Thomas et al. [R128]) (Pearce et al. [R199], [R211]) (Hayes et al. [R207]) (Cantor et al. [R268]) (Davis and Mostofi [R563]) (Tynes et al. [R570], [R605]) (Grayson [R592]) (Richter et al. [R747]) (Holly et al. [R838]) these studies are largely inconsistent with each other in terms of the adverse health endpoints affected, and often show no clear dose response with RF exposure. Many have serious flaws in their study design, contain limited or insufficient RF exposure assessment, and are generally inconsistent with the absence of findings of an association from other occupational studies (Tornqvist et al. [R131]) (Coleman [R142]) (Lilienfeld et al. [R146]) (Robinette and Silverman [R147], [R148]) (Siekierzynski et al. [R151], [R152]) (Wright et al. [R213]) (Coleman et al. [R214]) (Muhm [R506]) (Czerski et al. [R542]) (Hill [R568]) (Lagorio et al. [R616]) (Kaplan et al. [R647]) (Morgan et al. [R701]) (Gallagher et al. [R822]) (Groves et al. [R853]) (Wiklund [R1013]) (Armstrong et al. [R1014]). (IEEE C 95.1 – 2005, p.75)

Even allowing for restrictions of space for a discussion of the evidence, greater nonsense has not been produced so far in this field as condensed in these two sentences. Putting higgledy-piggledy all sorts of studies together and then wondering about endpoints being inconsistent is an intellectual masterpiece. Of the occupational studies mentioned, three (Thomas et al. 1987; Speers et al. 1988; Grayson 1996) were about brain cancer, three about hematopoietic cancers (Pearce et al. 1985; Kurt & Milham 1988; Pearce 1988), two about testicular cancer (Hayes et al. 1990; Davis & Mostofi 1993), one about male (Demers et al. 1991) and two about female breast cancer (Cantor et al. 1995, Tynes et al. 1996) the latter including other cancers as well,

and one about intraocular melanoma (Holly et al. 1996). Three further studies (Pearce et al. 1989; Tynes et al. 1992; Richter et al. 2000) investigated several or all malignancies. These studies differ not only in endpoints, study type (cohort, case-control, and cluster) but also in the methods of exposure assessment. Ignorance of the IEEE reviewers is underlined by the compilation of studies characterized by an "absence of findings of an association". Not only did several of these studies indeed indicate an association of cancer risk with EMF exposure (Lilienfeld et al. 1978; Robinette et al. 1980; Tornqvist et al. 1991; Armstrong et al. 1994; Lagorio et al. 1997; Groves et al. 2002) but two were no epidemiologic studies at all (Siekierzynski et al. 1974; Czerski et al. 1974) and several were rather addressing ELF exposure (Tornqvist et al. 1991; Wright et al. 1982; Coleman et al. 1983; Gallagher et al. 1991) and one (Wiklund 1981) was a cluster study in the telecommunication administration with uncertain type of exposure. Simply confronting studies finding an effect with others that were 'negative' is scientifically flawed and permits neither the conclusion that there is nor that there is no association between exposure and cancer risk. Even if all studies would have applied the same method, assessed the same endpoint and used the same exposure metric, studies reporting a significantly increased cancer risk are not outweighed by others that did not.

> *While micronuclei formation in workers occupationally exposed from broadcast antennas has been reported (Garaj-Vrhovac [R757]) (Lalic et al. [R791]), these findings were not verified in a larger study of more than 40 Australian linemen exposed under similar conditions (Garson et al. [R186]). (IEEE C 95.1 – 2005, pp.75-76)*

It goes without saying that also this statement is wrong. Garson et al. (1991) did not investigate micronuclei formation, their workers were considerably shorter exposed and it were not more than 40 linemen but 38 radio-lineman.

> *No clear association could be established between occupational exposures of parents to a number of agents, including RF, and effects (neuroblastoma) in their offspring (Spitz and Johnson [R289]) (De Roos et al. [R798]). (IEEE C 95.1 – 2005, p.76)*

What is meant by 'no clear association' is obscure. Spitz and Johnson (1985) found a significantly increased risk for paternal occupational exposure to electromagnetic fields, and also De Roos et al. (2001) found several jobs with paternal as well as maternal exposure to EMFs associated with an elevated risk for neuroblastoma in their children. However, broad groupings of occupations with ELF, RF EMF, as well as ionizing radiation (!) exposure did not reveal an increased risk.

One study reported a slight excess in brain tumors associated with combined exposure to RF and other exposures associated with electrical or electronic jobs, but not with RF alone (Thomas et al. [R128]). A study of a Polish military cohort reported a substantial excess of total cancer and several cancer sub-types with jobs associated with RF exposure (Szmigielski [R578]), (Szmigielski and Kubacki [R982]), although questions have been raised about severe bias in the exposure assessment of this study (Elwood [R665]) (Bergqvist [R1015]) (Stewart [R1133]). Studies by Milham of U.S. amateur radio operators reported an excess in one of nine types of leukemia assessed (see [R101], [R102], [R209], [R215], and [R569]), but not for total tumors, total leukemia, or brain tumors, and potential confounding factors might have included exposure to soldering fumes, degreasing agents and over-representation of a particular social class. (IEEE C 95.1 – 2005, p.76)

Again the evidence is incorrectly summarized for all cited investigations. Thomas et al. (1987) found a significantly elevated risk for brain tumors among all men exposed to RF fields and in particular in those exposed for 20 or more years. There were indications that this elevated risk is due to a subgroup with electrical or electronics jobs. The group of those exposed in other jobs is heterogeneous and may contain subjects with low or no exposure (e.g. some groups of welders) and therefore lack of an association could be due to a dilution effect from exposure misclassification.

As mentioned previously criticism of the Polish military cohort study about exposure assessment is unfounded. Bergqvist (1997), Elwood (1999) and Stewart (2000) criticized that the military health board assessed a number of potential risk factors only for cancer cases. However, they overlooked that the study was a cohort and not a case-control study and that at no stage information about these factors entered the analysis and therefore couldn't affect the results in any way.

The study by Milham (1988a, 1988b) of radio amateur operators revealed a significantly increased standardized mortality ratio (SMR) for acute myeloid leukemia while the overall mortality and cancer mortality was significantly reduced relative to the country mortality rates. As mentioned previously this points to a 'healthy worker' effect as well as to an influence of life-style factors (mortality related to smoking and overweight were reduced). From the mentioned nine types of leukemia three with expectancies below one and no case observed couldn't be assessed, from the six remaining types five had elevated SMRs with AML, the most frequent type in adults, being significantly elevated.

The last portion of the IEEE review of epidemiology studies is dedicated to mobile phone investigations that are discussed in another contribution.

The following citation presents the IEEE summary in its full length:

> *The epidemiological evidence to date does not show clear or consistent evidence to indicate a causal role of RF exposures in connection with human cancer or other disease endpoints. Many of the relevant studies, however, are weak in terms of their design, their lack of detailed exposure assessment, and have potential biases in the data. While the available results do not indicate a strong causal association, they cannot establish the absence of a hazard. They do indicate that for commonly encountered RF exposures, any health effects, if they exist, must be small. Even though epidemiological evidence cannot rule out a causal relationship, the overall weight-of-evidence is consistent with the results of the long term animal studies showing no evidence of physiological, pathological or disease-specific effects. (IEEE C95.1 - 2005; pp.76-77)*

As already pointed out earlier (Kundi 2006) there is an intolerable tendency in the past years that confronted with an undeniable epidemiologic evidence of an association between an agent and adverse health effects such as cancer, interested parties take their resort to the concept of causality based on the wrong assumption evidence to "indicate a causal role" is a lot more difficult to provide. Unprecedented, however, is the notion of "a strong causal association". Whatever the meaning of this exceptional statement, the conclusion that, if health effects of commonly encountered RF exposures exist, they must be small, is wrong. To the contrary: considering the "lack of detailed exposure assessment" and other potential biases that predominantly lead to an underestimation of the risk, the evidence points to a quite substantial hazard. While the animal studies reviewed in another section of the IEEE standard document cannot be discussed here it should be underlined that they are generally insufficient to support either an increased risk or the lack of health relevant effects. Therefore they cannot be used in a weight-of-evidence statement as has been made by IEEE, that there is no evidence for adverse health effects of RF exposure.

VI.   CONCLUSIONS

•   Only few studies of long-term exposure to low levels of RF fields and brain tumors exist, all of which have methodological shortcomings including lack of quantitative exposure assessment. Given the crude exposure categories and the likelihood of a bias towards the null hypothesis of no association the body of evidence is consistent with a moderately elevated risk.

•   Occupational studies indicate that long term exposure at workplaces may be associated with an elevated brain tumor risk.

•   Although in some occupations and especially in military jobs current exposure guidelines may have sometimes been reached or exceeded, overall the evidence suggest that long-term exposure to levels generally lying below current guideline levels still carry the risk of increasing the incidence of brain tumors.

•   Although the population attributable risk is low (likely below 4%), still more than 1,000 cases per year in the US can be attributed to RF exposure at workplaces alone. Due to the lack of conclusive studies of environmental RF exposure and brain tumors the potential of these exposures to increase the risk cannot be estimated.

•   Epidemiological studies as reviewed in the IEEE C95.1 revision (2006) are deficient to the extent that the entire analysis is professionally unsupportable. IEEEs dismissal of epidemiological studies that link RF exposure to cancer endpoints should be disregarded, as well as any IEEE conclusions drawn from this flawed analysis of epidemiological studies.

VII.   REFERENCES

References for Brain Tumor Epidemiological Studies

Ahlbom A, Green A, Kheifets L, Savitz D, Swerdlow A. 2004. Epidemiology of health effects of radiofrequency exposure. Environ Health Perspect 112: 1741–1754.

Altpeter ES, Krebs TT, Pfluger DH, von Kanel J, Blattmann R. 1995. Study on health effects of the short-wave transmitter station at Schwarzenburg, Berne, Switzerland," BEW Publication Series No. 55, University of Berne, Inst. for Social & Preventive Medicine.

Armstrong B, Theriault G, Guenel P, Deadman J, Goldberg M, Heroux P. 1994. Association between exposure to pulsed electromagnetic fields and cancer in electric utility workers in Quebec, Canada, and France. Am J Epidemiol 140: 805 – 820.

Berg G, Spallek J, Schüz J, Schlehofer B, Böhler E, Schlaefer K, Hettinger I, Kunna-Grass K, Wahrendorf J, Blettner M. 2006. Occupational exposure to radio frequency/microwave radiation and the risk of brain tumors: Interphone Study Group, Germany. Am J Epidemiol.

Bergqvist U. 1997. Review of epidemiological studies. In: Kuster N, Balzano Q, Lin JC (eds.), Mobile Communications Safety, London: Chapman & Hall, pp. 147 – 170

Bielski J. 1994. Bioelectrical brain activity in workers exposed to electromagnetic fields," Ann N Y Acad Sci 724: 435 – 437

Boscolo P. 2001. Effects of electromagnetic fields produced by radiotelevision broadcasting stations on the immune system of women. Sci Total Environ 273: 1 – 10

Cantor K, Stewart P, Brinton L, Dosemeci M. 1995. Occupational exposure and female breast cancer mortality in the United States. J Occup Environ Med 37: 336-348

Coleman M, Bell J, Skeet R. 1983. Leukaemia incidence in electrical workers. Lancet 1:982 – 983

Coleman M. 1985. Leukaemia mortality in amateur radio operators. Lancet 2: 106 – 107

Cooper DK, Hemmings K, Saunders P 2001. Cancer incidence near radio and television transmitters in Great Britain. I. Sutton Coldfield transmitter; II. All high power transmitters. Am J Epidemiol 153: 202 – 204

Czerski P, Siekierzynski M, Gidynski A. 1974. Health surveillance of personnel occupationally exposed to microwaves. I. Theoretical considerations and practical aspects. Aerospace Med 45: 1137 – 1142

Davis RL, Mostofi FK. 1993. Cluster of testicular cancer in police officers exposed to hand -held radar. Am J Ind Med 24: 231-233

De Roos AJ, Teschke K, Savitz DA, Poole C, Grufferman BH, Pollock BH. 2001. Parental occupational exposures to electromagnetic fields and radiation and the incidence of neuroblastoma in offsping. Epidemiol 12: 508 – 517

Demers PA, Thomas DB, Rosenblatt KA, Jimenez LM, McTiernan A, et al. 1991. Occupational exposure to electromagnetic fields and breast cancer in men. Am J Epidemiol 134: 340 – 347

Dolk H, Shaddick G, Walls P, Grundy C, Thakrar B, Kleinschmidt I, Elliott P. 1997a. Cancer incidence near radio and television transmitters in Great Britain, Part I. Sutton Coldfield Transmitter. Am J Epidemiol 145: 1-9.

Dolk H, Elliot P, Shaddick G, Walls P, Thakrar B. 1997b. Cancer incidence near radio television and transmitters in Great Britain, Part II. All high-power transmitters. Am J Epidemiol 145: 10-17.

Elwood MJ. 2003. Epidemiological studies of radiofrequency exposures and human cancer. Bioelectromagnetics Suppl 6: S63 - S73.

Finkelstein MM. 1998. Cancer incidence among Ontario police officers. Am J Ind Med 34: 157-162.

Gallagher RP, Band PR, Spinelli JJ, Threlfall WJ, Tamaro S. 1991. Brain cancer and exposure to electromagnetic fields. J Occup Med 33: 944 – 945

Garaj-Vrhovac V. 1999. Micronucleus assay and lymphocyte mitotic activity in risk assessment of occupational exposure to microwave radiation. Chemosphere 39: 2301 – 2312

Garson OM, McRobert TL, Campbell LJ, Hocking BA, Gordon I. 1991. A chromosomal study of workers with long-term exposure to radio-frequency radiation. Med J Australia 155: 289 – 292.

Grayson JK. 1996. Radiation exposure socioeconomic status and brain tumor risk in the US Air Force: a nested case-control study. Am J Epidemiol 143: 480-486.

Groves FD, Page WF, Gridley G, Lisimaque L, Stewart PA, Tarone RE et al. 2002. Cancer in Korean war navy technicians: mortality survey after 40 years. Am J Epidemiol 155: 810-818.

Hallberg O, Johansson O. 2002a. Melanoma incidence and frequency modulation (FM) broadcasting. Arch Environ Health 57: 32 – 40

Hallberg O, Johansson O. 2002b. Cancer trends during the 20th century. J Australian College Nutrtr Environ Med. 21: 3 – 8

Hayes RB, Brown LM, Pottern LM, Gomez M, Kardaun JWPF, Hoover RN, O'Connell KJ, Sutzman RE, Javadpour N. 1990. Occupation and risk of testicular cancer: a case-control study. Int J Epidemiol 19: 825-831

Hill DG. 1988. A longitudinal study of a cohort with past exposure to radar: the MIT Radiation Laboratory follow-up study. [Dissertation Manuscript], Johns Hopkins University, Baltimore, MD, UMI Dissertation Services, Ann Arbor, MI

Hocking B, Gordon IR, Grain ML, Hatfield GE. 1996. Cancer incidence and mortality and proximity to TV towers. Med J Aust 165: 601-605

Holly EA, Aston DA, Ahn DK, Smith AH. 1996. Intraocular melanoma linked to occupations and chemical exposures. Epidemiology 7: 55-61

Kaplan S, Etlin S, Novikov I, Modan B. 1997. Occupational risks for the development of brain tumors. Am J Ind Med 31: 15 – 20.

Krewski D, Byus CV, Glickman BW, Lotz WG, Mandeville R, McBride ML, Prato FS, Weaver DF. 2001. Potential health risks of radiofrequency fields from wireless telecommunication devices. J Tox Env Health Part B 4: 1-143.

Kundi M, Hansen Mild K, Hardell L, Mattsson MO. 2004. Mobile telephones and cancer - a review of epidemiological evidence. J Toxicol Envrion Health Part B 7: 351-384.

Kundi M. 2006. Causality and the interpretation of epidemiologic evidence. Environ Health Perspect 114: 969 – 974

Kurt TL, Milham S. 1988. Re: Increased mortality in amateur radio operators due to lymphatic and hematopoietic malignancies. [Letter and Reply] Am J. Epidemiol 128: 1384–1385

Lagorio S, Rossi S, Vecchia P, De Santis M, Bastianini L, Fusilli M, Ferrucci A, Desideri E, Comba P. 1997. Mortality of plastic-ware workers exposed to radiofrequencies. Bioelectromagnetics 18: 418-421

Lalic H, Lekic A, Radosevic-Stasic B. 2001. Comparison of chromosome aberrations in peripheral blood lymphocytes from people occupationally exposed to ionizing and radiofrequency radiation. Acta Medica Okayama 55: 117 – 127

Lilienfeld AM, Tonascia J, Tonascia S, Libauer CH, Cauthen GM, et al. 1978. Foreign Service Health Status Study: Evaluation of Status of Foreign Service and other Employees From Selected Eastern European Posts. NTIS Document No. PB-28B 163/9GA Dept. of State, Washington DC, Final Report, Dept. of Epidemiology, School of Hygiene Public Health, Johns Hopkins University, Baltimore, MD

Maskarinec G, Cooper J, Swygert L. 1994. Investigation of increased incidence in childhood leukemia near radio towers in Hawaii: preliminary observations. J Environ Pathol Toxicol Oncol 13: 33-37

McKenzie DR, Yin Y, Morrell S. 1998. Childhood incidence and acute lymphoblastic leukaemia and exposure to broadcast radiation in Sydney – a second look. Aust NZ J Public Health 22: 360-367

Michelozzi P, Capon A, Kirchmayer U, Forastiere F, Biggeri A, Barca A, Perucci CA. 2002. Adult and childhood leukemia near a high-power radio station in Rome, Italy. Am J Epidmiol 155: 1096-1103

Milham S. 1982. Mortality from leukemia in workers exposed to electrical and magnetic fields. [Letter] New England J Med 307: 249 – 249

Milham S. 1983. Occupational mortality in Washington State: 1950-1979. DHHS (NIOSH) Publication 83-116, October 1983, Contract No. 210-80-0088, U.S. Depart. of Health and Human Services, National Institute for Occupational Safety and Health, Cincinnati, OH

Milham S. 1985. Mortality in workers exposed to electromagnetic fields. Environ Health Perspect 62: 297 – 300

Milham S. 1988a. Increased mortality in amateur radio operators due to lymphatic and hematopoietic malignancies. Am J Epidemiol 127: 50-54

Milham S. 1988b. Mortality by license class in amateur radio operators. Am J Epidemiol 128: 1175 – 1176

Morgan RW, Kelsh MA, Zhao K, Exuzides KA, Heringer S, Negrete W. 2000. Radiofrequency exposure and mortality from cancer of the brain and lymphatic/hematopoietic systems. Epidemiology 11: 118-127

Moulder JE, Erdreich LS, Malyapa RS, Merritt JH, Pickard WF, Vijayalaxmi. 1999. Cell phones and cancer: what is the evidence for a connection? Radiat Res 151: 513 – 531

Muhm JM. 1992. Mortality investigation of workers in an electromagnetic pulse test program. J Occup Med 34: 287-292

Pearce N, Reif J, Fraser J. 1989. Case-control studies of cancer in New Zealand electrical workers. Int J Epidemiol 18: 55 – 59

Pearce NE, Sheppard RA, Howard JK, Fraser J, Lilley BM. 1985. Leukaemia in electrical workers in New Zealand. [Letter] Lancet 1: 811 – 812

Pearce NE. 1988. Leukemia in electrical workers in new Zealand: a correction. [Letter] Lancet 2: 48 Richter ED, Berman T, Ben-Michael E, Laster R, Westin JB. 2000. Cancer in radar technicians exposed to radiofrequency/microwave radiation: Sentinel episodes. Int J Occup Environ Health 6: 187 – 193

Robinette CD, Silverman C, Jablon S. 1980. Effects upon health of occupational exposure to microwave radiation radar. Am J Epidemiol 112: 39 – 53

Robinette CD, Silverman C. 1977. Causes of death following occupational exposure to microwave radiation (radar) 1950-1974. In Hazzard (ed), Symposium on Biological Effects and Measurement of radiofrequency Microwaves, Dept. of Health, Education, and Welfare, Washington, DC, HEW Publication No. (FDA) 77-8026: 338 – 344

Selvin S, Schulman J, Merrill DW. 1992. Distance and risk measures for the analysis of spatial data: a study of childhood cancers. Soc Sci Med 34: 769-777

Siekierzynski M, Czerski P, Milczarek H, Gidynski A, Czarnecki C, Dziuk E, Jedrzejczak W. 1974a. Health surveillance of personnel occupationally exposed to microwaves. II. Functional disturbances. Aerospace Med 45: 1143 – 1145

Siekierzynski M, Czerski P, Milczarek H, Gidynski A, Czarnecki C, Dziuk E, Jedrzejczak W. 1974b. Health surveillance of personnel occupationally exposed to microwaves. III. Lens translucency. Aerospace Med 45: 1146 – 1148

Speers MA, Dobbins JG, Miller VS. 1988. Occupational exposures and brain cancer mortality: a preliminary study of East Texas residents. Am J Ind Med 13: 629 – 638

Spitz MR, Johnson CC. 1985. Neuroblastoma and paternal occupation. A case-control analysis. Am J Epidemiol 121: 924 – 929

Stewart, Sir W. 2000. Mobile Phones and Health. Report by the UK Independent Expert Group on Mobile Phones. c/o UK National Radiological Protection Board, Chilton, Didcot, Oxon OX11 0RQ pp. 1 – 160.

Szmigielski S, Kubacki R. 1999. Analysis of cancer morbidity in Polish career military personnel exposed occupationally to RF and MW radiation. In: F. Bersani (ed.), Electricity and Magnetism in Biology and Medicine, Kluwer Academic/ Plenium, pp. 809 – 812.

Szmigielski S. 1996. Cancer morbidity in subjects occupationally exposed to high frequency (radiofrequency and microwave) electromagnetic radiation. Sci Total Environ 180: 9-17.

Thomas TL, Stolley PD, Stemhagen A, Fontham ETH, Bleeker ML, Stewart PA et al. 1987. Brain tumour mortality risk among men with electrical and electronic jobs: a case-control study. J Natl Cancer Inst 79: 233-238

Tornqvist S, Knave B, Ahlbom A, Persson T. 1991. Incidence of leukaemia and brain tumours in some 'electrical occupations'. Brit J Indust Med 48: 597 – 603

Tynes T, Andersen A, Langmark F. 1992. Incidence of cancer in Norwegian workers potentially exposed to electromagnetic fields. Am J Epidemiol 136: 81-88

Tynes T, Hannevik M, Andersen A, Vistnes AI, Haldorsen T. 1996. Incidence of breast cancer in Norwegian female radio and telegraph operators. Cancer Causes Control 7: 197-204

Wiklund K. 1981. An application of the Swedish cancer-environment registry: leukaemia among Telefone operators at the telecommunications administration in Sweden. Int J Epidemiol 10: 373 – 376

Wright WE, Peters JM, Mack TM. 1982. Leukaemia in workers exposed to electrical and magnetic fields. Lancet 307: 1160 – 1161

Brain Tumors And RF Fields

Lennart Hardell, MD, PhD, Professor

Department of Oncology, Örebro University Hospital, Sweden

Kjell Hansson Mild, PhD, Professor

Department of Radiation Physics, Umeå University, Sweden

Michael Kundi, PhD, med.habil, Professor

Institute of Environmental Health, Center for Public Health,

Medical University of Vienna, Austria

Table 1: Synopsis of epidemiologic studies of or including brain tumors (1987 – 2006)

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
Thomas et al. 1987	Northern New Jersey, Philadelphia, gulf coast of Lousiana/1979-1981/Case-control	Interviews with next-of-kin about occupational history – response rates: cases 74%, controls 63%; JEM (2 methods)	Death certificates verified through review of hospital records	age(m), (only males), year of death(m), area of residence(m), educational level, (lead, soldering fumes)	435/386	Cases: deaths of brain tumor or CNS tumors of white males (age>30) from death certificates Controls: deaths from other causes than brain tumors, epilepsy, etc.
Milham 1988	Washington, California/1979-1984/Cohort	Amateur radio operator license within 1/1979 to 6/1984	Mortality records	age, (only males), race, year of death	29	67829 operators, search of deaths in state registry through 1984
Selvin et al. 1992	San Francisco/1973-1988/Spatial cluster	Distance of center of census tract to microwave tower (Sutro tower)	SEER records	-	35	Search of cancer deaths of white individuals (age<21)
Tynes et al. 1992	Norway/1961-1985 /Occupational cohort	Job title in 1960 and 1970 censuses and expert categorization	Cancer registry	age, (only males)	119 overall, 6 in subgroup with possible RF exposure	Cohort of 37945 male workers identified that had jobs in 1960 with possible EMF exposure. among these 3017 with possible RF exposure
Grayson 1996	US Air Force/1970-	Detailed job	Screening of	age(m),	230/920	Cohort of ~880000

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
	1989/Nested case-control	history and classification based on JEM (RF/MW exposure from frequent measurements)	hospital discharge records	race(m), military rank, (ELF and ionizing radiation exposure)		US Air Force members with at least one completed year of service within the study period, no follow up after subjects left service
Szmigielski 1996	Poland (military)/1971-1985/Occupational cohort	Allocation to RF/MW exposure group based on service records, documented measurements of military safety groups	Incident cases from central and regional military hospitals and military health departments	age, (only males)	~46	Annual number of ~127800 military career personnel, ~3720 RF/MW exposed per year
Hocking et al. 1996	Sydney (Australia)/ 1972-1990/Ecological	Municipalities within ~4 km of 3 TV broadcasting towers considered higher exposed as compared to 6 further away	Incident and death cases from cancer registry	age, sex, calendar period	740 (incident) 606 (mortality) 64 age<15 (incident) 30 age<15 (mortality)	Study population: inner area ~135000, outer area ~450000
Tynes et al. 1996	Norway/1961-1991/ Occupational cohort	Certified radio and telegraph	Cancer registry	age, (only females)	5	2619 women certified as radio or telegraph

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
		operators 1920-1980 (98% worked on merchant ships); spot measurements on ships with old-fashioned equipment				operators by Norwegian Telecom
Dolk et al. 1997a	Birmingham (GB)/ 1974-1986/Ecological	Living near a TV/FM radio transmitter (Sutton Coldfield)	Cancer registry	age, sex, calendar year, SES	332	Population (age≥15) ~408000 within 10 km of the transmitter
Dolk et al. 1997b	GB/1974-1986/ Ecological	Living near a high power (≥500 kW erp) transmitter (overall 21)	Cancer registry	age, sex, calendar year, SES	244	Population (age<15) within 10 km of one of 20 high power transmitters
Lagorio et al. 1997	Italy/1962-1992/ Occupational cohort	Working as RF heat-sealer operator	Cancer deaths from registry	age, (only females), calendar period, region	1	302 women employed 1962-1992 in a plastic-ware manufacturing plant as RF sealers
Finkelstein 1998	Ontario (Canada)/ 1964-1995/ Occupational cohort	Working as a police officer (possible	Cancer registry	age, (only males), calendar year	16	20601 male officers of Ontario Police

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
		handheld radar exposure)				
Morgan et al. 2000	USA/1976-1996/ Occupational cohort	Jobs classified according to work with RF emitting devices with different output power	Death certificates from states' statistics offices	age, sex, period of hire	51	All U.S. Motorola employees with at least 1 day employment 1976-1996 (195775 workers, 2,7 million person-years)
Groves et al. 2002	USA/1950-1997/ Occupational cohort	6 occupational groups 3 with assumed low radar exposure (radar-, radio operator, aviation electrician's mate) and 3 with assumed high exposure (aviation electronics -, electronics -, fire control technician)	Death certificate from a state vital statistics office or National Death Index Plus	age at entry, (only males), attained age	88	40581 Navy Korean War veterans graduated 1950-54 from Navy technical schools; follow-up from graduation through 1997
Berg et al. 2006	Germany/2000-2003/ Case-control	JEM from occupational history collected in interview	Histological verified cases of glioma and meningioma	age(m), sex(m), region(m), SES, urban/rural, smoking,	Glioma 366/732 Meningioma 381/762	All histological confirmed cases of glioma and meningioma from 4

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
				ionizing rad. exposure		neurosurgical clinics (age: 30-69) (part.rate 84%); frequency matched controls from population registry (part.rate 63%)

SES…socio-economic status, JEM…job exposure matrix, erp…equivalent radiation power, RF/MW…radio frequency/microwaves, CNS…central nervous system, ELF…extremely low frequency

Table 2: Synopsis of main results of brain tumor studies (1987 – 2006)

Study	Endpoint	Exposure category	Meas.	Outcome [95% CI]
Thomas et al. 1987	Brain tumor deaths (ICD not specified)	Ever exposed to RF	OR	1.6 [1.0 – 2.4]
		Electrical/electronics job	OR	2.3 [1.3 – 4.2]
		Unexposed*		
		Ever exposed < 5 y	OR	1.0
		5-19 y	OR	2.3
		20+ y	OR	2.0
Milham 1988	Brain cancer deaths (ICD-8: 191)	All	SMR	1.39 [0.93 – 2.00]
		Novice[a]	SMR	0.34
		Technician	SMR	1.12
		General	SMR	1.75
		Advanced	SMR	1.74
		Extra	SMR	1.14
Selvin et al. 1992	Brain cancer deaths (ICD-O: 191.2)	> 3.5 km distance from tower*		
		≤ 3.5 km[b]	RR	1.16 [0.60 – 2.26]
Tynes et al. 1992	Incident brain cancer (ICD-7: 193)	All with possible EMF exposure	SIR	1.09 [0.90 – 1.41]
		Subgroup possible RF exposure[c]	SIR	0.49 [0.18 – 1.06]
Grayson 1996	Incident brain cancer (ICD-9: 191)	Never RF/MW exposed*		
		Ever exposed	OR	1.39 [1.01 – 1.90]
Szmigielski 1996	Incident nervous system & brain tumors	RF/MW exposed	OER	1.91 [1.08 – 3.47]
Hocking et al. 1996	Brain cancer (ICD-9: 191)	Outer area*		
		Inner area (incident, overall)	RR	0.89 [0.71 – 1.11]
		Inner area (mortality, overall)	RR	0.82 [0.63 – 1.07]
		Inner area (incident, age<15)	RR	1.10 [0.59 – 2.06]
		Inner area (mortality, age<15)	RR	0.73 [0.26 – 2.10]
Tynes et al. 1996	Incident brain cancer (ICD-7: 193)	All	SIR	1.0 [0.3 – 2.3]
Dolk et al. 1997a	Incident brain tumors (ICD-8/9: 191, 192)	0-2 km from transmitter	OER	1.29 [0.80 – 2.06]
		0-10 km from transmitter	OER	1.04 [0.94 – 1.16]

Study	Endpoint	Exposure category	Meas.	Outcome [95% CI]
Dolk et al. 1997b	Incident brain tumors (ICD-8/9: 191, 192)	0-2 km from transmitter	OER	0.62 [0.17 – 1.59]
		0-10 km from transmitter	OER	1.06 [0.93 – 1.20]
Lagorio et al. 1997	Brain cancer deaths (ICD-9: 191)	RF sealer operator	OER	1 : 0.1
Finkelstein 1998	Incident brain cancer (ICD-9: 191)	All police officers	SIR	0.84 [0.48 – 1.36]
Morgan et al. 2000	Incident brain cancer (ICD-9: 191)	No RF exposure*		
		Low[d]	RR	0.92 [0.43 – 1.77]
		Moderate	RR	1.18 [0.36 – 2.92]
		High	RR	1.07 [0.32 – 2.66]
Groves et al. 2002	Brain cancer deaths (ICD-9: 191)	Low radar exposure*		
		High radar exposure	RR	0.65 [0.43 – 1.01]
Berg et al. 2006	Incident glioma (ICD-O3: C71)	No occup. RF/MW exposure*		
		Probably no exposure	OR	0.84 [0.48 – 1.46]
		Probable exposure	OR	0.84 [0.46 – 1.56]
		High exposure	OR	1.22 [0.69 – 2.15]
		No high exposure*		
		High exposure <10 y	OR	1.11 [0.48 – 2.56]
		High exposure ≥ 10 y	OR	1.39 [0.67 – 2.88]
	Incident meningioma (ICD-O3: C70.0)	No occup. RF/MW exposure*		
		Probably no exposure	OR	1.11 [0.57 – 2.15]
		Probable exposure	OR	1.01 [0.52 – 1.93]
		High exposure	OR	1.34 [0.61 – 2.96]
		No high exposure*		
		High exposure <10 y	OR	1.15 [0.37 – 3.48]
		High exposure ≥ 10 y	OR	1.55 [0.52 – 4.62]

[a] From Milham 1988b, license classes as proxy for exposure duration

[b] Based on the assumption that exposure is higher near the microwave tower

[c] Computed based on Table 5 in Tynes et al. 1992

[d] Classification according to power output of equipment used for longest period of employment

OR…odds-ratio, SIR…standardized incidence ratio, SMR…standardized mortality ratio, RR…relative risk (rate ratio), OER…observed/expected ratio

_____

Use of Wireless Phones and Evidence for Increased Risk of Brain Tumors

2012 Supplement

Lennart Hardell, MD, PhD, Professor
Department of Oncology, University Hospital, Örebro, Sweden

Michael Carlberg, MSc
Department of Oncology, University Hospital, Örebro, Sweden

Kjell Hansson Mild, PhD, Professor
Department of Radiation Physics. Umeå University, Umeå, Sweden

I. INTRODUCTION

In May 2011 the International Agency for Research on Cancer (IARC) at WHO categorised the radiofrequency electromagnetic fields (RF-EMF) from mobile phones, and from other devices that emit similar non-ionising electromagnetic fields, as a Group 2B, i.e. a 'possible', human carcinogen (Baan et al., 2011, IARC, 2011). Nine years earlier IARC had also classified extremely low frequency (ELF) magnetic field as Group 2B carcinogen (IARC, 2002).

The IARC decision on mobile phones was based mainly on case-control studies from the Hardell group in Sweden and the IARC Interphone study. Both provided supportive results on positive associations between two types of brain tumors; glioma and acoustic neuroma, and exposure to RF-EMF from wireless phones.

The final IARC decision was confirmed by voting of 29 scientists (one not present during voting) at the meeting. A large majority of participants voted to classify RF-EMF radiation as 'possibly carcinogenic' to humans, Group 2B. The decision was also based on occupational studies. We present in this paper an updated review of evidence of the association between use of wireless phones and brain tumors including also papers published after the IARC evaluation.

The Nordic countries were among the first countries in the world to widely adopt the wireless telecommunications technology. Analogue phones (NMT; Nordic Mobile Telephone System) were introduced in the early 1980s using both 450 and 900 Megahertz (MHz) frequencies. NMT 450 was used in Sweden from 1981-2007, NMT 900 operated during 1986-2000.

The digital system (GSM; Global System for Mobile Communication) using dual band, 900 and 1800 MHz, started to operate in 1991 and dominates now the market. The third generation of mobile phones, 3G or UMTS (Universal Mobile Telecommunication System), using 1 900/2 100 MHz RF fields has been introduced worldwide in recent years, in Sweden in 2003. Currently the fourth generation, 4G (Terrestrial 3G), operating at 800/2600 MHz and Trunked Radio Communication (TETRA 380-400 MHz) are being established in Europe. Nowadays mobile phones are used more than landline phones in Sweden (http://www.pts.se/upload/Rapporter/Tele/2011/sv-telemarknad-halvar-2011-pts-er-2011-21.pdf). Worldwide, an estimate of 5.9 billion mobile phone subscriptions was reported at the

end of 2011 by the International Telecommunication Union (ITU; http://www.itu.int/ITU-D/ict/facts/2011/material/ICTFactsFigures2011.pdf).

Desktop cordless phones (DECT) have been used in Sweden since 1988, first using analogue 800-900 MHz RF fields, but since early 1990s using a digital 1900 MHz system. These cordless phones are becoming more common than traditional landlines. They emit RF-EMF radiation similar to that of mobile phones. Thus when human health risks are evaluated it is also necessary to consider the use of cordless phones along with mobile phones.

The real increase in use and exposure to radiation fields from wireless phones (mobile phones and cordless phones) in most countries has occurred since the end of the 1990s. The brain is the main target organ during use of the handheld phone (Cardis et al., 2008). Fear of an increased risk for brain tumors has dominated the debate during the last one or two decades. While RF-EMFs do not have sufficient energy to break chemical bonds like ionising radiation, at least not directly, they can nevertheless have harmful effects on biological tissues. Plausible biological mechanisms for these effects include DNA damage, impairment of DNA repair mechanisms, and epigenetic changes to DNA (see also chapters by H. Lai (Genotoxicity) and I. Belyaev (Physical and Biological Mechanisms).

Primary brain tumors (central nervous system; CNS) constitute of a heterogeneous group of neoplasms of different histological types depending on tissue of origin with different growth patterns, molecular markers, anatomical localisations, and age and gender distributions. The clinical appearance, treatment and prognosis are quite different depending on tumor type.

There are few established risk factors for brain tumors besides ionising radiation (Preston Martin et al., 2006). Higher socio-economic status tends to be related to higher incidence and some rare inherited cancer syndromes account for a small fraction of tumors (Preston Martin et al., 2006). Familial aggregation of glioma has also been reported (Scheurer et al., 2010).

We base this review primarily on the Hardell group papers and the WHO Interphone study (Interphone Study Group, 2010, 2011, Cardis et al., 2011). More discussion of the results and responses, agreements and disagreements of the findings for the Hardell group and Interphone studies can be found in Hardell et al., (2012, 2013).

II. MATERIALS AND METHODS

The PubMed database (www.ncbi.nlm.nih.gov) was used for an up-dated search of published studies in this area using mobile/cellular/cordless telephone and brain tumour/neoplasm/acoustic neuroma/meningioma/glioma as searching terms. Personal knowledge of published studies was also used in order to get as up-to-date review as possible.

III. RESULTS

Brain tumors overall

Exposure to the radiation from the phones is generally higher in the temporal lobe, the part of the brain that is near to the ear (Cardis et al., 2008). For tumors located in the temporal, occipital or temporoparietal lobe areas of the brain an increased risk was found for ipsilateral exposure, that is the telephone was mostly used on the same side of the head as the tumor appeared, yielding OR = 2.42, 95 % CI = 0.97-6.05 (Hardell et al., 2001). This was the first study in the world that indicated an association between use of mobile phones and an increased risk for brain tumors. However, the results were based on low numbers of exposed subjects and different histopathological types of brain tumors so no firm conclusions could be drawn. Furthermore, this first study did not include use of cordless phones, see also Hardell et al., (1999).

Glioma

Glioma is the most common malignant brain tumor and represents about 60 % of all central nervous system tumors. The most common glioma subtype is astrocytoma. Astrocytic tumors are divided in two groups depending on the malignant potential; low-grade (WHO grades I-II) and high-grade (WHO grades III-IV). Low-grade astrocytoma has a relatively favourable prognosis, whereas survival is shorter for patients with high-grade glioma. Glioblastoma multiforme (WHO grade IV) accounts for 60-75 % of all astrocytoma.

The Hardell group in Sweden studied the association between use of mobile and cordless phones and brain tumors diagnosed during 1997-2003. First, cases diagnosed during 1 January 1997 to 30 June 2000 were included (Hardell et al., 2002, 2003). The next study period included 1 July 2000 to 31 December 2003 (Hardell et al., 2005, 2006a). The methods were the same with the same inclusion criteria and an identical questionnaire in both studies.

In short, both men and women aged 20-80 years at the time of diagnosis were included and all were alive at the time of inclusion in the study. They were reported from cancer registries and had all a brain tumor verified by histopathology. The Swedish Population Registry was used for identification of matched controls. In addition to other exposures use of wireless phones was carefully assessed by a self-administered questionnaire supplemented over the phone. The ear that had mostly been used during calls with mobile phone and/or cordless phone was assessed by separate questions. This information was checked during the supplementary phone calls and finally also by a separate letter with good agreement between these three methods.

Use of the wireless phone was defined as ipsilateral ($\geq$ 50 % of the time), or contralateral ($<$ 50 % of the time) in relation to tumor side. The matched control was assigned the same side as the tumor of the respective case. Use of hands free devices was also assessed as well as use in a car with external antenna. Such use was not included in the calculation of cumulative number of hours for life time use. Latency time was defined as the period from the year of first use until diagnosis (corresponding year for the matched control).

Medical records including computer tomography (CT) and/or magnetic resonance imaging (MRI) were used to define tumor localisation in the brain. Further details can be found in the publications.

As a response to a critique from Boice and McLaughlin (2002) that the exclusion of deceased cases was a source of bias in our studies we performed a study on the cases with a malignant brain tumor that had died before inclusion in the case-control studies 1997-2003. These cases represented patients with a poor prognosis, mostly with astrocytoma WHO grade IV (glioblastoma multiforme). Controls were selected from the Death Registry in Sweden. The study encompassed 464 cases and 464 controls that had died from a malignant disease and 463 controls with other causes of death. Exposure was assessed by a questionnaire sent to the next of kin to each deceased case and control. The questionnaire was similar as in previous studies. This investigation confirmed the previous results of an association between use of mobile phones and malignant brain tumors (Hardell et al., 2010).

We have previously published pooled analysis of malignant brain tumors diagnosed during the period 1997-2003 (Hardell et al., 2006b). These results were updated including also results for the deceased cases with malignant brain tumors (Hardell et al., 2011a, Carlberg, Hardell 2012). The results on use of wireless phones were based on 1,251 cases with malignant brain tumor (response rate 85%) and 2,438 controls (response rate 84%). Most cases had glioma (n=1,148) so we present in the following results for that type of tumor. Latency was divided in three categories, >1-5 years, >5-10 years, and > 10 years from first use of a wireless phone until diagnosis of glioma.

Both use of mobile and cordless phone gave an increased risk overall, highest in the latency group >10 years, increasing further for ipsilateral use yielding for mobile phone OR = 2.9, 95 % CI = 1.8-4.7 and for cordless phone OR = 3.8, 95 % CI = 1.8-8.1 (Table 1). Highest ORs were found in the > 10 year latency group for total wireless phone use as well, OR = 2.1, 95 % CI = 1.6-2.8.

OR increased statistically significant for glioma for cumulative use of wireless phones per 100 h; OR = 1.014, 95 % CI = 1.008-1.019, and per year of latency; OR = 1.056, 95 % CI = 1.037-1.075 (Carlberg and Hardell, 2012). Separate calculations of mobile phone and cordless phone use yielded similar results with statistically significant increasing risks.

The Interphone study was conducted at 16 research centres in 13 countries during varying time periods between 2000 and 2004 under the guidance of IARC. An increased risk for brain tumor was found in some separate country studies and decreased risk in other studies as we have discussed elsewhere (Hardell et al., 2008, 2009). After several years of delay the overall Interphone results were finally published in May 2010 (Interphone Study Group, 2010).

In total 4,301 glioma cases were included in Interphone and the final results were based on 2,708 participating cases (response rate 64 %, range by centre 36-92 %). In total 14,354 potential controls were identified and interviews were completed with 7,658 (53 %, range 42-74 %). The low participation rates in some centres may have created selection bias, see Hardell et al., (2008).

Regular use of mobile phone in the past ≥ 1 year gave for glioma OR = 0.81, 95 % CI = 0.70-0.94 (Table 1). Subgroup analyses showed statistically significant increased risk in the highest

exposure group, i.e. those with cumulative mobile phone use ≥ 1,640 hours, OR = 1.40, 95 % CI = 1.03-1.89. The risk increased further for glioma in the temporal lobe yielding OR = 1.87, 95 % CI = 1.09-3.22. In the same exposure category, cumulative use ≥ 1,640 hours and ipsilateral exposure produced OR = 1.96, 95 % CI = 1.22-3.16 in total (no data given for temporal lobe).

In Appendix 2 (Interphone Study Group, 2010, available on the web) analysis was restricted to ever-regular users of mobile phones. Cumulative call time ≥ 1,640 hours gave OR = 1.82, 95 % CI = 1.15-2.89 compared with use < 5 hours. Time since start of regular use (latency) ≥ 10 years produced OR = 2.18, 95 % CI = 1.43-3.31; reference entity 1-1.9 years.

The Interphone study group concluded: "*However, biases and errors limit the strength of the conclusions we can draw from these analyses and prevent a causal interpretation.*" In an editorial accompanying the Interphone results the main conclusion of the Interphone results was described as "*both elegant and oracular…(which) tolerates diametrically opposite readings*" (Saracci and Samet 2010). Several methodological reasons why the Interphone results were likely to have underestimated the risks were discussed including the short latency period since first exposures became widespread; less than 10 % of the Interphone cases had more than 10 years exposure. "*None of the today's established carcinogens, including tobacco, could have been firmly identified as increasing risk in the first 10 years or so since first exposure*".

Estimated RF-EMF dose in the tumor area from mobile phone use was associated with an increased risk of glioma in parts of the Interphone study (Cardis et al., 2011). OR increased with increasing total cumulative dose of specific energy (J/kg) absorbed at the estimated tumor centre for more than 7 years before diagnosis giving OR = 1.91, 95 % CI = 1.05-3.47 (p trend = 0.01) in the highest quintile of exposure. A similar study based on less clear methods was later published by another part of the Interphone study group (Larjavaara et al., 2011). The results seemed not to support the findings of Cardis et al., (2011). However, only 42 cases had used a mobile phone for more than 10 years and no analysis was made of the most exposed group with longest duration of use.

Based on Hardell et al (2011b) and Interphone Study Group (2010) we made meta-analysis of glioma and use of mobile phones. Random-effects model was used based on test for heterogeneity in the overall (≥10 years and ≥1,640 hours) groups. We used published results in

Interphone since we do not have access to their database. Our results were recalculated to these groups of exposure. The meta-analysis yielded for mobile phone use OR = 1.71, 95 % CI = 1.04-2.81 for glioma in the temporal lobe in the ≥ 10 years latency group. Ipsilateral mobile phone use ≥ 1,640 h in total gave the highest risk, OR = 2.29, 95 % CI = 1.56-3.37 (Hardell et al 2012). This meta-analysis strengthens a causal association between use of mobile phones and glioma.

Meningioma

Meningioma is the most common benign brain tumor. It develops from the pia and arachnoid that covers the central nervous system. Meningioma is an encapsulated and well-demarked tumor more common in women than in men. It is rarely malignant.

A pooled analysis of benign brain tumors from the two case-control studies from the Hardell group as discussed above (Hardell et at., 2006c, Hardell and Carlberg, 2009) gave regarding meningioma and use of mobile phone OR = 1.1, 95 % CI = 0.9-1.3, and cordless phone OR = 1.1, 95 % CI = 0.9-1.4 (Table 2). Using > 10 year latency period OR increased; for mobile phone to OR = 1.5, 95 % CI = 0.98-2.4, and for cordless phone to OR = 1.8, 95 % CI = 1.01-3.2. Ipsilateral mobile phone use in the > 10 years latency group yielded OR = 1.6, 95 % CI = 0.9-2.9, and cordless phone OR = 3.0, 95 % CI = 1.3-7.2. These results were based on rather low numbers of exposed cases, however.

Regular use of mobile phone produced in the Interphone study (2010) a statistically significant decreased risk for meningioma, OR = 0.79, 95 % CI = 0.68-0.91, Table 2. The risk increased somewhat with cumulative use ≥ 1,640 hours and ipsilateral mobile phone use to OR = 1.45, 95 % CI = 0.80-2.61. Analysis restricted to tumors in the temporal lobe or to the group of ever-regular use did not change the overall pattern of no increased risk.

We performed meta-analysis of meningioma for use of mobile phone based on results in the Hardell group and Interphone results similarly as for glioma. No statistically significant decreased or increased risk was found (Hardell et al., 2012). These results support the conclusion that up to latency ≥ 10 years or cumulative use ≥1,640 hours there is no consistent pattern of an association between use of mobile phones and meningioma.

Acoustic neuroma

Acoustic neuroma or Vestibular Schwannoma is a slow growing benign tumor located in the eighth cranial nerve in the auditory canal. It grows gradually out into the cerebellopontine angle with potential compression of vital brain stem centres. Tinnitus and hearing problems are usual first symptoms of acoustic neuroma. The eighth cranial nerve is located close to the handheld wireless phone when used, so there is particular concern of an increased risk for neuroma development due to exposure to EMF-RF emissions during use of these devices.

The pooled analysis of the Hardell group studies yielded regarding use of mobile phones for acoustic neuroma OR = 1.7, 95 % CI = 1.2-2.3 increasing to OR = 2.9, 95 % CI = 1.6-5.5 with > 10 years latency period, Table 3. Ipsilateral use increased the risk further; in the > 10 years latency group to OR = 3.0, 95 % CI = 1.4-4.2 (Hardell and Carlberg, 2009). Cordless phone use gave OR = 1.5, 95 % CI = 1.04-2.0 increasing to OR = 1.7, 95 % CI =1.2-2.5 for ipsilateral use in the > 1 year latency group.

In the Interphone study (2011) 1,121 (82 %) acoustic neuroma cases participated, range 70-100 % by centre. Of the controls 7,658 (53 %) completed the interviews, range 35-74 % by centre. The final matched analysis (1:1 or 1:2) consisted of 1,105 cases and 2,145 controls. Overall no increased risk was found censoring exposure at one year or at 5 years before reference date, OR = 0.85, 95 % CI = 0.69-1.04 and OR = 0.95, 95 % CI = 0.77-1.17, respectively (Table 3).

Cumulative number of hours of ipsilateral mobile phone use ≥ 1,640 hours up to 1 year before reference date gave OR = 2.33, 95 % CI = 1.23-4.40 and contralateral use OR = 0.72, 95 % CI = 0.34-1.53 for acoustic neuroma, see Table 3 (Interphone Study Group, 2011). For cumulative number of hours of ipsilateral mobile phone use ≥ 1,640 hours up to 5 years before reference date OR = 3.53, 95 % CI = 1.59-7.82, and for contralateral use OR = 1.69, 95 % CI = 0.43-6.69 were obtained. The risk increased further for cumulative ipsilateral use ≥ 1,640 hours with start ≥ 10 years before reference date to OR = 3.74, 95 % CI = 1.58-8.83. Contralateral use in that group yielded OR = 0.48, 95 % CI = 0.12-1.94, however based on only 4 exposed cases and 9 exposed controls. Overall OR = 1.93, 95 % CI = 1.10-3.38 was obtained for long-term use with start ≥ 10 years before reference date and cumulative call time ≥ 1,640 hours.

Similar analyses of the data as in Appendix 2 for glioma (see Interphone Study Group, 2010) yielded highest OR for acoustic neuroma in the shortest latency group, 2-4 years before reference date, OR = 1.41, 95 % CI = 0.82-2.40. Lower OR was calculated in the ≥ 10 years group, OR = 1.08, 95 % CI = 0.58-2.04. Somewhat higher risk than in total, OR = 1.32, 95 % CI = 0.88-1.97, was found for cumulative mobile phone use ≥ 1,640 hours; OR = 1.74, 95 % CI = 0.90-3.36, in this analysis restricted to only regular users. No results were given for ipsilateral use.

We performed meta-analysis of the results for use of mobile phone and the association with acoustic neuroma based on results by the Hardell group and Interphone study (Hardell et al 2012). For the latency group ≥ 10 years highest risk was obtained for ipsilateral use, OR = 1.81, 95 % CI = 0.73-4.45. The risk increased further for cumulative use ≥ 1,640 hours yielding OR = 2.55, 95 % CI = 1.50-4.40 for ipsilateral use. The meta-analysis strengthens a causal association between use of mobile phones and acoustic neuroma.

A case-case study was performed in Japan (Sato et al., 2011). The cases were identified during 2000-2006 at 22 participating neurosurgery departments. The diagnosis was based on histopathology or CT/MRI imaging. Of 1,589 cases 816 (51 %) agreed to participate and answered a mailed questionnaire. The final analysis included 787 cases, Cases with ipsilateral use were regarded as exposed and those with contralateral use were assumed to be unexposed and were used as the reference category. Overall no increased risk was found. However, for average daily call duration > 20 minutes with reference date 1 year Risk Ratio (RR) = 2.74, 95 % CI = 1.18-7.85 was found increasing to OR = 3.08, 95 % CI = 1.47-7.41 with reference date 5 years before diagnosis (Table 3). Unfortunately no results were given for cumulative number of hours for use over the years. For cordless phones no increased risk was found but the analysis was not very informative.

Risks to children and adolescents

The developing brain is more sensitive to toxins (Kheifets et al., 2005) and it is still developing until about 20 years of age (Dosenbach et al., 2010). Children have smaller head and thinner skull bone than adults. Their brain tissue has also higher conductivity and these circumstances give higher absorption from RF-EMF than for adults (Cardis et al., 2008, Christ et al., 2010, Gandhi et al., 2012). Use of wireless phones is widespread among children and adolescents

(Söderqvist et al., 2007, 2008). The greater absorption of RF energy per unit of time, the greater sensitivity of their brains, and their longer lifetimes with the risk to develop a brain tumor leaves children at a higher risk than adults from mobile phone radiation.

We have published results regarding brain tumor risk for different age groups at the time of diagnosis (Hardell et al., 2004) or age at first use of wireless phones (Hardell and Carlberg, 2009, Hardell et al., 2011a, 2012, 2013). Three age groups for first use of a wireless phone were used: <20 years, 20-49 years and 50-80 years. Highest risk for glioma was found for first use of mobile phone or cordless phone before the age of 20 years (Table 4). Thus, mobile phone use yielded for glioma OR = 3.1, 95 % CI = 1.4-6.7 and cordless phone OR 2.6, 95 % CI = 1.2-5.5.

Also for acoustic neuroma the risk was highest in the youngest age group with OR = 5.0, 95 % CI = 1.5-16 for use of mobile phone. Only one case had first use of cordless phone before the age of 20, so no conclusions could be drawn for cordless phones. Regarding meningioma no clear pattern of age-dependent increased risk was seen.

A multi-centre case-control study was conducted in Denmark, Sweden, Norway, and Switzerland, CEFALO (Aydin et al., 2011). It included children and adolescents aged 7–19 years and has been commented elsewhere in detail since serious methodological problems exist in the study design and interpretation of the results (Söderqvist et al., 2011). In CEFALO a statistically non-significant increased risk for brain tumors among regular users (one call per week for at least 6 months) of mobile phones was found; OR = 1.36, 95 % CI = 0.92-2.02. This OR increased somewhat with cumulative duration of subscriptions and duration of calls (Aydin et al., 2011). No data for long-term use were given; the longest latency period was 5 years. Further support of a true association was found in the results based on operator-recorded use for 62 cases and 101 controls, which for time since first subscription >2.8 years yielded a statistically significant OR of 2.15, 95 % CI = 1.07-4.29, with a statistically significant trend (p=0.001).

Use of cordless phones was covered only in the first 3 years of use. No explanation was given for this most peculiar definition. Wireless phone use was not considered, that is use of both mobile phones and cordless phones as the relevant exposure category, as used by the Hardell group and adopted by IARC (Baan et al., 2011). Instead Aydin et al., (2011) included use of

cordless phones in the 'unexposed' category when risk estimates were calculated for mobile phone use. Similarly, regarding use of cordless phones RF-EMF emissions from mobile phones were regarded as 'no exposure'. Thus, an increased risk was potentially concealed.

The authors summarised that they "*did not observe that regular use of a mobile phone increased the risk for brain tumors.*" An editorial in the same journal accompanied that conclusion by stating by that the study showed "*no increased risk of brain tumors*" (Boice and Tarone, 2011). This was echoed by a news release from the Karolinska Institute in Stockholm claiming that the results of no increased risk were 'reassuring' (Karolinska Institute, 2011). However the results indicate a moderately increased risk, in spite of low exposure, short latency period and limitations in study design and analyses. Certainly it cannot be used as reassuring evidence against an association, see Söderqvist et al., (2011).

Danish cohort study on mobile phone subscribers

An attempt to establish a cohort of mobile phone users was made in Denmark in co-operation between the Danish Cancer Society and the International Epidemiology Institute (IEI), Rockville, MD, USA. It was financed by grants from two Danish telecom operation companies (TeleDenmark Mobil and Sonafon), IEI, and the Danish Cancer Society. The source of money for IEI has not been disclosed.

The Danish study on brain tumor risk among mobile phone subscribers has so far resulted in four publications (Johansen et al., 2001, Schüz et al., 2006, Frei et al., 2011, Schüz et al., 2011). It included subjects from January 1, 1982 until December 31, 1995 identified from the computerised files of the two Danish operating companies, TeleDenmark Mobil and Sonofon. A total of 723,421 subscribers were initially identified but the final cohort consisted of only 58 % of these subjects. Due to lack of names of individual users 200,507 corporate users were excluded.

We have discussed elsewhere several shortcomings in the Danish cohort study such as exclusion of corporate users, no individual exposure data, users of cordless phones are included in the reference category, no control for use of mobile phones in the population after the establishment of the cohort, and no operator-verified data on years of subscription is available (Söderqvist et al., 2012). These limitations are likely to have led to an underestimate of any risk in this study.

One would also expect considerable misclassification of mobile phone use both among subscribers and the reference population since no new subscribers were included in the exposed cohort after 1995.

The IARC working group concluded that the methods used could have resulted in considerable misclassification in exposure assessment in the Danish cohort study on mobile phone subscribers (Baan et al., 2011).

After the outcome of the IARC-evaluation was made public in June 2011 (Baan et al., 2011) two additional reports on the Danish cohort were published (Frei et al., 2011, Schüz et al., 2011). Both were new up-dates of the initial cohort and included more information on risk related to longer follow-up. One focused on acoustic neuroma (Schüz et al., 2011) while the other gave results both for all cancers and separately for glioma and meningioma (Frei et al., 2011). This time the number of the cohort was reduced to 358,403 (49.5 %) of the initially identified subscribers (n=723,421). The major additional exclusion (n=54,350) was due to record linkage with the Danish so-called CANULI cohort on socioeconomic factors (Dalton et al., 2008).

The authors of the Danish study have themselves pointed out the main causes of considerable exposure misclassifications (Frei et al., 2011). While at least non-response and recall bias can be excluded the study has serious limitations related to exposure assessment (Söderqvist et al., 2012). In fact, these limitations cloud the findings of the four reports to such an extent they are uninformative at best. At worst, they may be used in a seemingly solid argument against an increased risk; as reassuring results from a large nationwide cohort study.

Brain tumor incidence

It has been suggested that overall incidence data on brain tumors for countries show no increasing trends and may be used to disqualify the association between mobile phone use and brain tumors observed in the case-control studies (Aydin et al., 2011; Ahlbom, and Feychting, 2011; Deltour et al., 2012; Little et al., 2012).

However, by now several studies show increasing incidence of brain tumors. In Denmark a statistically significant increase in incidence rate per year for brain and central nervous system

tumors (combined) was seen during 2000-2009; in men +2.7 %, 95 % CI = +1.1 to 4.3 % and in women +2.9 %, 95 % CI = +0.7 to 5.2 % (NORDCAN). Updated results for brain and central nervous system tumors have been released in Denmark. The age-standardized incidence of brain and central nervous system tumors increased with 40 % among men and 29 % among women during 2001-2010 (Sundhedsstyrelsen, 2010). A more recent news release based on the Danish Cancer Register stated that during the last 10 years there has been an increasing number of cases with the most malignant glioma type, glioblastoma multiforme (astrocytoma WHO grade IV), especially among men (http://www.cancer.dk/Nyheder/nyhedsartikler/2012kv4/Kraftig+stigning+i+hjernesvulster.htm)
.

Little et al., (2012) studied the incidence rates of glioma during 1992-2008 in the United States and compared with ORs for glioma associated with mobile phone use in the 2010 Interphone publication (Interphone Study Group, 2010) and our pooled results published in 2011 (Hardell et al., 2011a). Since our results are discussed and questioned by Little et al their study needs to be reviewed in more detail. Our response to the journal (BMJ) was never accepted for publication in the journal and cannot be found via PubMed, only on the web (http://www.bmj.com/content/344/bmj.e1147/rr/578564).

First, one important methodological issue that was not stated in the abstract or in the article [Figures 2-4] by Little et al., (2012), but can be found in the web appendix, is that observed rates were based on men aged 60-64 years from the Los Angeles SEER registry as the baseline category. These data were used to estimate rates in the entire dataset, men and women aged ≥ 18 years and all 12 SEER registries. Thereby numerous assumptions were made as pointed out by Kundi (2012) and Davis et al., (2012).

Using only men, as Little et al., did, ignores the fact that women had less frequent use of mobile phones than men in our studies (Table 5). Overall 31 % of women reported such use *versus* 57 % of men. Furthermore, use varies with age group with a large difference according to age, as we have explored in our publications (Hardell and Carlberg, 2009, Hardell et al., 2011a). Thus, the age group 60-64 year old men is not valid to use for these calculations.

There are several other points that may be added. Another example is that the results for anatomical localisations and tumor grade [in Table 5 in the article] by Little et al are based on numerous assumptions from SEER data, Interphone and the Hardell group studies. The authors seem not to have paid attention to the fact that the fraction of mobile phone users differs for gender and age, see Table 5.

One interesting result that was not commented further by Little et al., (2012) was the finding of a statistically significant yearly increasing incidence of high-grade glioma (WHO grades III-IV) in the SEER data for 1992-2008, +0.64%, 95% CI = +0.33 to 0.95 %. On the contrary, the incidence of low-grade glioma (WHO grades I-II) decreased with –3.02 %, 95 % CI = –3.49 to –2.54 %. Little et al., (2012) found also a statistically significant increasing yearly trend for glioma in the temporal lobe, +0.73 %, 95 % CI = +0.23 to 1.23 %.

Zada et al., (2012) studied incidence trends of primary malignant brain tumors in the Los Angeles area during 1992-2006. The overall incidence of primary malignant brain tumors decreased over the time period with the exception of glioblastoma multiforme (astrocytoma WHO grade IV). The annual age adjusted incidence rate of that tumor type increased statistically significant in the frontal lobe with Annual Percentage Change (APC) +2.4 % to +3.0 % (p ≤ 0.001) and temporal lobe APC +1.3 % to +2.3 % (p ≤ 0.027) across all registries. In the California Cancer Registry the incidence of glioblastoma multiforme increased also in cerebellum, APC +11.9 % (p < 0.001). For lower grade astrocytoma decreases of annual age adjusted incidence rates were observed. The authors concluded that there was a real increase in the incidence of glioblastoma multiforme in frontal and temporal lobes and cerebellum, areas of the brain with the highest absorbed dose of RF-EMF emissions from handheld mobile phones (Cardis et al., 2008).

Of interest is also the report by de Vocht et al., (2011) from England that showed for the time period 1998 to 2007 a statistically significant increasing incidence of brain tumors, the majority glioma, in the temporal lobe for men and women (p < 0.01), and frontal lobe for men (p < 0.01). The incidence increased also for women in the frontal lobe, although not statistically significant (p = 0.07). The incidence decreased in other parts of the brain.

Deltour et al., (2012) reported increasing glioma incidence rates in Denmark, Finland, Norway, and Sweden for the time period 1979-2008. APC increased for men with +0.4 %, 95 % CI +0.1 to 0.6 % and for women with +0.3 %, 95 % CI +0.1 to 0.5 %. A study from Australia for the time period 2000-2008 showed that APC for malignant brain tumors increased statistically significant +3.9 %, 95 % CI +2.4 to 5.4 % (Dobes et al., 2011). An increase was seen among both men and women. The APC for benign tumors increased with +1.7 %, 95 % CI -1.4 to +4.9 %, thus not statistically significant.

From urban Shanghai an increasing incidence of brain and nervous system tumors for the time period 1983-2007 was reported with APC +1.2 %, 95 % CI +0.4 to 1.9 % in males and APC +2.8 %, 95 % CI +2.1 to 3.4 % in females (Ding and Wang, 2011).

We reported increasing incidence of astrocytoma WHO grades I-IV during 1970-2007 in Sweden. In the age group > 19 years the annual change was +2.16 %, 95 % CI +0.25 to 4.10 % during 2000-2007, for further details see Hardell and Carlberg (2009).

IV. DISCUSSION

As pointed out by IARC (Baan et al., 2011) the most comprehensive results on use of wireless phones and the association with brain tumors come from the Hardell group in Sweden and the international Interphone study. Results for latency time of 10 years or more have been published from both study groups.

Both were case-control studies and the cases were recruited during similar time periods, 1997-2003 in the Hardell group and during 2000-2004 in Interphone, with somewhat different years in the varying study regions. There was no overlapping of cases in the Hardell group studies and the Swedish part of Interphone.

The Hardell group included cases aged 20-80 years whereas eligible cases in Interphone were aged 30-59 years at diagnosis. One control subject matched on age, gender and geographical area (region) to each case in the Hardell group studies was drawn from the national population register. In Interphone one control was selected for each case from a 'locally appropriate population-based sampling frame'. In Germany two controls were selected and the centres used

individual matching or frequency matching. Regarding the Interphone study on acoustic neuroma some centres sampled special controls to the cases, other draw controls from the pool of controls in the glioma and meningioma studies, or used a mixture of both methods. In UK general practioners' lists (Hepworth et al 2006) and in Japan random digit dialling were used (Takebayashi et al., 2006, 2008). Certainly the methods used in Interphone may introduce selection bias.

Use of wireless phones and other exposures were carefully assessed by a self-administered questionnaire in the Hardell et al., studies. The information was supplemented over the phone by trained interviewers thereby using a structured protocol. This was done blinded as to case or control status. After the interviews all personal data like names and addresses were removed from the questionnaires so that only an id-number that did not disclose if it was a case or a control was shown. Thus, coding of the data for statistical analysis was performed without personal data of the individual.

On the contrary information on past mobile phone use was collected during face-to-face interviews in Interphone obviously disclosing if it was a case or a control that was interviewed. These interviews were performed by a large number of interviewers at different participating centres. Experienced interviewers were defined as those who conducted at least 20 interviews. In fact, in the sensitivity analysis the risk increased for glioma for cumulative mobile phone use $\geq$ 1,640 hours from OR = 1.40, 95 % CI 1.03-1.89 to OR = 1.50, 95 % CI = 1.10-2.06 if 'experienced interviewers only' were considered. The higher risk restricting analysis to 'experienced interviewers' in Interphone indicates observational bias during assessment of exposure decreasing the risk.

In the Hardell group studies few persons conducted all interviews of the 1,251 participating cases with malignant brain tumor, 1,254 cases with benign brain tumor, and 2,438 controls (total 4,942; note one case had both a malignant and a benign brain tumor). All interviewers were first educated; they used a defined protocol and gained considerable experience as interviewers. In fact, they were obliged to carry out the interviews extensively to fulfil the quality in data assessment according to the structured protocol. It is obvious that the few interviewers in the Hardell group study must have been much more experienced than the diversity of interviewers in Interphone.

In the personal interviews in Interphone a computer program that guided the interview with questions read by the interviewer from a laptop computer screen was used. The answers were entered directly into the computer by the interviewer. Using computer based face-to-face interviews may be a stressful situation for the patients. In fact patients scored significantly lower than controls due to recalling of words (aphasia), problems with writing and drawing due to paralysis in the Danish part of Interphone (Christensen et al., 2005). Furthermore, it has not been disclosed how the personal interviews were performed in sparsely populated areas, e.g. in the Northern Sweden. Did the interviewers travel long distances for interviews of controls in rural areas or were all controls living in the largest cities thereby creating selection bias?

In the Hardell group studies the response rate was 85 % (n=1,251) for cases with malignant brain tumor, 88 % (n=1,254) for cases with benign brain tumor, and 84 % (n=2,438) for controls (Hardell et al., 2006c, Carlberg and Hardell, 2012). Lower response rates were obtained in Interphone study, 64 %, range by centre 36-92 %, (n=2,765) for glioma cases, 78 %, range 56-92 %, (n=2,425) for meningioma cases, 82 %, range 70-100 % (n=1,121) for acoustic neuroma cases, and 53 %, range 42-74 %, (n=7,658) for controls (Interphone Study Group, 2010; 2011). These low response rates may have created the possibility of considerable selection bias (Hardell et al., 2008). Not responding controls in Interphone tended to be less frequent users of mobile phone than participating controls leading to underestimation of the risk.

The Hardell group studies included subjects aged 20-80 years, versus 30-59 years in Interphone. We have shown that restricting the age group to 30-59 years and considering subjects that used a cordless phone as unexposed in the Hardell group studies reduced the ORs and produced results quite similar to Interphone (Hardell et al., 2011b). Latency time > 10 years for glioma in the temporal lobe yielded OR = 1.40, 95 % CI = 0.70-2.81 in the Hardell group studies and OR = 1.36, 95 % CI = 0.88-2.11 in Interphone (latency ≥ 10 years). Thus, excluding exposure to RF-EMFs from cordless phones as in the Interphone study as well as excluding the younger and older subjects biased the ORs towards unity in Interphone, which likely dilutes the ability to see health risks.

By placing a strong emphasis on incidence data an association between use of wireless phones and brain tumors has been challenged (Swerdlow et al., 2011). The authors considered that if the

increased risks seen in case-control studies reflect a causal relationship, there would already be an increase in incidence of brain and central nervous system tumors. As discussed above by now increasing incidence rates, especially for certain brain tumor types and anatomical localisations of relevance, have been reported. The natural history of most glioma from earliest events to clinical manifestation is unknown, but most likely several decades. The exposure duration in most studies is thus incompatible with a tumor initiating effect. If the exposure on the other hand acts as a promoter, this would decrease latency time for already existing tumors, giving a temporary but not a continuous increase in incidence (Kundi, 2010).

The first case in the world on worker's compensation for a brain tumor after long-term use of wireless phones was the ruling 12 October 2012 by the Italian Supreme Court. A previous ruling that the Insurance Body for Work (INAIL) must grant compensation to a businessman who had used wireless phones for 12 years and developed a neurinoma in the brain was affirmed (http://www.applelettrosmog.it/public/news.php?id_news=44 ; www.microwavenews.com). He had used both mobile and cordless phones for five to six hours per day preferably on the same side as the tumour developed. The neurinoma was located in the trigeminal Gasser's ganglion in the brain. This 5th cranial nerve controls facial sensations and muscles. It is the same type of tumour as the acoustic neuroma in the 8th cranial nerve located in the same area of the brain. No further appeal of the Supreme Court decision is possible.

V. CONCLUSIONS

Based on epidemiological studies there is a consistent pattern of increased risk for glioma and acoustic neuroma associated with use of mobile phones and cordless phones. The evidence comes mainly from two study centres, the Hardell group in Sweden and the Interphone Study Group. No consistent pattern of an increased risk is seen for meningioma. A systematic bias in the studies that explains the results would also have been the case for meningioma. The different risk pattern for tumor type strengthens the findings regarding glioma and acoustic neuroma. Meta-analyses of the Hardell group and Interphone studies show an increased risk for glioma and acoustic neuroma. Supportive evidence comes also from anatomical localisation of the tumor to the most exposed area of the brain, cumulative exposure in hours and latency time that all add to the biological relevance of an increased risk. In addition risk calculations based on estimated absorbed dose give strength to the findings.

In summary:

- There is reasonable basis to conclude that RF-EMFs are bioactive and have a potential to cause health impacts.
- There is a consistent pattern of increased risk for glioma and acoustic neuroma associated with use of wireless phones (mobile phones and cordless phones) mainly based on results from case-control studies from the Hardell group and Interphone Final Study results.
- Epidemiological evidence gives that RF-EMF should be classified as a human carcinogen.
- Based on our own research and review of other evidence the existing FCC/IEE and ICNIRP public safety limits and reference levels are not adequate to protect public health.
- New public health standards and limits are needed.

Authors' contributions

VI. REFERENCES

Ahlbom A, Feychting M. 2011. Mobile telephones and brain tumours. BMJ 343:d6605.

Aydin D, Feychting M, Schüz J, Tynes T, Andersen TV, Schmidt LS, et al. 2011. Mobile phone use and brain tumors in children and adolescents: a multicenter case-control study. Journal of the National Cancer Institute 103(16):1264-1276.

Baan R, Grosse Y, Lauby-Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, et al. 2011. Carcinogenicity of radiofrequency electromagnetic fields. Lancet Oncology 12(7):624-626.

Boice JD Jr, McLaughlin JK. 2002. Epidemiologic Studies of Cellular Telephones and Cancer Risk - A Review. SSI Publication 2002:16, accessed at http://www.stralsakerhetsmyndigheten.se/Publikationer/Rapport/Stralskydd/2002/200216/

Boice JD Jr, Tarone RE. 2011. Cell phones, cancer, and children. Journal of the National Cancer Institute 103(16):1211-1213.

Cardis E, Deltour I, Mann S, Moissonnier M, Taki M, Varsier N, et al. 2008. Distribution of RF energy emitted by mobile phones in anatomical structures of the brain. Physics in Medicine and Biology 53(11):2771–2783.

Cardis E, Armstrong BK, Bowman JD, Giles GG, Hours M, Krewski D, et al. 2011. Risk of brain tumours in relation to estimated RF dose from mobile phones: results from five Interphone countries. Occupational and Environmental Medicine 68(9):631-640.

Carlberg M, Hardell L. 2012. On the association between glioma, wireless phones, heredity and ionising radiation. Pathophysiology 19(4):243-252.

Christ A, Gosselin MC, Christopoulou M, Kühn S, Kuster N. 2010. Age-dependent tissue-specific exposure of cell phone users. Physics in Medicine and Biology 5(7):1767-1783.

Christensen HC, Schüz J, Kosteljanetz M, Poulsen HS, Boice JD Jr, McLaughlin JK, et al. 2005. Cellular telephones and risk for brain tumors: a population-based, incident case-control study. Neurology 64(7):1189-1195.

Davis DL, Miller AB, Philips A. 2012. Association of mobile phone use with adult brain cancer remains plausible. BMJ 344:e3083.

Dalton SO, Steding-Jessen M, Gislum M, Frederiksen K, Engholm G, Schüz J. 2008. Social inequality and incidence of and survival from cancer in a population-based study in Denmark, 1994-2003: Background, aims, material and methods. European Journal of Cancer 44(14):1938-1949.

Deltour I, Auvinen A, Feychting M, Johansen C, Klaeboe L, Sankila R, et al. 2012. Mobile phone use and incidence of glioma in the Nordic countries 1997-2008: Consistency Check. Epidemiology 23(2):301-307.

de Vocht F, Burstyn I, Cherrie JW. 2011. Time trends (1998-2007) in brain cancer incidence rates in relation to mobile phone use in England. Bioelectromagnetics 32(5):334-339.

Ding L-X, Wang Y-X. 2011. Increasing incidence of brain and nervous tumours in urban Shanghai, China, 1983-2007. Asian Pacific Journal of Cancer Prevention 12(12):3319-3322.

Dobes M, Shadbolt B, Khurana VG, Jain S, Smith SF, Smee R, et al. 2011. A multicenter study of primary brain tumor incidence in Australia (2000-2008). Neuro-Oncology 13(7):783-790.

Dosenbach NU, Nardos B, Cohen AL, Fair DA, Power JD, Church JA, et al. 2010. Prediction of individual brain maturity using fMRI. Science 329(5997):1358-1361.

Frei P, Poulsen AH, Johansen C, Olsen JH, Steding-Jessen M, Schüz J. 2011. Use of mobile phones and risk of brain tumours: update of Danish cohort study. BMJ 343:d6387.

Gandhi OP, Morgan LL, de Salles AA, Han YY, Herberman RB, Davis, DL. 2012. Exposure limits: the underestimation of absorbed cell phone radiation, especially in children. Electromagnetic Biology and Medicine 31(1):34-51.

Hardell L, Näsman Å, Påhlson A, Hallquist A, Hansson Mild K. 1999. Use of cellular telephones and the risk for brain tumours: A case-control study. International Journal of Oncology 15(1):113-116.

Hardell L, Hansson Mild K, Påhlson A, Hallquist A. 2001. Ionizing radiation, cellular telephones and the risk for brain tumours. European Journal of Cancer Prevention 10(6):523-529.

Hardell L, Hallquist A, Hansson Mild K, Carlberg M, Påhlson A, Lilja A. 2002. Cellular and cordless telephones and the risk for brain tumours. European Journal of Cancer Prevention 11(4) 377-386.

Hardell L, Hansson Mild K, Carlberg M. 2003. Further aspects on cellular and cordless telephones and brain tumours. International Journal of Oncology 22(2):399-407.

Hardell L, Hansson Mild K, Carlberg M, Hallquist A. 2004. Cellular and cordless telephone use and the association with brain tumors in different age groups. Archives of Environmental Health 59(3):132-137.

Hardell, L, Carlberg M, Hansson Mild K. 2005. Case-control study on cellular and cordless telephones and the risk for acoustic neuroma or meningioma in patients diagnosed 2000-2003. Neuroepidemiology 25(3):120-128.

Hardell L, Carlberg M, Hansson Mild K. 2006a. Case-control study of the association between the use of cellular and cordless telephones and malignant brain tumors diagnosed during 2000-2003. Environmental Research 100(2):232-241.

Hardell L, Carlberg M, Hansson Mild K. 2006b. Pooled analysis of two case-control studies on use of cellular and cordless telephones and the risk for malignant brain tumours diagnosed in 1997-2003. International Archives of Occupational and Environmental Health 79(8):630-639.

Hardell L, Carlberg M, Hansson Mild K. 2006c. Pooled analysis of two case-control studies on the use of cellular and cordless telephones and the risk of benign brain tumours diagnosed during 1997-2003. International Journal of Oncology 28(2):509-518.

Hardell L, Carlberg M, Hansson Mild K. 2008. Methodological aspects of epidemiological studies on the use of mobile phones and their association with brain tumors. Open Environmental Sciences 2:54-61.

Hardell L, Carlberg M, Hansson Mild K. 2009. Epidemiological evidence for an association between use of wireless phones and tumor diseases. Pathophysiology 16(2-3):113-122.

Hardell L, Carlberg M. 2009. Mobile phones, cordless phones and the risk for brain tumours. International Journal of Oncology 35(1):5-17.

Hardell L, Carlberg M, Hansson Mild K. 2010. Mobile phone use and the risk for malignant brain tumors: a case-control study on deceased cases and controls. Neuroepidemiology. 35(2):109-114.

Hardell L, Carlberg M, Hansson Mild K. 2011a. Pooled analysis of case-control studies on malignant brain tumours and the use of mobile and cordless phones including living and deceased subjects. International Journal of Oncology 38(5):1465-1474.

Hardell L, Carlberg M, Hansson Mild K. 2011b. Re-analysis of risk for glioma in relation to mobile telephone use: comparison with the results of the Interphone international case-control study. International Journal of Epidemiology 40(4):1126-1128.

Hardell L, Carlberg M, Hansson Mild K. 2012. Use of mobile phones and cordless phones is associated with increased risk for glioma and acoustic neuroma. Pathophysiology. http://dx.doi.org/10.1016/j.pathophys.2012.11.001.

Hardell L, Carlberg M, Gee D. In press 2013. Mobile phone use and brain tumour risk: early warnings, early actions? In: Late Lessons from Early Warnings, part 2. European Environment Agency, Copenhagen, Denmark.

Hepworth SJ, Schoemaker MJ, Muir KR, Swerdlow AJ, van Tongeren MJ, McKinney PA. 2006. Mobile phone use and risk of glioma in adults: case-control study. BMJ 332(7546):883-887.

IARC. 2002. Non-Ionizing Radiation, Part 1: Static and Extremely Low-Frequency (ELF) Electric and Magnetic Fields. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 80, IARCPress, Lyon, France.

IARC. 2011. Non-Ionizing radiation, Part II: Radiofrequency Electromagnetic Fields [includes mobile telephones]. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 102, IARCPress, Lyon, France.

Interphone Study Group. 2010. Brain tumour risk in relation to mobile telephone use: results of the INTERPHONE international case-control study. International Journal of Epidemiology 39(3):675-694.

Interphone Study Group. 2011. Acoustic neuroma risk in relation to mobile telephone use: results of the INTERPHONE international case-control study. Cancer Epidemiology 35(5):453-464.

Johansen C, Boice J Jr, McLaughlin J, Olsen J. 2001. Cellular telephones and cancer--a nationwide cohort study in Denmark. Journal of the National Cancer Institute 93(3):203-207.

Karolinska Institute. 2011. Reassuring results from first study on young mobile users and cancer risk. (http://ki.se/ki/jsp/polopoly.jsp?d=130&a=125250&l=en&newsdep=130) accessed 5 August, 2012.

Kheifets L, Repacholi M, Saunders R, van Deventer E. 2005. The sensitivity of children to electromagnetic fields. Pediatrics 116(2):e303-313.

Kundi M. 2010. Essential problems in the interpretation of epidemiologic evidence for an association between mobile phone use and brain tumours. Comptes Rendus Physique 11(9-10):556-563.

Kundi M. 2012. Study of mobile phone use and glioma risk was fatally flawed. BMJ. 344:e3078.

Larjavaara S, Schüz J, Swerdlow A, Feychting M, Johansen C, Lagorio S, et al. 2011. Location of gliomas in relation to mobile telephone use: a case-case and case-specular analysis. American Journal of Epidemiology 174(1):2-11.

Little MP, Rajaraman P, Curtis RE, Devesa SS, Inskip P, Check DP, et al. 2012. Mobile phone use and glioma risk: comparison of epidemiological study results with incidence trends in the United States. BMJ 344:e1147.

NORDCAN, (http://www-dep.iarc.fr/NORDCAN/english/frame.asp) accessed 5 August, 2012.

Preston-Martin S, Munir R, Chakrabarti I. 2006. Nervous system, in: D. Schottenfeld, J.F. Fraumeni Jr (Eds.), Cancer Epidemiology and Prevention, Oxford University Press, 1173-1195.

Saracci R, Samet J. 2010. Commentary: Call me on my mobile phone...or better not?--a look at the INTERPHONE study results. International Journal of Epidemiology 39(3):695-698.

Sato Y, Akiba S, Kubo O, Yamaguchi N. 2011. A case-case study of mobile phone use and acoustic neuroma risk in Japan. Bioelectromagnetics 32(2):85-93.

Scheurer ME, Etzel CJ, Liu M, Barnholtz-Sloan J, Wiklund F, Tavelin B, et al. 2010.Familial aggregation of glioma: a pooled analysis. American Journal of Epidemiology 172(10):1099-1107.

Schüz J, Jacobsen R, Olsen JH, Boice JD Jr, McLaughlin JK, Johansen C. 2006. Cellular telephone use and cancer risk: update of a nationwide Danish cohort. Journal of the National Cancer Institute 98(23):1707-1713.

Schüz J, Steding-Jessen M, Hansen S, Stangerup SE, Cayé-Thomasen P, Poulsen AH, et al. 2011a. Long-term mobile phone use and the risk of vestibular schwannoma: a Danish nationwide cohort study. American Journal of Epidemiology 174(4):416-422.

Sundhedsstyrelsen. Cancerregisteret 2010. (http://www.sst.dk/publ/Publ2011/DAF/Cancer/Cancerregisteret2010.pdf) accessed 5 August, 2012.

Swerdlow AJ, Feychting M, Green AC, Kheifets L, Savitz DA. 2011. International Commission for Non-Ionizing Radiation Protection Standing Committee on Epidemiology. Mobile phones, brain tumors, and the interphone study: where are we now? Environmental Health Perspectives 119(11):1534-1538.

Söderqvist F, Hardell L, Carlberg M, Hansson Mild K. 2007. Ownership and use of wireless telephones: a population-based study of Swedish children aged 7-14 years. BMC Public Health 7:105.

Söderqvist F, Carlberg M, Hardell L. 2008. Use of wireless telephones and self-reported health symptoms: a population-based study among Swedish adolescents aged 15-19 years. Environmental Health 7:18.

Söderqvist F, Carlberg M, Hansson Mild K, Hardell L. 2011. Childhood brain tumour risk and its association with wireless phones: a commentary. Environmental Health 10(1):106.

Söderqvist F, Carlberg M, Hardell L. 2012. Review of four publications on the Danish cohort study on mobile phone subscribers and risk of brain tumors. Reviews on Environmental Health. 27(1):51-58.

Takebayashi T, Akiba S, Kikuchi Y, Taki M, Wake K, Watanabe S, et al. 2006. Mobile phone use and acoustic neuroma risk in Japan. Occupational and Environmental Medicine 63(12):802-807.

Takebayashi T, Varsier N, Kikuchi Y, Wake K, Taki M, Watanabe S, et al. 2008. Mobile phone use, exposure to radiofrequency electromagnetic field, and brain tumour: a case-control study. British Journal of Cancer 98(3):652-659.

Zada G, Bond AE, Wang Y-P, Giannotta SL, Deapan D. 2012. Incidence trends in the anatomic location of primary malignant brain tumors in the United States: 1992-2006. World Neurosurgery 77(3-4):518-524.

Table 1. Summary of studies on the use of wireless phones and glioma risk

Study	Years Study Type	Age	Tumour type	No. of exposed cases	Odds ratio, 95 % confidence interval	Comments
Hardell et al (2006b, 2010, 2011a) Carlberg, Hardell (2012) Sweden	1997-2003 Case-control	20-80 years	Glioma (n=1148)	123	OR **2.5** (1.8-3.3)	>10 year latency, mobile phone
				57	OR **2.9** (1.8-4.7)	>10 year latency, mobile phone, *ipsilateral*, only living
				50	OR **2.6** (1.7-4.1)	>10 year latency, *mobile phone only*
				45	OR **1.7** (1.1-2.6)	>10 year latency, cordless phone
				20	OR **3.8** (1.8-8.1)	>10 year latency, cordless phone, *ipsilateral*, only living
				9	OR 1.2 (0.5-2.9)	>10 year latency, *cordless phone only;* >5-10 year latency OR **1.9** (1.3-2.9; n=55)
				150	OR **2.1** (1.6-2.8)	>10 year latency, wireless phone (mobile and cordless phone)
			Astrocytoma, high grade (n=820)	102	OR **3.0** (2.1-4.2)	>10 year latency, mobile phone
				47	OR **3.9** (2.3-6.6)	>10 year latency, mobile phone, ipsilateral, only living
				37	OR **2.8** (1.7-4.6)	>10 year latency, *mobile phone only*
				36	OR **2.0** (1.2-3.2)	>10 year latency, cordless phone
				15	OR **5.5** (2.3-13)	>10 year latency, cordless phone, ipsilateral, only living
				6	OR 0.9 (0.3-2.6)	>10 year latency, *cordless phone only;* >5-10 year latency OR **2.4** (1.6-3,7; n=44)
				121	OR **2.5** (1.8-3.4)	>10 year latency, wireless phone (mobile and cordless phone)

Table 1. cont.

Study	Years Study Type	Age	Tumour type	No. of exposed cases	Odds ratio, 95 % confidence interval	Comments
Interphone Study Group (2010) 13 countries; Australia, Canada, Denmark, Finland, France, UK, Germany, Israel, Italy, Japan, New Zealand, Norway, Sweden	2000-2004, 2-4 years depending on study region. Case-control	30-59 years	Glioma (n=2708)	1666	OR **0.81** (0.70-0.94)	Regular use of mobile phone in the past ≥1 year
				210	OR **1.40** (1.03-1.89)	Cumulative hours mobile phone ≥ 1640 hours
				78	OR **1.87** (1.09-3.22)	Cumulative hours mobile phone ≥ 1640 hours, tumors in *temporal lobe*
				100	OR **1.96** (1.22-3.16)	Cumulative hours mobile phone ≥ 1640 hours, *ipsilateral* mobile phone use
Interphone Study Group (2010) Appendix 2			Glioma (n=1211)	460	OR **1.68** (1.16-2.41)	Restricted to *ever regular use* time since start 2-4 years; 1-1.9 years as reference entity
				468	OR **1.54** (1.06-2.22)	Restricted to *ever regular use* time since start 5-9 years; 1-1.9 years as reference entity
				190	OR **2.18** (1.43-3.31)	Restricted to *ever regular use* time since start 10+ years; 1-1.9 years as reference entity
				160	OR **1.82** (1.15-2.89)	Restricted to *ever regular use* ≥1640 hours, <5 hours as reference entity

Table 2. Summary of studies on the use of wireless phones and meningioma risk

Study	Years Study Type	Age	Tumour type	No. of exposed cases	Odds ratio, 95 % confidence interval	Comments
Hardell et al (2006c), Hardell, Carlberg (2009) Sweden	1997-2003 Case-control	20-80 years	Meningioma (n=916)	347	OR 1.1 (0.9-1.3)	> 1 year latency, mobile phone use
				38	OR 1.5 (0.98-2.4)	> 10 years latency of mobile phone use
				18	OR 1.6 (0.9-2.9)	> 10 years latency of ipsilateral mobile phone use
				294	OR 1.1 (0.9-1.4)	> 1 year latency, cordless phone
				23	OR 1.8 (1.01-3.2)	> 10 years latency of cordless phone use
				11	OR **3.0** (1.3-7.2)	> 10 years latency of ipsilateral cordless phone use
Interphone Study Group (2010) 13 countries; Australia, Canada, Denmark, Finland, France, UK, Germany, Israel, Italy, Japan, New Zealand, Norway, Sweden	2000-2004, 2-4 years depending on study region. Case-control	30-59 years	Meningioma (n=2409)	1262	OR **0.79** (0.68-0.91)	Regular use of mobile phone in the past ≥1 year
				130	OR 1.15 (0.81-1.62)	Cumulative hours mobile phone ≥ 1640 hours
				21	OR 0.94 (0.31-2.86)	Cumulative hours mobile phone ≥ 1640 hours, tumors in *temporal lobe*
				46	OR 1.45 (0.80-2.61)	Cumulative hours mobile phone ≥ 1640 hours, *ipsilateral* mobile phone use

Table 2. cont.

Study	Years Study Type	Age	Tumour type	No. of exposed cases	Odds ratio, 95 % confidence interval	Comments
Interphone (2010) Appendix 2	2000-2004, 2-4 years depending on study region. Case-control	30-59 years	Meningioma (n=842)	362	OR 0.90 (0.62-1.31)	Restricted to *ever regular use* time since start 2-4 years; 1-1.9 years as reference entity
				288	OR 0.75 (0.51-1.10)	Restricted to *ever regular use* time since start 5-9 years; 1-1.9 years as reference entity
				76	OR 0.86 (0.51-1.43)	Restricted to *ever regular use* time since start 10+ years; 1-1.9 years as reference entity
				96	OR 1.10 (0.65-1.85)	Restricted to *ever regular use* $\geq$1640 hours, <5 hours as reference entity

Table 3. Summary of studies on the use of wireless phones and acoustic neuroma risk

Study	Years Study Type	Age	Tumour type	No. of exposed cases	Odds ratio, 95 % confidence interval	Comments
Hardell et al (2006c), Hardell, Carlberg (2009) Sweden	1997-2003 Case-control	20-80 years	Acoustic neuroma (n=243)	130	OR **1.7** (1.2-2.3)	> 1 year latency of mobile phone use
				20	OR **2.9** (1.6-5.5)	> 10 years latency of mobile phone use
				13	OR **3.0** (1.4-6.2)	> 10 years of *ipsilateral* mobile phone use
				4	OR 1.3 (0.4-3.8)	> 10 years latency of cordless phone use
				3	OR 2.3 (0.6-8.8)	> 10 years latency of *ipsilateral* cordless phone use
Sato et al (2011) Japan	2000-2006 Case-case	All ages	Acoustic neuroma (n=787)	97	RR 1.08 (0.93-1.28)	Mobile phone, reference date 1 year before diagnosis, *ipsilateral*
				86	RR 1.14 (0.96-1.40)	Mobile phone, reference date 5 years before diagnosis, *ipsilateral*
				18	RR **2.74** (1.18-7.85)	Mobile phone, reference date 1 year before diagnosis, average daily call duration >20 min, *ipsilateral*
				28	RR **3.08** (1.47-7.41)	Mobile phone, reference date 5 years before diagnosis, average daily call duration >20 min, *ipsilateral*
				45	RR 0.93 (0.79-1.14)	Cordless phone, reference date 1 year before diagnosis, *ipsilateral;* mobile phone non-users
				125	RR 1.02 (0.91-1.17)	Cordless phone, reference date 5 years before diagnosis, *ipsilateral;* mobile phone non-users

Table 3 cont.

Study	Years Study Type	Age	Tumour type	No. of exposed cases	Odds ratio, 95 % confidence interval	Comments
Interphone Study Group (2011) 13 countries; Australia, Canada, Denmark, Finland, France, UK, Germany, Israel, Italy, Japan, New Zealand, Norway, Sweden	2000-2004, 2-4 years depending on study region. Case-control	30-59 years	Acoustic neuroma (n=1105)	643	OR 0.85 (0.69-1.04)	Mobile phone regular use up to 1 year before reference date
				304	OR 0.95 (0.77-1.17)	Mobile phone regular use up to 5 years before reference date
				77	OR 1.32 (0.88-1.97)	Cumulative hours mobile phone $\geq$ 1640 hours up to 1 year before reference date
				36	OR **2.79** (1.51-5.16)	Cumulative hours mobile phone $\geq$ 1640 hours up to 5 years before reference date
				47	OR **2.33** (1.23-4.40)	Cumulative hours mobile phone $\geq$ 1640 hours up to 1 year before reference date; *ipsilateral* use
				27	OR **3.53** (1.59-7.82)	Cumulative hours mobile phone $\geq$ 1640 hours up to 5 years before reference date; *ipsilateral* use
				37	OR **1.93** (1.10-3.38)	Cumulative hours mobile phone $\geq$ 1640 hours in the past start $\geq$10 years before reference date
				28	OR **3.74** (1.58-8.83)	Cumulative hours mobile phone $\geq$ 1640 hours in the past start $\geq$10 years before reference date, *ipsilateral*
				225	OR 1.41 (0.82-2.40)	Restricted to *ever regular use* time since start 2-4 years; 1-1.9 years as reference entity
				209	OR 1.38 (0.80-2.39)	Restricted to *ever regular use* time since start 5-9 years; 1-1.9 years as reference entity
				64	OR 1.08 (0.58-2.04)	Restricted to *ever regular use* time since start 10+ years; 1-1.9 years as reference entity
				72	OR 1.74 (0.90-3.36)	Restricted to *ever regular use* $\geq$1640 hours, <5 hours as reference entity

Table 4. Odds ratio (OR) and 95 % confidence interval (CI) for glioma, meningioma and acoustic neuroma in different age groups for first use of the wireless phone (Hardell et al 2006b,c, 2010, 2011a). Numbers of exposed cases (Ca) and controls (Co) are given. Adjustment was made for age, gender, SEI-code, year of diagnosis. For glioma adjustment was also made for vital status.

	Glioma (n=1148)		Meningioma (n=916)		Acoustic neuroma (n=243)	
	Ca/Co	OR, CI	Ca/Co	OR, CI	Ca/Co	OR, CI
Mobile phone	529/963	**1.3** (1.1-1.6)	347/900	1.1 (0.9-1.3)	130/900	**1.7** (1.2-2.3)
< 20 years old	17/14	**3.1** (1.4-6.7)	5/14	1.9 (0.6-5.6)	5/14	**5.0** (1.5-16)
20-49 years old	315/581	**1.4** (1.1-1.7)	210/555	1.3 (0.99-1.6)	86/555	**2.0** (1.3-2.9)
≥ 50 years old	197/368	**1.3** (1.01-1.6)	132/331	1.0 (0.8-1.3)	39/331	1.4 (0.9-2.2)
Cordless phone	402/762	**1.3** (1.1-1.6)	294/701	1.1 (0.9-1.4)	96/701	**1.5** (1.04-2.0)
< 20 years old	16/16	**2.6** (1.2-5.5)	2/16	0.5 (0.1-2.2)	1/16	0.7 (0.1-5.9)
20-49 years old	206/437	1.2 (0.9-1.5)	167/416	1.3 (0.98-1.6)	65/416	**1.7** (1.1-2.5)
≥ 50 years old	180/309	**1.4** (1.1-1.7)	125/269	1.1 (0.8-1.4)	30/269	1.3 (0.8-2.1)

Table 5. Gender and age distribution for use of mobile phones among cases aged 20-80 years in the Hardell group studies. Glioma (n=1148).

Age, diagnosis	Men		Women		Total	
	No use/≤1 year latency, mobile phones	Use >1 year latency, mobile phones	No use/≤1 year latency, mobile phones	Use >1 year latency, mobile phones	No use/≤1 year latency, mobile phones	Use >1 year latency, mobile phones
20-24	8	7 (47 %)	3	8 (73 %)	11	15 (58 %)
25-29	10	15 (60 %)	5	10 (67 %)	15	25 (63 %)
30-34	11	26 (70 %)	19	8 (30 %)	30	34 (53 %)
35-39	9	23 (72 %)	8	13 (62 %)	17	36 (68 %)
40-44	10	26 (72 %)	16	11 (41 %)	26	37 (59 %)
45-49	14	37 (73 %)	12	16 (57 %)	26	53 (67 %)
50-54	22	61 (73 %)	26	27 (51 %)	48	88 (65 %)
55-59	35	65 (65 %)	59	20 (25 %)	94	85 (47 %)
60-64	41	51 (55 %)	53	15 (22 %)	94	66 (41 %)
65-69	55	46 (46 %)	57	13 (19 %)	112	59 (35 %)
70-74	43	16 (27 %)	41	5 (11 %)	84	21 (20 %)
75-80	27	8 (23 %)	35	2 (5 %)	62	10 (14 %)
All	**285**	**381 (57 %)**	**334**	**148 (31 %)**	**619**	**529 (46 %)**

Evidence for Brain Tumors
(Epidemiological)
Supplement 2012

Prof. Michael Kundi, PhD med habil
Head: Institute of Environmental Health,
Medical University of Vienna
Vienna, Austria

Prepared for the BioInitiative Working Group

September 2012

I. INTRODUCTION

Primary central nervous system (CNS) tumors are a heterogeneous group of benign and malignant neoplasms localized in the brain, the spinal cord and their coverings. They differ in histological type, tissue of origin, anatomic site, growth pattern, age distribution, sex ratio, clinical appearance and many other features including molecular neuropathological markers. These features are not independent but little is known about the etiology of these tumors and the reason for the observed epidemiological patterns. The rapidly developing field of molecular neuropathology may provide clues to solve these problems in the future.

Annually about 57,000 new cases of CNS tumors are diagnosed in the US. The age distribution has two peaks: incidence is about 4.7 cases per 100,000 per year below 10 years of age (which is mainly due to astrocytoma of the juvenile pilocytic type, malignant glioma, medulloblastoma and tumors originating from mesodermal and embryonic tissues), and after age 15 there is a steady increase of incidence with increasing age reaching its second peak of about 68 cases per 100,000 per year at an age around 75 to 80 years (CBTRUS, 2011). The burden of CNS cancers is distinctly higher in children making up around 20% of all childhood malignancies, while in adults less than 2% of all cancers are primary brain cancers.

There are some rare cases of inherited cancer syndromes (e.g. von Hippel-Lindau disease, Li-Fraumeni syndrome) that are related to brain tumor risk, accounting for a small fraction of cases. Except for therapeutic x-rays no environmental or lifestyle factor has unequivocally been established as risk factor for brain tumors. Non-whites seem to have lower risk, and incidence tends to be higher with increasing socio-economic status. However, because of the rather advanced age of 75-80 years of peak incidence, such differences may partly be due to differences in life-expectancy. During the last decades of the 20th century some types of brain tumors show a steady increase of a few percent per year, which might to some extent be related to the introduction of computed tomography and other high-resolution neuroimaging methods. For most CNS tumors except meningioma and pituitary tumors the incidence is higher in males than females.

Since the report of Wertheimer and Leeper in 1979 of an increased incidence of brain tumors in children living in homes with an expected higher exposure to power-frequency electric and magnetic fields, exposure to electromagnetic fields have become an area of interest in the study of factors affecting brain tumor risk.

This review focuses on the radio frequency (RF) part of the electromagnetic spectrum (3 kHz to 300 GHz). However, because the epidemiology of mobile phone use is covered in another section, it will be restricted to RF exposure conditions other than microwaves from mobile phone use. Exposure to ELF magnetic fields and childhood brain tumors is covered in the chapter about childhood cancers.

II.   MATERIAL AND METHODS

Published articles of relevant studies restricted to the years 1987 to 2012 were obtained by searching PubMed using the following terms:

("radio frequency" OR electromagnetic* OR microwaves) AND ("brain cancer" OR brain tumor* OR "CNS cancer" OR CNS tumor* OR glioma* OR meningioma* OR neuroma*) NOT ("power frequency" OR "low frequency") AND epidemiolog*

The search resulted in 137 hits. After removing reviews and animal or in vitro studies as well as studies of mobile phone use, 10 articles remained. A hand search in review papers (Krewski et al. 2001; Elwood 2003; Ahlbom et al. 2004; Kundi et al. 2004) and reference lists of the articles found in PubMed revealed another 9 papers; hence the final body of evidence consists of 19 studies of exposure to various types of RF fields.

Of the 19 studies 8 were cohort studies, 5 case-control studies and 6 of an ecological type. The majority of studies (11) were occupational studies, four studies investigated children, and one ecological study investigated both, adults and children.

III.   EPIDEMIOLOGICAL STUDIES OF RF FIELDS AND BRAIN TUMORS

Table 10A-1 gives an overview of the 17 studies obtained by the literature search with respect to study type, assessment of exposure and outcome, confounders considered and matching variables used, number of cases included and selection method of study participants. Results are summarized in Table 10A-2.

Table 10A- 1: Synopsis of epidemiologic studies of or including brain tumors (1987 – 2007)

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
Thomas et al. 1987	Northern New Jersey, Philadelphia, gulf coast of Lousiana/1979-1981/Case-control	Interviews with next-of-kin about occupational history – response rates: cases 74%, controls 63%; JEM (2 methods)	Death certificates verified through review of hospital records	age(m), (only males), year of death(m), area of residence(m), educational level, (lead, soldering fumes)	435/386	Cases: deaths of brain tumor or CNS tumors of white males (age>30) from death certificates. Controls: deaths from other causes than brain tumors, epilepsy, etc.
Milham 1988	Washington, California/1979-1984/Cohort	Amateur radio operator license within 1/1979 to 6/1984	Mortality records	age, (only males), race, year of death	29	67829 operators, search of deaths in state registry through 1984
Selvin et al. 1992	San Francisco/1973-1988/Spatial cluster	Distance of center of census tract to microwave tower (Sutro tower)	SEER records	-	35	Search of cancer deaths of white individuals (age<21)

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
Tynes et al. 1992	Norway/1961-1985 /Occupational cohort	Job title in 1960 and 1970 censuses and expert categorization	Cancer registry	age, (only males)	119 overall, 6 in subgroup with possible RF exposure	Cohort of 37945 male workers identified that had jobs in 1960 with possible EMF exposure. among these 3017 with possible RF exposure
Grayson 1996	US Air Force/1970-1989/Nested case-control	Detailed job history and classification based on JEM (RF/MW exposure from frequent measurements)	Screening of hospital discharge records	age(m), race(m), military rank, (ELF and ionizing radiation exposure)	230/920	Cohort of ~880000 US Air Force members with at least one completed year of service within the study period, no follow up after subjects left service
Szmigielski 1996	Poland (military)/1971-1985/Occupational cohort	Allocation to RF/MW exposure group based on service records, documented measurements of military safety groups	Incident cases from central and regional military hospitals and military health departments	age, (only males)	~46	Annual number of ~127800 military career personnel, ~3720 RF/MW exposed per year

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
Hocking et al. 1996	Sydney (Australia)/ 1972-1990/Ecological	Municipalities within ~4 km of 3 TV broadcasting towers considered higher exposed as compared to 6 further away	Incident and death cases from cancer registry	age, sex, calendar period	740 (incident) 606 (mortality) 64 age<15 (incident) 30 age<15 (mortality)	Study population: inner area ~135000, outer area ~450000
Tynes et al. 1996	Norway/1961-1991/ Occupational cohort	Certified radio and telegraph operators 1920-1980 (98% worked on merchant ships); spot measurements on ships with old-fashioned equipment	Cancer registry	age, (only females)	5	2619 women certified as radio or telegraph operators by Norwegian Telecom
Dolk et al. 1997a	Birmingham (GB)/ 1974-1986/Ecological	Living near a TV/FM radio transmitter (Sutton Coldfield)	Cancer registry	age, sex, calendar year, SES	332	Population (age≥15) ~408000 within 10 km of the transmitter
Dolk et al. 1997b	GB/1974-1986/	Living near a	Cancer registry	age, sex,	244	Population (age<15)

6

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
	Ecological	high power (≥500 kW erp) transmitter (overall 21)		calendar year, SES		within 10 km of one of 20 high power transmitters
Lagorio et al. 1997	Italy/1962-1992/ Occupational cohort	Working as RF heat-sealer operator	Cancer deaths from registry	age, (only females), calendar period, region	1	302 women employed 1962-1992 in a plastic-ware manufacturing plant as RF sealers
Finkelstein 1998	Ontario (Canada)/ 1964-1995/ Occupational cohort	Working as a police officer (possible handheld radar exposure)	Cancer registry	age, (only males), calendar year	16	20601 male officers of Ontario Police
Morgan et al. 2000	USA/1976-1996/ Occupational cohort	Jobs classified according to work with RF emitting devices with different output power	Death certificates from states' statistics offices	age, sex, period of hire	51	All U.S. Motorola employees with at least 1 day employment 1976-1996 (195775 workers, 2,7 million person-years)

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
Groves et al. 2002	USA/1950-1997/ Occupational cohort	6 occupational groups 3 with assumed low radar exposure (radar-, radio operator, aviation electrician's mate) and 3 with assumed high exposure (aviation electronics -, electronics -, fire control technician)	Death certificate from a state vital statistics office or National Death Index Plus	age at entry, (only males), attained age	88	40581 Navy Korean War veterans graduated 1950-54 from Navy technical schools; follow-up from graduation through 1997
Ha et al. 2003	South Korea/1993-1996/Ecological	Area <2 km around 11 high power and 31 low power AM radio transmitter and control areas >2 km from any transmitter	Cancer cases from insurance records	age, sex (direct and indirect standardization)	45/not specified	Census and residents registration data 1995 (population size between 3152 and 126523 at the different sites)
Park et al. 2004	South Korea/1994-1995/Ecological	10 areas with a AM radio transmitter ≥100kW	Cancer deaths from death certificates	age, sex (direct standardization)	30/100	Census data from 1990

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
Berg et al. 2006	Germany/2000-2003/ Case-control	JEM from occupational history collected in interview	Histological verified cases of glioma and meningioma	age(m), sex(m), region(m), SES, urban/rural, smoking, ionizing rad. exposure	Glioma 366/732 Meningioma 381/762	All histological confirmed cases of glioma and meningioma from 4 neurosurgical clinics (age: 30-69) (part.rate 84%); frequency matched controls from population registry (part.rate 63%)
Schüz et al. 2006	Germany/2000-2003/ Case-control	Questionnaire about DECT cordless phone base station near the bed	Histological verified cases of glioma and meningioma	age(m), sex(m), region(m), SES, urban/rural, smoking, ionizing rad. exposure	Glioma 366/732 Meningioma 381/762	All histological confirmed cases of glioma and meningioma from 4 neurosurgical clinics (age: 30-69) (part.rate 84%); frequency matched controls from population registry (part.rate 63%)
Ha et al. 2007	South Korea/1993-1999/Case-control	Distance from 31 AM radio transmitters and 49 radio antennas, measurements and calculation of	Cases of brain cancer from verified by entry into cancer registry	age(m), sex(m), year of diagnosis(m), SES, population density	956/1020	All cases of brain cancer (age<15) from 14 hospitals and matched hospital controls with respiratory diseases

9

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
		total RF electric field strength				

SES…socio-economic status, JEM…job exposure matrix, erp…equivalent radiation power, RF/MW…radio frequency/microwaves, CNS…central nervous system, ELF…extremely low frequency

Table 10A- 2: Synopsis of main results of brain tumor studies (1987 – 2007)

Study	Endpoint	Exposure category	Meas.	Outcome [95% CI]
Thomas et al. 1987	Brain tumor deaths (ICD not specified)	Ever exposed to RF	OR	1.6 [1.0 – 2.4]
		Electrical/electronics job	OR	2.3 [1.3 – 4.2]
		Unexposed*		
		Ever exposed < 5 y	OR	1.0
		5-19 y	OR	2.3
		20+ y	OR	2.0
Milham 1988	Brain cancer deaths (ICD-8: 191)	All	SMR	1.39 [0.93 – 2.00]
		Novice[a]	SMR	0.34
		Technician	SMR	1.12
		General	SMR	1.75
		Advanced	SMR	1.74
		Extra	SMR	1.14
Selvin et al. 1992	Brain cancer deaths (ICD-O: 191.2)	> 3.5 km distance from tower*		
		≤ 3.5 km[b]	RR	1.16 [0.60 – 2.26]
Tynes et al. 1992	Incident brain cancer (ICD-7: 193)	All with possible EMF exposure	SIR	1.09 [0.90 – 1.41]
		Subgroup possible RF exposure[c]	SIR	0.49 [0.18 – 1.06]
Grayson 1996	Incident brain cancer (ICD-9: 191)	Never RF/MW exposed*		
		Ever exposed	OR	1.39 [1.01 – 1.90]
Szmigielski 1996	Incident nervous system & brain tumors	RF/MW exposed	OER	1.91 [1.08 – 3.47]
Hocking et al. 1996	Brain cancer (ICD-9: 191)	Outer area*		
		Inner area (incident, overall)	RR	0.89 [0.71 – 1.11]
		Inner area (mortality, overall)	RR	0.82 [0.63 – 1.07]
		Inner area (incident, age<15)	RR	1.10 [0.59 – 2.06]
		Inner area (mortality, age<15)	RR	0.73 [0.26 – 2.10]
Tynes et al. 1996	Incident brain cancer (ICD-7: 193)	All	SIR	1.0 [0.3 – 2.3]
Dolk et al. 1997a	Incident brain tumors (ICD-8/9: 191, 192)	0-2 km from transmitter	OER	1.29 [0.80 – 2.06]
		0-10 km from transmitter	OER	1.04 [0.94 – 1.16]
Dolk et al. 1997b	Incident brain tumors (ICD-8/9: 191, 192)	0-2 km from transmitter	OER	0.62 [0.17 – 1.59]
		0-10 km from transmitter	OER	1.06 [0.93 – 1.20]

Study	Endpoint	Exposure category	Meas.	Outcome [95% CI]
Lagorio et al. 1997	Brain cancer deaths (ICD-9: 191)	RF sealer operator	OER	1 : 0.1
Finkelstein 1998	Incident brain cancer (ICD-9: 191)	All police officers	SIR	0.84 [0.48 – 1.36]
Morgan et al. 2000	Incident brain cancer (ICD-9: 191)	No RF exposure*		
		Low[d]	RR	0.92 [0.43 – 1.77]
		Moderate	RR	1.18 [0.36 – 2.92]
		High	RR	1.07 [0.32 – 2.66]
Groves et al. 2002	Brain cancer deaths (ICD-9: 191)	Low radar exposure*		
		High radar exposure	RR	0.65 [0.43 – 1.01]
Ha et al. 2003	Brain cancer (ICD-10:C70-C72)	Low power transmitters*		
		High power transmitters	SIR	1.8 [0.8 – 11.1]
		Control sites (>2 km)*		
		100 kW transmitter	OER	2.27 [1.30 – 3.67]
		250 kW	OER	0.86 [0.41 – 1.59]
		500 kW	OER	1.47 [0.84 – 2.38]
		1500 kW	OER	2.19 [0.45 – 6.39]
Park et al. 2004	Brain cancer deaths (ICD-10:C69-C72)	Control area*		
		≥100 kW transmitter	SMR	1.52 [0.61 – 3.75]
Berg et al. 2006	Incident glioma (ICD-O3: C71)	No occup. RF/MW exposure*		
		Probably no exposure	OR	0.84 [0.48 – 1.46]
		Probable exposure	OR	0.84 [0.46 – 1.56]
		High exposure	OR	1.22 [0.69 – 2.15]
		No high exposure*		
		High exposure <10 y	OR	1.11 [0.48 – 2.56]
		High exposure ≥ 10 y	OR	1.39 [0.67 – 2.88]
	Incident meningioma (ICD-O3: C70.0)	No occup. RF/MW exposure*		
		Probably no exposure	OR	1.11 [0.57 – 2.15]
		Probable exposure	OR	1.01 [0.52 – 1.93]
		High exposure	OR	1.34 [0.61 – 2.96]
		No high exposure*		
		High exposure <10 y	OR	1.15 [0.37 – 3.48]

Study	Endpoint	Exposure category	Meas.	Outcome [95% CI]
		High exposure ≥ 10 y	OR	1.55 [0.52 – 4.62]
Schüz et al. 2006	Incident glioma (ICD-O3: C71)	DECT near bed	OR	0.82 [0.29 – 2.33]
	Incident meningioma (ICD-O3: C70.0)	DECT near bed	OR	0.83 [0.29 – 2.36]
Ha et al. 2007	All brain cancers (ICD-10: C70-C72)	≤2 km	OR	1.42 [0.38 – 5.28]
		2-4 km	OR	1.40 [0.77 – 2.56]
		4-6 km	OR	1.02 [0.66 – 1.57]
		6-8 km	OR	1.08 [0.73 – 1.59]
		8-10 km	OR	0.94 [0.67 – 1.33]
		10-20 km	OR	1.01 [0.77 – 1.34]
		>20 km*		

* Reference

[a] From Milham 1988b, license classes as proxy for exposure duration

[b] Based on the assumption that exposure is higher near the microwave tower

[c] Computed based on Table 5 in Tynes et al. 1992

[d] Classification according to power output of equipment used for longest period of employment

OR…odds-ratio, SIR…standardized incidence ratio, SMR…standardized mortality ratio, RR…relative risk (rate ratio), OER…observed/expected ratio

In the following paragraphs each study is briefly discussed with respect to its strengths and weaknesses.

A.   Thomas et al. 1987

This case-control study included 435 deaths from brain or CNS tumors and 386 deaths from other causes as controls. Only adult males were included. Basis of data collection on occupational history were interviews with next-of-kin. Two methods of classification were used: one method assigned subjects to one of three categories (never exposed to RF/ever exposed to RF in an electrical or electronics job/ever exposed to RF but not in an electrical or electronics job), the other method consisted of a classification of each job by an industrial hygienist for presumed exposure to RF, soldering fumes, and lead. Both methods revealed significantly increased brain tumor risks of presumed occupational exposure to RF fields. This increase was due to an association in electronics and electrical jobs with astrocytic tumors as the predominant outcome associated with employment in these categories. In addition a significant increase of brain tumor risk was found for increasing duration of exposure.

Although relying on information of next-of-kin could be a source of misclassification, one strength of this study is it's relying on occupational history only that could be assumed to be more accurate than recall of exposure to various agents. The two methods of classification led to almost the same results, which lends support to the hypothesis that indeed exposure in electrical and electronics jobs is associated with an increased brain tumor risk. Due to the relationship between RF exposure and exposure to lead, solvents or soldering fumes in these jobs, it is not possible to separate effects of these exposures. Soldering fumes were never investigates with respect to brain tumors, and the hypothesis of an association with sinonasal cancer could not be corroborated so far. However, analysis of exposure to lead did not show a consistent relationship with brain tumor risk, indicating that it may not confound the relationship to RF exposure.

Because this study is of dead cases only it is likely over-representing high grade brain tumors that may not all be associated with exposure leading to an effect dilution. Exposure misclassification, if it is non-differential in cases and controls, also reduces effect estimates.

A weakness of this study is obviously its lack of an exposure indicator other than the occupational category. While there is no doubt that in these jobs some exposure to RF fields occur quite regularly, specific characteristics including frequency ranges, modulation, intensity, duration and distance from the source vary considerably. Overall the study (as well as two earlier ones outside the search window: Lin et al. 1985 and Milham 1985) are sufficient to formulate a research hypothesis that can be tested in appropriately designed subsequent investigations. Unfortunately such studies have never been conducted.

B. Milham 1988

In this cohort study of 67,829 amateur radio operators holding a license within 1/1979 to 6/1984 in Washington and California 29 brain tumor deaths occurred during the follow up period with 21 expected.

It should be noted that there was a substantial and statistically significant lower number of overall deaths of less than three quarters of deaths expected from country mortality rates. This could be due to both a 'healthy-worker' effect as well as an effect of socio-economic status. In lieu of computing standardized mortality ratios (SMR) it may be instructive to look at the proportional mortality rates in the reference population and the amateur radio operators: 0.6% of all deaths are expected to be due to brain tumors in the reference population while in amateur radio operators twice as many occurred (1.2%). Whether or not this is an indication of an increased brain tumor risk due to RF exposure is difficult to assess. First of all, this study is a register only investigation and no information on intensity, frequency and duration of engagement in amateur radio operations were available. In a later analysis the author reported about results using a proxy of intensity and duration of exposure: the license class. In this analysis indications of an increase of risk with increasing license class were obtained.

This study could and should have started off a thorough follow up of amateur radio operators and nested case-control studies to address the problem of potential confounders and to narrow down the conditions that may be responsible for the increased mortality from some cancers. It is another loose end that leaves us without a clear message.

Although no risk factor for brain cancer except therapeutic ionizing radiation is known, there are some indications that risk increases with social class. The reason for this association is unknown but life-style factors may play a role as well as concomitant causes of death that

could lead to a spurious reduction of risk in lower class populations because brain tumors have their peak close to life-expectancy.

C. Selvin et al. 1992

The objective of this investigation was not primarily to study the relationship between RF exposure and childhood cancer but to address the general problem of how to assess disease incidence or mortality in relation to a point source. As the point source the Sutro Tower in San Francisco, the only microwaves emitting tower in this county, was chosen. A total of 35 brain tumor deaths occurred among 50,686 white individuals at risk aged less than 21 in the years 1973-88 in an area of approximately 6 km around the tower. The exact location of residence could not be obtained; therefore each case was located in the center of the census tract. Different methods of analysis were applied to assess a potential relationship between distance from the tower and brain tumor risk. Relative risk for brain tumors for a distance less than 3.5 km from Sutro Tower compared to more than 3.5 km was 1.162 and not significant.

The study explored different methodological procedures and has its merits from a methodological point of view. However, it starts from the wrong assumption: that distance to a point source is a valid proxy for intensity of exposure. Under ideal conditions of spherical symmetry of an emission this assumption holds, however, there are almost no real life situations where this assumption is sufficiently close to actual exposure levels. And it is definitely not true for the Sutro Tower. Radiations from the antennae are directed towards the horizon and the complex pattern of emission with main and side lobes results in a complex pattern of RF exposure at ground level. Furthermore, the area is topographically structured with hills and valleys such that areas of high exposure at the vertices are in close proximity to areas of low exposure at the shadowed side downhill.

Studying the relationship between a point source and disease is not only difficult due to the complex relationship between distance and exposure but also because of the fact that humans are not stable at a certain location. This is of greater importance for adults who may commute from and to work places and have generally a greater radius of activity as compared to children. Nevertheless, there is at least a high chance of one long-lasting stable location that is when people sleep in their beds. Therefore, studies in relation to a point source should attempt to assess exposure at the location of the bed. Because the objective of this study was not the

assessment of a potential brain tumor risk but the application of methods for the analysis of spatial data, no attempts were made to measure actual exposure.

D.   Tynes et al. 1992

In this study information on occupations obtained for all Norwegians every 10 years was used to assess cancer incidence in relation to job titles. In 1960 37,945 male workers were identified that had jobs with possible exposure to EMFs and among these 3,017 with possible RF exposure. Overall 119 brain tumor cases were found in the cancer registry between 1961 and 1985. Of these cases 6 occurred in the subgroup of workers possibly exposed to RF fields. The overall expected number of brain tumor cases was 109 and 12 for the subgroup with possible RF exposure. Hence no increased brain tumor risk could be detected.

Despite the long follow-up period of 25 years with an accumulated number of 65,500 person-years the expected number of brain tumors diagnosed during that period is too low to detect a moderately elevated risk of 1.3 to 1.5. Furthermore, the follow up period just reaches the median induction period for brain tumors as delineated from studies on ionizing radiation.

As mentioned above, all studies solely relying on job titles lead to exposure misclassification and, therefore, to a dilution of risk. For dichotomous exposure variables (exposed/not exposed) and assuming a negligibly small proportion of exposed in the reference population standardized incidence ratios (SIR) are biased by a factor $(1+f*(SIR-1))/SIR$, if f denotes the fraction of true exposed and SIR is the true incidence ratio. Hence a true SIR of 2.0 is reduced to 1.5 if only 50% in the cohort are actually exposed. The observed SIR is further reduced if the assumption of a negligible fraction of exposed in the reference population is wrong. In this case the bias factor given above is further divided by $(1+g*(SIR-1))$, where g is the fraction of exposed in the general population.

While a cohort study that is based on registry data has the advantage of independence from recall errors and selection bias due to possible differential participation, it has the disadvantage that registry data are generally insufficient to provide reliable exposure indicators. While no association with brain tumors could be detected in this study it revealed an increased number of leukemia cases in occupations with possible RF exposure. This could

be due to the higher incidence of leukemia or to a stronger association or to the shorter latency and various other reasons including chance.

E. Grayson 1996

In this case-control study nested within approx. 880,000 US Air Force personnel with at least one years of service during the study period of 1970-89, primary malignant brain tumor cases were ascertained by screening hospital discharge records. The study included only males and only as long as they were on Air Force records. From 246 cases detected 16 were dropped due to incomplete or ambiguous data. For each case four controls were randomly selected from the case's risk set matching it exactly on year of birth and race. Controls that were diagnosed with diseases possibly associated with EMF exposure (leukemia, breast cancer, malignant melanoma) were excluded from the risk set.

A strength of this study is the detailed job history filed for each cohort member that could be used for retrospective exposure assessment. Furthermore, Air Force files contained detailed data from personal dosimetry on ionizing radiation for the different posts and jobs. Classification of RF field exposure was based on a detailed job exposure matrix with over 1,950 entries, indexing 552 different job titles. One source of classification was recorded events of exposure to RF fields above 100 W/m^2. By this method probable exposure was assigned if for a job such events were recorded in the past as well as for closely related jobs. Possible exposure was assigned for jobs that required operation of RF emitters but without recorded overexposure.

A further strength is the thorough consideration of possible confounders. Because of the possible relationship of brain tumor risk with socio-economic status (SES), military rank was used as a surrogate for SES and included in the analysis as well as ionizing radiation exposure that has previously been shown to increase brain tumor risk.

Exposure to RF fields was associated with a moderate but statistically significant increased risk of OR=1.39. Investigation of duration of exposure was compromised by an ambiguity introduced due to the calculation of an exposure score as the product of exposure and months. Nevertheless, for those ever exposed there were indications of an increasing risk with increasing exposure duration.

A weakness of this investigation is its incomplete follow-up of cohort members. This could have resulted in an underestimation of the true risk. Leaving the Air Force could have been more likely in those exposed to RF fields and developing a brain tumor. Some malignant brain tumors have early signs that could be incompatible with the Air Force job especially if involving operation of RF equipment (like seizures, severe headaches, somnolence, and absences). Because the study did not involve personal contact it is free of other selection biases.

F.   Szmigielski 1996

In this military cohort study of cancer morbidity Polish military career personnel was assessed for occupational exposure to RF fields based on service records. The study covered 15 years (1971-85) including approx. 128,000 persons per year. Expected rates for 12 cancer types were calculated based on the age specific morbidity in those classified as unexposed.

For brain and nervous system tumors a significantly increased ratio of observed to expected (OER=1.91) was found. Other malignancies with significantly increased incidence in exposed were: esophageal and stomach cancers, colorectal cancers, melanoma, and leukemia/lymphoma.

A strength of this study is its substantial size with almost 2 million person-years of follow-up. Furthermore, accurate military records on job assignment and on exposure from military safety groups gives a unique opportunity to assess long-term exposure effects based on already filed data.

Some important data are missing because they were military classified information that could not be provided in the paper. This includes the exact number of cases of the different neoplasms. However, from the data presented an observed number of brain tumors of about 46 can be calculated.

The study has been criticized for an alleged bias because more information on risk factors was available for cancer cases. It is true that military medical boards collected data for cases such as life style factors and exposure to possible carcinogens during service, however, at no stage this information entered the analysis. Therefore, this criticism is unfounded. Such information could have been utilized within a nested case-control study applying the same methods of assessment of risk factors for controls as has been done for cases. Because some findings,

such as the increased risk for esophagus/stomach cancer, that are rarely reported in relation to RF exposure warrant further study, such a nested case-control approach is recommended. It could, albeit with some difficulties, even be successfully conducted retrospectively.

G.   Hocking et al. 1996

In an ecological study cancer incidence and mortality in nine municipalities of northern Sydney during 1972-90 three of which surround three TV towers were assessed. Population size in the three municipalities located within a radius of approx. 4 km around the TV towers amounts to 135,000, while population size in the six municipalities further away was 450,000. High-power transmission commenced in 1956, an additional 100 kW transmission started in 1965 and another 300 kV broadcast in 1980. Carrier frequencies varied between 63 and 533 MHz for TV broadcasting and were around 100 MHz for FM radio broadcast.

During the study period 740 primary malignant brain tumors were diagnosed in adults and 64 in children, 606 deaths due to brain cancer occurred in adults and 30 in children. While incidence of lymphatic leukemia was significantly higher in adults as well as in children inhabiting the three municipalities surrounding the transmission towers compared to the six districts further away, brain tumor incidence was not significantly elevated (RR=0.89 in adults and 1.10 in children).

As has been stated above, distance from a transmitter is a poor proxy for exposure. Some measurements done in the study area obtained levels much lower than those calculated from the power emitted and antenna gain. Several factors are responsible for this effect: multiple reflections, attenuation by buildings and vegetation, ground undulations, non-coincidence of maxima for the different signals as well as complex radiation characteristics of the broadcast antennae.

The exact location of the residence of cases could not be provided which reduces the potential of the study to relate incidences to measurements or calculations of RF fields. Authors discussed some potential sources of bias such as migration and other exposures in the different regions. However, the most important disadvantage in such studies is that individual risk factors cannot be adjusted for. Both spurious positive as well as false negative results can be obtained by disregarding such individual variables.

H. Tynes et al. 1996

In a historical cohort study 2,619 Norwegian female radio and telegraph operators certified between 1920 and 1980 were followed from 1961 through 1991 for entries in the cancer registry. During this period a total of 140 cases of cancer occurred which are about 20% more than expected from the Norwegian population. Among these were 5 brain tumor cases closely matching the number expected.

An excess for breast cancer was found in this study that may be related to a combination of RF field exposure and night work. For other cancers including brain cancer numbers of cases were too low to address exposure risk.

In this very thoroughly conducted study including a nested case-control approach for breast cancer, measurements at historical transmitters on ships, comparison with women at other jobs on sea, brain tumors were not distinctly higher than expected from the reference population. However, because of the limited cohort size a moderately increased risk cannot be excluded.

I. Dolk et al. 1997a

This ecological small area study of cancer incidence 1974-86 near the Sutton Coldfield TV/radio transmitter at the northern edge of the city of Birmingham (England) was initiated by an unconfirmed report of a 'cluster' of leukemias and lymphomas. The transmitter came into service in 1949. Transmission at 1 megawatt (effective radiated power erp) began in 1964, at 3 MW in 1969, and at 4 MW in 1982. The tower has a height of 240 m with no big hills in the surrounding area. The study area was defined by a circle of 10 km radius centered at the transmitter. The population within this area was about 408,000. All cancers, excluding non-melanoma skin cancer, were considered focusing on hematopoietic and lymphatic cancers, brain and nervous system cancers, eye cancer, and male breast cancer. Childhood cancers were restricted to all cancers and all leukemias.

In the study area a small but significant excess of all cancers was observed in adults. All leukemias and non-Hodgkin's lymphoma were particularly elevated and incidence within 2 to 4 km from the tower was about 30% higher than expected. Brain tumors were only analyzed for distances of within 2 km and the whole study area. Within 2 km an increased OER of 1.29

for all brain tumors and 1.31 for malignant brain tumors was calculated based on 17 and 12 cases, respectively.

Also this investigation suffers from using distance from the tower as proxy for intensity of exposure. The wrong assumption that exposure decreases with increasing distance invalidates the statistical trend test applied. Measurements conducted in the study area revealed the poor relationship with distance but without consequences on the evaluation of the data. Overall the study is consistent with a moderately increased risk of hematopoietic and lymphatic cancers as well as some other cancers including brain cancer in the vicinity of high-power transmitters that, if related to RF fields, must be substantially higher for actual exposure.

The Sutton Coldfield study was later continued (Cooper & Saunders 2001) to cover the period 1987-94. The study revealed, compared to the earlier period, an almost unchanged increase of leukemias and non-Hodgkin's lymphoma in adults and a slight increase in children.

J.   Dolk et al. 1997b

Because the Sutton Coldfield study was triggered by a cluster report and to provide independent test of hypotheses arising from that study, similar methods as applied in the previous study were used to study all high-power TV/radio transmitters ($\geq$ 500 kW ERP) in Great Britain. In adults leukemias, bladder cancer, and skin melanoma, and in children, leukemias and brain tumors were studied. The study period was 1974-86 for England and somewhat shorter in Wales and Scotland.

Although population density around transmitters was not always as high as in the case of the Sutton Coldfield tower, with an average population density of only about one third of that around Sutton Coldfield tower within 2 km from the towers, in the most important range of 2 to 4 km from the transmitters, where in many cases the maximum of radiated RF at ground level is reached, population density was similar. The study of all high-power transmitters essentially corroborated the findings for adult leukemias with an increase of incidence between 10 and 50% in the distance band of 2 to 4 km from the transmitters for the different transmitter types. Most of these increased incidences were statistically significant.

For children only the incidence in the whole study area and within a distance of 2 km was calculated, which is unfortunate because the area close to the towers is sparsely populated and

exposure is low. Number of brain tumors in children was slightly above expectation (244 observed and 231 expected).

In contrast to the interpretation by the authors, the study of all high power transmitters essentially replicated and supported the findings of an excess incidence of leukemias in relation to RF emission from TV/radio towers. Because the different heights and radiation characteristics of the transmitters result in different exposure patterns at ground level, the consistent increase in an area that is likely close to the maximum of exposure supports the hypothesis of an association.

K.   Lagorio et al. 1997

A mortality study of a cohort of 481 female plastic-ware workers employed between 1962 and 1992 in an Italian plant, 302 of which were engaged in the sealing department with exposure to RF fields, was reported by Lagorio et al. (1997). For RF-sealers 6,772 person-years of follow-up were accumulated and overall 9 deaths occurred, 6 of which were from malignant neoplasms (which are twice as many as expected from comparison with the local reference population). In the 31 years only one brain cancer occurred but only 0.1 were expected.

Although the small size of the cohort and the potential exposure to other agents except RF fields such as solvents and vinyl chloride prohibit far reaching conclusion, much more of such thorough follow-up studies of exposed cohorts are needed to accumulate a body of evidence that can provide a useful basis for analysis.

L.   Finkelstein 1998

A preliminary study intended to form the basis for an assessment of cancer risks associated with handheld radar devices was conducted among a cohort of 20,601 male Ontario police officers. The retrospective follow up covered the period of 1964-95. By linkage with the cancer registry and mortality database 650 cases of cancer were detected.

Testicular cancer and melanoma showed an excess incidence while overall cancer incidence was reduced as expected from a working cohort. Overall 16 cases of primary malignant brain tumors occurred which is slightly less than expected.

The author had difficulties to build up a proper cohort because some departments refused to participate and others couldn't spare the time to provide lists of all officers employed during the target period. Furthermore, while cancer sites of primary interest showed actually an increased incidence calling for a nested case-control approach, this study was never conducted due to lack of interest and support of the authorities.

M.   Morgan et al. 2000

In an occupational cohort study all US Motorola employees with at least 6 months cumulative employment and at least 1 day of employment in the period 1976-96 were included. A total of 195,775 workers contributing about 2.7 million person-years were available for the study. The cohort was compared to the SSA Master Mortality File and the National Death Index to obtain vital status. Death certificates were obtained by states' vital statistics offices and company records. Exposure was assessed by expert opinion. Four RF exposure groups were defined with increasing level of estimated RF exposure. Only about 5% of the total cohort was classified as highly exposed and more than 70% with only background exposure. Neither private nor occupational mobile phone use was included.

Overall 6,296 deaths occurred in the cohort in 21 years, which were only two thirds of deaths expected from mortality data of the four countries where most Motorola facilities are located. This reduction is too pronounced to be solely due to a healthy worker effect, other factors such as higher SES must have contributed, an interpretation supported by the substantial reduction of mortality from all life-style associated causes of death. Internal comparisons were done for mortality from brain cancer and hematopoietic and lymphatic cancers. Brain tumor mortality was slightly but insignificantly elevated in high and moderately high exposed workers as compared to those with no or low RF exposure.

This study of a huge cohort demonstrates the limitations of such a study design. The majority of the cohort (58%) consisted of retired or terminated workers that may or may not have accumulated further RF exposure at other companies. Furthermore, it can be assumed that Motorola employees were among the first that used mobile phones at the workplace and

privately. Neglecting mobile phone use may diminish the gradient of exposures between occupational groups studied. It would have been better to conduct nested case-control studies instead of using internal comparison that may be compromised by mobility bias, exposure misclassification and use of mobile phones.

N.   Groves et al. 2002

In this military cohort study of 40,581 men followed from the year of graduation (1950-1954) from Navy technical schools through 1997, known as the Korean War Veterans study, groups of sailors with imputed difference in likelihood and amount of exposure to radar waves were compared with respect to mortality. The original study, with a follow up through 1974, (Robinette et al. 1980) reported increased risks of cancer of the hematopoietic and lymphatic system, of the lung and digestive system for the high exposure group but was handicapped by the lack of information on date of birth of the cohort members. For the extended follow up study many missing birth dates were found in the Veterans Administration Master Index. Nevertheless, birth date remained unknown for over 8% of the cohort. Based on expert opinion low RF exposure was assigned to job classifications of radioman, radarman, and aviation electrician's mate, high exposure stratum included men with job classifications of electronics technician, aviation electronics technician, and fire control technician.

By matching against the Social Security Administration's Death Master File and the National Death Index 8,393 deceased subjects were identified through 1997. This number is substantially and significantly lower as expected from the male white US population. A healthy soldier effect may have been responsible for a lower mortality rate in the 1950ies but cannot explain the reduced mortality after 40 years. It has not been reported how long the cohort members stayed in service nor were life-style factors investigated; however, of more than 40% of the cohort no social security number could be obtained suggesting possible under-estimation of deaths.

Comparison of high- with low-exposure groups revealed significantly lower mortality from life-style associated causes of death (lung cancer, vascular diseases, diabetes mellitus, chronic obstructive pulmonary disease, and liver cirrhosis) and significantly higher mortality from all leukemias and external causes of death. Increased mortality from leukemias was found in all high exposure groups but the most pronounced increase was observed in aviation electronics

technicians. Brain cancer was less frequent in all high exposure groups compared to the low exposure category.

The long period of follow up of this large cohort with start of follow up almost at the same time (1950-54) and at a time when exposure commenced is a great advantage of this investigation. However, there are a number of shortcomings: follow up was possibly incomplete by unknown social security number of a substantial proportion of the cohort; almost half of all deaths in the first 20 years were from external causes which could have obscured an effect of exposure; duration and intensity of exposure is unknown as well as potential exposure after leaving the Navy; classification into low and high exposure groups may introduce substantial misclassification. In the earlier report, inspection of Navy records for a sample from the high exposure group revealed that 24% had no exposure to radar waves at all.

Concerning brain tumors, assuming an effect of radar exposure on tumor growth rate, exposure during the Korean War and no exposure afterwards would be expected to result in only a slightly increased risk during a period of about 10 years after the war. Sailors were about 20 to 25 years at that time. The fraction with an already initiated brain tumor during this age range is estimated to be less than 3 in 100,000 per year. Increase of growth rate even if substantial cannot result in an effect observable in a cohort of that size. If radar exposure increases the likelihood of malignant transformation this could increase the incidence during a time window of 10 to 30 years after the exposure period. Results of the Israeli study of x-ray treated tinea capitis (Sadetzki et al. 2005) suggests an average latency of about 20-25 years, however, risk decreased with increasing age at first exposure to x-rays. Taking the data on ionizing radiation as a guiding principle for brain tumor initiation, radar exposure of sailors during their twenties might result in an increase of brain tumor mortality of about 10 to 15%, i.e. a maximum of 8 additional cases among 20,000. Considering the biases of the study such a low risk is easily obscured. Hence neither tumor promotion nor initiation may be detected in this study even if there is an increased risk. Because of the mentioned limitation to a certain time window with possibly increased incidence due to exposures during service in the Korean War, it would have been instructive to compute Kaplan-Meier estimates for cumulative brain tumor mortality.

O.   Ha et al. 2002

An ecological study around 11 high-power AM transmitter study sites (i.e., 100–1,500-kW transmission power) and 31 low-power study sites (i.e., 50-kW transmission power) used for comparison was conducted in South Korea. For each high-power site four control areas located in the same or nearest adjacent province as the high-power site, but were at least 2 km from any of the transmitters were chosen. The incidence of cancer within a 2-km radius of each transmitter and within control districts was obtained from Korean medical-insurance records for the years 1993 through 1996. Standardized incidence ratios (SIR) of high- against low-power transmitter areas were reported and additionally observed-to-expected ratios for each type of transmitter. SIRs were elevated for all cancers and for female brain cancer. Concerning transmitter types, for all types except 250 kW elevated OER for brain cancer were obtained (statistically significant for 100 kW).

Due to the complex relationship between distance and field strength, depending on antenna type and characteristics, height above ground level, orographic conditions, electrical properties of the terrain, etc., choice of a 2-km radius for all transmitters might not have been the best option to select the highest exposure group.

P.   Park et al. 2004

A similar design as in the study of Ha et al. (2003) was applied in this ecological investigation of cancer deaths. Ten high-power (i.e., 100–1,500-kW transmission power) sites were chosen and compared to four control districts as in the previous study. Standardized mortality ratios were elevated for all single cancer sites but significant only for total cancer deaths. For brain cancer the ratio was 1.52 and statistically not significant.

The same criticism as for the study of Ha et al. (2003) applies to this study. Both studies share the limitations inherent in the ecological study design.

.

Q.   Berg et al. 2006

In the German part of the Interphone study special attention was paid to occupational history and exposure to RF fields at workplaces. Incident meningioma (n=381, response rate 88%) and glioma cases (n=366, response rate 80%) aged 30-69 years were selected from four

neurological clinics. Overall 1,535 (participation rate 63%) were randomly selected from population registries matched to the cases by sex, age, and region. Most cases were interviewed during their stay in hospitals, controls were interviewed at home. The interview contained several screening questions about occupations that are probably associated with RF exposure. If any of these screening questions were marked additional questions were asked about the job. Based on the literature and the evaluation by two industrial hygienists a classification into the following categories was performed: no RF exposure/not probably RF exposed/probably RF exposed/highly RF exposed. In total about 13% (299 cases and controls) were classified with at least possible RF exposure at the workplace. Analyses were adjusted for region, sex, age, SES, urban/rural residence, ionizing radiation exposure in the head/neck region. Mobile phone use was not considered as a confounder.

While overall RF exposure at workplaces showed no increased odds-ratios, high exposure and especially for durations of 10 years or more resulted in elevated risk estimates that were, however, not significant. This result was similar for meningioma (OR=1.55 for high exposure for 10 years or more) and glioma (OR=1.39).

The study tried to assess potential workplace exposure as precisely as possible in a personal interview, but still misclassification may have occurred especially in the probable and not probable categories while the high exposure group is likely to have had at least occasionally above average RF exposure. Odds ratios are in the range expected if exposure results in a substantial increase of growth rate. The small number of highly and long-term exposed cases (13 glioma and 6 meningioma) prohibit, however, far reaching conclusions.

R.   Schüz et al. 2006

In the same study as mentioned above also exposure to emissions from DECT (Digital Enhanced Cordless Telecommunications) base stations near the bed were analyzed. Both, for glioma and meningioma, not significantly decreased odds ratio were reported. There was also no increasing risk observed with duration of exposure to DECT cordless phone base stations. The study was limited due to the small number of exposed subjects and the short exposure duration. It is unlikely that after these short exposures periods an increased risk can be observed.

S. Hu et al. 2007

The study from South Korea that was a major improvement in investigating the possible association between RF EMF exposure and cancer risk applied not only instead of an ecological approach the case-control paradigm but also used an interesting method to estimate individual exposure. This method seems a reasonable compromise between effort and precision. The study included leukemia and brain cancer patients under age 15 years and controls with respiratory illnesses matched to cases on age, sex, and year of diagnosis (1993–1999). All were selected from 14 South Korean hospitals using the South Korean Medical Insurance Data System. Residential addresses were obtained from medical records so that no direct contact with the participants was necessary. Authors developed an exposure prediction program incorporating a geographic information system that was modified by the results of actual measurements carried out systematically at defined locations and during driving along specific trajectories. Furthermore, electrical characteristics of the environment were considered. This method was used to estimate RF EMF exposure from 31 AM radio transmitters with a power of 20 kW or more. A total of 1,928 leukemia patients, 956 brain cancer patients, and 3,082 controls were included.

A significantly increased odds ratio was obtained for childhood leukemia at a distance of 2 km or less from the transmitters relative to a distance of >20 km. In response to a critical comment by Schüz et al. (2008) authors recalculated the risk estimates for total and peak RF EMF exposure (Hu et al. 2008) and reported for the highest quartile of peak RF EMF exposure a significantly increased risk of ALL. For childhood brain cancers insignificantly increased risks of about 1.4 for ≤2 km and 2-4 km from the transmitter were obtained.

It seems that there were problems with the RF EMF estimates since peak and total field strengths had quite different results and also the correlation with peak exposure and distance was much higher than with total exposure suggesting that more distant transmitters led to a decrease in the gradient of exposures. The measurements are not reported for the different transmitter types and therefore it is difficult to assess their validity. For very high power transmitters (1,500 kW) the relationship is known to be not monotonous which cannot be discriminated in the figure shown in the article. Overall the study has an improved methodology due to the case-control and registry approach. However, the methods to assess actual exposure need to be further improved.

IV. EVALUATION OF THE EVIDENCE

Due to the varying endpoints, methods used and populations included the meta-analysis shown in fig.1 applied the random effects model and DerSimonian-Laird estimate of the overall risk and confidence interval. Only few studies found clear indications of an association between RF exposure and brain tumors: one cohort study (Szmigielski 1996) and two case-control studies (Thomas et al. 1987, Grayson 1996). None of the ecological studies except for Ha et al. (2003) for one of the AM transmitter types demonstrated a significantly increased risk in the vicinity of RF antennas.
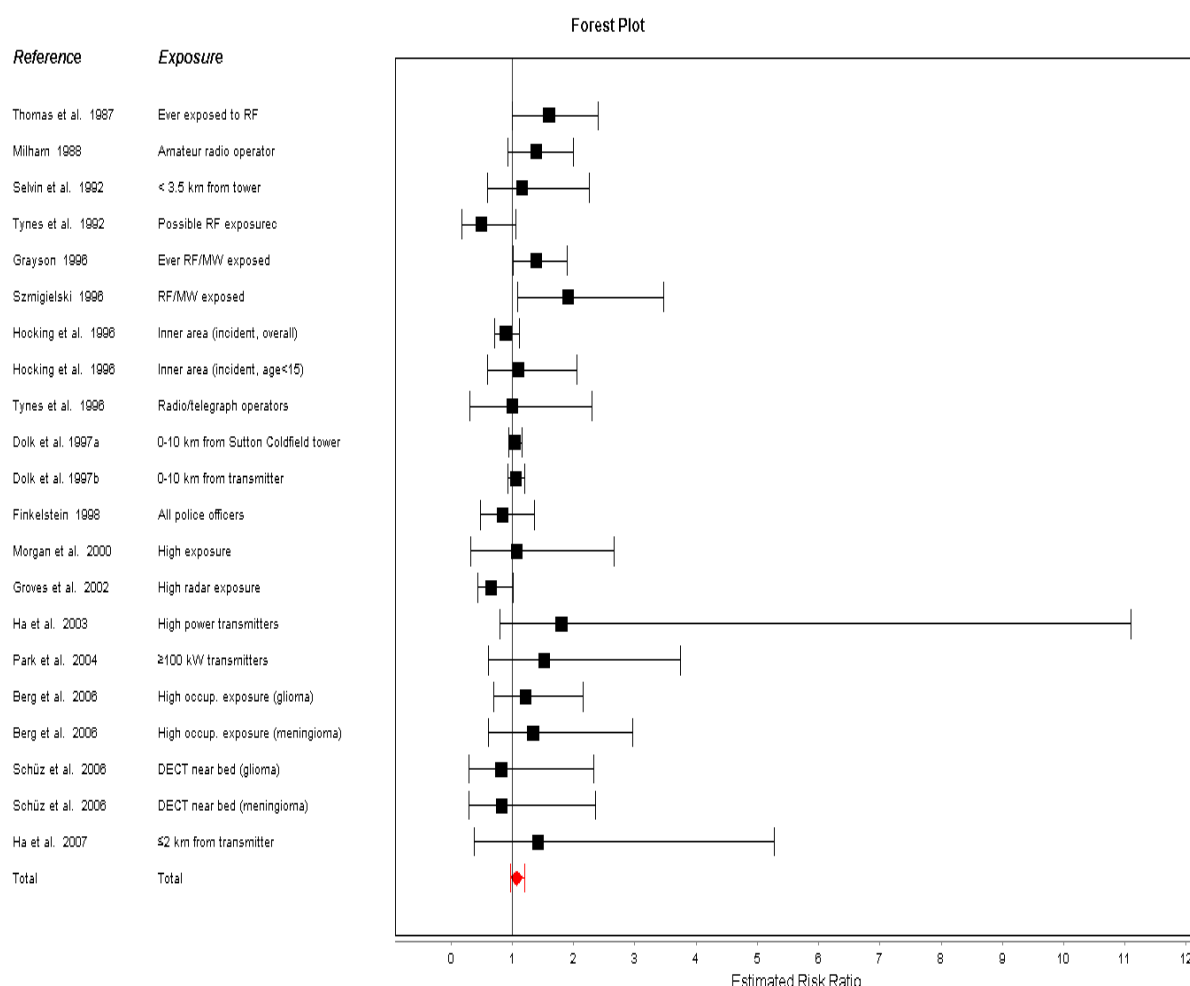

Fig. 1: Forest plot of risk estimates for RF exposure with respect to brain tumors and DerSimonian-Laird overall estimate

The meta-analytical estimate of the risk was 1.08 (95% confidence interval: 0.97 – 1.20). The discussion of the 19 published investigations revealed shortcomings in all studies. The

greatest problem was encountered in the difficulties to reliably assess actual exposure. Even if we don't know the relevant aspect of the exposure, if any, that is responsible for an increased risk, the type, duration and amount of exposure must be determined in order to use the studies in derivations of exposure standards. None of the studies included a useful quantitative indicator of intensity of exposure and even duration of exposure was rarely addressed. Concerning type of exposure only quite crude and broad categories were used.

In ecological studies, although for the studied population the exposure - despite considerable variations in time - is similar with respect to carrier frequency, modulation etc. it is quite different between various types of transmitters and hence results are not easily generalized. The ecological studies are not conclusive with respect to brain tumors but provide some evidence for hematopoietic malignancies that need to be further pursued. Investigating residential exposure to RF EMFs from broadcasting stations poses severe methodological problems mainly due to the small size of the exposed population because high exposure levels occur only in a small band around the radiation sources. Due to the transition to digital television many TV broadcasting antennas with high power are or will be disconnected leaving us with changing exposure conditions. Because brain tumors have long latencies it is hardly possible to produce conclusive evidence in the near future.

Considering the discussion of the different investigations and the fact that most biases encountered tend to dilute a potential risk, the compiled evidence from occupational cohorts is compatible with a moderately increased risk of RF exposure. Because of the lack of actual measurements but observing that exposure above guideline levels must have been a rare event a precautionary approach must result in a reduction of occupational exposure levels and organizational measures to avoid over-exposure and also environmental exposure levels should be given greater attention. Although brain tumors are rare and the population attributable risk is low (assuming 13% of adults being occupationally exposed to RF fields as inferred from Berg et al. 2006, and assuming a relative risk of 1.3, about 4% of brain tumors can be attributed to RF exposure, i.e. 2,200 cases per years in the US).

CONCLUSIONS

•   Only few studies of long-term exposure to low levels of RF fields and brain tumors exist, all of which have methodological shortcomings including lack of quantitative exposure assessment. Given the crude exposure categories and the likelihood of a bias towards the null hypothesis of no association the body of evidence is consistent with a moderately elevated risk.

•   Occupational studies indicate that long term exposure at workplaces may be associated with an elevated brain tumor risk.

•   Although in some occupations and especially in military jobs current exposure guidelines may have sometimes been reached or exceeded, overall the evidence suggest that long-term exposure to levels generally lying below current guideline levels still carry the risk of increasing the incidence of brain tumors.

•   Although the population attributable risk is low (likely below 4%), still more than 2,000 cases per year in the US can be attributed to RF exposure at workplaces alone. Due to the lack of conclusive studies of environmental RF exposure and brain tumors the potential of these exposures to increase the risk cannot be estimated. However, these figures are theoretical as long as the evidence is as weak as it is for the time being.

V. ASSESSMENT OF EPIDEMIOLOGICAL EVIDENCE BY IEEE (C95.1 REVISION)

Introduction

Before 1988 C95 standards were developed by Accredited Standards Committee C95, between 1988 and 1990, the committee was converted to Standards Coordinating Committee 28 (SCC 28) under the sponsorship of the IEEE Standards Board. In 2001 IEEE approved the name "International Committee on Electromagnetic Safety (ICES)" for SCC 28. Subcommittee 4 of ICES Technical Committee 95 is responsible for the revision of standard C95.1 "IEEE Standard for Safety Levels with Respect to Human Exposure to Radio Frequency Electromagnetic Fields, 3 kHz to 300 GHz". There are five TC95 subcommittees: 1) Techniques, Procedures, and Instrumentation; 2) Terminology, Units of Measurements and Hazard Communication; 3) Safety Levels with Respect to Human Exposure, 0-3 kHz; 4) Safety Levels with Respect to Human Exposure, 3 kHz-300 GHz; 5) Safety Levels with Respect to Electro-Explosive Devices.

The recommendations in standard C95.1 are intended to protect against scientifically established adverse health effects in human beings resulting from exposure to radio frequency electromagnetic fields in the frequency range of 3 kHz to 300 GHz. A "scientifically established adverse health effects" is defined as: "A biological effect characterized by a harmful change in health that is supported by consistent findings of that effect in studies published in the peer-reviewed scientific literature, with evidence of the effect being demonstrated by independent laboratories, and where there is consensus in the scientific community that the effect occurs for the specified exposure conditions." It is interesting that this definition does not only demand the effect being demonstrated by independent laboratories but also that a consensus must be reached in the scientific community. This is a strange definition. When is a consensus reached? If more than 50% of scientists in the scientific community agree? Or must all agree? Usually this term is used to describe a situation where there is no open or covert dissent. In decisions theory demanding consent is criticized as a policy that results in the preservation of the status-quo.

It might be instructive to contrast this definition with IARCs (International Agency for Research on Cancer) characterization of sufficient evidence for carcinogenicity in experimental animals: "The Working Group considers that a causal relationship has been established between the agent or mixture and an increased incidence of malignant neoplasms

or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols", and the characterization of sufficient evidence in humans: "The Working Group considers that a causal relationship has been established between exposure to the agent, mixture or exposure circumstance and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence." Clearly these definitions are incompatible with the definition by IEEE.

The scientific rationale for the derivation of the exposure standard of IEEE is presented in Annex C and Annex B "Identification of levels of RF exposure responsible for adverse effects: summary of the literature" which is based on "critical reviews of studies within the IEEE/WHO RF literature database". In this commentary I will address chapter 9) Epidemiological Studies of RF Exposures and Human Cancer.

Evaluation of Cancer-Related Endpoints (RF Exposure)

In their 2006 revision of the standard C95.1 IEEE has assessed the evidence from epidemiology for cancer related endpoints in chapter B.7.3. The assessment relies mainly on the reviews of Bergqvist (1997), Moulder et al. (1999) and Elwood (2003). These reviews and the IEEE overview share the same deficiencies. The main lines of argumentation would be impossible in any other field of environmental health and closely resemble the strategy used to dismiss a power frequency exposure/childhood leukemia association. In the following paragraphs the assessment by IEEE will be discussed. The text of IEEE C95.1 is presented in italics as blocked citation. References within the text of the citations are found by the Rnnn and Bnnn numbers in the Annexes F and G of the standard document, but are also included in the reference section of this overview.

> *Cluster studies, such as the one performed in Sutton Coldfield in the U.K. in response to a cluster of leukemia and lymphoma in adults living close to an RF broadcasting transmitter (Dolk et al. [R624]), are inherently difficult to interpret because of the impossibility of assessing all of the effects that chance variation might have contributed to the cluster. In the initial Sutton Coldfield study, the authors correctly concluded that no causal association could be drawn between the presence of the cluster and RF exposure from broadcasting towers (Dolk et al. [R625]) (Cooper et al. [R760]). (IEEE C 95.1 – 2005, p.75)*

34

First of all the Sutton Coldfield study was no cluster study but an ecological investigation. It only was initiated by an unconfirmed report of a cluster of leukemia and lymphoma in the vicinity of this broadcasting transmitter but it proceeded independently of this initial report and used registry data of the population living within a radius of 10 km around the transmitter. The statement that such studies are "inherently difficult to interpret because of the impossibility of assessing all of the effects that chance variation might have contributed to the cluster" is ridiculous not only because the study is no cluster study but because it is impossible for any study to "assess all effects that chance variation might have contributed" to the endpoint under investigation. It is not mentioned that the study was supplemented by a larger investigation of another 20 high-power transmitters in Great Britain. The difficulties of interpreting ecological studies is related to the fact that potential confounders can only be related to a segment of the population but not to individuals and that in general duration and intensity of exposure are not known for individual members of the different strata. While evidence for an effect on brain tumor incidence from both studies (Dolk et al. 1997a, 1997b) is weak, there is consistent evidence for a relation to hematopoietic cancers. This evidence has been overlooked by the authors due to their wrong assumption about the relation between proximity to the transmitter and exposure.

> *Inconsistent effects have been reported between residential proximity to other RF broadcast towers and adverse health endpoints (Bielski [R267]) (Maskarinec et al. [R579]) (Selvin and Merrill [R823]) (Michelozzi et al. [R858]) (Altpeter et al. [R977]) (Hallberg and Johansson [R995], [R996]) (Boscolo [R1012]), although many of these studies have significant flaws in their study design (making them difficult to interpret). (IEEE C 95.1 – 2005, p.75)*

Although it is not stated what these "inconsistent effects" might be, the statement is flawed in more than this respect. First of all the study by Bielski (1994) is an occupational investigation and not about residential proximity to RF broadcast towers, second three of these investigations (Selvin et al. 1992; Maskarinec et al. 1994; Michelozzi et al. 2002) included leukemia as an endpoint with indications of an increased incidence consistent with the studies from Great Britain (Dolk et al. 1997a, 1997b) and Australia (Hocking et al. 1996). Note that the study by Selvin et al. (1992), as stated in section 10, intended to compare different methods to assess the relationship between a point source and diseases and did erroneously assume a monotonous relationship between exposure and distance from a transmitter. Correcting this error there seems to be an increased probability of childhood leukemia in areas receiving the highest exposure from the Sutro tower. The other three investigations (Altpeter

et al. 1995; Boscolo 2001; Hallberg & Johansson 2002) have nothing in common and hence cannot be inconsistent.

> *An increased incidence and mortality rate of childhood leukemia was reported in Australia with residential proximity to a specific RF broadcasting tower (Hocking et al. [R633]), although subsequent reanalysis of the data showed the results may have been influenced by other confounding variables within the study location (McKenzie et al. [R669]). (IEEE C 95.1 – 2005, p.75)*

This is another example how carelessly and sloppy the evidence is dealt with by the IEEE committee. The study of Hocking et al. (1996) was not about "proximity to a specific RF broadcasting tower" but about an area where three broadcasting towers are located. While there is always the possibility of confounders influencing results of an epidemiologic investigation, the 'reanalysis' of McKenzie et al. (1998) is seriously flawed and cannot support the cited statement. Hocking et al. (1996) combined the districts near the broadcasting area and those further away based on homogeneity analyses, while McKenzie et al. (1998) omitted one area with high incidence (and highest exposure) based on inspection of data. Any statistical analysis subsequent to such data picking is useless.

> *While scattered reports of adverse health effects associated with occupational exposure to RF do exist (Demers et al. [R36]) (Kurt and Milham [R68]) (Pearce [R110]) (Speers et al. [R125]) (Thomas et al. [R128]) (Pearce et al. [R199], [R211]) (Hayes et al. [R207]) (Cantor et al. [R268]) (Davis and Mostofi [R563]) (Tynes et al. [R570], [R605]) (Grayson [R592]) (Richter et al. [R747]) (Holly et al. [R838]) these studies are largely inconsistent with each other in terms of the adverse health endpoints affected, and often show no clear dose response with RF exposure. Many have serious flaws in their study design, contain limited or insufficient RF exposure assessment, and are generally inconsistent with the absence of findings of an association from other occupational studies (Tornqvist et al. [R131]) (Coleman [R142]) (Lilienfeld et al. [R146]) (Robinette and Silverman [R147], [R148]) (Siekierzynski et al. [R151], [R152]) (Wright et al. [R213]) (Coleman et al. [R214]) (Muhm [R506]) (Czerski et al. [R542]) (Hill [R568]) (Lagorio et al. [R616]) (Kaplan et al. [R647]) (Morgan et al. [R701]) (Gallagher et al. [R822]) (Groves et al. [R853]) (Wiklund [R1013]) (Armstrong et al. [R1014]). (IEEE C 95.1 – 2005, p.75)*

Even allowing for restrictions of space for a discussion of the evidence, greater nonsense has not been produced so far in this field as condensed in these two sentences. Putting higgledy-piggledy all sorts of studies together and then wondering about endpoints being inconsistent is an intellectual masterpiece. Of the occupational studies mentioned, three (Thomas et al. 1987; Speers et al. 1988; Grayson 1996) were about brain cancer, three about hematopoietic cancers

36

(Pearce et al. 1985; Kurt & Milham 1988; Pearce 1988), two about testicular cancer (Hayes et al. 1990; Davis & Mostofi 1993), one about male (Demers et al. 1991) and two about female breast cancer (Cantor et al. 1995, Tynes et al. 1996) the latter including other cancers as well, and one about intraocular melanoma (Holly et al. 1996). Three further studies (Pearce et al. 1989; Tynes et al. 1992; Richter et al. 2000) investigated several or all malignancies. These studies differ not only in endpoints, study type (cohort, case-control, and cluster) but also in the methods of exposure assessment. Ignorance of the IEEE reviewers is underlined by the compilation of studies characterized by an "absence of findings of an association". Not only did several of these studies indeed indicate an association of cancer risk with EMF exposure (Lilienfeld et al. 1978; Robinette et al. 1980; Tornqvist et al. 1991; Armstrong et al. 1994; Lagorio et al. 1997; Groves et al. 2002) but two were no epidemiologic studies at all (Siekierzynski et al. 1974; Czerski et al. 1974) and several were rather addressing ELF exposure (Tornqvist et al. 1991; Wright et al. 1982; Coleman et al. 1983; Gallagher et al. 1991) and one (Wiklund 1981) was a cluster study in the telecommunication administration with uncertain type of exposure. Simply confronting studies finding an effect with others that were 'negative' is scientifically flawed and permits neither the conclusion that there is nor that there is no association between exposure and cancer risk. Even if all studies would have applied the same method, assessed the same endpoint and used the same exposure metric, studies reporting a significantly increased cancer risk are not outweighed by others that did not.

> *While micronuclei formation in workers occupationally exposed from broadcast antennas has been reported (Garaj-Vrhovac [R757]) (Lalic et al. [R791]), these findings were not verified in a larger study of more than 40 Australian linemen exposed under similar conditions (Garson et al. [R186]). (IEEE C 95.1 – 2005, pp.75-76)*

It goes without saying that also this statement is wrong. Garson et al. (1991) did not investigate micronuclei formation, their workers were considerably shorter exposed and it were not more than 40 linemen but 38 radio-lineman.

> *No clear association could be established between occupational exposures of parents to a number of agents, including RF, and effects (neuroblastoma) in their offspring (Spitz and Johnson [R289]) (De Roos et al. [R798]). (IEEE C 95.1 – 2005, p.76)*

What is meant by 'no clear association' is obscure. Spitz and Johnson (1985) found a significantly increased risk after paternal occupational exposure to electromagnetic fields, and also De Roos et al. (2001) found several jobs with paternal as well as maternal exposure to

EMFs associated with an elevated risk for neuroblastoma in their children. However, broad groupings of occupations with ELF, RF EMF, as well as ionizing radiation (!) exposure did not reveal an increased risk.

> *One study reported a slight excess in brain tumors associated with combined exposure to RF and other exposures associated with electrical or electronic jobs, but not with RF alone (Thomas et al. [R128]). A study of a Polish military cohort reported a substantial excess of total cancer and several cancer sub-types with jobs associated with RF exposure (Szmigielski [R578]), (Szmigielski and Kubacki [R982]), although questions have been raised about severe bias in the exposure assessment of this study (Elwood [R665]) (Bergqvist [R1015]) (Stewart [R1133]). Studies by Milham of U.S. amateur radio operators reported an excess in one of nine types of leukemia assessed (see [R101], [R102], [R209], [R215], and [R569]), but not for total tumors, total leukemia, or brain tumors, and potential confounding factors might have included exposure to soldering fumes, degreasing agents and over-representation of a particular social class. (IEEE C 95.1 – 2005, p.76)*

Again the evidence is incorrectly summarized for all cited investigations. Thomas et al. (1987) found a significantly elevated risk for brain tumors among all men exposed to RF fields and in particular in those exposed for 20 or more years. There were indications that this elevated risk is due to a subgroup with electrical or electronics jobs. The group of those exposed in other jobs is heterogeneous and may contain subjects with low or no exposure (e.g. some groups of welders) and therefore lack of an association could be due to a dilution effect from exposure misclassification.

As mentioned in section 10 criticism of the Polish military cohort study about exposure assessment is unfounded. Bergqvist (1997), Elwood (1999) and Stewart (2000) criticized that the military health board assessed a number of potential risk factors only for cancer cases. However, they overlooked that the study was a cohort and not a case-control study and that at no stage information about these factors entered the analysis and therefore couldn't affect the results in any way.

The study by Milham (1988a, 1988b) of radio amateur operators revealed a significantly increased standardized mortality ratio (SMR) for acute myeloid leukemia while the overall mortality and cancer mortality was significantly reduced relative to the country mortality rates. As mentioned in section 10 this points to a 'healthy worker' effect as well as to an influence of life-style factors (mortality related to smoking and overweight were reduced). From the mentioned nine types of leukemia three with expectancies below one and no case observed couldn't be assessed, from the six remaining types five had elevated SMRs with AML, the most frequent type in adults, being significantly elevated.

The last portion of the IEEE review of epidemiology studies is dedicated to mobile phone investigations that are discussed in another contribution.

The following citation presents the IEEE summary in its full length:

> *The epidemiological evidence to date does not show clear or consistent evidence to indicate a causal role of RF exposures in connection with human cancer or other disease endpoints. Many of the relevant studies, however, are weak in terms of their design, their lack of detailed exposure assessment, and have potential biases in the data. While the available results do not indicate a strong causal association, they cannot establish the absence of a hazard. They do indicate that for commonly encountered RF exposures, any health effects, if they exist, must be small. Even though epidemiological evidence cannot rule out a causal relationship, the overall weight-of-evidence is consistent with the results of the long term animal studies showing no evidence of physiological, pathological or disease-specific effects. (IEEE C95.1 - 2005; pp.76-77)*

As already pointed out earlier (Kundi 2006) there is an intolerable tendency in the past years that confronted with an undeniable epidemiologic evidence of an association between an agent and adverse health effects such as cancer, interested parties take their resort to the concept of causality based on the wrong assumption evidence to "indicate a causal role" is a lot more difficult to provide. Unprecedented, however, is the notion of "a strong causal association". Whatever the meaning of this exceptional statement, the conclusion that, if health effects of commonly encountered RF exposures exist, they must be small, is wrong. To the contrary: considering the "lack of detailed exposure assessment" and other potential biases that predominantly lead to an underestimation of the risk, the evidence points to a quite substantial risk. While the animal studies reviewed in another section of the IEEE standard document cannot be discussed here it should be underlined that they are generally insufficient to support either an increased risk or the lack of health relevant effects. Therefore they cannot be used in a weight-of-evidence statement as has been made by IEEE, that there is no evidence for adverse health effects of RF exposure.

REFERENCES FOR SECTIONS I – V
EVIDENCE FOR BRAIN TUMORS (EPIDEMIOLOGICAL)

Ahlbom A, Green A, Kheifets L, Savitz D, Swerdlow A. 2004. Epidemiology of health effects of radiofrequency exposure. Environ Health Perspect 112: 1741–1754.

Berg G, Spallek J, Schüz J, Schlehofer B, Böhler E, Schlaefer K, Hettinger I, Kunna-Grass K, Wahrendorf J, Blettner M. 2006. Occupational exposure to radio frequency/microwave radiation and the risk of brain tumors: Interphone Study Group, Germany. Am J Epidemiol.

CBTRUS (Central Brain Tumor Registry of the United States). 2011. CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2004-2007. Source: Central Brain Tumor Registry of the United States, Hinsdale, IL.

Cooper DK, Hemmings K, Saunders P 2001. Cancer incidence near radio and television transmitters in Great Britain. I. Sutton Coldfield transmitter; II. All high power transmitters. Am J Epidemiol 153: 202 – 204

Dolk H, Shaddick G, Walls P, Grundy C, Thakrar B, Kleinschmidt I, Elliott P. 1997a. Cancer incidence near radio and television transmitters in Great Britain, Part I. Sutton Coldfield Transmitter. Am J Epidemiol 145: 1-9.

Dolk H, Elliot P, Shaddick G, Walls P, Thakrar B. 1997b. Cancer incidence near radio television and transmitters in Great Britain, Part II. All high-power transmitters. Am J Epidemiol 145: 10-17.

Elwood MJ. 2003. Epidemiological studies of radiofrequency exposures and human cancer. Bioelectromagnetics Suppl 6: S63 - S73.

Finkelstein MM. 1998. Cancer incidence among Ontario police officers. Am J Ind Med 34: 157-162.

Grayson JK. 1996. Radiation exposure socioeconomic status and brain tumor risk in the US Air Force: a nested case-control study. Am J Epidemiol 143: 480-486.

Groves FD, Page WF, Gridley G, Lisimaque L, Stewart PA, Tarone RE et al. 2002. Cancer in Korean war navy technicians: mortality survey after 40 years. Am J Epidemiol 155: 810-818.

Ha M, Im H, Lee M, Kim BC, Gimm YM, Pack JK. 2007. Radio-frequency radiation exposure from AM radio transmitters and childhood leukemia and brain cancer. Am J Epidemiol 166(3):270-279.

Ha M, Im H, Lee M, Kim HJ, Kim BC, Gimm YM, Pack JK. 2008. Five authors reply. Am J Epidemiol 167(7):884-885

Hocking B, Gordon IR, Grain ML, Hatfield GE. 1996. Cancer incidence and mortality and proximity to TV towers. Med J Aust 165: 601-605

Krewski D, Byus CV, Glickman BW, Lotz WG, Mandeville R, McBride ML, Prato FS, Weaver DF. 2001. Potential health risks of radiofrequency fields from wireless telecommunication devices. J Tox Env Health Part B 4: 1-143.

Kundi M, Hansen Mild K, Hardell L, Mattsson MO. 2004. Mobile telephones and cancer - a review of epidemiological evidence. J Toxicol Envrion Health Part B 7: 351-384.

Kundi M. 2006. Causality and the interpretation of epidemiologic evidence. Environ Health Perspect 114: 969 – 974

Lagorio S, Rossi S, Vecchia P, De Santis M, Bastianini L, Fusilli M, Ferrucci A, Desideri E, Comba P. 1997. Mortality of plastic-ware workers exposed to radiofrequencies. Bioelectromagnetics 18: 418-421

Milham S. 1985. Mortality in workers exposed to electromagnetic fields. Environ Health Perspect 62: 297 – 300

Milham S. 1988a. Increased mortality in amateur radio operators due to lymphatic and hematopoietic malignancies. Am J Epidemiol 127: 50-54

Milham S. 1988b. Mortality by license class in amateur radio operators. Am J Epidemiol 128: 1175 – 1176

Morgan RW, Kelsh MA, Zhao K, Exuzides KA, Heringer S, Negrete W. 2000. Radiofrequency exposure and mortality from cancer of the brain and lymphatic/hematopoietic systems. Epidemiology 11: 118-127

Robinette CD, Silverman C, Jablon S. 1980. Effects upon health of occupational exposure to microwave radiation radar. Am J Epidemiol 112: 39 – 53

Schüz J, Böhler E, Schlehofer B, Berg G, Schlaefer K, Hettinger I, Kunna-Grass K, Wahrendorf J, Blettner M. 2006. Radiofrequency electromagnetic fields emitted from base stations of DECT cordless phones and the risk of glioma and meningioma (Interphone Study Group, Germany). Radiat Res 166(1 Pt 1):116-119.

Schüz J, Philipp J, Merzenich H, Schmiedel S, Brüggemeyer H. 2008. Re:"Radio-frequency radiation exposure from AM radio transmitters and childhood leukemia and brain cancer". Am J Epidemiol 167(7):883-884.

Selvin S, Schulman J, Merrill DW. 1992. Distance and risk measures for the analysis of spatial data: a study of childhood cancers. Soc Sci Med 34: 769-777

Szmigielski S. 1996. Cancer morbidity in subjects occupationally exposed to high frequency (radiofrequency and microwave) electromagnetic radiation. Sci Total Environ 180: 9-17.

Tynes T, Andersen A, Langmark F. 1992. Incidence of cancer in Norwegian workers potentially exposed to electromagnetic fields. Am J Epidemiol 136: 81-88

Tynes T, Hannevik M, Andersen A, Vistnes AI, Haldorsen T. 1996. Incidence of breast cancer in Norwegian female radio and telegraph operators. Cancer Causes Control 7: 197-204

REFERENCES FOR SECTION VI

ASSESSMENT OF EPIDEMIOLOGICAL EVIDENCE BY IEEE (C95.1 REVISION)

Altpeter ES, Krebs TT, Pfluger DH, von Kanel J, Blattmann R. 1995. Study on health effects of the short-wave transmitter station at Schwarzenburg, Berne, Switzerland," BEW Publication Series No. 55, University of Berne, Inst. for Social & Preventive Medicine.

Armstrong B, Theriault G, Guenel P, Deadman J, Goldberg M, Heroux P. 1994. Association between exposure to pulsed electromagnetic fields and cancer in electric utility workers in Quebec, Canada, and France. Am J Epidemiol 140: 805 – 820.

Bergqvist U. 1997. Review of epidemiological studies. In: Kuster N, Balzano Q, Lin JC (eds.), Mobile Communications Safety, London: Chapman & Hall, pp. 147 – 170

Bielski J. 1994. Bioelectrical brain activity in workers exposed to electromagnetic fields," Ann N Y Acad Sci 724: 435 – 437

Boscolo P. 2001. Effects of electromagnetic fields produced by radiotelevision broadcasting stations on the immune system of women. Sci Total Environ 273: 1 – 10

Cantor K, Stewart P, Brinton L, Dosemeci M. 1995. Occupational exposure and female breast cancer mortality in the United States. J Occup Environ Med 37: 336-348

Coleman M, Bell J, Skeet R. 1983. Leukaemia incidence in electrical workers. Lancet 1:982 – 983

Coleman M. 1985. Leukaemia mortality in amateur radio operators. Lancet 2: 106 – 107

Cooper DK, Hemmings K, Saunders P 2001. Cancer incidence near radio and television transmitters in Great Britain. I. Sutton Coldfield transmitter; II. All high power transmitters. Am J Epidemiol 153: 202 – 204

Czerski P, Siekierzynski M, Gidynski A. 1974. Health surveillance of personnel occupationally exposed to microwaves. I. Theoretical considerations and practical aspects. Aerospace Med 45: 1137 – 1142

Davis RL, Mostofi FK. 1993. Cluster of testicular cancer in police officers exposed to hand - held radar. Am J Ind Med 24: 231-233

De Roos AJ, Teschke K, Savitz DA, Poole C, Grufferman BH, Pollock BH. 2001. Parental occupational exposures to electromagnetic fields and radiation and the incidence of neuroblastoma in offsping. Epidemiol 12: 508 – 517

Demers PA, Thomas DB, Rosenblatt KA, Jimenez LM, McTiernan A, et al. 1991. Occupational exposure to electromagnetic fields and breast cancer in men. Am J Epidemiol 134: 340 – 347

Dolk H, Shaddick G, Walls P, Grundy C, Thakrar B, Kleinschmidt I, Elliott P. 1997a. Cancer incidence near radio and television transmitters in Great Britain, Part I. Sutton Coldfield Transmitter. Am J Epidemiol 145: 1-9.

Dolk H, Elliot P, Shaddick G, Walls P, Thakrar B. 1997b. Cancer incidence near radio television and transmitters in Great Britain, Part II. All high-power transmitters. Am J Epidemiol 145: 10-17.

Elwood MJ. 2003. Epidemiological studies of radiofrequency exposures and human cancer. Bioelectromagnetics Suppl 6: S63 - S73.

Gallagher RP, Band PR, Spinelli JJ, Threlfall WJ, Tamaro S. 1991. Brain cancer and exposure to electromagnetic fields. J Occup Med 33: 944 – 945

Garaj-Vrhovac V. 1999. Micronucleus assay and lymphocyte mitotic activity in risk assessment of occupational exposure to microwave radiation. Chemosphere 39: 2301 – 2312

Garson OM, McRobert TL, Campbell LJ, Hocking BA, Gordon I. 1991. A chromosomal study of workers with long-term exposure to radio-frequency radiation. Med J Australia 155: 289 – 292.

Grayson JK. 1996. Radiation exposure socioeconomic status and brain tumor risk in the US Air Force: a nested case-control study. Am J Epidemiol 143: 480-486.

Groves FD, Page WF, Gridley G, Lisimaque L, Stewart PA, Tarone RE et al. 2002. Cancer in Korean war navy technicians: mortality survey after 40 years. Am J Epidemiol 155: 810-818.

Hallberg O, Johansson O. 2002a. Melanoma incidence and frequency modulation (FM) broadcasting. Arch Environ Health 57: 32 - 40

Hallberg O, Johansson O. 2002b. Cancer trends during the 20th century. J Australian College Nutrtr Environ Med. 21: 3 – 8

Hayes RB, Brown LM, Pottern LM, Gomez M, Kardaun JWPF, Hoover RN, O'Connell KJ, Sutzman RE, Javadpour N. 1990. Occupation and risk of testicular cancer: a case-control study. Int J Epidemiol 19: 825-831

Hill DG. 1988. A longitudinal study of a cohort with past exposure to radar: the MIT Radiation Laboratory follow-up study. [Dissertation Manuscript], Johns Hopkins University, Baltimore, MD, UMI Dissertation Services, Ann Arbor, MI

Hocking B, Gordon IR, Grain ML, Hatfield GE. 1996. Cancer incidence and mortality and proximity to TV towers. Med J Aust 165: 601-605

Holly EA, Aston DA, Ahn DK, Smith AH. 1996. Intraocular melanoma linked to occupations and chemical exposures. Epidemiology 7: 55-61

Kaplan S, Etlin S, Novikov I, Modan B. 1997. Occupational risks for the development of brain tumors. Am J Ind Med 31: 15 – 20.

Kundi M. 2006. Causality and the interpretation of epidemiologic evidence. Environ Health Perspect 114: 969 – 974

Kurt TL, Milham S. 1988. Re: Increased mortality in amateur radio operators due to lymphatic and hematopoietic malignancies. [Letter and Reply] Am J. Epidemiol 128: 1384–1385

Lagorio S, Rossi S, Vecchia P, De Santis M, Bastianini L, Fusilli M, Ferrucci A, Desideri E, Comba P. 1997. Mortality of plastic-ware workers exposed to radiofrequencies. Bioelectromagnetics 18: 418-421

Lalic H, Lekic A, Radosevic-Stasic B. 2001. Comparison of chromosome aberrations in peripheral blood lymphocytes from people occupationally exposed to ionizing and radiofrequency radiation. Acta Medica Okayama 55: 117 – 127

Lilienfeld AM, Tonascia J, Tonascia S, Libauer CH, Cauthen GM, et al. 1978. Foreign Service Health Status Study: Evaluation of Status of Foreign Service and other Employees From Selected Eastern European Posts. NTIS Document No. PB-28B

163/9GA Dept. of State, Washington DC, Final Report, Dept. of Epidemiology, School of Hygiene Public Health, Johns Hopkins University, Baltimore, MD

Maskarinec G, Cooper J, Swygert L. 1994. Investigation of increased incidence in childhood leukemia near radio towers in Hawaii: preliminary observations. J Environ Pathol Toxicol Oncol 13: 33-37

McKenzie DR, Yin Y, Morrell S. 1998. Childhood incidence and acute lymphoblastic leukaemia and exposure to broadcast radiation in Sydney – a second look. Aust NZ J Public Health 22: 360-367

Michelozzi P, Capon A, Kirchmayer U, Forastiere F, Biggeri A, Barca A, Perucci CA. 2002. Adult and childhood leukemia near a high-power radio station in Rome, Italy. Am J Epidmiol 155: 1096-1103

Milham S. 1982. Mortality from leukemia in workers exposed to electrical and magnetic fields. [Letter] New England J Med 307: 249 – 249

Milham S. 1983. Occupational mortality in Washington State: 1950-1979. DHHS (NIOSH) Publication 83-116, October 1983, Contract No. 210-80-0088, U.S. Depart. of Health and Human Services, National Institute for Occupational Safety and Health, Cincinnati, OH

Milham S. 1985. Mortality in workers exposed to electromagnetic fields. Environ Health Perspect 62: 297 – 300

Milham S. 1988a. Increased mortality in amateur radio operators due to lymphatic and hematopoietic malignancies. Am J Epidemiol 127: 50-54

Milham S. 1988b. Mortality by license class in amateur radio operators. Am J Epidemiol 128: 1175 – 1176

Morgan RW, Kelsh MA, Zhao K, Exuzides KA, Heringer S, Negrete W. 2000. Radiofrequency exposure and mortality from cancer of the brain and lymphatic/hematopoietic systems. Epidemiology 11: 118-127

Moulder JE, Erdreich LS, Malyapa RS, Merritt JH, Pickard WF, Vijayalaxmi. 1999. Cell phones and cancer: what is the evidence for a connection? Radiat Res 151: 513 – 531

Muhm JM. 1992. Mortality investigation of workers in an electromagnetic pulse test program. J Occup Med 34: 287-292

Pearce N, Reif J, Fraser J. 1989. Case-control studies of cancer in New Zealand electrical workers. Int J Epidemiol 18: 55 – 59

Pearce NE, Sheppard RA, Howard JK, Fraser J, Lilley BM. 1985. Leukaemia in electrical workers in New Zealand. [Letter] Lancet 1: 811 – 812

Pearce NE. 1988. Leukemia in electrical workers in new Zealand: a correction. [Letter] Lancet 2: 48

Richter ED, Berman T, Ben-Michael E, Laster R, Westin JB. 2000. Cancer in radar technicians exposed to radiofrequency/microwave radiation: Sentinel episodes. Int J Occup Environ Health 6: 187 – 193

Robinette CD, Silverman C, Jablon S. 1980. Effects upon health of occupational exposure to microwave radiation radar. Am J Epidemiol 112: 39 – 53

Robinette CD, Silverman C. 1977. Causes of death following occupational exposure to microwave radiation (radar) 1950-1974. In Hazzard (ed), Symposium on Biological

Effects and Measurement of radiofrequency Microwaves, Dept. of Health, Education, and Welfare, Washington, DC, HEW Publication No. (FDA) 77-8026: 338 – 344

Selvin S, Schulman J, Merrill DW. 1992. Distance and risk measures for the analysis of spatial data: a study of childhood cancers. Soc Sci Med 34: 769-777

Siekierzynski M, Czerski P, Milczarek H, Gidynski A, Czarnecki C, Dziuk E, Jedrzejczak W. 1974a. Health surveillance of personnel occupationally exposed to microwaves. II. Functional disturbances. Aerospace Med 45: 1143 - 1145

Siekierzynski M, Czerski P, Milczarek H, Gidynski A, Czarnecki C, Dziuk E, Jedrzejczak W. 1974b. Health surveillance of personnel occupationally exposed to microwaves. III. Lens translucency. Aerospace Med 45: 1146 – 1148

Speers MA, Dobbins JG, Miller VS. 1988. Occupational exposures and brain cancer mortality: a preliminary study of East Texas residents. Am J Ind Med 13: 629 – 638

Spitz MR, Johnson CC. 1985. Neuroblastoma and paternal occupation. A case-control analysis. Am J Epidemiol 121: 924 – 929

Stewart, Sir W. 2000. Mobile Phones and Health. Report by the UK Independent Expert Group on Mobile Phones. c/o UK National Radiological Protection Board, Chilton, Didcot, Oxon OX11 0RQ pp. 1 – 160.

Szmigielski S, Kubacki R. 1999. Analysis of cancer morbidity in Polish career military personnel exposed occupationally to RF and MW radiation. In: F. Bersani (ed.), Electricity and Magnetism in Biology and Medicine, Kluwer Academic/ Plenium, pp. 809 – 812.

Szmigielski S. 1996. Cancer morbidity in subjects occupationally exposed to high frequency (radiofrequency and microwave) electromagnetic radiation. Sci Total Environ 180: 9-17.

Thomas TL, Stolley PD, Stemhagen A, Fontham ETH, Bleeker ML, Stewart PA et al. 1987. Brain tumour mortality risk among men with electrical and electronic jobs: a case-control study. J Natl Cancer Inst 79: 233-238

Tornqvist S, Knave B, Ahlbom A, Persson T. 1991. Incidence of leukaemia and brain tumours in some 'electrical occupations'. Brit J Indust Med 48: 597 – 603

Tynes T, Andersen A, Langmark F. 1992. Incidence of cancer in Norwegian workers potentially exposed to electromagnetic fields. Am J Epidemiol 136: 81-88

Tynes T, Hannevik M, Andersen A, Vistnes AI, Haldorsen T. 1996. Incidence of breast cancer in Norwegian female radio and telegraph operators. Cancer Causes Control 7: 197-204

Wiklund K. 1981. An application of the Swedish cancer-environment registry: leukaemia among Telefone operators at the telecommunications administration in Sweden. Int J Epidemiol 10: 373 – 376

Wright WE, Peters JM, Mack TM. 1982. Leukaemia in workers exposed to electrical and magnetic fields. Lancet 307: 1160 – 1161

_____

Evidence for Childhood Cancers (Leukemia)

2012 Supplement

(Replaces 2007 Chapter)

Prof. Michael Kundi, PhD med habil

Head: Institute of Environmental Health

Medical University of Vienna

Vienna, Austria

I. INTRODUCTION

The International Agency for Research on Cancer (IARC) concluded in 2001 that power-frequency magnetic fields are a possible human carcinogen (Group 2B). This classification was based on the evidence from epidemiological studies of childhood leukemia. The panel rated the evidence from all other types of cancer, from long-term animal experiments and mechanistic studies as inadequate. The IARC working group decided that the association between power frequency magnetic fields and childhood leukemia can be interpreted as only limited evidence because bias and confounding cannot be ruled out.

Since the seminal work of Wertheimer and Leeper (1979) many epidemiological studies of childhood cancer and residential exposure to power-frequency EMFs were published, not counting some studies about electrical appliances and cluster observations. Although these studies make up an impressive body of evidence, there is an ongoing discussion whether the observed relationships between exposure to power-frequency EMFs and childhood cancer (in particular leukemia) can be causally interpreted. Based on the comparatively few empirical studies virtually hundreds of commentaries, reviews and meta-analyses have been produced, more often than not increasing confusion instead of clarifying the issue. In 2000 two pooled analyses of childhood leukemia, the endpoint most often studied, have been published, one (Ahlbom et al., 2000) that was restricted to 9 studies that fulfilled a number of strict inclusion criteria (a defined population base for case ascertainment and control selection and using measurements or historical magnetic field calculations for exposure assessment), and another (Greenland et al., 2000) including also wire-code studies. Both pooled analyses got essentially the same result: a monotonously increasing risk with increasing power-frequency (50Hz/60Hz) magnetic field levels. These pooled analyses were the bases for the IARC working group decision.

Typically, if an agent is classified as a Group 2B carcinogen, precautionary measures are taken at workplaces and special care is recommended if it is present in consumer products (e.g. lead, styrene, benzofuran, welding fumes). Concerning power-frequency EMFs the WHO International EMF Program made the following exceptional statement: "In spite of the large number data base, some uncertainty remains as to whether magnetic field exposure or some other factor(s) might have accounted for the increased leukaemia incidence." (WHO Fact Sheet 263, 2001). This is the line of arguments that has been unswervingly followed by the electrical power industry since the early 1980's. An endless chain of factors allegedly

responsible for the 'spurious' positive association between power-frequency EMF exposure and cancer has been put forward, leading to nothing except waste of energy and money. The statement of WHO is scientifically flawed because there is no finite number of empirical tests to refute it. It is always possible that some factor not yet tested could be responsible, however low the probability that it remained obscure for such a long time. In the last years, due to the fact that no confounding factor has been found that explains the increased leukemia risk, a slight change of arguments can be discerned that consists of pointing out the very low proportion of children (less than 1%) exposed to power frequency fields associated with a significantly increased risk. In fact, both pooled analyses concluded that there is little indication of an increased risk below 3 to 4 mG magnetic flux density.

Since the evaluation of IARC several other epidemiological studies have been published that corroborate the earlier findings and strengthen the evidence of an association. It becomes increasingly less likely that confounding factors exist that operate all over the world and still remained undetected.

In the following chapters we will present the epidemiological evidence, discuss potential biases and demonstrate that from a worst-case scenario the evidence compiled so far is consistent with the assumption of a much greater proportion of leukemia cases attributable to power frequency field exposure than previously assumed. The key problem identified is the lack of a bio-physical model of interaction between very weak ELF EMFs and the organism, tissues, cells, and biomolecules.

II.   EPIDEMIOLOGICAL STUDIES OF POWER-FREQUENCY EMF AND CHILDHOOD CANCER

Table 11-4 gives a synopsis of studies on childhood cancer and exposure to power-frequency EMF, Table 11-5 presents the main findings of these investigations. Most often assessment of exposure was by measurements with 16 studies measuring for at least 24 hours up to 7 days, and 9 studies with spot measurements. Eleven studies used distance from power lines as a proxy (some in combination with spot measurements) and 11 studies used wire codes (solely or in addition to other methods) classified according to the Wertheimer-Leeper or Kaune-Savitz methods or some modifications thereof accounting for specific power grid conditions. Several investigations covered more than one endpoint with hematopoietic cancers the most

frequently included malignancies (overall 37 studies), followed by nervous system tumors (13 studies) and other cancers (10 studies). All childhood cancer cases were assessed by 9 investigations.

The most restrictive criteria for combining the evidence for an association between ELF magnetic fields (MF) exposure and childhood leukemia were applied by Ahlbom et al., (2000) that included 9 investigations. Table 11-1 shows the results of these investigations for the exposure category ≥ 4 mG (against < 1 mG as reference category). The studies included 3,203 children with leukemia, 44 of which were exposed to average flux densities of 4 mG or above. Thus only 1.4% of children with leukemia and less than 1% of all children in the studies were exposed that high in accordance with measurement samples from the general population in Europe, Asia and America (Brix et al., 2001; Decat et al., 2005; Yang et al., 2004; Tomitsch et al. 2010; Zaffanella, 1993; Zaffanella & Kalton, 1998).

Meta-analyses of wire-code studies (Greenland et al., 2000; Greenland 2003; Wartenberg, 2001) revealed similar results for childhood leukemia with estimates of risks around 2 for very high current codes but with considerable heterogeneity across studies.

Table 11- 1: Results from nine studies included in Ahlbom et al. (2000) updated according to Schüz (2007) of residential MF exposure and risk of childhood leukemia

Country	Odds-Ratio[*] (95%-CI)	Observed Cases
Canada	1.55 (0.65−3.68)	13
USA	3.44 (1.24−9.54)	17
UK	1.00 (0.30−3.37)	4
Norway	0 cases / 10 controls	0
Germany	3.53 (1.01−12.3)	7
Sweden	3.74 (1.23−11.4)	5
Finland	6.21 (0.68−56.9)	1
Denmark	2 cases / 0 controls	2
New Zealand	0 cases / 0 controls	0
Overall	2.08 (1.30 – 3.33)	49

[*] 24-h geometric mean MF flux density of ≥ 4 mG against <1 mG

In 2010 Kheifets et al. published a pooled analysis of studies that appeared after the analyses of Ahlbom et al. (2000) and Greenland et al. (2000). This analysis included data from Bianchi et al. (2000) , Kabuto et al. (2006), Kroll et al. (2010), Lowenthal et al. (2007), Malagoli et al. (2010), Schüz et al. (2001), and Wünsch-Filho et al. (2011). For this pooled analysis the data from Bianchi et al. (2000) were extended by 5 years. Table 11-2 gives an overview of the results of this pooled analysis.

Table 11- 2: Results from the pooled analysis of 7 (6) studies of residential MF exposure and risk of childhood leukemia (Kheifets et al. 2010a) and of the earlier pooled analysis of 9 other studies (Ahlbom et al. 2000). Shown are odds ratios (95% confidence interval) adjusted for age, sex, SES and study.

Exposure category	Kheifets et al. 2010a	Kheifets et al. 2010a without Brazil	Ahlbom et al. 2000
<1 mG (ref)			
1-2 mG	1.07 (0.81 – 1.41)	1.15 (0.83 – 1.61)	1.08 (0.89 – 1.31)
2-4 mG	1.22 (0.78 – 1.89)	1.20 (0.67 – 2.17)	1.11 (0.84 – 1.47)
≥4 mG	1.46 (0.80 – 2.68)	2.02 (0.87 – 4.69)	2.00 (1.27 – 3.13)
>200 m (ref)			
100-200 m	1.20 (0.90, 1.59)		
50-100 m	1.30 (0.89, 1.91)		
≤50 m	1.59 (1.02, 2.50)		

In addition to studies investigating the risk of leukemia in relation to power frequency MF the hypothesis has been examined that effects on relapse and survival in newly diagnosed acute lymphoblastic leukemia occur (Foliart et al. 2006, 2007). There was a significantly increased hazard ratio for death at exposures ≥3 mG that was based on four deaths only.

The only other endpoint except leukemia and other hematopoietic diseases that has been investigated in several studies is nervous system tumors. The number of cases studied is too low to allow a differentiation according to diagnostic subgroups. Several papers have investigated childhood CNS tumors amongst other endpoints, including leukemia (Wertheimer & Leeper, 1979; Tomenius, 1986; Savitz et al., 1988; Feychting & Ahlbom, 1993; Olsen et al., 1993; Verkasalo et al., 1993; Tynes & Haldorsen, 1997; UKCCS, 1999; 2000; Draper et al., 2005; Kroll et al., 2010), whereas others have solely investigated CNS tumors (Gurney et al., 1996; Preston-Martin et al., 1996; Schüz et al., 2001b; Saito et al., 2010). In most cases the time window was restricted to the postnatal period. Exposure was assessed based on residential proximity to overhead power lines, measurements and wiring

configurations of houses. In a meta-analysis of childhood brain tumor studies (Wartenberg et al., 1998) estimates of risk were similar whether based on calculated fields (OR 1.4, 95% CI: 0.8 – 2.3), measured fields (OR 1.4, 95% CI: 0.8 – 2.4), wire codes (OR 1.2, 95% CI: 0.7 – 2.2), or proximity to electrical installations (OR 1.1, 95% CI: 0.7 – 1.7). The few studies published after this review do not change these figures substantially. Kheifets et al. (2010) report a pooled analysis of 10 studies using measured or calculated fields. The results are summarized in Table 11-3.

Table 11- 3: Summary of results from a pooled analysis of 10 studies of residential MF exposure and risk of childhood brain tumors (Kheifets et al. 2010b). Shown are odds ratios (95% confidence interval) adjusted for age and sex.

Exposure category	Type of measurement		
	Long-term	Calculated fields	Spot
<1 mG (ref)			
1-2 mG	1.13 (0.69 - 1.87)	1.06 (0.53 - 2.11)	1.16 (0.79 - 1.72)
2-4 mG	0.94 (0.43 - 2.06)	0.56 (0.19 - 1.60)	1.21 (0.67 - 2.18)
≥4 mG	1.35 (0.39 - 3.71)	1.21 (0.53 - 2.78)	0.68 (0.26 - 1.80)
Exposure category	Type of home exposure		
	Home at diagnosis	Longest lived-in	Birth home
<1 mG (ref)			
1-2 mG	0.89 (0.60 - 1.31)	1.42 (0.79 - 2.56)	1.03 (0.59 - 1.80)
2-4 mG	0.77 (0.44 - 1.36)	0.86 (0.28 - 2.65)	0.79 (0.34 - 1.80)
≥4 mG	1.08 (0.54 - 2.16)	2.19 (0.57 - 8.44)	1.14 (0.52 - 2.49)

III. DISCUSSION

With overall 42 epidemiological studies published to date power frequency EMFs are among the most comprehensively studied environmental factors. Except ionizing radiation no other environmental factor has been as firmly established to increase the risk of childhood leukemia, but for both there are ongoing controversies. Although data from atomic bomb survivors and radiotherapy of benign diseases (ringworm, ankylosing spondylitis, and thymus enlargement) clearly indicate a causal relationship between exposure and leukemia, for other conditions like living in the vicinity of nuclear power plants, diagnostic x-rays, exposure secondary to the Chernobyl incident evidence is less clear and therefore no agreement has been reached so far. Concerning power frequency EMFs few deny that the relationship is real and not due to chance, but still there is a discussion whether or not this association can be causally interpreted. Still the possibility that confounding, exposure misclassification, and selection and other biases are responsible for the observed relationship is mentioned as an argument against a causal interpretation. Furthermore, it is often claimed that even if the exposure is causally related, due to the low attributable fraction no expensive measures to reduce exposure are warranted.

The Environmental Health Criteria 238 (WHO 2007) summarizes:

> Scientific evidence suggesting that everyday, chronic low-intensity (above 0.3–0.4 µT) power-frequency magnetic field exposure poses a health risk is based on epidemiological studies demonstrating a consistent pattern of increased risk for childhood leukaemia. Uncertainties in the hazard assessment include the role that control selection bias and exposure misclassification might have on the observed relationship between magnetic fields and childhood leukaemia. In addition, virtually all of the laboratory evidence and the mechanistic evidence fail to support a relationship between low-level ELF magnetic fields and changes in biological function or disease status. Thus, on balance, the evidence is not strong enough to be considered causal, but sufficiently strong to remain a concern.
> Although a causal relationship between magnetic field exposure and childhood leukaemia has not been established, the possible public health impact has been calculated assuming causality in order to provide a potentially useful input into policy. However, these calculations are highly dependent on the exposure distributions and other assumptions, and are therefore very imprecise. Assuming that the association is causal, the number of cases of childhood leukaemia worldwide that might be attributable to exposure can be estimated to range from 100 to 2400 cases per year. However, this represents 0.2 to 4.9% of the total annual incidence of leukaemia cases, estimated to be 49 000 worldwide in 2000. Thus, in a global context, the impact on public health, if any, would be limited and uncertain. (pp.11-12)

Concerning preventive measures with respect to long-term effects it is stated:

> Implementing other suitable precautionary procedures to reduce exposure is reasonable and warranted. However, electric power brings obvious health, social and economic benefits, and precautionary approaches should not compromise these benefits. Furthermore, given both the weakness of the evidence for a link between exposure to ELF magnetic fields and childhood leukaemia, and the limited impact on public health if there is a link, the benefits of exposure reduction on health are unclear. Thus the costs of precautionary measures should be very low. (p.13)

The sequence of arguments is as follows:

- There are possible biases, exposure misclassification and confounding that could lead to spuriously increased risks

- There is no support from animal experiments and mechanistic studies for the association found in epidemiological investigations

- Therefore the association cannot be causal interpreted

- Even if the association is causal the number of attributable cases is low because of the small proportion of exposed children

- Therefore only low-cost precautionary measures are warranted.

In the following sections we will challenge these arguments.

A.   The association between power frequency MF and childhood leukemia

After the pooled analyses of Ahlbom et al. (2000) and Greenland et al. (2000) were published several other epidemiological investigations were conducted that did not change the conclusions of an association between power frequency MF and childhood leukemia. Seven of these additional investigations were included in a pooled analysis by Kheifets et al. (2010a). Seven other studies were excluded for several reasons: because only distance to power lines was assessed, because data were not available in time etc. Overall the results of all studies taken together speak in favor of an association between exposure to power frequency MF and childhood leukemia (see Table 11-5).

B.   Confounding

A confounder is a factor that is associated with the agent in question as well as with the disease. Hence a confounder must be a risk factor for the disease. Concerning childhood leukemia it was clear from the very beginning that any suggested confounder must be purely

speculative since there is no established environmental risk factor except ionizing radiation. Even if a condition can be found that is strongly associated with exposure to power frequency fields, if it is not associated with childhood leukemia it cannot confound the relationship. In the homogenous case, i.e. the association between EMF exposure and the confounder does not depend on disease status, and the confounder - leukemia association is independent of exposure to power frequency EMFs, even a stronger assertion can be proven: power frequency EMF remains a risk factor if the risk associated with the confounder is smaller than that associated with power frequency EMFs. Equation (1) gives the bias-factor for the homogenous case and dichotomous exposure variables (that can, however, easily be extended to categorical or continuous exposure variables):

$$B_F = \frac{1 + \pi_F(\Psi_{AF}\Psi_{DF} - 1)}{[1 + \pi_F(\Psi_{AF} - 1)][1 + \pi_F(\Psi_{DF} - 1)]} \tag{1}$$

(π_F is the prevalence of the confounder, Ψ_{DF} is the odds ratio for the confounder with respect to the disease, and Ψ_{AF} is the odds ratio of the agent in question with respect to the confounder). From this equation it is immediately clear that if either Ψ_{DF} or Ψ_{AF} or both are 1 there is no bias (i.e. the confounder is no risk factor for the disease and/or the agent in question is not associated with the confounder). This equation can be used to obtain limiting conditions for the odds ratio of the confounder given specific associations with power frequency fields. This has been done by Langholz (2001).

Langholz (2001) investigated factors that have been proposed as possible confounders based on data from Bracken et al. (1998). None of these factors on their own explain the power frequency EMF - leukemia relationship. It has been criticized (Greenland, 2003) that too far reaching conclusions have been drawn based on the failure to discover a single factor that may explain the relationship, because combinations of such factors have not been addressed. However, even considering combinations of confounders it is unlikely that confounding alone explains the relationship between power frequency EMFs and childhood leukemia.

Because of the rather small relative risks of around two for average exposure to $\geq$ 3 to 4 mG magnetic flux density or very high current codes there is, however, a possibility that bias due to a combination of confounding and other errors account for the increased risk. It will be shown in the last section that the most important aspect is the exposure metric. A much higher risk may be associated with exposure to power frequency fields. If this is actually the case the problem of bias of other provenience disappears.

Because the increased risk from high levels of exposure to power frequency EMFs is found all over the world a confounder explaining this increased risk must not be quite strong and associated with magnetic fields of various sources but must also be present everywhere in the world. It is virtually impossible that such a risk factor has not yet been detected. Therefore, confounding alone as an explanation for the relationship with leukemia can practically be ruled out.

C.   Exposure misclassification

Disregarding chance variations, non-differential exposure misclassification (i.e. misclassification that does not depend on disease status) always leads to an underestimation of the risk. The methods applied to calculate or measure MF in the residences of children are unlikely producing a bias that depends on the disease status (they have usually been done blinded to the case or controls status). Hence, if exposure misclassification was present this will rather have reduced the overall risk estimate. Different effects must be considered whether sensitivity (the probability that a child that was exposed is correctly classified as exposed) or specificity (the probability that a child that was not exposed is correctly classified as not exposed) is affected by the assessment method. The bias depends on six parameters (the exposure prevalence, the true odds ratio, the sensitivity and specificity in cases and controls). A thorough analysis of the effect of different types of exposure misclassification reveals that the vast majority of cases result in a bias towards the zero hypothesis. For low exposure prevalence the impact of a lack of specificity is greater than that of a lack of sensitivity, while for large exposure prevalence the opposite is the case. Considering that high levels of magnetic fields have a low prevalence an increase of specificity (i.e. reducing the number of false positives) has a greater impact on the reduction of bias than of increasing sensitivity (i.e. reducing the number of false negatives). This could explain why odds ratios tend to increase if longer measurements are applied.

Overall, exposure misclassification is a very unlikely cause of a bias in the direction of a higher odds ratio.

D.   Selection bias

In studies that were relying on individual measurements selection bias may have played an important role. Participation rates were sometimes lower in controls and especially for families with lower SES. Schüz et al. (2001b) calculated in a simulation study that about two

thirds of the increased risk could be due to selection bias. Although Wartenberg (2001) applying a meta-regression could not establish any aspect of study methodology that could account for the variation across studies, it is possible that the proportion of children exposed to high levels of MF has been underestimated in some studies.

The biased odds ratio can be factored into the true odds ratio and a bias factor. The bias factor is often called the selection odds ratio. It can be estimated if there are some data on exposure for non-participants. In the study from Brazil (Wünsch-Filho et al. 2011) measurements of magnetic flux density at the front door of participating and non-participating cases and controls have been conducted that allow computation of the bias factor. It turned out to be 1.08, which indicates a slight bias towards an increased risk. The specific conditions of the study in Brazil (e.g. restriction to cases and controls that did not move to a district outside Sao Paulo, inclusion of children less than 9 years, differences in age distribution of participants and non-participants) do not allow generalization to other studies. However, due to the fact that studies that were registry based obtained essentially the same results speak against a distorting selection bias.

E. Exposure metric

After measurements of MF over 24 hours or even longer periods were introduced lower risk estimates for measured fields as compared to estimates from wire codes were noted. This observation was termed the "wire code paradox". Although much of the discrepancies disappeared after the pooled analyses (Ahlbom et al., 2000; Greenland et al., 2000), and also the comprehensive meta-analysis of Wartenberg (2001) could find no support for a systematic effect, still in some investigations there was indeed a stronger relationship to estimates from wire codes as compared to measurement. Bowman et al. (1999) and Thomas et al. (1999) published a thorough analysis of this aspect based on data of the Californian childhood leukemia study (London et al., 1991). They correctly noted the different error structure associated with measured fields and calculated fields from the wire codes that are more stable over time. They further pointed to the fact that the bias introduced by basing the risk estimate on exposure variables that are unbiased but prone to statistical variation will be towards the null. It can be shown that this bias is inversely related to the conditional variance of the exposure metric. Hence the higher the variance of the used exposure metric, conditional on the true one, the greater the bias of the risk estimate.

Up to now most considerations put forward were directed towards identification of factors and methodological issues that would explain a spurious relationship between power frequency EMFs and childhood leukemia. Hardly anyone asked the question: "Why is the risk estimated so low?" This question should, however, been asked because there are a number of intriguing facts: First of all, in developing countries with low levels of electrification childhood leukemia incidence is manifold lower as compared to industrialized regions (Parkin et al., 1998). Although registry data in developing countries are less reliable and sparse the difference is too pronounced to be due to underreporting. The time trend of childhood leukemia in industrialized countries suggests that childhood leukemia in the age group below 4 to 5 years of age is essentially a new phenomenon that emerged in the 1920s. Milham and Ossiander (2001) suggest that the acute lymphoblastic leukemia peak is due to electrification. Given the evidence of the pooled analyses, risk increases as a function of average MF flux density reaching significance at the far end of the exposure distribution for children exposed to an average of 3 to 4 mG. This result is clearly not in line with the hypothesis that much if not all of childhood leukemia (at least for the most prevalent ALL type in the age group of 2 to 4 years) is due to power frequency EMFs. Obviously there are two conclusions possible: either the hypothesis is wrong or the data must be reinterpreted.

Another difficulty arises due to the fact that animal studies and in vitro tissue culture investigations provided equivocal evidence for a causal relationship between power frequency EMFs and cancer. There is a fundamental problem in clarifying the etiological role of the exposure in the development of leukemia. According to present theory (Greaves 1999; 2002; 2003; 2006; Wiemels et al., 1999) childhood leukemia is a consequence of several (at least two) genetic events one of which already occurred before birth. Factors affecting childhood leukemia may therefore be related to different critical exposure windows: the preconceptional, the prenatal, and the postnatal period. Preconceptional factors may affect the mother and the grandmother during pregnancy with the mother, as well as the father during spermatogenesis. During the prenatal period exposure of the mother during pregnancy and exposure of the fetus may differentially affect the first stage of the disease. In fact, there is evidence that at birth around 1% of children show genetic deviations in cord blood cells (Wiemels et al., 1999; Eguchi-Ishimae et al., 2001; Mori et al., 2002) that could lead to leukemia conditional on them surviving and on additional genetic or epigenetic events. While the frequency of these deviations at birth might have been overestimated it is still manifold higher than the cumulative probability of childhood leukemia. Given this higher incidence of

early genetic events, a causal factor for childhood leukemia need not be directly genotoxic and not even mutagenic. A slight but continuous shift of the balance towards survival and proliferation of deviating clones will be sufficient to dramatically increase the incidence. Experimental investigations were generally insufficient to cover such effects.

Assuming that there is an exposure metric, intimately connected to average magnetic flux densities, and actually related to that condition responsible for the increased incidence of childhood leukemia, how does such a metric look like? Actually it is easy to derive the necessary conditions for such an exposure metric from bias considerations. There are only two such conditions that must be met:

    a. The conditional expectancy $E(x|z) = z$ (or equal to a linear function of z); where x is the unknown exposure metric and z is the logarithm of the true average magnetic flux density the child is exposed to.

    b. The conditional variance $V_{x|z}$ must be inversely related to z.

Based on the pooled analysis of Ahlbom et al. (2000) and assuming average magnetic flux density follows a log-normal distribution with mean 0.55 mG and a geometric standard deviation of 1, using the complete data set of cases and controls, the results of the pooled analysis can be reconstructed. However, *by varying the magnitude of the variance and the slope of the logistic function relating the purported exposure metric to the probability of developing childhood leukemia up to 80% of all cases can be attributed to the exposure.*

Fig.1 shows one of such Monte Carlo analyses. It can be seen that the bias of the risk estimate related to average MF flux density decreases as the level increases, however, the bias with respect to the assumed exposure metric reaches a factor of about 25 at levels above the third quartile. Of course, the precision of the actual measurements is much lower than indicated in the figure that is constructed by sampling from a theoretical log-normal distribution. However, this does not affect the validity of the argument since imprecisions in the average flux density lead to a bias towards 1. Therefore, the argument even holds in the absence of a relevant imprecision in measurements. The simulation was performed in such a way that exactly the same number of cases and controls are allocated to the average flux density categories as reported in Ahlbom et al. (2000) while varying the relationship between the theoretical alternative exposure metric that has the features a. and b. outlined above. Assuming that this correct metric is causally related to childhood leukemia, attributable

fractions between 1% and 80% are calculated dependent on the relationship between the average MF flux density and this assumed metric.

While of course this analysis does not prove the assumption that most of childhood leukemia is due to electrification, it demonstrates that the data obtained so far do not contradict this assumption. It is of crucial importance to analyze existing measurement data for aspects of the exposure that are in line with conditions a. and b. stated above. These exposure conditions may be analyzed by in vitro studies to asses their potential the facilitate transformation of already genetically damaged cells.
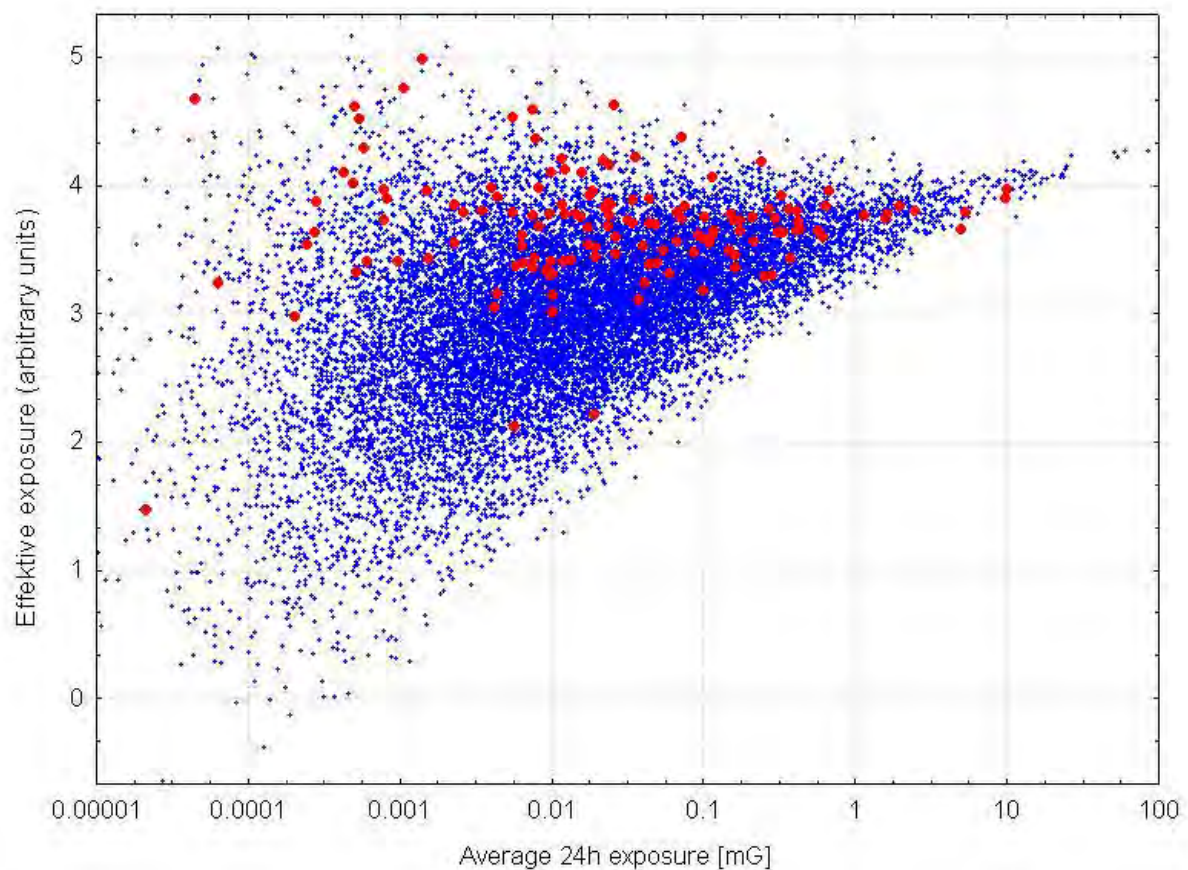

Fig. 1: Results of Monte Carlo simulation under the assumption of a log-normal distribution of average magnetic flux densities in the homes of children that are related to an assumed ‚effective' exposure metric that follows the conditions a. and b. mentioned in the text. Blue are controls and red children with leukemia. The purported ‚effective' exposure metric is associated with an attributable fraction of 80% and the odds-ratio for the highest quartile is around 50.

IV. CONCLUSIONS

The only endpoint studied so far in sufficient detail is childhood leukemia. Brain and nervous system tumors were also studied in some detail but due to the diversity of these tumors no conclusions can be drawn.

Childhood leukemia is the most frequent childhood malignancy that peaks in the age group of 2 to about 5 years. This peak seems to have been newly evolved in the early quarter of the 20th century and may be due to electrification. This assumption is supported by the absence of this peak or it being much less pronounced in developing countries.

An overview of existing evidence from epidemiological studies indicates that there is a continuous increase of risk with increasing levels of average magnetic field exposure. Risk estimates reach statistical significance at levels of 3 to 4 mG. A low number of children are exposed at these or higher levels.

As an alternative interpretation of the association of leukemia with power frequency MF contact currents have been put forward (Kavet et al. 2000). Indeed, considering that a correlation between the magnitude of contact currents in the homes (e.g. in the bathtub) has been found and dosimetry indicates that high levels of internal fields could exist in the bone marrow of children touching metallic water fixtures, the hypothesis has some empirical support. However, a report from an epidemiological investigation in California (Does et al. 2011) could find no indication that contact currents play a decisive role while results for MF flux densities are in line with the previous findings of an increased risk with increasing exposure to power frequency MF in the homes.

I have pointed out (Kundi 2006) that under four conditions (temporal relation, association, environmental equivalence, and population equivalence) epidemiological evidence alone is sufficient to suggest disease causation. This is in line with the hazard assessment of IARC that specifies the default rule for assessing an agent as carcinogenic if there is sufficient evidence from epidemiological studies. Support from animal experiments or mechanistic studies is not necessary in these cases. Evidence from epidemiological studies is considered sufficient if a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence.

In the studies of childhood leukemia and residential exposure to power frequency magnetic fields measurements have been conducted after diagnosis. This is a violation of the condition

of temporal relation. However, these measurements can be considered an estimate of the exposure during the etiologically relevant period. But still it would result in some exposure misclassification. Because this type of misclassification is non-differential it can only reduce the observed association. Furthermore, support comes from studies with calculated fields that cover the relevant period. Therefore, the epidemiological evidence can be considered to fulfill the criterion.

Due to the small fraction of homes with very high exposure levels single studies have often insufficient power to detect an effect of the assumed magnitude of a doubling of the risk at levels around 3-4 mG. Therefore, meta-analyses and pooled analyses are important to investigate whether the association is due to chance. These analyses show a statistically significant association. There is no indication of a threshold but some investigations found reduced risks at intermediate levels, which might be due to inconsistencies in the sources that account for these exposure levels. There is sufficient evidence of an association that is apparent based on measurements, calculations, wire codes and other proxies for exposure.

Most studies used matching by at least sex and age, some added other potential confounders like region, SES, number of siblings etc. Care has been applied in most investigations to have the same population base for cases and controls. Studies investigating potential confounders did not reveal any factor other than exposure to power frequency MF that could be responsible for the observed association. There is only one cohort study (Verkasalo et al. 1993). This study, although with only 140 childhood cancer cases, is in line with the assumption of an association. An important analysis using the case-specular method supports the assumption of population and environmental equivalence (Ebi et al. 1999). Because the etiology of childhood leukemia is still not clear it is difficult to directly test the features most relevant for assessing the ceteris paribus condition. One investigation (Yang et al. 2008) indicates that power frequency MF may interact with specific genetic conditions. These results can be interpreted in two ways: the risk of leukemia from exposure to MF may be increased only in individuals harboring some specific polymorphism, on the other hand it is possible that exposure increases the genetic instability independently of an already increased instability due to a genetic polymorphism leading to a greater probability of developing the disease. At present there is no evidence to discriminate between these possibilities. If the first interpretation is valid different fractions of children harboring the relevant genetic condition would result in differences in the observed risk and thus some studies could have violated the population equivalence principle. Only in this case, it would be failure to detect an effect and

not a spuriously increased risk. Overall, there is no reason to assume that the principles of population and environmental equivalence has been violated in such a way that spuriously increased risks could have resulted.

For all these reasons it can be concluded that there is sufficient evidence from epidemiological studies of an increased risk from exposure to power frequency MF that cannot be attributed to chance, bias or confounding. Therefore, according to the rules of IARC such exposures can be classified as a group 1 carcinogen.

It has to be stressed, however, that according to the rules of IARC the working groups may up- or down-grade the classification upon consideration of the overall evidence. The IARC working group considered the lack of supporting evidence from animal experiments and in vitro studies as sufficient to down-grade the classification to 2B. Although it is not possible to discuss this aspect in this context, there are several problems with this view: first, there is no animal model for ALL, the most frequent childhood leukemia type; second, animal studies are difficult due to the fact that procedures usually applied, i.e. exposure levels just below the acute toxicity level, cannot be followed for MFs due to muscle and nerve excitations accompanying such exposures; third, at levels relevant for human long-term exposure in vitro experiments would have to detect extremely rare cellular events to account for the increased risk observed in epidemiological investigations, which is impossible using methods available to date. Therefore, strong and consistent support from such studies can neither be expected nor demanded. Consequently, lack of support from such evidence cannot be used as an argument to down-grade the classification based in epidemiology.

Considering the possibility that aspects of exposure to power frequency EMFs that have not yet been detected may account for a greater proportion of cases than assumed there are two necessary steps to be taken: Concerted efforts must be undertaken to scrutinize existing data and collect new ones that should reveal whether or not exposure metrics exist that show the necessary conditions for an effective exposure metric; and, second, precautionary measures must be delineated that result in a reduction of all aspects of exposure to power frequency EMFs.

Exposure guidelines of IEEE and ICNIRP are solely derived from immediate effects such as nerve and muscle excitations. These guidelines are indeed sufficient to protect from such acute effects (although indirect effects from contact currents cannot be ruled out). Evidence for long-term chronic effects has been collected in the past decades and has reached a state

that it cannot longer be denied that these effects are real. Only under very exceptional and remote conditions of a combination of several unknown confounders, selection bias and differential exposure misclassification the established relationship could be spurious. These combinations must have been present all over the world. There is no other risk factor identified so far for which such unlikely conditions have been put forward to postpone or deny the necessity to take steps towards exposure reduction. As one step in the direction of precaution, measures should be implemented to guarantee that exposure due to transmission and distribution lines is below an average of about 1 mG. This value is arbitrary at present and only supported by the fact that in many studies this level has been chosen as a reference.

- The balance of evidence suggests that childhood leukemia is associated with exposure to power frequency EMFs either during early life or pregnancy

- Considering only average MF flux densities the population attributable risk is low to moderate, however, there is a possibility that other exposure metrics are much stronger related to childhood leukemia and may account for a substantial proportion of cases. The population attributable fraction ranges between 1-4% (Kheifets et al., 2007) 2-4% (Greenland & Kheifets 2006), and 3.3% (Greenland 2001) assuming only exposures above 3 to 4 mG are relevant. However, if not average MF flux density is the metric causally related to childhood leukemia the attributable fraction can be much higher. Calculating a guideline level based on the unit-risk approach leads to a level close to 1 mG.

- Other childhood cancers except leukemia have not been studied in sufficient detail to allow conclusions about the existence and magnitude of the risk

- IEEE guideline levels are designed to protect from short-term immediate effects, long-term effects such as cancer seem to be evoked by levels several orders of magnitudes below current guideline levels

- Precautionary measures are warranted that should reduce all aspects of exposure, because at present we have no clear understanding of the etiologically relevant aspect of the exposure

V.   REFERENCES

Abdul Rahman HI, Shah SA, Alias H, Ibrahim HM. 2008. A case-control study on the association between environmental factors and the occurrence of acute leukemia among children in Klang Valley, Malaysia. Asian Pac J Cancer Prev 9(4): 649 – 652

Ahlbom A, Day N, Feychting M, Roman E, Skinner J, Dockerty J, Linet M, McBride M, Michaelis J, Olsen JH, Tynes T, Verkasalo PK. 2000. A pooled analysis of magnetic fields and childhood leukaemia. Br J Cancer 83:692– 698.

Bowman JD, Thomas DC, Jiang L, Jiang F, Peters JM. 1999. Residential magnetic fields predicted from wiring configurations: I. Exposure model. Bioelectromagnetics 20: 399-413.

Bracken MB, Belanger K, Hellebrand K, Adesso K, Patel S, Trich E, Leaderer B. 1998. Correlates of residential wiring configurations. Am J Epidemiol 148: 467–474.

Brix J, Wettemann H, Scheel O, Feiner F, Matthes R. 2001. Measurement of the individual exposure to 50 and 16 2/3 Hz magnetic fields within the Bavarian population. Bioelectromagnetics 22: 323–332.

Coghill RW, Steward J, Philips A. 1996. Extra low frequency electric and magnetic fields in the bedplace of children diagnosed with leukemia: A case-control study. Europ J Cancer Prev 5: 153–158.

Coleman MP, Bell CM, Taylor H-L, et al. 1989. Leukaemia and residence near electricity transmission equipment: a case-control study. Br J Cancer 60:793-798.

Decat G, Van den Heuvel I, Mulpas L. 2005. Final Report of the BBEMG Research Contract, June 10, 2005.

Dockerty JD, Elwood JM, Skegg DCG, Herbison GP. 1998. Electromagnetic field exposures and childhood cancers in New Zealand. Cancer Causes Control 9: 299–309; Erratum 1999; 10:641.

Does M, Scélo G, Metayer C, Selvin S, Kavet R, Buffler P. 2011. Exposure to electrical contact currents and the risk of childhood leukemia. Radiat Res 175(3):390-396

Draper G, Vincent T, Kroll ME, Swanson J. 2005. Childhood cancer in relation to distance from high voltage power lines in England and Wales: a case-control study. Brit Med J 330: 1290-1294.

Ebi KL, Zaffanella LE, Greenland S. 1999. Application of the case-specular method to two studies of wire codes and childhood cancers. Epidemiology 10 (4): 398 – 404.

Eguchi-Ishimae M, Eguchi M, Ishii E, Miyazaki S, Ueda K, Kamada N, Mizutani S. 2001. Breakage and fusion of the TEL (ETV6) gene in immature B lymphocytes induced by apoptogenic signals. Blood 97: 737–743.

Fajardo-Gutierrez A, Garduno-Espinosa J, Yamamoto-Kimura L, Hernandez-Hernandez DM, Gomez-Delgado A, Meija-Arangure M, Cartagena-Sandoval A, del Carmen Martinez-Garcia M. 1993. Close residence to high electric voltage lines and its association with children with leukemia (in Spain). Bol Med Hosp Infant Mex 50: 32-37.

Feizi AA, Arabi MA. 2007. Acute childhood leukemias and exposure to magnetic fields generated by high voltage overhead power lines – a risk factor in Iran. Asian Pac J Cancer Prev 8(1): 69 – 72

Feychting M, Ahlbom A. 1993. Magnetic fields and cancer in children residing near Swedish high-voltage power lines. Am J Epidemiol 138:467– 481.

Foliart DE, Pollock BH, Mezei G, Iriye R, Silva JM, Ebi KL, Kheifets L, Link MP, Kavet R. 2006. Magnetic field exposure and long-term survival among children with leukaemia. Br J Cancer 94(1):161-164.

Foliart DE, Mezei G, Iriye R, Silva JM, Ebi KL, Kheifets L, Link MP, Kavet R, Pollock BH. 2007. Magnetic field exposure and prognostic factors in childhood leukemia. Bioelectromagnetics 28(1):69-71.

Fulton JP, Cobb S, Preble L, Leone L, Forman E. 1980. Electrical wiring configuration and childhood leukemia in Rhode Island. Am J Epidemiol 111: 292-296.

Greaves M. 1999. Molecular genetics, natural history and the demise of childhood leukaemia. Eur J Cancer 35:1941–1953.

Greaves M. 2002. Childhood leukaemia. BMJ 324:283–287.

Greaves M. 2003. Pre-natal origins of childhood leukemia. Rev Clin Exp Hematol 7: 233–245.

Greaves M. 2006. Infection, immune responses and the aetiology of childhood leukaemia. Nat Rev Cancer 6:193–203.

Green LM, Miller AB, Agnew DA, Greenberg ML, Li J, Villeneuve PJ, Tibshirani R. 1999a. Childhood leukemia and personal monitoring of residential exposures to electric and magnetic fields in Ontario, Canada. Cancer Causes Control 10:233-243.

Green LM, Miller AB, Villeneuve PJ, Agnew DA, Greenberg ML, Li J, Donnelly KE. 1999b. A case-control study of childhood leukemia in southern Ontario Canada and exposure to magnetic fields in residences. Int J Cancer 82: 161–170.

Greenland S, Kheifets L. 2006. Leukemia attributable to residential magnetic fields: results from analyses allowing for study biases. Risk Anal 26:471– 481.

Greenland S, Sheppard AR, Kaune WT, Poole C, Kelsh MA. 2000. A pooled analysis of magnetic fields, wire codes, and childhood leukemia. Childhood Leukemia–EMF Study Group. Epidemiol 11:624–634.

Greenland S. 2001. Estimating population attributable fractions from fitted incidence ratios and exposure survey data with an application to electromagnetic fields and childhood leukemia. Biometrics 57: 182–188.

Greenland S. 2003. The impact of prior distributions for uncontrolled confounding and response bias: A case study of the relation of wire codes and magnetic fields to childhood leukemia. J Am Statist Ass 98: 47–54.

Gurney JG, Mueller BA, Davis S, Schwartz SM, Stevens RG, Kopecky KJ. 1996. Childhood brain tumor occurrence in relation to residential power line configurations, electric heating sources, and electric appliance use. Am J Epidemiol 143:120-128.

IARC (International Agency for Research on Cancer) 2002. Monographs on the evaluation of carcinogenic risks to humans: Volume 80. Non-ionizing radiation, Part 1: Static and extremely lowfrequency (ELF) electric and magnetic fields. Lyon, France: IARC Press.

Kabuto M, Nitta H, YamamotoS, Yamaguchi N, Akiba S, Honda Y, Hagihara J, et al. 2006. Childhood leukemia and magnetic fields in Japan: A case-control study of childhood

leukemia and residential power-frequency magnetic fields in Japan. Int J Cancer 119: 643–650.

Kavet R, Zaffanella LE, Daigle JP, Ebi KL. 2000. The possible role of contact current in cancer risk associated with residential magnetic fields. Bioelectromagnetics 21(7):538-553.

Kheifets L, Afifi AA, Shimkhada R. 2007. Public health impact of extremely low frequency electromagnetic fields. Environ Health Perspect.

Kheifets L, Ahlbom A, Crespi CM, Draper G, Hagihara J, Lowenthal RM, Mezei G, Oksuzyan S, Schüz J, Swanson J, Tittarelli A, Vinceti M, Wünsch-Filho V. 2010a. Pooled analysis of recent studies on magnetic fields and childhood leukaemia. Br J Cancer 103(7):1128-1135.

Kheifets L, Ahlbom A, Crespi CM, Feychting M, Johansen C, Monroe J, Murphy MF, Oksuzyan S, Preston-Martin S, Roman E, Saito T, Savitz D, Schüz J, Simpson J, Swanson J, Tynes T, Verkasalo P, Mezei G. 2010b. A Pooled Analysis of Extremely Low-Frequency Magnetic Fields and Childhood Brain Tumors. Am J Epidemiol 172(7):752-761.

Kroll ME, Swanson J, Vincent TJ, Draper GJ. 2010. Childhood cancer and magnetic fields from high voltage power lines in England and Wales: a case-control study. Br J Cancer 103(7):1122-1127

Langholz B. 2001. Factors that explain the power line configuration wiring code-childhood leukemia association: what would they look like? Bioelectromagnetics Suppl.5:S19 – S31.

Li C-Y, LeeW-C, Lin RS. 1998. Risk of leukemia in children living near high-voltage transmission lines. J Occup Environ Med 40:144-147.

Linet MS, Hatch EE, Kleinerman RA, Robison LL, Kaune WT, Friedman DR, Severson RK, Haines CM, Hartsock CT, Niwa S, Wacholder S, Tarone RE. 1997. Residential exposure to magnetic fields and acute lymphoblastic leukemia in children. N Engl J Med 337:1–7.

London SJ, Thomas DC, Bowman JD, Sobel E, Cheng T-C, Peters JM. 1991. Exposure to residential electric and magnetic fields and risk of childhood leukemia. Am J Epidemiol 134: 923–937.

Lowenthal RM, Tuck DM, Bray IC. 2007. Residential exposure to electric power transmission lines and risk of lymphoproliferative and myeloproliferative disorders: a case-control study. Intern Med J 37(9): 614 – 619

Malagoli C, Fabbi S, Teggi S, Calzari M, Poli M, Ballotti E, Notari B, Bruni M, Palazzi G, Paolucci P, Vinceti M. 2010. Risk of hematological malignancies associated with magnetic fields exposure from power lines: a case-control study in two municipalities of northern Italy. Environ Health 9: 16

Maslanyj M, Simpson J, Roman E, Schuz J. 2009. Power frequency magnetic fields and risk of childhood leukaemia: misclassification of exposure from the use of the 'distance from power line' exposure surrogate. Bioelectromagnetics 30(3): 183 – 188

McBride ML, Gallagher RP, Theriault HG, Armstrong BG, Tamaro S, Spinelli JJ, et al. 1999. Power-frequency electric and magnetic fields and risk of childhood cancer. Am J Epidemiol 149: 831–842.

Mejia-Arangure JM, Fajardo-Gutierrez A, Perez-Saldivar M-L, Gorodezky C, Martinez-Avalos A, Romero-Guzman L, Campo-Martinez MA, et al. 2007. Magnetic fields and acute leukemia in children with Down syndrome. Epidemiology 18: 158-161.

Michaelis J, Schüz J, Meinert R, Menger M, Grigat J, Kaatsch P, Kaletsch U, Miesner A, Stamm A, Brinkmann K, et al. 1997a. Childhood leukemia and electromagnetic fields: Results of a population-based case-control study in Germany. Cancer Causes Control 8: 167–174.

Michaelis J, Schüz J, Meinert R, Semann E, Grigat JP, Kaatsch P, et al. 1997b. Combined risk estimates for two German population-based case-control studies on residential magnetic fields and childhood leukemia. Epidemiology 9: 92–94.

Milham S, Ossiander EM. 2001. Historical evidence that residential electrification caused the emergence of the childhood leukemia peak. Medical Hypoth 56: 290–295.

Mizoue T, Onoe Y, Moritake H, Okamura J, Sokejima S, Nitta H. 2004. Residential proximity to high-voltage power lines and risk of childhood hematological malignancies. J Epidemiol 14: 118-123.

Mori H, Colman SM, Xiao Z, Ford AM, Healy LE, Donaldson C, Hows JM, Navarrete C, Greaves M. 2002. Chromosome translocations and covert leukemic clones are generated during normal fetal development. Proc Natl Acad Sci USA 99: 8242–8247.

Myers A, Clayden A, Cartwright R, Cartwright S. 1990. Childhood cancer and overhead powerlines: A case-control study. Brit J Cancer 62: 1008–1014.

Olsen JH, Nielsen A, Schulgen G. 1993. Residence near high voltage facilities and risk of cancer in children. Brit Med J 307: 891–895.

Parkin DM, Kramarova E, Draper GJ, Masuyer E, Michaelis J, Neglia J, Qureshi S, Stiller CA. 1998. International incidence of childhood cancer, Vol II. Lyon, France: IARC; Scientific Publication No. 144.

Perez CB, Pineiro RG, Diaz NT. 2005. Campos electromagneticos de baja frecuencia y leucemia infantil en Cuidad de La Habana. Rev Cubana Hig Epidemiol 43(3): 1–10.

Petridou E, Trichopoulos D, Kravaritis A, Pourtsidis A, Dessypris N, SkalkidisY, Kogevinas M, Kalmanti M, Koliouskas D, Kosmidis H, Panagiotou JP, Piperopoulou F, Tzortzatou F, Kalapothaki V. 1997. Electrical power lines and childhood leukemia: a study from Greece. Int J Cancer. 73(3):345-348.

Preston-Martin S, Navidi W, Thomas D, Lee PJ, Bowman J, Pogoda J. 1996. Los Angeles study of residential magnetic fields and childhood brain tumors. Am J Epidemiol 143: 105-119.

Savitz DA, Wachtel H, Barnes FA, John EM, Tvrdik JG. 1988. Case-control study of childhood cancer and exposure to 60-Hz magnetic fields. Am J Epidemiol 128: 21–38.

Svendsen AL, Weihkopf T, Kaatsch P, Schüz J. 2007. Exposure to magnetic fields and survival after diagnosis of childhood leukemia: a German cohort study. Cancer Epidemiol Biomarkers Prev 16(6):1167-1171.

Schüz J, Grigat JP, Brinkmann K, Michaelis J. 2001a. Residential magnetic fields as a risk factor for acute childhood leukemia: Results from a German population-based case-control study. Int J Cancer 91: 728–735.

Schüz J, Kaletsch U, Kaatsch P, Meinert R, Michaelis J. 2001b. Risk factors for pediatric tumors of the central nervous system: results from a german population-based case-control study. Med Pediatr Oncol 36: 274-282.

Schüz J. 2007. Implications from epidemiologic studies on magnetic fields and the risk of childhood leukemia on protection guidelines. Health Phys 92: 642-648.

Schüz J, Svendsen AL, Linet MS, McBride ML, Roman E, Feychting M, Kheifets L, Lightfoot T, Mezei G, Simpson J, Ahlbom A. 2007. Nighttime exposure to electromagnetic fields and childhood leukemia: an extended pooled analysis. Am J Epidemiol 166(3):263-269

Thomas DC, Bowman JD, Jiang L, Jiang F, Peters JM. 1999. Residential magnetic fields predicted from wiring configurations: II. Relationships to childhood leukemia. Bioelectromagnetics 20: 414-422.

Tomenius L.1986. 50-Hz electromagnetic environment and the incidence of childhood tumors in Stockholm County. Bioelectromagnetics 7: 191–207.

Tomitsch J, Dechant E, Frank W. 2010. Survey of electromagnetic field exposure in bedrooms of residences in lower Austria. Bioelectromagnetics 31(3):200-208.

Tynes T, Haldorsen T. 1997. Electromagnetic fields and cancer in children residing near Norwegian high-voltage power lines. Am J Epidemiol 145:219-226.

UKCCS (UK Childhood Cancer Study Investigators). 1999. Exposure to power-frequency magnetic fields and the risk of childhood cancer. Lancet 354: 1925–1931.

UKCCS (UK Childhood Cancer Study Investigators). 2000. Childhood cancer and residential proximity to power lines. Brit J Cancer 83: 1573–1580.

Verkasalo PK, Pukkala E, Hongisto MY, Valjus JE, Järvinen PJ, Heikkilä KK, Koskenvuo M. 1993. Risk of cancer in Finnish children living close to power lines. Brit Med J 307: 895–899.

Wartenberg D, Dietrich F, Goldberg R, Poole C, Savitz D. 1998. Meta-analysis of childhood cancer epidemiology. Final report. Philadelphia: Information Ventures, Inc. Order Number PR-702871.

Wartenberg D. 2001. Residential EMF exposure and childhood leukemia: meta-analysis and population attributable risk. Bioelectromagnetics Suppl.5: S86-S104.

Wertheimer N, Leeper E. 1979. Electrical wiring configurations and childhood cancer. Am J Epidemiol 109:273-284.

Wertheimer N, Leeper E. 1997. An exchange on the use of wire codes in the NCI study. Microwave News 1997 July/August: 12-14.

WHO (World Health Organization). 2001. Fact Sheet 263.

WHO (World Health Organization). 2007. Environmental Health Criteria 238: Extremely Low Frequency Fields.

Wiemels JL, Cazzaniga G, Daniotti M, Eden OB, Addison GM, Masera G, Saha V, Biondi A, Greaves MF. 1999. Prenatal origin of acute lymphoblastic leukaemia in children. Lancet 354: 1499–1503.

Yang KH, Ju MN, Myung SH. 2004. Sample of Korean's occupational and residential exposures to ELF magnetic field over a 24-hour period. In Abstracts of 26th Annual Meeting of the Bioelectromagnetics Society (pp. 188–189). Washington, DC.

Yang Y, Jin X, Yan C, Tian Y, Tang J, Shen X. 2008. Case-only study of interactions between DNA repair genes (hMLH1, APEX1, MGMT, XRCC1 and XPD) and low-frequency electromagnetic fields in childhood acute leukemia. Leuk Lymphoma 49(12): 2344 – 2350

Zaffanella LE, Kalton GW. 1998. Survey of Personal Magnetic Field Exposure Phase II: 1000-Person Survey.EMFRapid Program Engineering Project No.6 Lee MA: Enertech Consultants. http://www.emf-data.org/rapid6-report.html.

Zaffanella LE. 1993. Survey of residential magnetic field sources. Vol 1. Goals, results, and conclusions. (Report no. TR-102759-VI). Palo Alto, CA: Electric Power Research Institute.

Table 11- 4: Synopsis of childhood cancer epidemiologic studies (1979 – 2012)

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Wertheimer & Leeper 1979	Greater Denver area, Colorado/ 1950-1973/ Case-control	wire-codes by inspection (not blinded) of surroundings of residences occupied at birth and time of death	retrospective (1976-1977) assessment	all assessments within 22 days	age (m), sex, urbanization, SES, family pattern, traffic	344 cancer deaths (age<19) from files, matched controls from next entry in birth register or from alphabetical list
Fulton et al. 1980	Rhode Island/1964-1978/Case-control	power lines (<45.72m from residences) assessed and MF calculated as combined weighted average (based on Wertheimer-Leeper measurements)	retrospective (1979) assessment	all assessments within same period	age(m), SES	119 leukemia patients (age<20) from Rhode Island hospital files; 240 control addresses from birth register

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Tomenius 1986	Stockholm county/ 1958-1973/ Case-control	inspection of visible electrical constructions within 150m of dwellings occupied at birth and diagnosis date; spot measurements at the door of the dwellings (blinded to case status)	retrospective (~1981) assessment	all assessments within same period	age(m), sex(m), district(m)	716 tumor cases (660 malignant, 56 benign) from cancer registry (age<19), matched controls from entry into birth register just before or after index case from same church district
Savitz et al. 1988	Five-county Denver area, Colorado/1976-1983/Case-control	wire-code of homes occupied prior to diagnosis (blinded to case status); spot measurements at the front door, in child's and parent's bedrooms and other rooms of frequent occupancy; interviews of mothers (in some cases fathers or adopted mothers)	retrospective (~1985) assessment	all assessments within same period	age±3y (m), sex(m), area(m), SES, traffic density, maternal age, maternal smoking	356 cancer cases (age<15) from cancer registry (71% interviewed, 36% measurements, 90% wire codes); 278 controls (79% resp.rate) from RDD (80% interviewed, 75% measurements, 93% wire codes)

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Coleman et al. 1989	Four boroughs near London/1965-1980/ Case-control	historical exposure by type and distance of electricity supply within 100 m of residences; distance to center of building assessed blinded to case status; calculations according to peak winter load of the power lines	retrospective assessment	all assessments within same period	age(m), sex(m), year of diagnosis(m)	84 leukemia cases (age<18) and 141 cancer controls from cancer registry
Myers et al. 1990	Yorkshire/1970-1979/ Case-control	assessment of overhead power lines within a distance depending on type of power line (100-500m) of home at birth; flux densities calculated from line load data and distance to center of dwelling	retrospective (1981-1989) assessment	all assessments within same period	age(m), sex(m), district(m), house type	374 cancer cases (age<15) from registries; 588 controls from nearest entry in birth register of the same district
London et al.	Los Angeles County,	24-h MF	measurements	all	age±1 or 2 or	232 leukemia cases

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
1991	CA/1980-1987/Case-control	measurements (IREQ/ EMDEX) at location of child's bed; EF, MF and static magnetic field spot measurements; Wertheimer-Leeper wire code (all facilities within 46m; blinded to case status); interviews with parents about use of appliances etc.	1987-1989	assessments within same period	3y(m), sex(m), ethnicity(m), indoor pesticides, hair dryers, black&white TV, fathers occupational exposure to chemicals	(70% part.rate) from LA County Cancer Surveillance Program (age<11); 232 matched controls (90% part.rate) – 65 as friends of cases, others by RDD (5 digits cases, last 2 random)
Verkasalo et al. 1993	Finland/ 1970-1989/ Retrospective Cohort	estimated magnetic flux density from high-voltage power lines in the center of the building	cumulative and max. flux density any time between birth and diagnosis	n.a.	age, sex, calendar period	68300 boys and 66500 girls (age<20) identified having lived any time after birth in a house with a distance < 500m from a 110, 220, or 400 kV power line and an estimated flux density exceeding 0.1mG; 140 cancer cases from follow-up in cancer registry through 1990.
Feychting &	Sweden/1960-	calculations (blinded)	the year	all	age(m), sex(m),	142 cancer cases within

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Ahlbom 1993	1985/Nested Case-control	based on historical load data, wire configuration and distance from 220 and 400kV power lines and spot measurements (several rooms, 5-min measurements, main current turned on and off)	closest to date of diagnosis	assessments within same period	parish(m), year of diagnosis, apartment/single house, traffic (NO$_2$)	the study base of children (age<16) living on a property <300m from any 220 or 400kV power line; 558 matched controls from the study base.
Olsen et al. 1993	Denmark/1968-1986/ Case-control	calculations based on estimated historical load of overhead transmission lines, transmission cables, and substations (50-400 kV)	retrospective up to 9 mo before birth	all assessments within same period	age(m), sex(m)	1707 cancer cases from registry (age<15) and 4788 matched controls from population register
Fajardo-Gutierrez et al. 1993	Mexico City/not specified/Case-control	interview with parents including assessment of distance and type of transmission and distribution lines, power substations etc.	n.a.	n.a.	age±2y(m), SES	81 leukemia cases from two hospitals; 77 controls from orthopedics or traumatology department

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Coghill et al. 1996	England/1986-1995/ Case-control	E- and H-field probes designed for the study measured 24 h in the bedroom; data used only for the period 20:00 to 08:00	retrospective	parallel measurements in case and control homes	age(m), sex(m)	56 leukemia cases (age<15) from various sources (media advertising, self-help groups, Wessex Health Authority) and 56 controls
Gurney et al. 1996	Seattle area, Washington/1984-1990/Case-control	wire-code by inspection of homes (blinded for case status) occupied within 3 y before diagnosis, electrical appliances by interview with mothers and mailed questionnaire	retrospective (1989-1994) assessment	all assessments within same period	age±2y(m), sex(m), area of residence(m), race, mothers education, family history of brain tumors, ETS, living on a farm, head/neck x-ray, head injury, epilepsy, fits	133 brain-tumor cases (age<20) (74% part.rate) by Cancer Surveillance System; 270 controls by RDD (79% part.rate)

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Preston-Martin et al. 1996	Los Angeles County, California/1984-1991/ Case-control	wire-code and outside spot measurements of homes occupied from conception to diagnosis (blinded for case status); 24h measurements in child's bedroom and another room for a subset; electrical appliances, occupation etc. by interviews with mothers	retrospective (1990-1992) assessment	all assessments within same period	age±1y(m), sex(m), year of diagnosis, SES, parents occupation, building type	298 brain tumor cases (age<20) (68% part.rate); 298 controls by RDD (70% part.rate)
Tynes & Haldorsen 1997	Norway/1965-1989/Nested Case-control	cohort (age <15) living in a ward crossed by a high-voltage power line (≥45kV in urban, ≥100kV in rural areas) in at least one of the years 1960, 1970, 1980, 1985, 1987, 1989.	Calculated historical fields	n.a.	age(m), sex(m), muni-cipality(m), SES, type of building, number of dwellings	500 cancer cases (94%) from cancer registry; 2004 controls (95%) randomly selected from cohort

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Petridou et al. 1997	Greece/1993-1994/Case-control	distance to transmission and distribution lines, field calculation	n.a.	n.a.	age(m), sex(m), region(m), maternal age, education etc.	117 childhood leukemia cases (age<15) (77% of eligible) and 202 controls (68% of eligible)
Michaelis et al. 1997a	Lower Saxony, Germany/1988-1993/ Case-control	24h measurements (EMDEX II) in the child's bedroom and living room in dwellings where the child lived longest (not blinded to case status); perimeter measurements (measurement wheel) with recordings every foot (~30cm) when walking through the rooms and outside the house where the child lived for at least 1 y.	measurements 1992-1995	all measurements within same period	age±1y(m), sex(m), SES, urbanization	129 leukemia cases (age<15) (59% part.rate) from register; 328 controls (167 from same district, 161 from random district) (53% part.rate) from government registration files

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Michaelis et al. 1997b	Berlin/1991-1994/ Case-control (pooled with data from Michaelis et al. 1997a)	as above	not specified	not specified	age±1y(m), sex(m), SES, urbanization, age at diagnosis, West/East Germany	47 leukemia cases (age<15) (59% part.rate) from register; 86 controls (28% part.rate) from government registration files
Linet et al. 1997	Illinois, Indiana, Iowa, Michigan, Minnesota, New Jersey, Ohio, Pennsylvania, and Wisconsin/1989-1994/Case-control	24h measurements (EMDEX C) in child's bedroom (blinded to case status); spot measurements in the residences and at the front door; wire coding of residences of residentially stable case-control pairs	~2 years	all measurements within same period	age(m), ethnicity(m), 8-digits phone number(m), sex, SES, time of measurem., urbanization, type of residence, birth order, birth weight, mother's age, medical x-ray	638 ALL cases (age<15) from register of Children's Cancer Group (78% part.rate); 620 controls from RDD (63% part.rate).

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Li et al. 1998	Taipei Metropol. Area (3 districts), Taiwan/ 1987-1992/ Ecological	high voltage transmission lines (69 -345kV) were mapped to 124 administrative regions; households with ≥50% intersecting a buffer zone of 100m around transmission lines	n.a.	n.a.	age (5y groups), calendar year	28 leukemia cases from registry in a study base of ~121.000 children (age<15); 7 cases within 21 cases outside a 100m corridor each side of a transmission line
Dockerty et al. 1998	New Zealand/1990-1993/Case-control	24h measurements (Positron) in child's bedroom and another room (only for leukemia cases); interview with mothers	1-2 years	all measurements within same period	age(m), sex(m), SES, maternal smoking, living on a farm	303 cancer cases (age<15) from 3 registries (88% part.rate) – 121 leukemia cases; 303 controls from birth register (68% part.rate)

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
UKCCS 1999	England, Scotland & Wales/1991(92)-1994(96)/Case-control	spot measurements (EMDEX II) in child's bedroom, 90 min measurements in main family room, 48h measurements (20% of case-control pairs) at child's bedside; school measurements; weighted averages from info obtained by questionnaire; adjustments from historical load data	~2 years	<4 months in 98% of case-control pairs (spot), within 4 weeks (48h measurem.)	age (m), sex(m), district(m), deprivation index	2226 cancer cases (age<15) from registry (59% part.rate); 2226 matched controls from registry
McBride et al. 1999	Canada (5 provinces)/ 1990-1994(95)/Case-control	48h personal measurements (Positron), 24h measurements in child's bedroom	9 months average	2 months average	age±3-6mo (m), sex(m), area(m), maternal age, maternal education,	399 leukemia cases (age<15) (90% part.rate) from treatment centers and registry; 399 matched

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
		(75% cases, 86% controls); wire codes (78% cases, 85% controls) and residence perimeter and front door measurements (64% cases, 74% controls) (blinded to case status) (EMDEX C); interviews with parents			income, ethnicity, number of residences	controls (76% part.rate) from health insurance/family allowance rolls
Green et al. 1999a	Greater Toronto Area, Canada/1985-1993/ Case-control	48h personal measurements (Positron); spot measurements in child's bedroom and two other rooms; wire codes; interviews with parents	2-3 y average	~5 mo average	age±1y (m), sex(m), family income, siblingship, residential mobility, insecticides, mother's medication and exp. prior or during pregn.	201 leukemia cases (age<15) from hospital record (64% part.rate); 406 controls from telephone marketing list (10,000 residences) (63% part.rate)
Green et al. 1999b	Greater Toronto Area, Canada/1985-1993/ Case-control	as above	2-3 y average	~5 mo average	as above	88 leukemia cases (age<15) from hospital record; 133 controls

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
						from telephone marketing list (10,000 residences)
Schüz et al. 2001a	West Germany/1993(90)-1997(94)/Case-control	24h measurements (FW2a) under mattress of child's bed; 24h measurements (EMDEX II) in living room; perimeter measurements with recordings every foot (~30cm) when walking through the rooms			age(m), sex(m), community(m), SES, year of birth, urbanization, residential mobility, season, type of residence	514 leukemia cases (age<15) from cancer registry (61% of eligible) and 1301 controls from population registry (61% of eligible)
Schüz et al. 2001b	Lower Saxony/1988 – 1993 & Western Germany/1992-1994/ Case-control	as above			age(m), sex(m), community(m), SES, urbanization	64 cases of CNS tumors (age<15) from registry and 414 controls from population registry

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Mizoue et al. 2004	Japan/1992-2001/Ecological	classification of 294 districts according to their proximity to high voltage power lines (66 and 220V); proportion of area of district (0%, <50%, >50%) within ±300m of a power line	n.a.	n.a.	age (5y groups)	14 cases (age<15) of hematopoietic malignancies identified from two hospitals (all that treated these malignancies)
Draper et al. 2005	England & Wales/ 1962-1995/Case-control	computed distance from nearest overhead power line (132kV, 275kV, 400kV) of residence at birth	n.a.	n.a.	age±6mo(m), sex(m), district(m), SES	29081 cancer cases (age<15) identified from several registries (88% of total); 29081 controls from birth registers
Perez et al. 2005	Cuba (Habana)/1996-2000/Case-control	spot measurements inside and outside (Bell 4090), measurement of ionizing radiation	not specified	not specified	age(m), sex(m), school(m)	unknown number of leukemia cases (age<15) and controls

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Kabuto et al. 2006	Tokyo, Nagoya, Kyoto, Osaka and Kitakyushu metropolitan areas (Japan)/1999-2001/Case-control	7 days continuous MF measurement (EMDEX Lite) in child's bedroom; spot measurements in- and outside the house (EMDEX II)	~13 mo	~3 days	age±(≤)1y(m), sex(m), region(m), population size(m), father's and mother's education	321 ALL/AML cases (age<15) from several registries of childhood cancer study groups (49% part.rate); 634 controls from residential registry (29% part.rate)
Mejia-Arangure et al. 2007	Mexico-City/1995-2003/Case-control	spot measurements (EMDEX II) at the front door; wire coding (blinded to case status)	not specified	not specified	age, sex, SES, birth weight, maternal age, traffic, district, family history of cancer	42 ALL/AML cases (age<16) with Down syndrome from 4 (all) treating hospitals; 124 healthy controls with Down syndrome from 2 centers
Feizi & Arabi 2007	Iran (Tabriz)/1998-2004/Case-control	distance and calculated fields	n.a.	n.a.	age(m), sex(m), SES(m), race(m), district(m)	60 AL cases (83% of eligible) (age<15) and 59 hospital controls (79% of eligible)
Lowenthal et al. 2007	Tasmania/1972-1980/Case-control	distance from power line	n.a.	n.a.	age(m), sex(m)	783 adult and 71 childhood cases of MPD or LPD and matched controls

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Yang et al. 2008	Shanghai/2006-2007/Case-only	distance from transformer or power lines	n.a.	n.a.	age, gender, parental education, pesticides, television set etc. in children's room, chemical factory, telecom transmitter <500 m	123 AML cases (age<15) with or without XRCCI Ex9p16A
Abdul-Rahman et al. 2008	Malaysia/2001-2007/Case-control	distance from power lines and substations (GPS)	n.a.	n.a.	not specified	128 AL cases (age<15) and 128 hospital controls
Malagoli et al. 2010	Italy (Modena, Reggio Emilia)/1986-2007/	calculated fields from power lines ≥132 kV	n.a.	n.a.	age(m), sex(m), municipality(m), parent education, income	64 cases (age<14) of hematological malignancies and 256 controls
Kroll et al. 2010	England, Wales/1962-1995/Case-control	calculated fields from overhead power line (132kV, 275kV, 400kV) of residence at birth	n.a.	n.a.	age(m), sex(m), district(m)	28968 cancer cases (age<15)
Sohrabi et al. 2010	Iran (Teheran)/2007-2009/Case-control	distance to power lines (123, 230, 400 kV) using GPS	n.a.	n.a.	age(m), sex(m)	300 ALL cases (age<18) and 300 hospital controls

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Saito et al. 2010	Japan/1999-2002/Case-control	1-week measurement (EMDEX Lite) near bedside	Not specified	12.4 days	age(m), sex(m), region(m), population size(m), mother education	55 childhood brain tumor cases (age<15) and 99 controls
Does et al. 2011	California/2004-2007/Case-control	30 min measurement of contact current in the bathtub , indoor spot measurements (EMDEX Lite)	28 months	8 months	age, sex, race, income	245 leukemia cases (95% of eligible) (age<8) and 269 controls (92% of eligible)
Wünsch-Filho et al. 2011	Brazil (Sao Paulo)/2003-2009/Case-control	24 h measurements (EMDEX II) under the child's bed, distance to power lines	Not specified	Not specified	age(m), sex(m), city of birth(m),race, mobility,etc.	179 ALL cases (age<9) (90% of contacted) and 565 controls (88% of contacted)

RDD…Random Digit Dialing, n.a…not applicable, MF…magnetic field, SES…socio-economic status, ALL…acute lymphoblastic leukemia, AML…acute myeloid leukemia, AL…acute leukemia, LPD…lymphoproliferative disorders, MPD…myeloproliferative disorders

Table 11- 5: Synopsis of main results of childhood cancer studies (1979 – 2012)

Study	Endpoint	Exposure category	Outcome [95% CI]
Wertheimer & Leeper 1979[a]	Leukemia	LCC* (birth address)	
		HCC	OR 2.28 [1.34 – 3.91]
	Lymphoma	LCC*	
		HCC	OR 2.48 [0.73 – 8.37]
	Nervous system tumors	LCC*	
		HCC	OR 2.36 [1.03 – 5.41]
	Others	LCC*	
		HCC	OR 2.38 [0.93 – 6.06]
	All hematopoietic	LCC*	
		HCC	OR 2.31 [1.41 – 3.77]
	All cancers	LCC*	
		HCC	OR 2.33 [1.59 – 3.42]
Fulton et al. 1980	Leukemia	Very low*[c]	
		Low	OR 1.1 [0.5 – 2.4]
		High	OR 1.2 [0.6 – 2.6]
		Very high	OR 1.0 [0.5 – 2.3]
Tomenius 1986	Leukemia	no 200 kV-line*	
		200 kV-line<150m	OR 1.09 [0.29 – 4.12]
	Lymphoma	no 200 kV-line*	
		200 kV-line<150m	OR 1.48 [0.35 – 6.35]
	Nervous system tumors	no 200 kV-line*	
		200 kV-line<150m	OR 3.96 [0.85 – 18.52]
	Others	no 200 kV-line*	
		200 kV-line<150m	OR 2.59 [0.70 – 9.66]
	All hematopoietic	no 200 kV-line*	
		200 kV-line<150m	OR 1.26 [0.47 – 3.34]
	All cancers	no 200 kV-line*	
		200 kV-line<150m	OR 2.15 [1.12 – 4.11]

Study	Endpoint	Exposure category	Outcome [95% CI]
	All cancers	<3mG birth dwelling*	
		≥3mG	OR 2.67 [1.18 – 6.08]
	All cancers	<3mG diagn. dwelling*	
		≥3mG	OR 2.60 [1.20 – 5.67]
Savitz et al.1988	Leukemia	<2mG low power use*	
		2+ mG	OR 1.93 [0.67 – 5.56]
	Lymphoma	<2mG low power use*	
		2+ mG	OR 2.17 [0.46 – 10.31]
	Brain tumors	<2mG low power use*	
		2+ mG	OR 1.04 [0.22 – 4.82]
	Others	<2mG low power use*	
		2+ mG	OR 0.96 [0.31 – 2.98]
	All hematopoietic	<2mG low power use*	
		2+ mG	OR 1.99 [0.57 – 5.14]
	All cancers	<2mG low power use*	
		2+ mG	OR 1.35 [0.63 – 2.90]
	Leukemia	<2mG high power use*	
		2+ mG	OR 1.41 [0.57 – 3.50]
	Lymphoma	<2mG high power use*	
		2+ mG	OR 1.81 [0.48 – 6.88]
	Brain tumors	<2mG high power use*	
		2+ mG	OR 0.82 [0.23 – 2.93]
	Others	<2mG high power use*	
		2+ mG	OR 0.75 [0.30 – 1.92]
	All hematopoietic	<2mG high power use*	
		2+ mG	OR 1.51 [0.68 – 3.35]
	All cancers	<2mG high power use*	
		2+ mG	OR 1.04 [0.56 – 1.95]

Study	Endpoint	Exposure category	Outcome [95% CI]
	All cancers	0-0.64 mG low power use*	
		0.65-0.99 mG	OR 1.28 [0.67 – 2.42]
		1.0-2.49 mG	OR 1.25 [0.68 – 2.28]
		2.5+ mG	OR 1.49 [0.62 – 3.60]
	All cancers	0-0.64 mG high power use*	
		0.65-0.99 mG	OR 1.13 [0.61 – 2.11]
		1.0-2.49 mG	OR 0.96 [0.56 – 1.65]
		2.5+ mG	OR 1.17 [0.54 – 2.57]
	Leukemia	LCC*	
		HCC	OR 1.41 [0.57 – 3.50]
	Lymphoma	LCC*	
		HCC	OR 1.81 [0.48 – 6.88]
	Brain tumors	LCC*	
		HCC	OR 0.82 [0.23 – 2.93]
	Others	LCC*	
		HCC	OR 0.75 [0.30 – 1.92]
	All hematopoietic	LCC*	
		HCC	OR 1.51 [0.68 – 3.35]
	All cancers	LCC*	
		HCC	OR 1.04 [0.56 – 1.95]
	All cancers	UG 2y before diagnosis*	
		VLCC	OR 0.96 [0.39 – 2.34]
		OLCC	OR 1.17 [0.65 – 2.08]
		OHCC	OR 1.40 [0.71 – 2.75]
		VHCC	OR 5.22 [1.18 – 23-09]
	All cancers	VLCC/OLCC*[b]	
		UG	OR 0.89 [0.51 – 1.55]
		OHCC	OR 1.25 [0.67 – 2.31]
		VHCC	OR 4.66 [0.95 – 22.76]

Study	Endpoint	Exposure category	Outcome [95% CI]
Coleman et al. 1989	Leukemia	≥100 m nearest substation*	
		50-99 m	OR 0.75 [0.40 – 1.38]
		25-49 m	OR 1.49 [0.61 – 3.64]
		0-24 m	OR 1.63 [0.32 – 8.38]
Myers et al. 1990	All cancers	<0.1mG*	
		0.1-0.3mG	OR 0.96 [0.37 – 2.51]
		≥0.3mG	OR 1.73 [0.59 – 5.07]
London et al. 1991	Leukemia	<0.68mG* (24h.measurem.)	
		0.68-1.18mG	OR 0.68 [0.39 – 1.17]
		1.19-2.67mG	OR 0.89 [0.46 – 1.71]
		≥2.68mG	OR 1.48 [0.66 – 3.29]
		<0.32mG (spot bedroom)*	
		0.32-0.67mG	OR 1.01 [0.61 – 1.69]
		0.68-1.24mG	OR 1.37 [0.65 – 2.91]
		≥1.25mG	OR 1.22 [0.52 – 2.82]
		UG/VLCC*	
		OLCC	OR 0.95 [0.53 – 1.69]
		OHCC	OR 1.44 [0.81 – 2.56]
		VHCC	OR 2.15 [1.08 – 4.26]
Verkasalo et al. 1993	Leukemia	≥4mG any time	SIR 1.55 [0.32 - 4.54]
	Lymphoma	≥4mG any time	SIR [0.00 - 4.19]
	Nervous system tumors	≥4mG any time	SIR 2.31 [0.75 - 5.40]
	Others	≥4mG any time	SIR 1.24 [0.26 - 3.62]
	All hematopoietic	≥4mG any time	SIR 1.49 [0.74 - 2.66]
	All cancers	≥4mG any time	SIR 1.66 [0.34 - 4.84]
Feychting & Ahlbom 1993	Leukemia	<1mG* (calculated)	
		1-2mG	OR 2.1 [0.6 – 6.1]

Study	Endpoint	Exposure category	Outcome [95% CI]
		≥2mG	OR 2.7 [1.0 – 6.3]
	Lymphoma	<1mG* (calculated)	
		1-2mG	OR 0.9 [0.0 – 5.2]
		≥2mG	OR 1.3 [0.2 – 5.1]
	Nervous system tumors	<1mG* (calculated)	
		1-2mG	OR 1.0 [0.2 – 3.8]
		≥2mG	OR 0.7 [0.1 – 2.7]
	Others	<1mG* (calculated)	
		1-2mG	OR 1.6 [0.6 – 4.3]
		≥2mG	OR 0.2 [0.0 – 1.7]
	All hematopoietic	<1mG* (calculated)	
		1-2mG	OR 1.7 [0.6 – 4.5]
		≥2mG	OR 2.2 [1.0 – 4.7]
	All cancers	<1mG* (calculated)	
		1-2mG	OR 1.5 [0.7 – 2.9]
		≥2mG	OR 1.1 [0.5 – 2.1]
Olsen et al. 1993	Leukemia	<1mG* (calculated)	
		1-4mG	OR 0.3 [0 – 2.0]
		≥4mG	OR 6.0 [0.8 – 44]
	Lymphoma	<1mG* (calculated)	
		1-4mG	OR 5.0 [0.7 – 36]
		≥4mG	OR 5.0 [0.3 – 82]
	CNS tumors	<1mG* (calculated)	
		1-4mG	OR 0.4 [0.1 – 2.8]
		≥4mG	OR 6.0 [0.7 – 44]
	All three combined	<1mG* (calculated)	
		1-4mG	OR 0.7 [0.2 – 2.0]
		≥4mG	OR 5.6 [1.6 – 19]

Study	Endpoint	Exposure category	Outcome [95% CI]
Fajardo-Gutierrez et al. 1993	Leukemia	Transformer station[d]	OR 1.56 [0.73 – 3.30]
		High voltage power line	OR 2.63 [1.26 – 5.36]
		Electric substation	OR 1.67 [0.65 – 4.35]
		Transmission line	OR 2.50 [0.97 – 6.67]
Coghill et al. 1996	Leukemia	< 5 V/m E-field *	
		5-9 V/m	OR 1.49 [0.47 – 5.10]
		10-19 V/m	OR 2.40 [0.79 – 8.09]
		≥20 V/m	OR 4.69 [1.17 – 27.78]
Gurney et al.1996	Brain tumors	UG*	
		VLCC	OR 1.25 [0.74 – 2.13]
		OLCC	OR 0.74 [0.34 – 1.61]
		OHCC	OR 1.07 [0.55 – 2.06]
		VHCC	OR 0.51 [0.16 – 1.60]
		LCC*	
		HCC	OR 0.86 [0.50 – 1.48]
Preston-Martin et al. 1996	Brain tumors	0.09-0.51 mG Md 24h *	
		0.52-1.02 mG	OR 1.5 [0.7 – 3.2]
		1.03-2.03 mG	OR 1.8 [0.7 – 4.5]
		2.04-10.4 mG	OR 1.2 [0.4 – 3.2]
		VLCC/OLCC*	
		UG	OR 1.9 [1.0 – 3.6]
		OHCC	OR 0.8 [0.6 – 1.2]
		VHCC	OR 1.2 [0.6 – 2.1]
Tynes & Haldorsen 1997	Leukemia	<0.5mG (TWA birth-diagn)*	
		0.5-1.4mG	OR 1.8 [0.7 – 4.2]
		≥1.4mG	OR 0.3 [0.0 – 2.1]
	Lymphoma	<0.5mG (TWA birth-diagn)*	
		0.5-1.4mG	OR 1.0 [0.1 – 8.7]
		≥1.4mG	OR 2.5 [0.4 – 15.5]

Study	Endpoint	Exposure category	Outcome [95% CI]
	Nervous system tumors	<0.5mG (TWA birth-diagn)*	
		0.5-1.4mG	OR 1.9 [0.8 – 4.6]
		≥1.4mG	OR 0.7 [0.2 – 2.1]
	Others	<0.5mG (TWA birth-diagn)*	
		0.5-1.4mG	OR 2.9 [1.0 – 8.4]
		≥1.4mG	OR 1.9 [0.6 – 6.0]
	All hematopoietic	<0.5mG (TWA birth-diagn)*	
		0.5-1.4mG	OR 1.4 [0.7 – 3.1]
		≥1.4mG	OR 0.7 [0.2 – 2.4]
	All cancers	<0.5mG (TWA birth-diagn)*	
		0.5-1.4mG	OR 1.9 [1.2 – 3.3]
		≥1.4mG	OR 1.0 [0.5 – 1.8]
Petridou et al. 1997	Leukemia	Very Low*	
		Low	OR 0.99 [0.54–1.84]
		Medium	OR 1.84 [0.26–12.81]
		High	OR 4.26 [0.94–19.44]
		Very high	OR 1.56 [0.26–9.39]
Michaelis et al. 1997a	Leukemia	<2mG (Median 24h)*	
		≥2mG	OR 3.2 [0.7 – 14.9]
		<2mG (Median night)*	
		≥2mG	OR 3.9 [0.9 – 16.9]
Michaelis et al. 1997b (pooled with previous)	Leukemia	<2mG (Median 24h)*	
		≥2mG	OR 2.3 [0.8 – 6.7]
		<2mG (Median night)*	
		≥2mG	OR 3.8 [1.2 – 11.9]

Study	Endpoint	Exposure category	Outcome [95% CI]
Linet et al. 1997	ALL	<0.65mG (TWA)*	
		0.65-1mG	OR 0.96 [0.65 – 1.40]
		1-2mG	OR 1.15 [0.79 – 1.65]
		2-3mG	OR 1.31 [0.68 – 2.51]
		3-4mG	OR 1.46 [0.61 – 3.50]
		4-5mG	OR 6.41 [1.30 – 31.7]
		≥5mG	OR 1.01 [0.26 – 3.99]
Li et al.1998	Leukemia	≥100m from transm.line	
		<100m	SIR 2.43 [0.98 – 5.01]
		Total population<15y	
		≥100m from transm.line	SIR 1.05 [0.64 – 1.58]
		<100m	SIR 2.69 [1.08 – 5.55]
Dockerty et al. 1998	Leukemia	<1mG (24h bedroom AM)*	
		1-2mG	OR 1.4 [0.3 – 7.6]
		≥2mG	OR 15.5 [1.1 – 224]
		<1mG (24h daytime room)*	
		1-2mG	OR 3.7 [0.7 – 18.8]
		≥2mG	OR 5.2 [0.9 – 30.8]
UKCCS 1999	Leukemia	<1mG (estim.AM exp.)*	
		1-2mG	OR 0.78 [0.55 – 1.12]
		2-4mG	OR 0.78 [0.40 – 1.52]
		≥4mG	OR 1.68 [0.40 – 7.10]
	Central nervous system cancers	<1mG (estim.AM exp.)*	
		1-2mG	OR 2.44 [1.17 – 5.11]
		2-4mG	OR 0.70 [0.16 – 3.17]
		≥4mG	OR --
	Others	<1mG (estim.AM exp.)*	

Study	Endpoint	Exposure category	Outcome [95% CI]
		1-2mG	OR 0.81 [0.52 – 1.28]
		2-4mG	OR 1.08 [0.45 – 2.56]
		≥4mG	OR 0.71 [0.16 – 3.19]
	All cancers	<1mG (estim.AM exp.)*	
		1-2mG	OR 0.93 [0.72 – 1.19]
		2-4mG	OR 0.87 [0.53 – 1.42]
		≥4mG	OR 0.89 [0.34 – 2.32]
McBride et al. 1999	Leukemia	<0.8mG (lifetime predicted)*	
		0.8-1.5mG	OR 0.74 [0.48 – 1.13]
		1.5-2.7mG	OR 1.15 [0.70 – 1.88]
		≥2.7mG	OR 1.02 [0.56 – 1.86]
		Low (Kaune-Savitz)*	
		Medium	OR 1.12 [0.77 – 1.64]
		High	OR 1.17 [0.74 – 1.86]
Green et al. 1999a	Leukemia	<0.4mG (spot measurem.)*	
		0.4-0.9mG	OR 0.47 [0.12 – 1.89]
		0.9-1.5mG	OR 0.75 [0.19 – 3.02]
		≥1.5mG	OR 1.47 [0.44 – 4.85]
Green et al. 1999b	Leukemia	<0.3mG (48h measurem.)*	
		0.3-0.7mG	OR 2.0 [0.6 – 6.8]
		0.7-1.4mG	OR 4.0 [1.1 – 14.4]
		≥1.4mG	OR 4.5 [1.3 – 15.9]
		<0.4mG (spot measurem.)*	
		0.4-0.8mG	OR 1.8 [0.5 – 6.1]

Study	Endpoint	Exposure category	Outcome [95% CI]
		0.8-1.6mG	OR 2.8 [0.8 – 10.4]
		≥1.6mG	OR 4.0 [1.2 – 13.6]
Schüz et al. 2001a	Leukemia	<1mG (Md 24h)*	
		1-2mG	OR 1.15 [0.73 – 1.81]
		2-4mG	OR 1.16 [0.43 – 3.11]
		≥4mG	OR 5.81 [0.78 – 43.2]
		<1mG (Md night-time)*	
		1-2mG	OR 1.42 [0.90 – 2.23]
		2-4mG	OR 2.53 [0.86 – 7.46]
		≥4mG	OR 5.53 [1.15 – 26.6]
Schüz et al. 2001b	CNS tumors	<2mG (Md 24h)*	
		≥2mG	OR 1.67 [0.32 – 8.84]
		<2mG (Md night-time)*	
		≥2 mG	OR 2.60 [0.45 – 14.9]
Mizoue et al. 2004	All hematopoietic	0% area intersection*	
		<50%	IRR 1.6 [0.5 – 5.1]
		>50%	IRR 2.2 [0.5 – 9.0]
Draper et al.2005	Leukemia	≥600m (from power line)*	
		200-600m	RR 1.22 [1.01 – 1.47]
		<200m	RR 1.68 [1.12 – 2.52]
	Brain tumors	≥600m (from power line)*	
		200-600m	RR 1.18 [0.95 – 1.48]
		<200m	RR 0.74 [0.47 – 1.15]
	Others	≥600m (from power line)*	
		200-600m	RR 0.96 [0.82 – 1.12]
		<200m	RR 0.88 [0.62 – 1.25]
Perez et al. 2005	Leukemia	<1mG*	
		1 mG	OR 1.46

Study	Endpoint	Exposure category	Outcome [95% CI]
		5 mG	OR 6.72
		10 mG	OR 45.15
Kabuto et al. 2006	ALL+AML	<1mG (1wk TWA)*	
		1-2mG	OR 0.93 [0.51 – 1.71]
		2-4mG	OR 1.08 [0.51 – 2.31]
		≥4mG	OR 2.77 [0.80 – 9.57]
	ALL+AML	<1mG (1wk night-time)*	
		1-2mG	OR 0.97 [0.52 – 1.79]
		2-4mG	OR 1.08 [0.47 – 2.47]
		≥4mG	OR 2.87 [0.84 – 9.88]
	ALL	<1mG (1wk TWA)*	
		1-2mG	OR 0.87 [0.45 – 1.69]
		2-4mG	OR 1.03 [0.43 – 2.50]
		≥4mG	OR 4.67 [1.15 – 19.0]
Mejia-Arangure et al. 2007	ALL+AML	<1mG (spot)*	
		1-4mG	OR 0.94 [0.37 – 2.4]
		4-6mG	OR 0.88 [0.15 – 5.1]
		≥6mG	OR 3.7 [1.05 – 13]
		Low (Kaune-Savitz)*	
		Medium	OR 5.8 [0.92 – 37]
		High	OR 4.1 [0.66 – 25]
Feizi & Arabi 2007	Leukemia	≤4.5mG*	
		>4.5mG	OR 3.60 [1.11 – 12.39]
Lowenthal et al. 2007	LPD+MPD	>300 m from power line*	
		0-300 m (at age 0-15)	OR 3.23 [1.26 – 8.29]
Yang et al. 2008	AL with XRCC1 Ex9 + 16A allele	>500 m from power line*	

Study	Endpoint	Exposure category	Outcome [95% CI]
		0-500 m	OR 2.37 [0.94–5.97]
		>100 m from power line*	
		0-100 m	OR 4.31 [1.54–12.08]
		>50 m from power line*	
		0-50 m	OR 4.39 [1.42–13.54]
Abdul-Rahman et al. 2008	Leukemia	>200 m from power line*	
		0-200 m	OR 2.30 [1.18–4.49]
Malagoli et al. 2010	All hematological malignancies	<1mG*	
		≥1mG	OR 2.4 [0.4-15.0]
	Leukemia	<1mG*	
		≥1mG	OR 6.7 [0.6-78.3]
	ALL	<1mG*	
		≥1mG	OR 5.3 [0.7-43.5]
Kroll et al. 2010	Leukemia	<1mG*	
		1-2mG	OR 2.00 [0.50–7.99]
		2-4mG	0 case/ 2 controls
		≥4mG	OR 2.00 [0.18–22.04]
	CNS/brain tumors	<1mG*	
		1-2mG	OR 0.50 [0.09–2.73]
		2-4mG	1 case/ 0 control
		≥4mG	OR 0.33 [0.03–3.20]
	Other cancers	<1mG*	
		1-2mG	OR 0.33 [0.07–1.65]
		2-4mG	OR 1.00 [0.14–7.10]
		≥4mG	OR 5.00 [0.58–42.80]
Sohrabi et al. 2010	ALL	>400 m from power line*	
		0-400 m	OR 2.75 [1.59 – 4.76]
Saito et al. 2010	Brain tumors	<1mG bedroom*	
		1-2mG	OR 0.74 [0.17–3.18]
		2-4mG	OR 1.58 [0.25–9.83]

Study	Endpoint	Exposure category	Outcome [95% CI]
		≥4mG	OR 10.9 [1.05–113]
Does et al. 2011	Leukemia	<0.25mV contact current	
		0.25-1.5mV	OR 0.98 [0.63 – 1.53]
		≥1.5mV	OR 0.99 [0.65 – 1.52]
		<0.1mG*	
		0.1-0.2mG	OR 0.96 [0.57 – 1.62]
		0.2-0.5mG	OR 1.23 [0.74 – 2.04]
		≥0.5mG	OR 1.18 [0.71 – 1.96]
Wünsch-Filho et al. 2011	ALL	≥600 m from power line*	
		200-600 m	OR 0.69 [0.28–1.71]
		100-200 m	OR 1.67 [0.49–5.75]
		<100 m	OR 1.54 [0.26–9.12]
		≥600 m from power line*	
		200-600 m (never moved)	OR 0.91 [0.25–3.25]
		100-200 m	OR 3.68 [0.68–19.82]
		<100 m	OR 1.52 [0.11–21.24]

*Reference category

[a] Computed from table 5 of the original publication (could be biased due to not considering individual matching)

[b] Computed from table 5 of the original publication

[c] Quartiles of exposure distribution of controls (exposure calculated)

[d] Reference categories: Without the respective appliance near the residence

OR…odds-ratio, SIR…standardized incidence ratio, RR…relative risk, IRR…incidence rate ratio, LCC…low-current code, HCC…high-current code, UG…underground cable, VLCC…very low current code, OLCC…ordinary low current code, OHCC…ordinary high current code, VHCC…very high current code, Md…median, TWA…time weighted average, AM…arithmetic mean, ALL…acute lymphoblastic leukemia, AML…acute myeloid leukemia, LPD…lymphoproliferative disorders, MPD…myeloproliferative disorders

SECTION 13

_____

ELF MF – Melatonin Production – Alzheimer's Disease and Breast Cancer

2012 Updated Chapter

Zoreh Davanipour, DVM, PhD

Eugene Sobel, PhD

TABLE OF CONTENTS

SECTION 1: UPDATE INTRODUCTION

It has been over 5 years since the publication of the initial BioInitiative in 2007. During that time the BioInitiative web site has been accessed by a considerable number of individuals worldwide: **_(Provide viewing figures.)_** Unfortunately, "pro-industry" representatives from industry itself, from government, and from academia have continued their campaign, despite all evidence to the contrary, against any possible serious ill effects of exposure to extremely low frequency (ELF) magnetic fields (MF) at levels experienced in occupational and residential settings. These pro-industry representatives simply argue that the evidence is insufficient because some epidemiologic studies are negative and some are positive and that there are no biologically confirmed causal pathways. As we showed in the earlier 2007 original BioInitiative publications, the negative studies have serious flaws while the positive studies do not have such flaws. In addition, we discussed two biological pathways related to Alzheimer's disease and breast cancer, which have plausibility based on scientific studies. A third suggested pathway is discussed in this update.

In this chapter update, we provide the following:

1. descriptions and evaluations of newly published epidemiologic studies relating occupational ELF MF exposure to the risk of (a) Alzheimer's disease (AD) and/or dementia, (b) breast cancer;
2. updates related to the three proposed or suggested pathways from ELF MF exposure to AD or dementia:
    a. increased peripheral and brain production of amyloid beta;
    b. decreased production of melatonin; and
    c. ELF MFs may cause chromosome instability, resulting in chromosome segregation errors and increased mutational loads;
3. a discussion of the potential increase in cellular production of amyloid beta (associated with the risk of AD) due to low melatonin production;
4. an update of the relationship between low melatonin production and the risk of breast cancer;

STRUCTURE OF THE UPDATED REPORT

New material is incorporated into the body of the Report. New and revised text and table additions are presented with a red text color.

EXECUTIVE SUMMARY

Melatonin Production

Melatonin is a hormone produced primarily by the pineal gland, located in the center of the brain. Melatonin is evolutionarily conserved and is found in nearly all organisms. It has numerous properties which indicate that it helps prevent both Alzheimer's disease and breast cancer. There is strong evidence from epidemiologic studies that high ($\geq$ 10 milligauss or mG)* that long-term exposure to extremely low frequency (ELF, $\leq$ 60 Hz) magnetic fields (MF) is associated with a decrease in melatonin production(Section II.)

Alzheimer's Disease

Amyloid beta ($A\beta$) protein is generally considered the primary neurotoxic agent causally associated with Alzheimer's disease (AD). $A\beta$ is produced by both brain and peripheral cells and can pass through the blood brain barrier.

1. There is longitudinal epidemiologic evidence that high peripheral blood levels of $A\beta$, particularly $A\beta_{1-42}$, is a risk factor for Alzheimer's disease (AD). (Section III.A.)
2. There is epidemiologic evidence that extremely low frequency (ELF, 50-60 Hz) magnetic field (MF) exposure up-regulates peripheral blood levels of $A\beta$. (Section III.A.)
3. There is evidence that melatonin can inhibit the development of AD and, thus, low melatonin may increase the risk of AD (Section III.B.)
4. There is strong epidemiologic evidence that significant (i.e., high), occupational ELF MF exposure can lead to the down-regulation of melatonin production. The precise components of the magnetic fields causing this down-regulation are unknown. Other factors which may influence the relationship between ELF MF exposure and melatonin production are unknown, but certain medications may play a role. (Section II.)
5. There is strong epidemiologic evidence that high occupational ELF MF exposure is a risk factor for AD, based on case-control studies which used expert diagnoses and a restrictive classification of ELF MF exposure. (Section III.C.)
6. There are no epidemiologic studies of AD and radiofrequency MF exposure, only one epidemiology study of non-acute radiofrequency MF exposure and melatonin. There are studies of "AD mice" and radiofrequency exposure (Sections III.D and II.) So, no conclusions concerning health consequences due to exposure are currently possible.

Breast Cancer

The only biological hypothesis which has been epidemiologically investigated to explain the relationship between ELF MF exposure and breast cancer is that high* ELF MF exposure can lower melatonin production, which in turn can lead to changes in the various biological systems which melatonin influences, including increased estrogen production and subsequent deleterious interactions with DNA, decreased antiproliferative activities, increased oxidative DNA damage, and immune response capabilities. Thus lowered melatonin production can be expected to lead to increased risk of breast cancer.

1. *In vitro* and animal studies have demonstrated that (i) melatonin is a potent scavenger of oxygen and nitrogen radicals that cause DNA damage, (ii) melatonin interferes with

estrogen's deleterious interactions with DNA, and (iii) melatonin inhibits the development of mammary tumors. (Section IV.A.)

2. A study published in 2009 (Davanipour et al.) evaluated guanine DNA/RNA damage in relation to melatonin production among 55 mother-father-adult daughter triples who were relatively healthy for their age. The lower melatonin production among the mothers was associated with higher guanine DNA damage. Lower melatonin production among the fathers was marginally associated with guanine damage in either DNA or RNA.

3. Human studies indicate that ELF MF exposure can decrease melatonin production. (Section II.)

3. Human studies have found that low melatonin production is a likely risk factor for breast cancer. (Section IV.B.)

4. Human studies have shown that light-at-night and night shift work reduce melatonin production and are both risk factors for breast cancer. (Section IV.D.)

5. Occupational studies indicate that high ELF MF exposure increases the risk of breast cancer. This is particularly true for a recent, large, and well-designed study from Poland (funded by the NCI, administered for the NCI by Westat, and conducted by Polish scientists).

6. A recent, large, and well-designed, Swedish case-control study used a new ELF MF job exposure matrix, developed by the same group, which is nearly completely at odds with earlier exposure classifications. The female occupation generally thought to be the one with the highest ELF MF exposure (seamstress) was considered to have medium-low exposure, while several lower ELF MF exposed occupations were considered high. The case-control study consequently found no risk associated with high ELF MF occupations as rated by the new matrix, but did find that seamstresses had a statistically elevated risk of breast cancer. This job exposure matrix is likely inappropriate in many important instances and needs to be thoroughly reviewed. (Section IV.E.)

7. Studies of residential ELF MF exposure and breast cancer have been generally negative. Measured residential ELF MF exposure may not be related to actual individual exposure. Residential exposure is most often low, is usually not measured in residences that may be related to the latency period of breast cancer, does not take into consideration point sources of strong magnetic fields which may be related to real exposure, and thus often does not relate to actual exposure. Residential exposure studies are therefore not considered to be of importance for the purposes of this report. (Section IV.F.)

8. Quality radiofrequency studies are lacking. (Section IV.G.)

Seamstresses

As a group, seamstresses have proven to constitute an important occupation for the demonstration of a relationship between ELF MF exposure and both Alzheimer's disease and breast cancer. Seamstresses who use industrial sewing machines have very high and relatively constant ELF MF exposure, particularly those seamstresses working in the apparel industry. This is because the motors of older AC machines are large and produce high levels of ELF MFs, and are on and producing such fields even when no sewing is being done. The AC/DC transformers of DC industrial machines always produce a high field even when the machine is turned off (but not unplugged). In addition, rooms, in which a large number of such machines are used, even have relatively high ambient ELF MF levels. Home sewing machines generally produce smaller ELF MFs, but even these weaker ELF MFs are substantial.

RECOMMENDATION Using the Precautionary Principal, mitigating exposure is a proper goal. Mean occupational exposures over 10 mG or intermittent exposures above 100 mG should be lowered to the extent possible. In situations where this is not feasible, the daily length of exposure should curtailed. Lowering ELF MF exposure can be done by improved placement of the source(s) of magnetic fields (e.g., electric motors in sewing machines, AC/DC converters), shielding, and redesign. It is clear that re-engineering products can greatly lessen ELF MF exposure, and possibly result in important innovations. It is noted that certain automotive models produce medium to high ELF MFs, as do steel-belted radial tires (Milham *et al.*, 1999).

I. INTRODUCTION

All of the studies discussed have based exposure classifications using magnetic field (MF) measurements, not electric field (EF) measurements. We separately discuss extremely low frequency (ELF, ≤ 60 Hz) MFs and radiofrequency (RF) MFs. Furthermore, the discussion is primarily limited to investigations related to ELF MF exposure as a possible risk factor for Alzheimer's disease (AD), female breast cancer (BC), and the possible biological pathways linking ELF MF exposure to AD and BC incidence, e.g., reduction in the production of melatonin.

Exposure Concerns

Epidemiologic investigations are sensitive to errors in exposure assessment and errors in case-control designation. This is particularly true for ELF MF exposure and for AD classification. With respect to occupational exposures, all job exposure matrices (JEM) are based on the measurement of a relatively small number of subjects in each job type. However, extensive measurements have been performed for workers in the electric utility industry and for seamstresses. Note, however, that the Swedish breast cancer study by Forssén *et al.* (2005) used only 5 essentially part-time seamstresses to determine exposure classification (Forssén *et al.* (2004).

The geometric mean ELF MF exposure over the time period of observation is generally used for classification. For ordinal classifications, individual subjects in jobs with mean ELF MF exposure measured close to a boundary value, e.g., between low and medium or between medium and high ELF MF exposure, will frequently be incorrectly classified. This misclassification will generally lead to bias in the estimated risk towards 1, i.e., no risk.

For residential exposures, which do not include living near high power lines, measurements of necessity need to be taken at the current residence. Measurements are usually taken in several rooms at various locations, sometimes with and without electrical equipment turned on, but rarely (if ever) with water lines turned on. Thus, individualized exposures, e.g., sitting near a fuse box, being near one or more AC/DC transformers, use of specific brands and models of home sewing machines, being near a microwave oven in operation, and a myriad of other point sources are missed. Previous residences are usually not available for measurements. Consequently, exposure classification is problematic for studies interested in risk associated with residential ELF MF exposure.

* Unless otherwise specified, "high" ELF MF exposure as used in this report means an exposure of at least 10 mG or (relatively frequent) intermittent exposure above 100 mG,

while "medium" exposure is an average exposure of between 2 and 10 mG or (relatively frequent) intermittent exposure above 10 mG. "Long-term exposure" means exposure over a period of years. Often, other researchers use a cut-point of around 2-3 mG, or sometimes even less, as a "high" average. The reviews of each study presented here detail the specific cut-point(s) used.

** Also, unless otherwise specified, "high" ELF MF exposure as used in this report means an exposure of at least 10 mG, while exposure means exposure over a period of years. **

Diagnostic Concerns

AD is difficult to correctly diagnose. Non-specialists frequently incorrectly diagnose a patient as having AD. Exposure assessment and case-control classification errors bias the odds ratio (OR) estimator, when based on dichotomous exposure classification, towards the null hypothesis. When based on three (3) or more classification groups, exposure assessment and case-control classification errors in the types of analyses used most likely also lead to bias towards the null hypothesis.

> With respect to AD, unless the diagnosis is made by experts, there is a very large false positive rate. That is, community-based physicians often incorrectly diagnose dementia (versus depression, for example) and are particularly poor at determining the correct differential diagnosis of dementia. Most subjects with a diagnosis of dementia are simply assumed to have AD. This means that around 40% of all AD diagnoses by physicians who are not experts are incorrect. Diagnostic information on death certificates is even worse. Such a large error in caseness clearly biases the OR estimator towards the null hypothesis. (Many cases of AD go undiagnosed, especially early stage AD. However, this likely does not lead to a significant error rate in classification of controls.)

> With respect to breast cancer, the sub-type of breast cancer is generally recorded, e.g., estrogen receptor positive (ER+) or negative (ER-), which may very well be important with respect to ELF MF exposure. However, sub-group analyses have not usually been performed.

Therefore, in reviewing published studies, particular emphasis is placed on these errors or caveats. Studies which assessed occupational exposures and those which assessed residential exposures are both discussed. Various algorithms for "ELF MF exposure" have been used, and these will also be discussed. Not all studies, exposure data, and exposure algorithms are of equal value.

For both AD and BC, a possible biological pathway of particular importance is down-regulation of melatonin production as a result of long-term ELF MF exposure. This is discussed in detail in this review.

A second possible biological pathway relates specifically to Alzheimer's disease. Long-term ELF MF exposure may increase the production of amyloid beta (Aβ), both in the brain and peripherally. Aβ, particularly the form with 42 amino acids (Aβ1-42), is considered the primary neurotoxic compound causing AD. This pathway was proposed by Sobel and Davanipour (1996a). Recent epidemiologic studies have provided some degree of confirmation. A third

pathway has been proposed: genomic instability.   Thus, ELF MF exposure may be a risk factor for AD through possibly three complementary biological pathways. (See Sections III.A. and III.B.)

There may certainly be other potential biological pathways that will be identified. For example, melatonin interacts with certain cytokines which appear to affect immune responses. This may be relevant to the early elimination of cells which are either pre-malignant or malignant, thus preventing the development of overt breast or other cancers. However, the two primary pathways outlined above can most easily be evaluated in human studies, both population-based studies and clinical trials.

There are also several epidemiologic studies of melatonin production among workers with long-term occupational exposure to magnetic fields and a single study of women with high (vs low) residential ELF MF exposure. These studies generally indicate that long-term ELF MF exposure can lead to lowered melatonin production.

II.   ELF Magnetic Field EXPOSURE and MELATONIN ACTIVITY AND PRODUCTION

A. Melatonin Production

Conclusion: Eleven (11) of the 13 published epidemiologic residential and occupational studies are considered to provide (positive) evidence that high ELF MF exposure can result in decreased melatonin production. The two negative studies had important deficiencies that may certainly have biased the results. There is sufficient evidence to conclude that long-term relatively high ELF MF exposure can result in a decrease in melatonin production. It has not been determined to what extent personal characteristics, e.g., medications, interact with ELF MF exposure in decreasing melatonin production.

Eightly-five percent (85%) to 90% of pineal melatonin production is at night. Laboratory-based studies, using pure sinusoidal magnetic fields under experimental conditions have not found an effect on melatonin production (Graham *et al.*, 1996, 1997; *Brainard et al.*, 1999). However, several studies among subjects chronically exposed in occupational and residential environments have found an effect, while a few have not. The lack of an effect in laboratory settings may be because the ELF MF exposure was too "clean" or because the duration of exposure was not sufficiently long, e.g., days, weeks, months.

The evidence indicates that high and ELF MF exposures may lead to a decrease in melatonin production. Whether this decrease is reversible with a cessation of exposure is unknown. The extent of the decrease is hard to evaluate. It is also not yet possible to identify individual susceptibility to such a decrease in melatonin production.

Melatonin production is generally measured using its primary urinary metabolite, 6-sulphatoxymelatonin (aMT6s). Total overnight melatonin production is best estimated using complete overnight urine samples. Creatinine-adjusted aMT6s is slightly more correlated with cumulative melatonin estimates obtained from sequential overnight blood samples than is unadjusted aMT6s (Cook *et al.*, 2000; Graham *et al.*, 1998).

The human studies in occupational or residential environments which identified an effect are

summarized below.

Positive Studies

- <u>Assessment in the Finnish Garment Industry</u> As a follow-up component to a Finnish study of ELF MF exposures among garment factory workers, a small study of nighttime melatonin production was carried out (Juutilainen *et al.*, 1999). aMT6s excretion and creatinine were measured using complete overnight urine samples. Seamstresses (n=31), other garment workers (n=8), and non-exposed outside workers (n=21) participated. Observations were taken using complete overnight urine collections beginning on a Thursday night through the first morning void on Friday and on the subsequent Sunday night through the first morning void on Monday. There was very little variation between the two time period observations within each group, indicating that if there is an effect of ELF MF exposure, it does not disappear over the weekend, at least among seamstresses using older industrial alternating current machines. The average Thursday-Friday non-adjusted aMT6s excretion level and the average aMT6s excretion level adjusted for creatinine were both statistically significantly lower (p< 0.05) among the workers in the garment factory compared to the controls, even after controlling for other factors associated with a lowering of melatonin levels: creatinine-adjusted aMT6s - 16.4 vs 27.4 ng/mg; unadjusted aMT6s - 5.1 vs 10.0 ng. There was no indication of a dose-response relationship among the garment factory workers.

  In a follow-up study, Juutilainen and Kumlin analyzed the same data in conjunction with a dichotomization of a measure of light-at-night (LAN), obtained from items in the original study questionnaire concerning use of a bedroom light at night, street lights outside the bedroom windows, and use of curtains which do or do not let light filter through. There was a significant interaction between the dichotomized ELF MF exposure (high/low, i.e., cases vs controls) and LAN (yes/no). aMT6s was significantly lower for subjects with high ELF MF with or without LAN. In addition, aMT6s was significantly lower among subjects with high ELF MF and LAN exposure versus subjects with high ELF MF and no LAN exposure. Alternatively, aMT6s was essentially identical for subjects with low ELF MF exposure, regardless of the LAN status.

- <u>Washington State Residential ELF MF Exposure and Melatonin Study</u> Women, aged 20 to 74, were selected for a study of the relationship of bedroom 60 Hz magnetic field levels and melatonin production (Kaune *et al.*, 1997a,b; Davis *et al.*, 2001a). Approximately 200 women were recruited based on magnetic field exposure information from a case-control study of breast cancer (PI: S Davis). About 100 women were sought whose bedrooms were at the high end of magnetic field level in the original study and about 100 were sought who were at the low end. Concurrent measurements of light at night in the bedrooms of these women were also obtained using a specially modified EMDEX II system. Mean magnetic field levels in the two groups differed by less than 1 mG. Thus, compared to ELF MF exposures in many occupations, the women had quite low ELF MF exposures. However, there was an inverse association between bedroom magnetic field levels and urinary aMT6s adjusted for creatinine levels on the same night, after adjusting for time of year, age, alcohol consumption, and use of medications. The association was strongest at those times of the year with the longest length of daylight and in women who were using medications that themselves were expected to attenuate melatonin production,

e.g., beta and calcium channel blockers and psychotropic drugs.

- Crossover Trial of ELF MF Exposure at Night and Melatonin Production Davis *et al.* (2006) conducted a randomized crossover trial among 115 pre-menopausal women with regular periods between 25 and 35 days apart, a body mass index between 18 and 30 kg/m^2, not using hormonal contraceptives or other hormones for at least 30 days before the study period, no history of breast cancer, no history of chemotherapy or tamoxifen therapy, not having been pregnant or breast-feeding within the previous year, not working any night shifts, not taking supplemental melatonin, phytoestrogens or isoflavones, and not eating more than 5 servings of soy-based foods within any one week. ELF MF exposure or sham exposure was for 5 consecutive days. A random half of these women received ELF MF exposure and then sham exposure one month later. The other random half had the exposures reversed. Ovulation was determined in the first, second and third months. The initial exposure (ELF MF or sham) was in the second month during days 3-7 post-ovulation. The second exposure (sham or ELF MF) was during the same days in the third month. The charging base of an electric toothbrush which produced a steady magnetic field was used. It was placed under the subject's bed at the head level so that the subject's head received 5-10 mG exposure above baseline. Complete overnight urine samples were collected on the night of the last exposure (ELF MF or sham) in each of the two exposure periods. There were 2 subjects who did not ovulate during either exposure month and 13 who did not ovulate in one of the two months. Statistical adjustment was made for age, hours of darkness, body mass index, medication use, any alcohol consumption, and number of alcoholic beverages consumed. Because each subject was her own control, these adjustments probably did not affect the point estimates much. A regression analysis was undertaken. The 95% confidence interval (CI) of the regression slope was [-3.0 – +0.7] for all subjects and [-4.1 – -0.2] when the 15 subjects with "minor" protocol violations were eliminated from the analysis. These violations were (a) more than 40 days between the two assessments, (b) urine collections not on the same post-ovulation day, and (c) menstrual period started early. Only (b) appears to be really relevant because these subjects could have had less ELF MF exposure. However, this information is not provided. Separate analyses were conducted for "medication users" (n=14) and non-users (n=101). The slope point estimate for the users was numerically smaller (-3.1) than for the non- users (-1.0). The authors state that the study "found that nocturnal exposure to 60-Hz magnetic fields 5 to 10 mG greater than ambient levels in the bedroom is associated with decreased urinary concentrations of (aMT6s)". It should be noted that the p-value of the slope estimate in the primary analysis (all participants) was greater than 0.05. However, the 95% CI, [-3.0 – +0.7], was quite unbalanced, with 0 being much closer to the upper end of the CI than the lower end. Also, the 95% CI, when the 15 subjects with minor protocol violations are eliminated is entirely below 0, and thus the point estimate is statistically significant at the 0.05 level. The authors also state the following: "(t)he more pronounced effect of magnetic field exposure on melatonin levels seen in medication users and in those with an anovulatory cycle suggest {sic} that individuals who have decreased melatonin levels already may be more susceptible to the effects of magnetic field exposure in further decreasing melatonin levels." The justification for this statement is not based on statistical testing.

- Residential High Power Lines, ELF MF Exposure and aMT6s in the Quebec City Study Levallois *et al.* (2001) evaluated aMT6s among 221 women living near 735-kV power lines

compared to 195 age matched women who live far away from such lines. The subjects wore magnetic field dosimeters for 36 consecutive hours to measure their actual ELF MF exposure. The geometric mean 24-hour ELF MF exposure was 3.3 mG among women living near a high power line and 1.3 mG among those who did not live near a high power line. Similarly, geometric mean exposure during sleep was 2.9 mG versus 0.8 mG for the two groups. No direct effect of ELF MF exposure on creatinine-adjusted aMT6s was identified. However, living near a high power line and ELF MF exposure interacted with age and body mass index (BMI; kg/m^2). Living near a high power line was associated with a significant decline in creatinine-adjusted aMT6s among older subjects and subjects with higher BMI. There were similar significant decreases related to age and BMI for women in the lowest quartile versus highest quartile. All analyses were adjusted for age, BMI, alcohol consumption in the previous 24 hours, medication use in the previous 24 hours, light at night, and education.

- <u>Assessment in the Electric Utility Industry</u> Burch *et al.* (1996, 1998, 1999, 2000, 2002) have reported on the association between levels of occupational daytime magnetic field exposure, non-work ELF MF exposure, and the excretion of total overnight and daytime aMT6s among electric utility workers in several studies. These studies are among the largest to evaluate the relationships between ELF MF exposure and melatonin production in humans, and are the only studies to use personal exposure monitoring of both ELF MF and ambient light with a repeated measures design.

  ✓ In their 1996 abstract, analyses were conducted for 35 of 142 electric utility workers enrolled in a larger study. ELF MF exposure was assessed continuously at 15 second intervals for three 24-hour periods, with logs kept to identify work, sleep and other non-work time periods. Ambient light intensity was also individually measured. Complete overnight urine samples and post-work spot urine samples were collected at the same times over the 3 days. There were statistically significant inverse relationships between nocturnal aMT6s levels and log- transformed worktime mean ELF MF exposure (p=0.013), geometric work-time mean ELF MF exposure (p=0.024), and cumulative work-time ELF MF exposure (p=0.008). There was no association, however, between sleep time and other time ELF MF exposure levels and aMT6s levels during the daytime or nighttime, even though average cumulative ELF MF levels were only somewhat higher during work: 18.3 mG-hours (work); 13.1 mG-hours (non-work); 12.6 mG-hours (sleep).

  ✓ In their 1998 study, further results related to nocturnal aMT6s urinary excretion in relation to ELF MF exposure were presented, using all 142 electric utility workers. The ELF MF exposure metrics were geometric mean intensity, a rate-of-change metric (RCM), and the standardized rate-of-change metric (RCMS). RC was used as a measure of intermittence, while RCMS was used as a measure of the temporal stability of the serially recorded personal ELF MF exposures. Statistical adjustments were made for age, month, and personal ambient light exposure. 24-hour mean ELF MF exposure intensity, RCM, and RCMS were not associated with either nocturnal aMT6s or creatinine-adjusted aMT6s. However, there was an inverse relationship between residential RCMS and nocturnal aMT6s. The interaction between residential intensity and RCMS was inversely associated with total overnight urinary aMT6s excretion and with

creatinine-adjusted nocturnal aMT6s excretion. There was a "modest" reduction in nocturnal aMT6s with more temporally stable ELF MF exposures at work. The effect on nocturnal aMT6s was greatest when residential and workplace RCMS exposures were combined. The authors concluded that their study provides evidence that temporally stable ELF MF exposure (i.e., lower RCMS) are associated with decreased nocturnal urinary aMT6s levels. Given the strong correlation between cumulative overnight serum melatonin levels and both total overnight urinary aMT6s and creatinine-adjusted aMT6s levels, these results indicate a reduction in overnight melatonin production.

✓ In their 1999 study, data from the same 142 electric utility workers were further analyzed. Personal exposure to workplace geometric mean and RCMS were evaluated for their effect on post-work urinary aMT6s measurements. No association between creatinine-adjusted aMT6s and the geometric mean ELF MF exposure, before or after adjustment for age, calendar month and light exposure was found. However, ELF MF temporal stability was associated with a statistically significant reduction in adjusted mean post-work aMT6s concentrations on the second (p=0.02) and third (p=0.03) days of observation. Light exposure modified the ELF MF exposure effect. Overall, there was a significant (p=0.02) interaction between RCMS and ambient light exposure. Reductions in post-work aMT6s levels were associated with temporally stable ELF MF exposures among workers in the lowest quartile of ambient light exposure (mostly office workers), whereas there was no RCMS effect among workers with intermediate or elevated ambient light exposure.

✓ In their 2000 study, Burch *et al.* examined aMT6s levels among a completely different population of 149 electrical workers, 60 in substations, 50 in 3-phase environments, and 39 in other jobs, using the same data collection strategy as was used in the previous study, but with the added characterization of specific work environments. The rationale for this study was based on previous observations in experimental animals suggesting that non-linear field polarization was critical in the reduction of melatonin production. These types of fields were expected to be present within substations and in the vicinity of 3-phase electrical conductors. Other conductors (1-phase, linear polarization) were selected as a control condition because they had not previously been associated with an alteration of melatonin production in laboratory animal studies. Thus, participating workers recorded the times they spent in these environments over the 3-day data collection period. Comparisons were made separately for subjects working in substation or 3-phase environments, or among those working in 1-phase environments. Adjusted mean aMT6s levels were compared statistically among workers in the lowest and highest tertiles of ELF MF exposure, using either the geometric mean or the RCMS measurements. Among workers in either a substation or 3-phase environment for more than 2 hours, nocturnal aMT6s decreased 43% (p=0.03) when tertiles were based on geometric mean exposure and decreased 42% (p=0.01) when tertiles were based on RCMS. With RCMS tertiles, total overnight aMT6s excretion also decreased 42% (p=0.03) and post-work creatinine-adjusted aMT6s decreased 49% (p=0.02). With geometric mean tertiles, total overnight aMT6s excretion decreased 39% and post-work creatinine-adjusted aMT6s

decrease 34%. However, neither of these decreases was statistically significant. No ELF MF-related effects were observed among workers with less than 2 hours time spent in substation/3-phase environments. Similarly, no reduction in aMT6s levels were observed among workers in 1-phase environments.

✓ In 2002, Burch *et al.* studied two consecutive cohorts of electric utility workers using the same data collection strategy to evaluate the effects of cellular telephone use and personal 60 Hz ELF MF exposure on aMT6s excretion. The sample sizes were 149 for Cohort 1 (from the 2000 study) and 77 for Cohort 2. Total overnight and post-work urine samples and self-reported workplace cell phone use were obtained over three (3) consecutive workdays. ELF MF and ambient light exposure were also measured with specially adapted personal dosimeters. The outcome of interest was melatonin production as measured by aMT6s. The cut- point for high versus low cell phone use was 25 minutes per day. Only 5 worker- days of cell phone use more then 25 minutes were reported in Cohort 1 versus 13 worker-days in Cohort 2. No differences in aMT6s production were found in Cohort 1. However, for Cohort 2 there were significant linear trends of decreasing overnight aMT6s and creatinine-adjusted aMT6s levels with increasing cell phone use. There was also a marginally significant increasing trend in post-work creatinine-adjusted aMT6s with increasing cell phone use. Finally, there was a combined effect of cell phone use and ELF MF exposure on aMT6s excretion: among workers in the highest tertile of ELF MF exposure, those who used a cell phone for more than 10 minutes had the lowest overnight aMT6s and creatinine-adjusted aMT6s levels compared to those with lower ELF MF exposure or cell phone use. All analyses used a repeated measures method and were adjusted for age, month of participation, and light exposure.

- <u>Swiss Railway Worker Study</u> Pfluger and Minder (1996) studied 66 railway engineers operating 16.7 Hz electric powered locomotives and 42 "controls". Mean ELF MF exposure at the thorax for the engineers was above 150 mG and approximately 10 mG for the controls. Thus most controls also had high ELF MF exposure, certainly compared to residential and most occupational ELF MF exposures. Morning and early evening (post-work) urine samples were used to measure aMT6s. Evening aMT6s values were significantly lower following work periods (early, normal or late shifts) compared to leisure periods for the engineers, but not for the controls. Also, morning samples did not differ between leisure and work mornings. This indicates that there was at least somewhat of a recovery from the work-time ELF MF exposures. Evening aMT6s values did not differ between work time and leisure time for either engineers or controls. However, there was a rebound in morning aMT6s between a work period and leisure period. Pfluger and Minder did not report the results of a comparison of nighttime aMT6s levels between engineers and controls.

- <u>Video Display Unit Studies</u> Non-panel video display screens, e.g., computer monitors, produce significant ELF MF exposure despite improvements over the last decade or so. Arnetz and Berg (1996) studied 47 Swedish office workers who used video display units (VDU) in their work in the 1980s. Circulating melatonin levels significantly decreased during work, but not during a day of "leisure" in the same environment.

Nighttime melatonin production was not observed. In 2003, Santini *et al.* conducted a similar, but quite small, study of 13 young female office workers, 6 of whom worked for at least 4 hours per day in front of a video screen. Overnight urine samples were used to measure aMT6s. The aMT6s values of the exposed workers was 54% lower (p<0.01) compared to the non- exposed workers.

Negative Studies

- Italian Study of Workers Gobba *et al.* (2006) recruited 59 workers, 55.9% of whom were women, for a study of melatonin production and ELF MF exposure. Actually more workers were recruited, but urine samples for only those subjects who did not get up to urinate during sleep time were assayed. Creatinine-adjusted aMT6s was measured using a Friday morning urine sample and the following Monday morning urine sample. Mean age was 44.4 years (standard deviation, 9.2). Exposure during worktime was measured over a three-day period. The logarithm of the time weighted average (TWA) and the percent of time above 2 mG were used as the measures of exposure. 2 mG was the cut-point between low and high exposure. 52.5% were in the low exposed group; a larger percentage of men than women were in the low exposed group. Occupations included clothing production (n=26), utility companies (14), teachers (6), engineering industry (5), and miscellaneous (8). There were no significant differences in creatinine-adjusted aMT6s values based on the logarithm of the TWA or percent of observations above 2 mG.

- Occupational ELF MF Exposures among 30 Males Subjects in France Touitou *et al.* (2003) studied 15 men exposed to occupational magnetic fields for between 1 and 20 years and age-matched 15 controls. All subjects were free of acute or chronic diseases, had regular sleep habits, did not do night work, took no transmeridian airplane flights during the preceding 2 months, took no drugs, were nonsmokers, and used alcohol and coffee in moderate amounts. Furthermore, they did not use electric razors or hair dryers during the study or in the 24 hours prior to blood sampling. All of the 15 ELF MF exposed men worked in high voltage electrical substations. They also lived near substations. None of the controls had an occupation associated with ELF MF exposure. Exposed subjects had a mean exposure of 6.4 mG during work and 8.2 mG during other times. For the control subjects, the mean exposure was 0.04 mG, both during the day and at other times. Blood samples were taken hourly from 8:00 pm until 8:00 am in a standard manner. All urine between these times was collected. Melatonin concentration (pg/ml) was measured in each blood sample. The study was done in the autumn. The 12 hour melatonin blood concentration curves for the exposed and non-exposed subjects are almost identical. The creatinine-adjusted aMT6s levels are also nearly identical. No analyses were conducted based on length of time in the occupation.

B. **Melatonin Activity and ELF MF**

Conclusion: New research indicates that ELF MF exposure, in vitro, can significantly decrease melatonin activity through effects on MT1, an important melatonin receptor.

Girgert et al. (2010) studied the effects of 12 mG 50 Hz ELF MF exposure on signal transduction of MT1 in parental MCF-7 cells and MCF-7 cells transfected with the MT1 gene. MT1 is a high-affinity melatonin receptor and is responsible for many of melatonin's activities. 12 mG is an

exposure experienced by individuals in many occupations, e.g., seamstresses and welders. Melatonin, as discussed in this chapter, has many important properties related to cancer prevention and growth, particularly breast cancer, and to the delay or prevention of AD. For proliferation tests, the MT1-negative and MT1-transfected cells were placed in a medium with and without an estradiol solution – estradiol concentrations ranged from 10^{-12} to 10^{-10} moles. 4×10^{-9} moles of melatonin were used in a parallel series of estradiol concentrations to evaluate the effect of melatonin. Cell proliferation assays demonstrated that (i) melatonin inhibited cell growth and (ii) 12 mG ELF MF exposure nearly eliminated the effect of melatonin on cell growth. Furthermore, melatonin's growth inhibitory effect was more prominent in the MCF cells transfected with the MT1 receptor than in the cells which were not transfected.

> Girgert et al. (2010) note that several studies designed to evaluate the effects of melatonin in breast cancer cells were negative. They measured the ELF MF produced by various cell incubators and found several that generated approximately 12 mG. They suggest that negative findings may be due to the use of incubators which produce these relatively high fields.

III. ALZHEIMER'S DISEASE

A. Possible Biologic Pathways from ELF MF Exposure to Alzheimer's Disease

A.1. Over-Production of Peripheral Amyloid Beta Caused by ELF MF Exposure

Conclusion: There is now evidence that (i) high levels of peripheral amyloid beta are a risk factor for AD and (ii) medium to high ELF MF exposure can increase peripheral amyloid beta. High brain levels of amyloid beta are also a risk factor for AD and medium to high ELF MF exposure to brain cells likely also increases these cells' production of amyloid beta.

Sobel and Davanipour (1996a) have published a biologically plausible hypothesis relating ELF MF exposure to AD, based on the unrelated work of many researchers in several different fields. The hypothesized process involves increased peripheral or brain production of amyloid beta (Aβ) as a result of ELF MF exposure, and subsequent transportation of peripheral Aβ across the blood brain barrier. Figure 1 provides a schematic outline of the hypothesis. Each step in the proposed pathway is supported by *in vitro* studies.

Two versions of the amyloid beta protein have been identified. They are identical, except one is longer, 42 versus 40 amino acids. These are specified, respectively, by $A\beta_{1-42}$ and $A\beta_{1-40}$. $A\beta_{1-42}$ is considered the more neurotoxic of the two.

This hypothesis has not yet been fully tested. However, two recent studies of elderly subjects and electrical workers, respectively, have provided important initial support. The Mayeux *et al.* (1999, 2003) papers demonstrate that higher levels peripheral $A\beta_{1-42}$ are a risk factor for AD. The Noonal et al. (2002a) paper demonstrates that ELF MF exposure can increase the peripheral levels of $A\beta_{1-42}$ and that contemporaneous blood levels of melatonin are inversely associated with peripheral levels of $A\beta_{1-42}$.

- Mayeux *et al.* (1999, 2003, 2011) conducted a population-based, longitudinal study of

elderly subjects who were cognitively normal at baseline and found that higher peripheral blood levels of $A\beta_{1-42}$ were prognostic of subsequent development of AD. The 2003 paper had a longer follow-up period and 282 additional subjects (169 vs 451).

In the first paper, 105 subjects, cognitively normal at baseline, were followed for an average of 3.6 years. The mean age at baseline was 74.3 +/- 5.3 years. Sixty-four (64) subjects developed AD. Table 1 provides the baseline and follow-up means for age, education, $A\beta_{1-42}$, $A\beta_{1-40}$, and the ratio $A\beta_{1-42}/A\beta_{1-40}$. The subjects who developed AD were older at baseline, had nearly two years less education, and higher $A\beta_{1-42}$, $A\beta_{1-40}$, and $A\beta_{1-42}/A\beta_{1-40}$. All mean differences were significant at the $p=0.001$ level, except for the ratio, which was significant at the $p=0.05$ level.

For $A\beta_{1-42}$, the OR for AD, based on the actual $A\beta_{1-42}$ values, was 1.0114, $p = 0.006$. Thus, for example, the OR for an individual with an $A\beta_{1-42}$ value 10 pg/ml above the cutpoint for the 1st quartile (24.6 pg/ml) is estimated to be $(1.0114)^{10} = 1.12$, an increase of 12%; for an individual with an $A\beta_{1-42}$ value 40 points above this cutpoint, the estimated increase in risk is 57%. A similar analysis for $A\beta_{1-40}$ did not yield a significant result.

Subjects were then divided into quartiles based on their $A\beta_{1-42}$ values. For $A\beta_{1-42}$ there was a highly significant ($p=0.004$) trend across quartiles. The adjusted odds ratios (OR) for the $2^{nd} - 4^{th}$ quartiles were 2.9, 3.6, and 4.0, using logistic regression. The latter two were statistically significant at the 0.05 level. The ranges for the 3rd and 4th quartiles were 45.9 $- 85.0$ pg/ml and > 85.0 pg/ml, respectively. For the 2nd quartile, the significance level of the OR was not provided; however, the 95% confidence interval (CI) was $[0.9 - 6.8]$. Perhaps because the per unit analysis was not significant for $A\beta_{1-40}$, an analysis using quartiles was not reported.

In the second paper (Mayeux *et al.*, 2003), follow-up of patients was up to 10 years and there were 451 patients who were cognitively normal at baseline, versus 169 in the initial paper. Table 2 contains the same information for this study as is provided in Table 1 for the initial study. Eighty-six (86) of the 451 subjects developed AD. Presumably, the additional subjects had had their peripheral amyloid beta assayed after the submission of the original paper. Again, the $A\beta_{1-42}$ values were divided into quartiles, based on the 451 subjects who were cognitively normal at their last follow-up. The adjusted relative risk (RR) estimates for the $2^{nd} - 4^{th}$ quartiles were 1.3, 1.9, and 2.4, using Cox survival analysis. The latter two were statistically significant at the 0.05 and 0.006 levels, respectively. The ranges for the 3rd and 4th quartiles were $60.2 - 84.15$ pg/ml and ≥ 84.15 pg/ml, respectively. For the 2nd quartile, the significance level of the OR was again not provided; however, the 95% confidence interval (CI) was $[0.6 - 2.1]$.

The mean levels of $A\beta_{1-40}$, $A\beta_{1-42}$, and $A\beta_{1-42}/A\beta_{1-40}$ at baseline in the second paper were 133.9 pg/ml, 62.2 pg/ml, and 0.50. In the initial paper, the comparable figures were 120.5 pg/ml, 63.2 pg/ml, and 0.57. The means for $A\beta_{1-42}$ and $A\beta_{1-42}/A\beta_{1-40}$ are quite similar in the two studies. However, the means for $A\beta_{1-40}$ are quite different, so there were most likely several subjects who were not in the initial report, and who had $A\beta_{1-40}$ assays which were very high. These subjects were evidently almost all in the cognitively normal group. This is because in the AD groups, the $A\beta_{1-40}$ means were 134.7 and 136.2 pg/ml. However, in the cognitively normal group, the means were

111.8 and 133.3 pg/ml. Thus, the additional 260 subjects with did not develop AD (365-105=260) had an average $A\beta_{1-40}$ of 142.0 pg/ml. Such a large difference is left unexplained in the Mayeux *et al.* (2003) paper.

Mayeux *et al.* (1999) comment that "cerebral deposition of $A\beta_{1-42}$ is unlikely to result directly from increased plasma $A\beta_{1-42}$". However, studies by Zlokovic and colleagues provide a basis for concluding that, in fact, peripheral $A\beta_{1-42}$ is likely to cross the blood brain barrier, perhaps chaperoned by apolipoprotein E (ApoE), particularly the ε4 isoform (see Sobel & Davanipour, 1996a). Currently, the relative amounts of peripheral and cerebral $A\beta_{1-42}$ or $A\beta_{1-40}$ which aggregate are unknown.

> Two newly developed PET scan techniques, however, provide the ability to investigate the relative amounts in humans (Klunk *et al.*, 2004; Ziolko *et al.*, 2006; Small *et al.*, 2006). It is also straightforward to use labeled amyloid beta to determine the rate at which peripheral amyloid beta is transported to the brain, at least in animal models and perhaps also in humans.

In 2011, Mayeux and Schupf further discussed their and other researchers findings and their hypothesis that a high blood level of $A\beta_{1-42}$ is a risk factor for late onset AD, but the $A\beta_{1-42}$ blood levels decline with advancing dementia. Similarly, blood levels of $A\beta_{1-40}$ may also decline with disease progression.

- Schupf et al. (2008) studied a sample of 1021 non-demented subjects at least 65 years old at baseline. Plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ levels were assayed at baseline. One hundred and four (104; 10.2%) subjects developed AD within 4.6 years. Higher plasma $A\beta_{1-42}$ at baseline was associated with a 3-fold increase in the risk of AD. On the other hand, development of AD was associated with a significant decline in plasma $A\beta_{1-42}$ and a decrease in the $A\beta_{1-42}/A\beta_{1-40}$ ratio as dementia progressed.
- Cosentino et al. (2010) studied a sample of 880 subjects, 65 or older and dementia free at the first of two plasma Aβ measurements. High baseline plasma for both $A\beta_{1-42}$ and $A\beta_{1-40}$, and decreasing or stable $A\beta_{1-42}$ were associated with faster decline in multiple cognitive areas.
- Schupf et al. (2010) studied the relationship between plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ levels and the occurrence of dementia among a community-based cohort of 225 Down syndrome adults, dementia-free at baseline. Sixty-one (61, 27.1%) developed AD during follow-up. The mean length of follow-up was 4.1 years. The increase in plasma $A\beta_{1-40}$, decrease in plasma $A\beta_{1-42}$, and decrease in $A\beta_{1-42}/A\beta_{1-40}$ levels were significantly associated with development of dementia. This study was an extension of the follow-up time of an earlier study (Schupf et al., 2007).
- Devanand et al. (2011) studied a small number of patients (n=20) with amnestic mild cognitive impairment (MCI), a harbinger of AD development in the majority of cases, and 19 cognitively normal controls. Plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ levels were assayed. In addition PET scans determined Pittsburgh compound B (PiB) binding in various brain locations and in the total brain. The plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio was decreased in the MCI patients compared to the controls, but $A\beta_{1-42}$ and $A\beta_{1-40}$ did not differ between the two groups. PiB binding levels were significantly higher in the cingulate and parietal brain areas and in the entire brain among the MCI patients compared to the

controls. However, in the prefrontal cortex and parahippocampal gyrus the differences were only marginally significant, but the sample size was relatively small. Low $A\beta_{1-42}/A\beta_{1-42}$ and $A\beta_{1-40}$ were associated with high cingulate, parietal and total brain PiB binding, using regression analyses which included age, gender, and cognitive test scores.

- For completeness, we provide the results of a meta-analysis by Song et al. (2011) of 12 cross-sectional and 7 longitudinal studies of plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ levels related to AD. The results were as follows:
  - ✓ Longitudinal studies: cognitively normal subjects who developed AD had higher baseline plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ (p=0.0001 and 0.006, respectively), but non-significantly increased $A\beta_{1-42}/A\beta_{1-40}$ (p=0.10).
  - ✓ Cross-sectional studies: AD patients had marginally significant (p=0.08) lower plasma $A\beta_{1-42}$. The $A\beta_{1-40}$ levels were not significantly different (p=0.69).

- Noonan *et al.* (2002a) examined 60 electric utility workers in studying the relationship between measured ELF MF exposure during the work day and serum $A\beta_{1-42}$ and $A\beta_{1-40}$ (square root transformed) levels. ELF MF exposure was individually determined by wearing a dosimeter at the waist during work time. Blood samples were obtained between 2:50 pm and 4:50 pm. The primary findings were as follows:
  - **i.** there was an inverse association between physical work and A $A\beta$ levels;
  - **ii.** there was an apparent trend for the $A\beta_{1-42}$, $A\beta_{1-40}$, and $A\beta_{1-42}/A\beta_{1-40}$ levels to be higher for higher magnetic field exposure (significance not provided); and
  - **iii.** the differences (Table 3) in $A\beta$ levels between the highest (≥ 2 milliGauss (mG), n=7) and lowest (< 0.5 mG, n=20) exposure categories were 156 vs 125 pg/ml (p=0.10) for $A\beta_{1-40}$, 262 vs 136 pg/m (p=0.14) for $A\beta_{1-42}$, and 1.46 vs 1.03 for $A\beta_{1-42}/A\beta_{1-40}$ (significance not provided).

There was a 93% increase in $A\beta_{1-42}$, a 25% increase in $A\beta_{1-40}$, and a 42% increase in the ratio $A\beta_{1-42}/A\beta_{1-40}$ between the lowest and highest ELF MF exposure categories. The 2 mG cutpoint for the highest category is the cutpoint generally used for medium (or at times high) ELF MF exposure in epidemiologic studies. Thus, while the sample size was small, this study provides some evidence that ELF MF exposure may result in higher peripheral production of $A\beta$ for exposures above 2mG.

Melatonin production was estimated using urinary 6-sulphatoxymelatonin (aMT6s) adjusted for creatinine (Graham *et al.*, 1998). aMT6s is the primary urinary metabolite of melatonin. A complete overnight urine sample was used to estimate overnight melatonin production, normally about 85-90% of total 24-hour production. A post-work urine sample, taken on the same day as the post-work blood sample, was used to estimate work time melatonin blood levels. The overnight creatinine-adjusted aMT6s levels were, on average, about 5 times higher than the post-work creatinine-adjusted aMT6s levels. Noonan *et al.* state that the correlations between overnight creatinine-adjusted aMT6s and amyloid beta levels were not significant. No data were provided. However, post-work creatinine-adjusted aMT6s levels were negatively correlated with both the $A\beta_{1-42}$ and the $A\beta_{1-42}/A\beta_{1-40}$ post-work levels. The Spearman correlation coefficients were -0.22 (p=0.08) and -0.21 (p=0.10), respectively. With adjustment for age and physical work, the correlation with $A\beta_{1-42}$ was marginally stronger (-0.25, p=0.057). The timing of the urine sample with respect to the blood sample appears to be important. Table 4 provides

the Spearman correlations, adjusted for age and physical work, based on the time difference between blood and urine samples, which were all obtained after the blood draw. Some of the workers had their urine sample in the early evening. It is clear that the correlation is strongest when the samples are taken close to one another in time.

In an unadjusted analysis, the post-work creatinine-adjusted aMT6s levels were split into tertiles. Subjects in the highest tertile had the lowest levels of $A\beta_{1-42}$, $A\beta_{1-40}$, and $A\beta_{1-42}/A\beta_{1-40}$ (Table 5). However, subjects in the middle tertile had higher levels than subjects in the lowest tertile.

- In an *in vitro* study, Del Giudice *et al.* (2007) used human neuroglioma cells (H4/APPswe), which stably overexpress a specific human mutant amyloid precursor protein (APP, to examine the effect of ELF MF exposure. ELF MF or sham exposure was 3.1 mT (31,000 mG) for 18 hours. Total Aβ and total $A\beta_{1-42}$ production was statistically significantly elevated among the ELF MF exposed cells compared to the cells with sham exposure. No gross morphological changes or changes in viability were observed in the ELF MF exposed cells. The 3.1 mT exposure level is 2-3 orders of magnitude higher than the highest occupational mean exposures. The authors state that such high levels were administered because occupational exposures are "much more prolonged than the one described in our experimental setting". There was no indication that any longer duration exposure at lower levels was studied.

A.2. Lowered Melatonin Production: An Alternative/Complementary Pathway

Conclusion: There is considerable in vitro and animal evidence that melatonin protects against AD. Therefore it is certainly possible that low levels of melatonin production are associated with an increase in the risk of AD.

Several *in vitro* and animal studies indicate that melatonin may be protective against AD and thus low or lowered melatonin production may be a risk factor for AD. These studies have generally found that supplemental melatonin has the following effects:
- the neurotoxicity and cytotoxicity of A□ is inhibited, including mitochondria (Pappolla *et al.*, 1997, 1999, 2002; Shen YX *et al.*, 2002a; Zatta *et al.*, 2003; Jang *et al.*, 2005);
- the formation of β-pleated sheet structures and Aβ fibrils is inhibited (Pappolla *et al.*, 1998; Poeggeler *et al.*, 2001; Skribanek *et al.*, 2001; Matsubara *et al.*, 2003; Feng *et al.*, 2004; Cheng and van Breemen, 2005);
- the profibrillogenic activity of apolipoprotein E ε4, an isoform conferring increased risk of AD, is reversed (Poeggeler *et al.*, 2001);
- oxidative stress *in vitro* and in transgenic mouse models of AD is inhibited if given early (Clapp-Lilly *et al.*, 2001a; Matsubara *et al.*, 2003; Feng *et al.*, 2006), but not necessarily if given to old mice (Quinn *et al.*, 2005);
- survival time is increased in mouse models of AD (Matsubara *et al.*, 2003);
- oxidative stress and proinflammatory cytokines induced by A□$_{1-40}$ in rat brain are reduced *in vitro* and *in vivo* (Clapp-Lilly *et al*, 2001b; Shen YX *et al.*, 2002b; Rosales-Corral *et al.*, 2003);
- the prevalence of $A\beta_{1-40}$ and $A\beta_{1-42}$ in the brain is decreased in young and middle aged mice (Lahiri *et al.*, 2004);

- memory and learning is improved in rat models of AD pathology (Shen YX *et al.*, 2001; Weinstock and Shoham, 2004), but not necessarily in Aβ-infused rat models (Tang *et al.*, 2002).

Note that transgenic mouse models of AD mimic senile plaque accumulation, neuronal loss, and memory impairment. See Pappolla *et al.* (2000), Cardinali *et al.* (2005), Srinivasan *et al.* (2006), Cheng *et al.* (2006), and Wang and Wang (2006) for reviews. Thus, chronic low levels of melatonin production may be etiologically related to AD incidence.

A.3.   Cytogenetic Hypothesis Relating ELF MF Exposure to Alzheimer's Disease

Conclusion: This is an interesting hypothesis and is deserving of research efforts.

Maes and Verschaeve (2011) review evidence that genomic instability, including aneuploidy, telomere shortening, and gene amplification, is associated with an increased risk of early-onset familial AD and perhaps sporadic AD. The authors then discuss possible genetic effects of ELF MF (or electromagnetic field (EMF)) exposure. Further, directed research into this hypothesis is warranted.

D.   Epidemiologic Studies of Alzheimer's Disease/Dementia and ELF MF Exposure

Conclusion: There is strong epidemiologic evidence that exposure to ELF MF is a risk factor for AD. There are now twelve (12) studies of ELF MF exposure and AD or dementia which . Nine (9) of these studies are considered positive and three (3) are considered negative. The three negative studies have serious deficiencies in ELF MF exposure classification that results in subjects with rather low exposure being considered as having significant exposure. There are insufficient studies to formulate an opinion as to whether radiofrequency MF exposure is a risk or protective factor for AD.

D.1.   Introduction

First, it is necessary to point out that there are no case-control studies of melatonin as a risk factor for AD. This is primarily because AD results in a precipitous decline in melatonin production due to the destruction of specific neuronal structures and therefore it is inappropriate to use "current" melatonin production of cases as a surrogate estimate of the pre-AD melatonin production. Also there have yet to be any longitudinal studies of melatonin production. This is probably because neither urine nor blood have been collected appropriately to measure nocturnal melatonin production.

If ELF MF exposure is a true risk factor, there are several problematic areas in evaluation and comparison of epidemiologic studies related to occupational ELF MF exposure and Alzheimer's disease, particularly the following.

1. Diagnosis – false positive diagnoses will bias the odds ratio estimator towards 1.0
2. Occupational exposure assessment – inclusion of subjects with low exposure in the "exposed" categories likely biases the odds ratio estimator towards 1.0
    - Definition of ELF MF exposure – published studies have differing definitions

of ELF MF exposure, potentially resulting in "exposure" categories with significant proportions of subjects with low exposure

- Cut-points for non-exposure/exposure categories – some studies use numerical estimates of exposure developed from earlier exposure studies (job exposure matrices) in certain occupations and use average estimates and/or low cut-points to determine "medium" exposure
- Ever versus never exposed – at least one study used ever exposed, with a low threshold for exposure
- Categorized occupational data – categorized data from governmental databases leads to relatively large variation in "exposure" within occupational categories, which results in subjects with low exposure being classified as having been exposed.

Table 6 provides the data on the percentages of ELF MF exposed subjects in the published studies to date. There is a wide range of percentages, due primarily to variation in exposure definition, use of average or mean job-specific estimates, and secondarily to the use of varying job exposure matrices. Table 7 provides the odds ratio estimates of studies discussed in some detail below. The studies which used death certificates or other non-expert databases for the identification of AD cases are not included in Table 7.

The role of seamstresses among workers with high occupational ELF MF exposure in the two *et al.* studies (1995, 1996b) and the Davanipour *et al.* study (2007) is discussed.

D.2.   **Death Certificates-Governmental Databases: Alzheimer's Disease Diagnosis**

The use of death certificates or governmental databases to identify AD cases is certainly problematic. False positive diagnoses tend to bias the OR estimator towards 1.0. Most diagnoses of AD have been and still are made by physicians who are not experts in AD, and who seldom have sufficient clinical time to make a proper diagnosis. The determination of dementia and subsequent differential diagnosis of AD by someone other than an expert has a high false positive rate. In addition, many physicians do not think that AD is a "cause of death", which results in an increase in the false negative rate.

Therefore the recent "positive" Feychting *et al.* (2003), Håkansson *et al.* (2003), and Park *et al.* (2005) studies and the "negative" Savitz *et al.* (1998a,b) and Noonan *et al.* (2002b) studies have been excluded from the discussion below of individual studies. The Johansen *et al.* study (2000) has also been excluded because it depended upon the clinical hospital discharge diagnoses of an historical cohort to determine a "diagnosis" of "presenile" AD or "dementia". Evidently, in that study, late-onset (age at least 65) AD was included under "dementia". (It should be noted that Johansen *et al.* found an increased risk of "dementia", but not "presenile" AD, associated with higher ELF MF exposure.)

D.3.   ELF **MF Exposure Assessment Rates and Analytic Results**

The Sobel *et al.* (1995, 1996b), the Davanipour *et al.* (2007), and the Harmanci *et al.* (2003) studies have followed nearly the same protocol for ELF MF exposure assessment and classification into low, medium and high ELF MF occupations. In these studies, medium exposure was defined as mean ELF MF occupational exposure above 2 mG, but less than 10 mG, or intermittent exposures above 10 mG, while high exposure was defined as mean ELF M F exposure above 10 mG or

intermittent exposures above 100 mG. The rates of medium or high (M/H) exposure in these studies are considerably lower than the rates in the Feychting *et al.* (1998a), Graves *et al.* ((1999), Qiu *et al.* (2004), and Savitz *et al.* (1998b) studies and somewhat lower than the Feychting *et al.* (2003) study. The remaining three studies (Häkansson *et al.*, 2003; Savitz *et al.*, 1998a; Johansen, 2000) utilized subjects from electrical industries and therefore understandably have high rates of ELF MF exposure. (See Table 6 for these rates.)

Thus, it is likely that a substantial percentage of ELF MF "exposed" subjects in 4 of the 6 comparable studies (Feychting *et al.*, 1998a; Graves *et al.*, 1999; Qiu *et al.*, 2004) (Table 7) had a high rate of somewhat minimal exposure in the "exposed" category, due to classification methodologies, compared to the "exposed" categories in the Davanipour *et al.* (2007), Harmanci *et al.* (2003), and the Sobel *et al.* (1995, 1996b) studies. This would tend to lead to an OR estimate closer to 1.0 in the 4 former studies.

D.3.1. **Sobel *et al.* (1995) Study – <u>Positive Study</u>**

The initial publication of an apparent association between AD and having worked in occupations with likely ELF MF exposure consisted of three case-control studies, two from Helsinki, Finland, and one from Los Angeles, USA (Sobel *et al.*, 1995). Control groups varied: the first case-control study analyzed used VaD patients; the second (and largest study) used non-neurologic hospital patients; and the third (and second largest study) used non-demented well subjects. The study-specific ORs were 2.9, 3.1, and 3.0, while the combined OR was 3.0 (95% CI = [1.6 – 5.4], p < 0.001), with no confounder adjustments necessary. The occupational information was apparently primarily related to the last occupation, e.g., judge, high ranking military officer. A total of 386 cases and 575 controls was analyzed in these studies. 9.3% of the cases and 3.4% of the controls were judged to have had an occupation with likely medium or high ELF MF exposure. Among women, 31 (5.3%) were exposed to M/H occupational ELF MF, of whom 29 (95%) were seamstresses, who were classified as having high exposure based on measurements taken during the study. Seamstresses have subsequently been shown to have very high ELF MF exposures (e.g., Hansen *et al.*, 2000; Kelsh *et al.*, 2003; Szabó *et al.*, 2006).

D.3.2. **Sobel *et al.* (1996b) and Davanipour *et al.* (2007) Studies – <u>Positive Studies</u>**

These two studies utilized the databases of the nine (9) State of California funded Alzheimer's Disease Diagnosis and Treatment Centers (ADDTC). Sobel *et al.* (1996b), the second published study of occupational ELF MF and AD, used the Rancho Los Amigos (RLA) ADDTC database. There were 316 cases and 135 controls. Twelve percent (12%) of the cases and 5.3% of the controls had had a medium or high "primary" exposed (ELF MF) occupation. The Davanipour *et al.*, 2007) study used the databases of the other 8 ADDTCs. Seven and one-half percent (7.5%) of the cases and 3.8% of the controls had had a medium or high ELF MF "primary" occupation. Among the women in the RLA ADDTC study, 26 (8.4%) had M/H exposure, of whom 17 (65.4%) were seamstresses. In the Davanipour *et al.* study, among women, 50 (3.8%) had M/H ELF MF exposure, of whom 34 (68%) were seamstresses. This difference is statistically significant (p < 0.001). Among the men in the RLA ADDTC study, 14.8% had a medium or high ELF MF exposed occupation, while in the Davanipour *et al.* ADDTC study, 13.5% had a medium or high ELF MF exposed occupation. This difference is not significant. It thus appears that the women in the combined populations from which the ADDTCs in the Davanipour *et al.* study have drawn their patients have a lower rate of ELF MF exposed occupations than the population from

which the RLA ADDTC draws its patients. This is not too surprising because Los Angeles has a large apparel manufacturing industry.

The OR (adjusted for age-at-onset, gender, and education) for medium or high ELF MF exposure in the RLA ADDTC study was 3.9 (95% CI = [1.5 – 10.6], p = 0.006). The ORs for medium or high ELF MF exposure in the Davanipour *et al.* ADDTC study were lower: 2.2 (p < 0.02; 95% CI = [1.2 – 3.9]) and 1.9 (p < 0.04; 95% CI = [1.04 – 3.6]), using age-at-exam and age-at-onset, respectively, plus gender and history of stroke in the model. These ORs are all statistically significant. In the two studies, the 95% CIs greatly overlap and, under the assumption of normality of the natural logarithms of the odds ratios estimators and a straightforward hypothesis test that the means of two independent normally distributed variables are equal, the null hypothesis that the corresponding ORs are equal cannot be rejected at the 0.05 level.

D.3.3.   **Other AD/Dementia and Occupational ELF MF Exposure Studies**

Studies with (at least some) Positive Results

Qiu et al. (2004) *Study* Qiu *et al.* (2004) studied a Swedish cohort of 931 subjects, aged 75+ at baseline, followed for up to 7 years. Job history was usually obtained from the next-of-kin, but only after 4 years of follow-up. ELF MF exposure assessment was estimated using previous occupational exposure studies, specific measurements (e.g., seamstresses and tailors), and expert opinion. During the follow-up period, 265 subjects developed dementia, with 202 receiving an AD diagnosis. Numerical exposure estimates were obtained using both the longest held occupation, last occupation, and any occupation. The estimated average daily ELF MF exposure was used to classify individual exposure.

Exposure for a sample of seamstresses and tailors was measured at the head. They were classified as having low exposure. Exposures of seamstresses who used industrial sewing machines and workers who used home sewing machines likely were under estimated by Qiu *et al.* (2004): 5.5 mG for "industrial seamstresses" and 1.9 for tailors. Qui *et al.* only considered home sewing machines, which at the head had a mean exposure of 10 mG. For "industrial seamstresses, they assumed that 50% of the workday was at a 10 mG exposure and 50% was at background, 1 mG. This gives an average exposure of 5.5 mG. For tailors, they assumed that only 10% of the workday was spent sewing, so the mean exposure was 1.9 mG. There are several problems with this determination of exposure for seamstresses and tailors:

1. exposures to the head are among the lowest body exposures and are not necessarily the sole important exposure;
2. even in Sweden, it is unlikely that home sewing machines were exclusively used. It is more likely that most of the machines were industrial machines, which produce much higher fields constantly, even when sewing is not occurring;
3. seamstresses have exposure most of the workday;
4. ambient exposure levels in industrial settings have been measured at up to 6 mG (Sobel and Davanipour, unpublished Finnish data);
5. tailors would not make a living sewing only 0.8 hours per day.

Hansen *et al.* (2000) found that, at the side of the waist, mean full-shift exposure for industrial machines was approximately 30 mG, while Qiu used a figure of 10 mG. Based on unpublished measurements on AC home sewing machines, Sobel and Davanipour (1996c) found that exposures

to the head were usually the lowest measurements, while the chest, pelvic area, thigh, knee, right arm and hand had much higher exposures (Table 8). In addition, foot pedals can produce high magnetic fields (Table 8). Also, AC/DC converters in the handles (right side) of computerized home sewing machines constantly produce high magnetic fields – about 75 mG at 2 inches away from the handle. The right hand, lower right arm, and knee regularly receive high exposures (Table 8). Thus, the 10% sewing time assumed by Qiu *et al.* (2004) does not mean that significant exposure is not over a longer time period. The biological plausibility of hypotheses discussed above provides an argument that exposure to other body parts may also be deleterious. The numbers or percentages of industrial seamstresses and/or home sewing machine workers were not provided by Qui *et al.* Note: seamstress' exposure assessment is discussed further in Section V.B.

Nevertheless, for the principal occupation, but not for the last occupation or cumulative lifetime exposure, Qiu *et al.* (2004) found statistically significant ORs: OR=2.3 (95% CI = [1.0 – 5.1]) for AD and OR=2.0 (95% CI = [1.1 – 3.7]) for any dementia for men with average exposures greater than 2 mG. For women, no increase in risk was found for the principal occupation, last occupation, and all occupations combined. The average lengths of time in the last and principal occupations were not provided. Thus, comparison with the Feychting *et al.* study (1998a) could not be made.

The proportions of subjects with at least 2 mG exposure were 28.2% for AD cases and 28.8% for controls for the principal occupation (Table 6). For all occupations combined, the proportions were higher. For men, with cases and controls combined, the proportions were 43.1% and 33.0%, respectively, for principal occupation and all occupations combined. For women, the proportions were 24.3% and 32.1%. In the Sobel *et al.* (1995, 1996b) and Davanipour *et al.* (2007) studies, the proportion of female cases and controls with medium or high exposure (considered above 2 mG) was only 5.5%, 80% of whom were seamstresses or had allied professions with significant ELF MF exposure, e.g., cutter. Thus, in these three publications, the exposure category for women contained a higher percentage of subjects with very high exposure. This may explain the lack of findings among women. The occupations which were in the exposure categories 'at least 2 mG' (dichotomized exposure) or 'at least 1.8 mG' (trichotomized) were not provided by Qiu *et al.*(2004).

Harmanci et al. (2003) *Study* Harmanci *et al.* (2003) conducted a cross-sectional, population-based study of Alzheimer's disease by selecting a random sample of 1067 subjects at least age 70, among whom 1019 (96%) agreed to participate in the study. AD was determined in a two-step process: a screening exam using the Turkish version of the Mini-Mental State Exam MMSE, followed by an expert clinical exam among those whose MMSE scored indicated cognitive impairment. Two hundred twenty three (223) were asked to have a clinical exam, and 155 (69.5%) agreed. Among the subjects with a "normal" score on the MMSE, 126 were randomly selected for a clinical examination. Among these 281 subjects, 57 were clinically diagnosed as having possible AD, and 127 were determined to be cognitively normal. These subjects were included in the case-control study. M/H ELF MF exposed occupations were stenographers and typists, carpenters and joiners, metal molders and core makers, tailors, dressmakers, and hatters. Except for stenographers, these occupations were considered to result in medium or high ELF MF exposure in the Sobel *et al.* (1995, 1996b) and current study. A stepwise backwards logistic regression analysis was used. Medium/high ELF MF exposure occupations had an adjusted OR of 4.0, with a 95% CI of [1.02 – 15.78]. It is interesting to note that use of electrical residential heating was also a risk factor (OR = 2.8, 95% CI = [1.1 – 6.9]).

Feychting et al. (1998a) *Study* In the case-control study by Feychting *et al.* (1998a), ELF MF exposure during the last occupation, but not during the longest held occupation, was a risk factor

for dementia not caused by a single stroke. The last occupation was held an average of 24.8 years among cases and 25.9 and 25.1 years among subjects within the two control groups. Consequently exposure during the last occupation was over a significant period of time. Using the two control groups, the ORs for dementia were 3.3 and 3.8 with 95% CIs of [1.3 – 8.6] and [1.4 – 10.2] for occupations with geometric mean ELF MF exposures estimated to be at least 2 mG. Housewives were excluded from the analyses. The ORs for Alzheimer's disease were somewhat lower (2.4 and 2.7). When the analysis was restricted to subjects aged 75 and below at onset or examination, the ORs (5.0 and 4.8) for AD were statistically significant. Also, for subjects of all ages with occupations likely to have resulted in an average ELF MF exposure above 5 mG, the ORs for AD were both high, but significant for one referent group (OR = 8.3), and not for the other (OR = 4.1). The Feychting *et al.* study was small: 44 dementia cases had occupational data, 29 of whom were diagnosed with AD. 43% of the cases were in the ELF MF exposed group, while 23% and 19% of the controls were in this exposure group. Given these high percentages, it is clear that some lower ELF MF exposed occupations were classified in the exposed category than were classified in this study and the earlier Sobel *et al.* studies (1995, 1996b).

Chang et al. (2004) Study Chang et al. (2004) studied exposure to ELF MFs and other possible risk factors for AD among 62 AD patients and 124 controls, all of whom were elderly ex-military personnel, aged 66 to 102. (The published paper is in Chinese and we only have the PubMed English translation of the article's abstract.) Cases and controls were matched for age. Univariate and multivariate logistic regression models were analyzed. "Early" exposure to ELF MFs had an odds ratio of 2.49, with a 95% CI of (0.96-6.45).

Röösli et al. 2007 Study (Röösli et al. 2007) used records from the Swiss Federal Railway on employees who were employed or retired between January 1, 1972 and December 31, 2002. Employees in the following categories were used in analyses: train drivers, shunting yard engineers, train attendants, and station masters. "Average" ELF MF exposure for each year was assessed, based on measurements and "modeling". Five (5) ELF MF exposure indices were used: train drivers vs the other 4 occupations; cumulative work-time exposure (microtesla [μT] years); cumulative time above 10 μT; cumulative exposure up to 10 years prior to death or study closure; exposure within 20 years before death or study closure. Death certificates were used to determine disease status: AD (not coded in ICD-8 and only for subjects whose death was from 1995-2002); senile dementia (including AD); Parkinson's disease (PD); amyotrophic lateral sclerosis (ALS); cardiovascular disease (CVD); and respiratory tumor (RT). The total sample size for analysis was 20,141. Cox proportional hazards models were used to estimate the hazard ratio (HR) with station masters as the referent group. Station masters had, by far, the lowest ELF MF exposure.

Generally, train drivers experienced a very much higher ELF MF exposure than shunting yard engineers, train attendants, or station masters. ELF MF exposure was not associated with death due to (or with) CVD, PD, ALS, or RT. For senile dementia, which included AD, the HR for train drivers was 1.96, with a 95% CI of (0.98-3.92). For AD only, the HR was 3.15 with a 95% CI of (0.90-11.04). It should be noted that the number of deaths due to or with senile dementia or AD were small among the train drivers, shunting yard engineers, train attendants, and station masters, respectively: 30, 3, 17, 11 for senile dementia; 14, 2, 6, 3 for AD. This leads to wide confidence intervals.

Risks associated with increasing cumulative ELF MF exposure were assessed by determining hazard ratios related to exposure tertiles, with the lowest tertile as the referent group. There was an apparent possible increase in risk for subjects in the highest tertile, although the 95% CIs

included 1.0.

Risks were also assessed by determining the HR for the number of years of exposure at or above 10 μT. In this analysis, risk increased by 5.7% for senile dementia and 9.4% for AD. Both figures are statistically significant at the 0.05 level: 95% CIs were above 1.0.

Studies with Only or Mostly Negative Results

Graves et al. (1999) Study Graves *et al.* (1999) studied 89 matched case-control pairs. Complete occupational histories were obtained. ELF MF exposure in a given occupation was defined as having at least "probable intermittent exposures (a few minutes)" above 3 mG. A high exposure category was defined as exposure of "1 to several hours" above 3 mG. Two industrial hygienists rated the occupations. Thus, many exposed subjects likely had a low average exposure. 19.1% and 21.4% of the cases were considered to have been 'ever' exposed, while 21.4% and 22.5% of the controls were considered 'ever' exposed. An unknown number of subjects, classified as having experienced ELF MF exposure, would not have been so classified in most or all of the other studies of neurodegenerative diseases or cancer. The estimated adjusted ORs for 'ever' having been exposed were 0.74 and 0.95, depending upon which industrial hygienist's classification was used (Graves *et al.*, 1999).

As noted above, the Feychting *et al.* (1998a) study found elevated odds ratios associated with the last occupation, and in the Sobel *et al.* studies (1995, 1996b) and the Davanipour *et al.* (2007) study, occupational information most likely related to the last occupation. Also, Feychting *et al.* (1998a) did not find an increased risk associated with measures which included earlier occupations, e.g., highest exposed occupation and longest held occupation. Qui *et al.* (2004) found elevated risk associated with the principal occupation for males. Consequently, 'ever' vs 'never' exposed, as used by Graves *et al.* (1999), may not be an appropriate comparison.

Graves *et al.* (1999) also used a cumulative exposure index, the weighted sum of the numbers of years in each occupation with the weights being 0, 1 and 2 for no exposure, only "intermittent exposures" above 3 mG, and exposure for "1 to several hours" above 3 mG, respectively. Using the non-zero cumulative index values, exposure was dichotomized at the median as 'low' or 'high'. Adjusted ORs for 'low' or 'high' cumulative exposure versus no exposure were also close to 1.0. The last or the primary occupation was not separately analyzed.

In summary, the non-significance of the ORs in the Graves *et al.* (1999) study may be due to three reasons: (1) less restrictive definitions of magnetic field exposure resulting in minimally exposed subjects being classified as having been 'ever exposed' or even highly exposed; (2) equal weight given to exposure during any age period, e.g., age 25-45 and age 45-65; (3) a cumulative exposure metric which equates what can be negligible exposure with significant exposure, e.g., negligible exposure for 20 years equals significant exposure for 10 years. In addition, there were no seamstresses among their subjects, who were from an HMO established primarily for union families. Seamstresses are seldom in a union.

Seidler et al. (2007) Seidler et al. (2007) conducted a case-control study by recruiting dementia-diagnosed cases, all 65 or older, from 23 general practices located in Frankfurt-on Main and neighboring cities. Recruitment was primarily based on the Mini-Mental State Examination. The Hachinski Ischemic Score was used in an attempt to differentiate between AD and vascular dementia (VaD). 195 cases (45 men and 150 women) were obtained: 108 were thought to have

"possible" AD, 59 "possible" VaD, 25 had "secondary" dementia, and 3 an "unclassified" dementia.   Imaging studies were also used for differential diagnostic purposes, if available. Population controls were randomly selected among those 65+ years of age who scored at least 27 on the MMSE. A second control group was selected from the general practices which contributed dementia cases. These controls needed to be ambulatory and also were required to have a MMSE of 27 or above. The authors state, but do not provide any other information, that "preliminary" analyses using the control groups separately produced "comparable results" with one exception: the ORs for blue collar work were "markedly" higher (p<0.1) for ambulatory controls than for population controls. Based on these unpublished analyses, the control groups were combined for "final" analyses. There were 229 controls in these latter analyses: 75 men and 154 women.

Analyses are conducted for dementia, possible AD, and possible VaD cases. However, the diagnostic methods used were really quite insufficient. For example, subjects with depression often have a low MMSE score.

Occupational histories were obtained by interview. Informational items obtained were job phase, job title, industry, and specific job tasks for every job that lasted at least one year. Next-of-kin were used for the dementia subjects, unless there was no next-of-kin and the subject was in the "first signs of dementia". These cases were not excluded in the published results because the results were not "fundamentally" different without them. Only jobs prior to the date of symptom onset or more than 4 years prior to dementia diagnosis if symptom onset timing was unknown were considered. Again, exclusion of these cases did not "substantially" alter the study results. The median time interval between the end of the last job and dementia diagnosis was 17 years for men and 24 years for women, while the for the controls the medians were 10 and 21 years, respectively.

Job titles were coded by experienced members the Frankfurt Institute for Occupational Medicine according to the Classification of the Federal Statistical Office in Germany and the Occupational Classification of the Finnish Censuses. Two-digit occupational codes were used. ELF MF exposure levels for each job were estimated by an "expert" co-author from the German Federal Institute for Occupational Safety and Health, blinded to case-control status. Exposure categories were specified as follows: < 1 mG; 1-2 mG; 2-10 mG, 10-100 mG,; 100-1000 mG, and > 1000 mG. (It is not clear in which category the lower and upper limits of each of the middle 4 categories belong.)

Analyses were based on cumulative exposure and maximum exposure to ELF MF, as determined by the expert co-author. ORs were determined for the 15 primary occupational two-digit categories (ever vs never worked in the category and per 10 years work) and for estimated cumulative exposure and maximum exposure. ORs were adjusted for age, region, gender, dementia in parents, and pack-years of smoking. The referent group consisted of subjects who never worked in the given category and who held white-collar jobs as their main occupation

Statistically significant findings among the ever vs never analyses were as follows:

    Dementia Cases

- food & beverage processors; tobacco product makers - OR=4.1, 95% CI = (1.4 , 11.8);

- laborers (unskilled workers) – OR=7.6; 95% CI = (1.7 , 34.2);
- blue-collar work as the main occupation – OR=1.6; 95% CI = (1.0 , 2.5)

AD Cases

- blue-collar work as the main occupation – OR=1.7; 95% CI = (1.0 , 3.1)

VaD Cases

- food & beverage processors; tobacco product makers - OR=7.3, 95% CI = (2.0, 27.3);
- laborers (unskilled workers) – OR=6.3; 95% CI = (1.0 , 39.2).

Analyses based on "per 10 years" of work which were statistically significant or nearly so for possible AD were as follows:

- metal workers (machinery fitters, machine assemblers, mechanics, manufacturers of precision instruments, plumbers, welders, sheet metal and structural metal preparers and erectors – OR=2.2; 95% CI = (1.0 , 5.1),
- electrical and electronics workers – OR=2.7; 95% CI = (0.9 , 8.1),
- spinners, weavers, knitters, dyers, tailors, dressmakers – OR=1.4; 95% CI = (0.9 , 2.2),
- construction workers, including structural engineers, civil engineers) – OR=12.9; 95% CI = (0.9 , 186).

The "ever" versus "never" analyses are really quite inappropriate because the duration of time in the specific and general occupational categories can be quite low. The "per 10 years" analyses are thus more appropriate, but the sample sizes within job categories are quite small, except for "spinners, weavers, knitters, dyers, tailors, and dressmakers". However, it is not clear what the actual ELF MF exposures for spinners, weavers, knitters, and dyers might be.

The categories of (1) metal workers, (2) electrical and electronics workers, (3) spinners, weavers, knitters, dyers, tailors, and dressmakers; and (4), construction workers contain many of the occupations classified as medium or high ELF MF exposed occupations in the Sobel, Davanipour et al. papers and the papers by those who have essentially used the same classification methodology. One of the problems in the Seidel el al. (2007) paper is that the higher classification categories contain may occupations with low exposure.

The authors have available to them the actual specific occupations of each subject. They could therefore classify subject ELF MF exposure using the Sobel-Davanipour et al. methodology to reanalyze their data and determine if their findings for presumptive dementia (cognitive dysfunction) or AD patients replicate (or not) the Sobel, Davanipour et al. findings.

Andel et al. (2010) Study This study uses subjects from the Swedish Twin Registry. All subjects were 65 years or older in 1998. In all, 9,508 subjects had both a dementia/AD diagnostic workup and ELF MF occupational exposure estimates. 27.9% of the subjects were classified as having high exposure – above 2 mG. Among the subjects diagnosed as having dementia, 33.8% were classified as having had high exposure. The figure for subjects diagnosed with dementia was 34.0%. Among

the controls, the corresponding figure was 27.8%. Dementia and AD were diagnosed in a structured, presumably appropriate manner : 216 (2.27%) with dementia; 141 (1.49%) with AD. Age at dementia onset (≤ 75 vs > 75) was determined by informants, presumably family members. Analyses were adjusted for covariates: gender, education, coronary disease, and stroke. Subjects were classified into three (3) exposure groups: < 1.2 mG, 1.2 to < 2.0 mG, and ≥ 2.0 mG. The referent group consisted of subjects with estimated exposure below 1.2 mG. Note that in the manuscript microTesla (μT) units were used: 1 mG = 0.1 μT. For all subjects, the dementia adjusted odds ratios (AORs) were 1.41 (p=0.079) for exposure between 1.2 and <2.0 mG and 1.38 (p=0.108) for exposure ≥ 2.0 mG. The AD AORs were 1.35 (p=0.211) and 1.38 (p=1.53). For age of onset ≤ 75, the AORs were 1.94 (p=0.03) and 2.01 (p=0.022) for all types of dementia and 1.69 (p=0.215) and 1.94 (p=0.090) for AD. For age of onset greater than 75, the AORs were much closer to 1.0 and clearly not significant. Analyses were conducted also for manual and non-manual workers separately. AORs for non-manual workers were clearly non-significant. For manual workers, the AORs for dementia and AD had p-values below 0.05, except for exposure ≥ 2.0 mG for AD when the p-value was 0.056.

It is our opinion that the ELF MF exposure assessment is not accurate in this study and other studies (e.g., breast cancer) which use the same exposure assessment methods and data. Specific occupational information was obtained by interview and then sent to "Statistics Sweden for coding according to categories from the 1980 Swedish Population and Housing Census". For men, occupational exposure assessment was based on measurements of a sample of 1098 Swedish men (Floderus et al., 1996). For women, the results of a study of 49 occupations by Forssén et al. (2004) have been used. This latter paper is also discussed below in our discussion of breast cancer, primarily in Section IV.E. We have two major concerns with the occupational classifications with respect to ELF MF exposure:

1. Generally, government classifications of occupation are wider than occupational determination based on individual subject information. Individual ELF MF exposure classification based on government classifications is therefore not likely to be particularly accurate. This will result in many individuals being misclassified as having exposures above 2 mG. The exposure classification methodology used by Davanipour, Sobel et al. and others has, we believe, much lower misclassification rates for 2.0 mG and above. For example in Davanipour et al. (2007) the rates of classification were 7.5% and 3.8% for AD cases and controls, respectively. As stated above, the comparable classification rate in the Andel et al. (2010) study was 27.9%.

2. The Forssén et al. (2004) measurements for women classified seamstresses as having low ELF MF exposure. This is very much out of line with our experience in Finland and in California and with the experiences of other researchers. Davanipour & Sobel measured ELF MF exposures in two clothing manufacturing companies in Finland. The ambient exposure, except during lunch time, among seamstresses and associated workers (e.g., cutters) in the same areas was over 6 mG. Exposures of individual seamstresses, all of whom used AC current industrial sewing machines, were much higher at every body location. We personally measured scores of seamstresses. The lowest exposure to any body part was 20 mG (e.g., Hansen et al., 2000). The usual work pattern was as follows: (1) the seamstress sits at a U-shaped table; (2) clothes to be sewed are folded on the right hand side; (3) the seamstress selects an article, sews it as specified; and (4) refolds the article, placing it on the left hand side of the desk.

All this time, the sewing machine is producing ELF MFs. This is because the motor is always on and a clutch needs to be engaged in order to move the needle. The seamstresses are doing this work for 6-8 hours per day. Seamstresses who work in drycleaners stores certainly do not sew all day long, so their exposure would be lower.

E.   **RF Exposure and Alzheimer's Disease**

We found no human studies of AD and RF to discuss. The single published epidemiologic study of RF and melatonin is discussed in Section II (Burch *et al.*, 2002).

E.1. Transthyretin Studies

There have, however, been studies related to the effect RF exposure on transthyretin (TTR), also referred to as prealbumin. TTR is found in the brain, cerebrospinal fluid (CSF), and blood. Based on earlier research related to Aβ deposition (discussed below), Söderqvist et al. (2009a,b) investigated the effect(s) of RF on TTR in two studies. Söderqvist et al. (2010) discusses these same studies. In these studies, serum TTR levels are used as indicators for CSF and (presumably) brain TTR levels. However, there is apparently no study demonstrating that this assumption is valid.

1.   In the 2009a study, 500 females and 500 males, aged 18-65, were randomly recruited from the municipality of Örebro, Sweden. Consenting subjects initially completed a questionnaire which included employment history, use of specific types of wireless telephones, X-ray, chemical, and radiation exposures (e.g., in medical therapy), and health and lifestyle questions, including physical exercise and disease history. An initial blood sample was collected from each subject as close to the end of a work week as possible. TTR concentrations (g/L) were determined using "standard immunoephelometric techniques". 133 (26.6%) of the male and 184 (36.8%) of the female subjects who were "recruited" fully participated. TTR assay results were log-transformed in all statistical analyses. Short-term wireless telephone use was determined by cumulative use (minutes) on the day the blood sample was delivered. Long-term use had two categories: "cumulative use" in total hours; and years since initial use. These short- and long-term figures were presumably guestimates by the study subjects. High TTR was chosen as the highest quartile (> 0.31 g/L. Low TTR was ≤ 0.31 g/L.

There was no indication that wireless telephone use for at least 5 years or at least 10 years affected TTR levels as dichotomized. However, using the TTR levels themselves, for cumulative use, among men, there was an indication of increased risk with increasing use of mobile telephones (both analogue and digital). That is, the p-values were between 0.05 and 1.0. For years since first use, among men, the results were stronger. The p-values were below 0.05 for mobile telephones (all phones and analogue only). However, among men, for Universal Mobile Telecommunications System *(*UMTS*)* telephones there was declining risk with higher use (p=0.02).

For short-term use, there were no findings of significance or, evidently, marginal significance, except in one instance. Among women, the shorter the time between last use of a mobile telephone and blood samples, the lower the TTR value (p-0.03).

There is no indication that the statistically significant or marginally significant finding have any biological importance.

2. Based on these short-term use finding, Söderqvist et al. conducted a "provocation" study, exposing volunteers to an 890 MHz mobile "phone-like" signal. Forty-four volunteers, aged 18-30 were recruited. Exposures occurred during the working day: 8 am – 5 pm. Exposures were over a 2 hour period, with blood samples collected prior to exposure, after a 30 minutes "rest" period, immediately following the provocation, and 60 minutes after the provocation. The provocation exposure had an average $kSAR_{1G}$ of 1.0 watts/kg. Seemingly the study design did not work out very well. The biggest mean change was a decrease between sample 1 and sample 2, when presumably nothing much was happening, except that the subjects were told to rest. The mean changes were very minimal between sample 2 and post-exposure samples 3 and 4, especially compared to the between subject values. There was also a control group who did not have any exposure. Their TTR measurements were not much different from the experimental groups measurements. However, no statistical comparison was presented.

In short, this study seems to have provided <u>no</u> useful information.

The questions of importance here are (i) whether TTR concentrations in serum are indicative of concentrations in the CSF and brain and (ii) whether TTR inhibits or increases the aggregation and neurotoxicity of Aβ.

i.   As mentioned above, we could find no studies of the relationship(s) between serum and CSF or brain levels of TTR.

ii. In *in vitro* studies, Schwarzman et al. (1994, 1996) found that CSF TTR binds to Aβ, possibly preventing or limiting amyloid formation within the brain. Their conclusion was that perhaps TTR helps prevent or delay AD onset. Serot et al. (1997) studied elderly AD patients and controls with ages between 2 and 90. TTR concentrations in CSF increased with age among the controls. TTR concentrations among the AD cases were similar to those controls in middle age and lower than the elderly controls (20.02 mg/l (sd=2.45) vs 17.49 mg/l (sd=2.02), p<0.001). The authors suggest that AD development may result in a lowering of TTR secretion. Lovell et al. (2008) studied the "aberrant" protein complex prostaglandin-d-synthase (PSD) and TTR in the CSF of autopsy verified late-onset AD patients, patients with mild cognitive impairment (MCI), and controls. They found that complexed PDS/TTR was significantly increased in the ventricular CSF of the AD and MCI patients compared to normal controls. This possibly explains the results of Serot et al. (1997). Animal and cell studies have found that TTR infusion leads to a reduction in Aβ deposits (Link, 1995), lack of neurodegeneration in the transgenic mouse AD model Tg2576 (Stein and Johnson, 2002), inhibition of Aβ aggregation, toxicity, and induced apoptotic changes in cultured cells (Giunta et al., 2005).

Wati et al. (2009) then studied TTR and vascular Aβ deposition in two (2) transgenic mouse models of AD: Tg2576/TTR^{-/-} which lacks endogenous TTR, but produces human variant amyloid precursor protein (APP), and Tg2576/TTR^{+/-}, which does not lack endogenous TTR. The Tg2576/TTR^{-/-} mice had a significantly reduced Aβ burden compared to the Tg2576/TTR^{+/-} mice, contrary to the researchers expectations. Their result indicates that, in their animal model, TTR appears to be associated with increased

risk of amyloid burden.

On the other hand, using a different mouse model ceAPPswe/PSIΔE9/TTR$^{+/-}$ versus ceAPPswe/PSIΔE9/TTR$^{+/+}$, Choi et al. (2007) found that amyloid deposition in the hippocampus and cortex was elevated in the brains and "accelerated" in the hippocampus and cortex of the ceAPPswe/PSIΔE9/TTR$^{+/-}$ mice compared to the ceAPPswe/PSIΔE9/TTR$^{+/+}$.

Thus, results may be dependent upon differences between experimental species or sub-species. This suggests that (1) replication is warranted and (2) concentration on studies involving humans is appropriate if animal model replications continue to demonstrate differing results.

E.2. RF and Mitochondrial DNA (mtDNA) Oxidative Damage

Coskun et al. (2010) have demonstrated that mutations in the control region of mtDNA accumulate in the brain with age, with AD patients having a significant elevation of these mutations. These mutations in AD patients are associated with a reduced mtDNA copy number. They found that these mutations generally increase with age, both within the brain and in peripheral blood DNA and lymphoblastoid cell DNA. They argue that the mtDNA mutation level is inversely correlated with mtDNA copy number and positively correlated with beta-secretase activity, an indicator of increasing amyloid beta. Consequently, mtDNA damage may be associated with increased risk of AD.

Xu et al. (2010) studied oxidative damage to mitochondrial DNA related to 1800 MHz RF exposure in primary cultured cortical neurons. The neurons were exposed to 1800 MHz modulated by 217 Hz, using an average special absorption rate of 2 watts/kg for 24 hours. Examination of the neurons demonstrated a significant increase in 8-hydrodeoxyguanosine (8-oxodG), an indication of increased DNA damage. In addition, there was a clear reduction in the copy number of mtDNA and in the level of mtRNA after RF exposure. Xu et al. (2010) also conducted replicate assays, but with the addition of melatonin. The effects of RF exposure were reversed, but not completely.

IV. BREAST CANCER

Figure 2 provides a schematic outline of the areas of study providing evidence that ELF MF exposure can lead to breast cancer through an effect on melatonin production levels, and, of course, possible but unknown other pathways. Section references are provided in Figure 2.

There is now accumulating evidence that low melatonin production may increase the risk of breast cancer (BC). This evidence comes from *in vitro*, animal, and two longitudinal human studies. The *in vitro* and animal study literature is quite extensive, so only a highlight review is provided. There are numerous published case-control studies of residential and occupational ELF MF exposure as a risk factor for breast cancer. No epidemiologic studies of radiofrequency MF exposures and breast cancer have been published, which do not include ELF MF exposure, and which have reasonable data on RF exposure.

For a review of melatonin from basic research to cancer treatment, see Vjayalaxmi *et al.*, 2002.

- *Conclusion: There is sufficient evidence from in vitro and animal studies, from human biomarker studies, and from occupational and light at night studies to conclude that high ELF MF exposure may certainly be a risk factor for breast cancer. Most of the residential ELF MF exposure studies have been negative. This may be because "high" residential exposures are actually not very high. Individual exposures may be of importance, e.g., home sewing machines, hair dryers, AC/DC converters near the head of the bed, water pipes causing intermittent high exposures near living room or TV room sofas and easy chairs.*

As with Alzheimer's disease, we provide the results of a meta-analysis for breast cancer (Chen et al., 2010) despite our antipathy for such analyses, due primarily to varying study design components, exposure assessments, and subject differences. Chen et al. (2010) chose 15 studies published between 2000 and 2009. They found no associations between ELF MF exposure and (female) BC, including subgroup analyses based on exposure modes, menopausal status, and estrogen receptor status. These results are said to be in agreement with results by Erren (2001). Chen et al. (2010) found no statistically significant association between ELF MF exposure (residential, electric blanket, or occupational) and BC in general or BC based on menopausal status or ER status. There was substantial heterogeneity between studies. On the other hand, Erren (2001) found, using earlier studies not included in Chen et al. (2010), a slightly increased risk (referred to as RR) of BC in general: 1.12, 95% CI = (1.09 , 1.15). This is clearly statistically significant due to the very large sample size. Erren (2001) remarks that the results are quite variable between studies and "in part contradictory". He found that the primary methodologic problems were "probable misclassification of exposure" and "possible misclassification of the disease itself". Thus Chen et al.'s (2010) claims that (1) their results suggest no association between ELF MF exposure and BC and (2) are "in accordance" with Erren's results (2001) should be taken with a grain of salt.

A.   ***In Vitro* and Animal Studies Relating to Melatonin as a Protective Factor against Breast Cancer**

A.1.   ***In Vitro* Studies Related to Prevention of Oxidative Damage; Comparative *in vivo* Studies with Vitamin C and Vitamin E**

Melatonin has been found to neutralize hydroxyl radicals and to reduce oxidative damage in over 800 publications (Reiter et al., 1995; Tan *et al.*, 2002). Melatonin has also been shown to act synergistically with vitamin C, vitamin E and glutathione (Tan *et al.*, 2000) and stimulates the antioxidant enzymes superoxide dismutase, glutathione peroxidase and glutathione reductase (Reiter *et al.*, 2002).

- Using a cell-free system, Tan et al. and others have demonstrated that melatonin neutralizes hydroxyl radicals more efficiently than does reduced glutathione Tan *et al.*, 1993a; Bromme *et al.*, 2000).
- Melatonin reduces oxidative damage to macromolecules in the presence of free radicals (Reiter *et al.*, 1997, 2001a). One mode of action is as a free radical scavenger (Reiter *et al.*, 2001b).
- Melatonin increases the effectiveness of other antioxidants, e.g., superoxide dismutase, glutathione peroxidase, and catalase (Antolin *et al.*, 1996; Kotler *et al.*, 1998; Pablos *et al.*,

1995; Barlow-Walden *et al.*, 1995; Montilla *et al.*, 1997).

- Melatonin has protective effects against ultraviolet and ionizing radiation (e.g., Vijayalaxmi *et al.*, 1995). Vijayalaxmi et al. studied the effects of melatonin on radiation induced chromosomal damage in human peripheral blood lymphocytes (Vijayalaxmi *et al.*, 1996). Blood from human volunteers was collected before and after administration of a single 300 mg oral dose of melatonin. The post-administration samples of both serum and leukocytes had increased concentration of melatonin compared to the samples prior to melatonin administration. After gamma radiation and mitogen exposure, a sample of cells was cultured for 48-72 hours. Lymphocytes from the sample after melatonin was administered had significantly fewer chromosomal aberrations and micronuclei. Primary DNA damage was reduced. Vijayalaxmi et al. hypothesized that melatonin, in addition to its hydroxyl radical scavenging, may also stimulate or activate DNA repair processes (Vijayalaxmi *et al.*, 1998).

Melatonin has been found to be a more potent protector from oxidative injury than vitamin C or vitamin E (micromoles/kg) in several *in vivo* studies (for a review, see: Tan *et al.*, 2002). Melatonin was also found *in vitro* to scavenge peroxyl radicals more effectively than vitamin E, vitamin C or reduced glutathione (Pieri *et al.*, 1994; Reiter *et al.* 1995), although melatonin is not a very strong scavenger of peroxyl radicals (Reiter *et al.*, 2001b).

A.2. **Animal Studies of Mammary Tumor Prevention with Melatonin**

Several studies have found that melatonin inhibits the incidence of mammary tumors in laboratory animals either prone to such tumors or exposed to a carcinogen (e.g., Tamarkin *et al.*, 1981; Shah *et al.*, 1984; Kothari *et al.*, 1984; Subramanian and Kothari, 1991a,b; Blask *et al.*, 1991). In 1981, Tamarkin et al. found that supplemental melatonin, given on the same day as 7,12-dimethylbenz(alpha)-anthracene (DMBA) and continued for 90 days, lowered the incidence of mammary tumors from 79% in controls to 20% (p<0.002) in the melatonin treated Sprague-Dawley rats (Tamarkin *et al.*, 1981). When they treated pinealectomized rats with DMBA, the incidence of mammary tumors increased to 88%, indicating a possible effect on endogenous melatonin on tumor incidence. Similar results, but with somewhat different study designs, using female Holtzman rats given the carcinogen 9,10-dimethylbenzanthracene have been found (Shah *et al.*, 1984; Kothari *et al.*, 1984). Subramanian and Kothari studied the suppressive effect by melatonin in rats treated similarly with DMBA under varying light:dark schedules and time of melatonin administration in both intact and pinealectomized female Holtzman rats (Subramanian and Kothari, 1991a). They found that when administered during the initiation phase, melatonin only suppressed tumor development in intact animals. However, when administered during the promotion phase, melatonin had suppressive effects regardless of the presence or absence of the pineal gland. Subramanian and Kothari (1991b) also studied C3H/Jax mice and spontaneous mammary tumor development. Mammary tumors developed in 23.1% of mice provided with melatonin from 21 to 44 days of age, but in 62.5% of control mice (p<0.02). Furthermore, there was a decrease in serum 17-beta-estradiol levels in the melatonin treated mice (p<0.05). In a N-methyl-N-nitrosourea (NMU) model of hormone-responsive Sprague-Dawley rat mammary carcinogenesis, Blask *et al.* (1991) found that melatonin, given during the promotion phase, reduced the incidence of tumors and antagonized estradiol's stimulation of NMU-induced tumor incidence and growth. They, however, did not find a decrease in estradiol in the melatonin treated rats.

In two studies, Tan *et al.* (1993b, 1994) found that melatonin protected Sprague-Dawley rats from safrole induced liver DNA adduct formation. The protection was found at both physiological and pharmacological levels of supplementation. The level of protection was dose dependent. Intraperitoneal injection of paraquat causes lipid peroxidation, a decrease in total glutathione, and an increase in oxidized glutathione in Sprague-Dawley rats. Melchiorri et al. found that melatonin inhibits these effects (Melchiorri *et al.*, 1995). In addition, melatonin and retinoic acid appear to act synergistically in the chemoprevention of animal model tumors (Teplitzky *et al.*, 2001) and *in vitro* systems (e.g., Eck-Enriquez *et al.*, 2000).

A.3.   **Animal Studies Related to Prevention of Oxidative DNA Damage by Estradiol and Radiation**

Karbownik *et al.* (2001) found that melatonin protects against DNA damage in the liver and kidney of male hamsters caused by estradiol treatment. They also found that in the testes, estradiol did not increase DNA damage, but that melatonin was protective against the natural level of oxidative DNA damage, as indicated by 8-hydrodeoxyguanosine (8-oxodG) levels. Several studies have found that laboratory animals are protected by melatonin from lethal doses of ionizing radiation (e.g., Blickenstaff *et al.*, 1994; Vijayalaxmi *et al.*, 1999; Karbownik *et al.*, 2000). Vijayalaxmi *et al.* (1999) and Karbownik *et al.* (2000) investigated markers of oxidative DNA damage and found that significant decreases in these markers in the melatonin treated animals.

A.4.   **Melatonin: Scavenger of $^\bullet$OH and Other ROS**

Melatonin is a powerful, endogenously produced scavenger of reactive oxygen species (ROS), particularly the hydroxyl radical ($^\bullet$OH). Other ROS which melatonin scavenges include hydrogen peroxide (H_2O_2), nitric oxide ($NO^\bullet$), peroxynitrite anion ($ONOO^-$), hypochlorous acid (HOCl), and singlet oxygen ($^1O_2$) (Reiter, 1991; Tan *et al.*, 2000, Hardeland *et al.*, 1995; Antolin *et al.*, 1997; Stasica *et al.*, 1998). $^\bullet$OH is produced at high levels by natural aerobic activity. ROS are also produced by various biological activities or result from certain environmental and lifestyle (e.g., smoking) exposures.

Hydrogen peroxide does not appear to react directly with DNA (Halliwell, 1998), but does undergo chemical reactions within the cell nucleus which produce $^\bullet$OH, e.g., with Fe^{+2}. On the other hand, $^1O_2$ readily oxidizes the guanine base and causes HOCl, $ONOO^-$, and $NO^\bullet$ damage in various patterns (Halliwell, 1998).

However, $^\bullet$OH is the most reactive and cytotoxic of the ROS (Halliwell *et al.*, 1986). $^\bullet$OH appears not to be removed by antioxidative enzymes, but is only detoxified by certain direct radical scavengers (Tan *et al.*, 1999) such as melatonin.

Melatonin is found in every cell of the body and readily crosses the blood-brain barrier. It scavenges ROS at both physiologic and pharmacologic concentrations. In the literature, "physiologic" refers to blood level concentrations of melatonin, while "pharmacologic" indicates 2-3 orders of magnitude higher concentration. Recently, intracellular levels of melatonin, especially within the nucleus, have been shown to be naturally at "pharmacologic" levels for all cellular organelles studied to date (Maestroni, 1999; Reiter *et al.*, 2000).

Tan *et al.* (2002) review the underlying basis for melatonin's scavenging of ROS, which is briefly discussed here. From the known structure-activity relationships, the reactive center of the interaction between oxidants and the melatonin molecule is its indole moiety. This is due to its high resonance stability and quite low activation energy barrier towards free radical reactions. In addition, the methoxy and amide side chains contribute significantly to melatonin's antioxidant activity. The methoxy group in the C5 component of the molecule appears to prevent prooxidative activity. If this methoxy group is replaced by a hydroxyl group, under some *in vitro* conditions, melatonin may exhibit prooxidant capability. The mechanisms of melatonin's scavenging ROS appear to involve the donation of an electron to form a melatoninyl cation radical or a radical addition at site C3 of the melatonin molecule. (There are other possibilities also.) All known intermediates generated by the scavenging of a ROS by melatonin are also free radical scavengers. This is known (by some) as the 'free radical scavenging cascade reaction', which allows one melatonin molecule to scavenge 4 or more ROS. (See Tan *et al.*, 2007, for details).

A.5.   Melatonin and Oxidatively Damaged Guanine in DNA

Davanipour et al. (2009) published the results of a study relating overnight melatonin production (as measured by aMT6s/creatinine levels in complete overnight urine samples) to the levels of oxidatively damaged guanine in DNA (as measured by urinary guanine damage/repair guanine products 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydro-guanine (8-oxoGua). 8-oxodG is a product of the damage/repair of DNA guanine, while 8-oxoGua is a product of the damage/repair of either DNA or RNA guanine. Fifty-five (55) mother-father-oldest adult daughter families were recruited. All were healthy for their age. The age ranges were as follows: mothers – 43-80; fathers – 46-81; daughters – 18-51. The results were as follows:

- with or without adjustment for BMI or weight, among the mothers there was an inverse relationship between creatinine-adjusted aMT6s and 8-oxodG (p=0.02);
- among the mothers older than the oldest daughter (age 51.6) the significance level of the inverse relationship between creatinine-adjusted aMT6s and 8-oxodG fell to 0.009;
- among the fathers older than the oldest daughter, the inverse relationship between 8-oxoGua and creatinine-adjusted aMT6s was significant at the 0.03 level;
- among the oldest daughters, there was an increase in 8-oxoGua with increasing age.

This study appears to be the only research published to date on the relationship between melatonin production and DNA damage/repair in humans.

B.   Longitudinal Human Studies of Low Overnight Melatonin Production as a Risk Factor for Breast Cancer

Conclusion: Five longitudinal studies have now been conducted of low melatonin production as a risk factor for breast cancer. Two of the studies collected urine samples in an optimal manner to estimate the important component of melatonin production – overnight production. However, two (2) used first morning void, which is close to optimal and one (1) had to use 24-hour collection, which hides possible non-circadian rhythm, which can be deleterious. One study, which used first morning void urine, was limited to premenopausal BC. The study which used 24-hour urine samples was negative. Of the remaining 4 studies, three were positive and the one limited to premenopausal BC was problematic, perhaps due to lag times and the likely adverse effect of BC in its very early stage on melatonin production.

Thus, there is increasingly strong longitudinal evidence that low melatonin production is a risk factor for at least post-menopausal breast cancer.

There have been five (5) longitudinal studies, two of which were from the Nurses' Health Study cohort, of low melatonin production as a risk factor for breast cancer. Note that many breast cancers are associated with a decrease in melatonin production (Bartsch *et al.*, 1997). There is often a "rebound" after excision of the tumor, but it is not known if post-excision melatonin production is near the pre-tumor production level (Bartsch *et al.*, 1997). Thus, as with AD, it is not appropriate to use post-tumor melatonin levels in a case-control study of low melatonin as a risk factor for breast cancer.

DNA damage is the pathway through which normal cells become malignant. Thus, the greater the amount of DNA, the greater the probabilities of a malignant transformation and the development of cancer. Davanipour *et al.* (2009) have conducted a study on the association between endogenous melatonin levels and oxidative guanine DNA damage among mothers and their oldest sampled daughters. The mothers' age range was 43-80, while the oldest daughter's age range was 18-51. Nearly all of the mothers, but few of the daughters were postmenopausal. Complete overnight urine samples were obtained. Creatinine-adjusted aMT6s and 6- hydrodeoxyguanosine (8-oxodG) were assayed. 8-oxodG is a measure of the level of oxidative DNA damage. Creatinine-adjustment is not necessary because the 8-oxodG level using complete overnight urine is a measure of the total repair of oxidized DNA guanine during the night. There was a statistically significant (p=0.02) inverse association between the level of nocturnal melatonin production (aMT6s/creatinine) and 8-oxodG for the mothers, but not for the daughters. Statistical adjustment was made for age and weight; however, there was little difference in the results with or without adjustment. The correlation between creatinine-adjusted aMT6s and 8- oxodG was 0.35 (p=0.01).

Positive Studies

Schernhammer and Hankinson (2005) reported on the association between urinary melatonin levels and breast cancer risk in the Nurses' Health Study II. The study had collected first morning void urine samples prior to the diagnosis of any cancer in a sub-sample of the women in the study. Assays of aMT6s and creatinine for 147 women who developed invasive breast cancer, and 291 age-matched controls, plus 43 women who developed in situ breast cancer and 85 matched controls were analyzed. Analyses were based on quartiles of creatinine-adjusted aMT6s developed from the control data, with subjects in the lowest quartile as the referent group. (Thus, the analyses were conducted with a view that higher levels of melatonin production might be protective.) Unadjusted analyses, estradiol level adjusted analyses, and analyses adjusted for age-at-menarche, parity, age-at-first birth, family history of BC and benign breast disease, alcohol use, antidepressant use, and body mass index were conducted. It should be noted that low levels of melatonin are causally associated with earlier age-at-menarche (e.g., Cohen *et al.*, 1978; Sizonenko, 1987). Thus, inclusion of age-at-menarche in the adjustment is perhaps not appropriate. Analyses of cases and controls from the lowest and the highest quartile were statistically significant for each level of adjustment. The odds ratios (OR) were all 0.59. (In terms of risk associated with low melatonin production, the OR was 1/0.59 = 1.69.) Inclusion of the the cases with in situ breast cancer led to OR between 0.68 and 0.70. Significance levels were not provided. However, the 95% CI's for invasive breast cancer did not contain 1.0, while the 95% CIs when in situ breast cancer cases were included just

barely contained 1.0.

In 2008, Schernhammer and Hankinson used the Hormones and Diet in the Etiology of Breast Cancer Risk (ORDET) cohort to study low overnight melatonin production as a possible risk factor for postmenopausal breast cancer. The ORDET study was conducted in northern Italy and included 10,786 healthy women aged 35-69 at baseline, 3966 of whom were postmenopausal. Complete 12-hour overnight urine samples were obtained. There were 178 subjects who developed postmenopausal BC prior to the Schernhammer et al. study analysis and met inclusion criteria, e.g., BC as the initial cancer, urine sample availability. Seven hundred ten (710) women were selected as controls, matched on age at enrollment (± 3 years), date of recruitment (± 180 days) and laboratory assay batch. Conditional regression models were used for analyses, adjusting for thirteen (13) known BC risk factors and circulating testosterone, which was a BC risk factor in the ORDET study. Analyses were performed using both aMT6s and creatinine-adjusted aMT6s. Analyses were done by quartiles of aMT6s. 95% CIs and trend p-values were calculated. Trend p-values were 0.05 or below when the analyses excluded in situ BC and below 0.10 when in situ BC was included. When analyses were conducted without current smokers, the trend p-values were below 0.005. Comparing the highest versus lowest quartile of aMT6s, the p-values were at or below 0.05 for invasive BC, including or excluding testosterone. When only non-current smokers were analyzed, the p-values were smaller. (Note: only 95% CIs were actually published.) Results were similar for creatinine-adjusted aMT6s analyses.

In 2009, Schernhammer and Hankinson used to Nurses' Health Study cohort to further investigate the relationship between urinary melatonin levels and postmenopausal BC. Spot morning urine assays for aMT6s were available for 357 postmenopausal women who developed incident BC after recruitment into the cohort and 533 matched controls. The analysis methods were much the same as in the previous paper. Quartiles of aMT6s among the controls were analyzed. In multi-variable adjusted analyses, the subjects in the lowest quartile of aMT6s had an increased risk (p < 0.05) of developing BC compared to subjects in the highest quartile. This was true for all BC, for in situ BC only, and for invasive BC only. Subjects in the lowest quartile also had an increased risk compared to subjects in the 3rd (highest) quartile for all BCs and for in situ BC only. Trend p-values were below 0.05 for all three groups: all BCs, invasive BC, in situ BC.

** It should be noted that the first morning void, especially when the subject has had urine voids during sleep time, is not as good as complete overnight urine collection in estimating nocturnal melatonin production. **

Negative Study

Travis *et al.* (2004) conducted a study of melatonin and breast cancer using the Island of Guernsey or Guernsey III longitudinal study. This study recruited women for an eight and one-half year period, ending in 1985. During the follow-up period, 127 women developed breast cancer. Three hundred fifty three (353) controls were selected with matching based on age, recruitment date, menopausal status, day of menstrual cycle (if applicable) when the urine sample was obtained, and number of years post-menopausal (if applicable). Twenty-four (24) hour urine samples were collected. These samples were evidently not divided between overnight and other time-of-day sub-samples. None of the analyses (all cases-

controls, only pre-menopausal cases-controls, or only post-menopausal cases-controls) showed any hint of an increase risk associated with low 24-hour melatonin production.

** It is unfortunate that the 24-hour urine samples were not subdivided by time of day. It is the nocturnal blood level of melatonin that is important. About 85%-90% of pineal melatonin is produced nocturnally. The circadian rhythm appears to be vital for the effects of melatonin in regulation of important biologic functions, including immune response. This particular problem with the study makes the results suspect. (See Hrushesky and Blask, 2004, for further details.) **

Problematic/Peculiar Study

In 2010, Schernhammer et al. used the ORDET cohort to investigate premenopausal BC. There were 180 premenopausal BC cases, with 683 controls selected – nearly 4 per case – using the same matching criteria as was previously used. The urine samples were 12 hour, overnight (7:00 pm – 7:00 am) samples. There was a statistically significant trend towards **increasing risk** with higher baseline aMT6s. This was the opposite of what was likely anticipated. However, when current smokers were excluded, the increasing risk completely disappeared. On the other hand, among non-current smokers, a BC diagnosis within 3 years of urine collection was much more likely for subjects in the highest aMT6s quartile compared to subjects in the lowest quartile. Lag time from urine collection to BC diagnosis was also investigated among non-current smokers. Only after 8 years of lag time was there a statistically significant difference between the lowest and highest quartiles of aMT6s: an increase in risk associated with low production. Thus, this study's results are clearly perplexing. The authors recognize this and suggest that perhaps very early BC is causing an increase in melatonin production.

C.   **No Case-Control Studies of Low Melatonin Production as a Risk Factor for Breast Cancer**

As mentioned previously, breast cancer itself often causes a decrease in melatonin production, e.g., Bartsch *et al.* (1997). It is therefore inappropriate to use current levels of melatonin production of breast cancer cases in a case-control study of whether low levels of melatonin are a risk factor for breast cancer, and none have been published.

D.   **Light-at-Night and Night Shift Work Studies as a Risk Factor for Breast Cancer – Surrogates for Low Melatonin Production**

Conclusion: There is moderately strong evidence that both long-term light-at-night and night shift work increase the risk of breast cancer. Five (5) studies are reviewed, 4 of which are positive. The negative study did find an increased risk for light-at-night, but not shift work. This study classified subjects as having had rather short shift work as exposed. Only very few subjects had at least 8 years of shift work: 8 (1.6%) of cases and 19 (3.7%) of controls.

Several studies have found an increase in risk of breast cancer among women who have rotating night shift work or who otherwise experience light at night. Light at night (LAN) is well-known to cause a decrease in nocturnal melatonin production (e.g., Lewy *et al.*, 1980; Lowden *et al.*, 2004; Schernhammer *et al.*, 2004). Note that occupational studies of ELF MF exposure

(Section E, below) have included jobs with night shift work, e.g., flight attendant and radio/telegraph operators.

<u>Positive Studies</u>

- Lie *et al.* (2006) studied the occurrence of breast cancer among Norwegian nurses. All data were obtained from government registers. Among a cohort 44,835 nurses, who graduated from a 3-year nursing program between 1914 and 1980 and who were alive on January 1, 1953, or born after this date, 537 breast cancer cases which occurred between 1960 and 1982 were identified. (1960 was chosen because that was the first year for which fertility data were available.) Four (4) controls, alive and cancer free, for each case were selected from the nurse cohort, matched by year of birth (± 1 year). Controls were required to have graduated or started their initial job no later than the year the corresponding case was diagnosed with BC. Number of years of night shift work was estimated from work history and work locations. Statistical adjustments in OR estimates included total employment time and parity. The OR for 30+ years of night shift employment versus 0 years, was 2.21 (p<0.05), 95% CI = [1.10 – 4.45]. The p-value for trend was 0.01. When the analysis was limited to nurses aged 50+, the OR was 2.01 (p>0.05), 95% CI = [0.95 – 4.26]. The number of cases without night shift work was only 50 for all ages, and was 29 for nurses over age 50. The number of cases with at least 30 years of night shift work was 24. (No case below age 50 had 30+ years of night shift work.)

- Schernhammer *et al.* (2001) examined rotating night shift work as a possible risk factor for breast cancer in the Nurses' Health Study. The total number of years in which a subject had worked rotating night shifts of at least 3 nights per month was obtained in 1988. The sample was quite large: 31,761 nurses had not had any years meeting the night shift criterion; 40,993 had had 1-14 years; 4,426 had had 15-29 years; and 1,382 had had 30+ years. During the following 10 year period, 2,441 incident cases of breast cancer were identified. Compared to nurses who had had no qualifying years, the adjusted relative risk (RR) for nurses with 30+ years of rotating night shift work was 1.36, with a 95% CI of [1.04 – 1.78]. All subjects with 30+ of rotating night shift work were post-menopausal Analyses were also conducted within pre- and post-menopausal groups. The RR and 95% CI were the same for 30+ years of exposure, because the number of nurses with no exposure decreased slightly (from 925 down to 801). While not statistically significant, perhaps due to sample size, pre-menopausal nurses who had at least 15 years of shift work had an adjusted RR of 1.34, 95% CI = [0.77 – 2.33], essentially the same RR as post-menopausal women (RR=1.36, 95% CI = [1.04 – 1.78]) who worked night shift for at least 30 years. There were only 14 pre-menopausal nurses with 15+ years of exposure. The trend in RR for increasing years of exposure was statistically significant for post-menopausal nurses and all nurses. Adjustments were made for age, weight change between age 18 and menopause, and many other variables associated with breast cancer. The increase in risk was almost totally due to hormone-receptor positive breast cancers. This was the first prospective night shift and breast cancer study.

- Davis *et al.* (2001b) studied 813 breast cancer patients, aged 20-74, and 793 controls. The controls were obtained through random digit dialing and were frequency matched

by 5-year age intervals. Lifetime occupational history, bedroom lighting, and sleep habits were obtained by interview for the 10 years prior to diagnosis. Not sleeping during nocturnal periods (when melatonin production is usually at its peak) had an OR of 1.14 for each night per week. The 95% CI was [1.01 – 1.28]. Night shift work had an OR of 1.6, 95% CI = [1.0 – 2.5]. There was a significant upward trend (p = 0.02) in the OR with increasing years and more hours per week in night shifts. Statistical adjustments were made for parity, family history of BC, oral contraceptive use (ever), and recent (but discontinued) use of hormone replacement therapy.

- Hansen (2001) studied BC risk among younger Danish women whose work was mostly at night. All women born between 1935 and 1959, and 30-54 years of age, were identified though the Danish Cancer Registry. The number of such women was 7,565. One control per case was randomly selected from the Danish Central Population Registry. Controls were (i) living, (ii) apparently cancer free, and (iii) working before the date of diagnosis of the corresponding case. Work history was obtained from the Danish pension fund database. No work history was found for 530 cases, so the number of case-control pairs for the study was 7,035. Using a national survey (1976) of women and working conditions, 4 occupational categories were identified in which at least 60% of the female employees so some work at night. These were manufacturing of beverages, land transport services, catering, and air transport services. For hospitals, furniture manufacturing, water transport services, and cleaning services, between 40% and 59% of the women work some night shifts. Comparisons were made between occupations in which 60%+ of the women work night shifts and occupations in which less than 40% work night shifts. Only occupations within 5 years of diagnosis were considered. This limit was based on suspected induction time for breast cancer. To be placed in the "exposed" category a women had to have worked at least 6 months in a night shift occupation. Statistical adjustments were made for age, social class, ages at birth of first and last child, and parity. The OR for all "exposed" occupations was statistically significant (p<0.05): OR=1.5, 95% CI = [1.3 – 1.7]. For women who worked at least 6 years in "exposed" occupations, the OR was 1.7 (p<0.05). The results were essentially driven by the catering and air transport service occupations. (It should be noted that these two occupations may also result in higher ELF MF exposure, compared to manufacture of beverages and land transport services.) The authors state that "(w)hen the 5-year induction time was ignored, the ORT decreased marginally".

Negative Study

- O'Leary *et al.* (2006) studied night shift work, light-at-night and BC in Long Island, NY, as part of the Electromagnetic Fields and Breast Cancer on Long Island Study (EFBCLIS) Group. There were 487 cases and 509 population-based controls, frequency matched to the expected age distribution of the cases in the study. These subjects had to have participated in the earlier Long Island Breast Cancer Study Project (LIBCSP). Each case had to have lived in the same home for at least 15 years prior to the diagnosis of breast cancer, while each control had to have lived in the same residence for at least 15 years prior to recruitment. Cases had to have had their BC diagnosis within the 12 month period beginning August 1, 1996. Controls were concurrently recruited. The LIBCSP had collected, via direct interview, complete job history information, including shift work – all jobs held for at least 6 months beginning at age 16, full time or part-time. The EFBCLIS repeated the job history interview, without the shift work

information, for the period 15 years prior to the date of BC diagnosis (cases) or recruitment (controls). Military assignments were included. Light-at-night information was obtained by interview, and included information about sleep hours, frequency and length of having lights on during sleep time for the 5 year period prior to the reference date.

Exposure to shift work was defined as ever having had a job ($\geq$ 6 months, either part or full time) with at least 1 day per week of shift work, during the 15 years prior to the reference date. Sub-groups were defined as follows: ever had an evening shift job; ever had an overnight shift job; ever had an evening shift, but never an overnight job; ever had an overnight shift; but never an even shift job. Statistical analyses were adjusted for reference date, parity, family history of BC, education, history of benign breast disease.

For any of the various categories of shift work during the 15 years prior to the reference date, there was no elevated risk of BC. However, 'any overnight shift work' had a statistically significant OR below one. The referent group included subjects with a jobs having less than 1 shift work day per week. Such a job could have been held for many years. The OR for at least 8 years of overnight shift work was statistically significantly below 1. For light-at-night within 5 years prior to the reference date, the only statistically significant finding was an OR = 1.65 for waking up and turning on lights at least 2 times per night versus doing so no more than 3 times per month.

The authors conclude that their study "provides mixed evidence for the light-at-night hypothesis". Analyses of shift work within 5 years of the reference date, the "induction" period used by Hansen (2001), were not presented. Overnight shift work was in the work history of only 26 cases and 50 controls; a duration of at least 8 years of overnight shift work was experienced by only 6 cases and 19 controls. Thus, the effective, "exposed" sample size was quite small. Information as to when this shift work occurred relative to the reference date was not provided.

E. **Occupational Case-Control Studies of** ELF **MF Exposure as a Risk Factor for Breast Cancer**

Conclusion: There is rather strong evidence from case-control studies that long-term, high occupational exposure to ELF magnetic fields is a risk factor for breast cancer. Six (6) independent studies are reviewed. Four (4) have positive conclusions, while two (2) are negative. The latest study is particularly strong. The two negative studies have serious shortcomings in exposure classification and come from the same research group.

There have been several case-control studies of occupations with more or less high ELF MF exposure and the risk of breast cancer. These studies have been generally positive, in the sense that there appears to be an increased risk. Earlier studies generally lack appropriate exposure information (e.g., Wertheimer and Leeper, 1994).

Positive Studies

- Peplonska *et al.* (2007) have conducted a large, population-based, case-control study of

breast cancer and 73 occupational categories. All incident cases of cytologically or histologically confirmed breast cancer among women aged 20-74 in Warsaw and Lódź, Poland, in 2000-2002 were identified. 2,502 controls were randomly selected using the Polish Electronic System of Population Evidence, which maintains records on all citizens of Poland. Controls were matched to cases by city of residence and age ± 5years. A structured questionnaire was completed by 79% of the cases and 69% of the controls. The questionnaire included items related to demographics, reproductive and menstrual history, hormone use history, physical activity, occupational history for all jobs held at least 6 months, smoking, alcohol use, diet, cancer history in female relatives, medical and screening history, prenatal exposures, and history of weight and height development. Occupational information included job title, start and stop dates, employer, company products and/or services, work activities and duties, physical activity related to work, passive smoking, and exposures to a list of chemicals. The study was funded by the U.S. National Cancer Institute (NCI) and managed by Westat (Rockville, MD).

Statistical adjustment was made for age, age-at-menarche (≤ 12; 13-14; ≥ 15), menopausal status; age-at-menopause, parity ≤ 1; 2; ≥ 3), body mass index (< 25; 25-30; ≥ 30 kg/m^2), first degree female family history of BC, education ($<$ high school; high school; some college or professional training; college degree), previous mammographic screening, and city of residence. Oral contraceptive use, marital status, tobacco and alcohol use, age-at-first full term birth, breastfeeding, recreational and occupational history were not used for adjustment in the final analyses because they had "little impact" on the results.

In the primary analyses, for each specific job category/industry, the referent group consisted of all subjects who did not work in that job/industry for at least 6 months. For each specific "white-collar" occupation, additional analyses using all other white-collar jobs as the referent group were conducted. This was thought to provide at least a partial account for socio-economic factors not accounted for by education. Similar blue-collar job analyses were not conducted. Several job categories containing occupations with elevated ELF MF exposure had statistically significantly elevated ORs.

** These ORs were significantly elevated despite the fact that all other occupations with elevated ELF MF exposure were placed in the referent group. **

ELF MF exposure was determined using a job exposure matrix developed within NCI for a brain cancer study. No, low, medium and high categories were developed by "experienced industrial hygienists". (No reference was provided.) The highest ELF MF exposure category of all jobs for an individual was used in analyses. 99% of the high exposed subjects were so ranked due to employment as machine operators and tenders in the textile apparel and furnishing industry. Information on which occupations were classified as low or medium ELF MF exposure were not provided.

** It should be noted that (1) 'tenders' generally provide maintenance to machinery and (2) operators of machines other than sewing machines, e.g., cutters, both have lower ELF MF exposure than seamstresses. **

The OR for high ELF MF exposure versus no exposure was significant: OR = 1.5,

95% CI = [1.1 – 2.0]. For low exposure, the OR was also significant: OR = 1.2, 95% CI = [1.0 – 1.5]. For medium exposure the OR was also 1.2, but the 95% CI was [0.9 – 1.5]. Additional data analyses were not provided. The OR for high exposure among textile apparel machine operators and tenders is in line with the statistically significantly increased OR for seamstresses in the Forssén *et al.* (2005) study (see below under "negative studies") discussed below. In the Forssén *et al.* study (2004), seamstresses were classified as having medium-low ELF MF exposure.

Specific ORs for occupations classified (surprisingly and for some likely incorrectly) as having high (as opposed to low or at most medium) ELF MF exposure by Forssén *et al.* (2004) (see below) were calculated: cooks (OR=1.0); computer scientists (OR=1.3); computer and peripheral equipment operators (OR=0.7); data entry keyers (OR=0.3); dentists (OR=0.6); dental nurses (OR=1.0); counter clerks and cashiers (OR=1.1); and telephone operators (OR=0.9).

- Labréche *et al.* (2003) studied occupational ELF MF exposure and post-menopausal breast cancer. Cases and controls were identified through pathology department records at 18 hospitals in Montreal, Canada. These hospitals treat most of the breast cancer cases in the area. Age was restricted to 50-75 at the time of initial diagnosis of primary BC. Cases had to be residents of the region and the diagnosis had to have been in 1996 or 1997. Controls had one of 32 other cancer diagnoses and were frequency matched by age and hospital. The following cancers were excluded: liver, intrahepatic bile duct, pancreas, lung, bronchus, trachea, brain, central nervous system, leukemia, lymphoma, and non-melanoma skin cancer, but not gastrointestinal (Schernhammer *et al.*, 2003) or colorectal cancer (Bubenik, 2001).

Complete occupational history, including task descriptions, and other personal information was obtained by personal interview, either of the subject or a surrogate if the subject was deceased or otherwise unavailable. Specialized occupational questionnaires were used for specific occupations, including sewing machine operators, cooks and nurses. The development of these questionnaires was led by Jack Siemiatycki. See, for example, Siemiatycki *et al.* (1991, 1997). ELF MF exposures were estimated from detailed descriptions of tasks, equipment used, and the work environment by industrial hygienists intimately familiar with Montreal workplaces. The ELF MF exposure categories and primary occupations were as follows: no exposure (< 2 mG; low exposure (2-5 mG, "typical jobs", including VDT operators, electric typewriter operators); medium exposure (5-10 mG; denturists, machinists); and high exposure (≥ 10 mG; sewing machine operators, textile workers). The industrial hygienists "confidence" in each subject's exposure assessment was obtained as definitely no exposure, or low, medium, and high confidence of exposure.

Exposures to benzene, perchloroethylene, and alphatic aldehyes, chemicals found in the textile industry, were also considered.

Statistical adjustments were made for age at diagnosis, family history of breast cancer, education, ethnicity, age-at-bilateral oophorectomy, age-at-menarche, age-at-first full-term pregnancy, oral contraception use, duration of HRT, total duration of breast feeding, alcohol use, smoking, and body mass index, as appropriate. Adjustment was also made for proxy versus personal responses because proxies tend to report fewer

jobs. In addition, duration of employment in the textile industry was an adjustment variable. As mentioned previously, adjustment for age-at-menarche is probably not appropriate due to melatonin's causal relationship with age-at-menarche.

In addition to the categorical analyses, the number of hours of medium or high exposure was used as a risk factor. The number of hours from the lower limit of the second quartile to the upper limit of the third quartile of medium/high exposure was 6000 hours. ORs were presented for a difference of 6000 hours.

All analyses, e.g., no exposure vs ever exposed, prior to 10 years before diagnosis, or before age 35, were non-signficant and non-elevated except for the following ones, adjusted for textile industry employment and other factors:

- ✓ No exposure vs medium-to-high exposure – OR = 1.90, 95% CI = [0.99 – 3.85];
- ✓ 6000 hour increase in medium-to-high exposure – OR = 1.21, 95% CI = [0.97 – 1.49];
- ✓ 6000 hour increase in medium-to-high exposure prior to 10 years before diagnosis – OR = 1.31 (p<0.05);
- ✓ 6000 hour increase in medium-to-high exposure prior to age 35 – OR = 1.54 (p<0.05).

The significant results appear to be primarily due to ELF MF association with progesterone positive and/or estrogen positive breast cancers.

The use of a 10 year lag eliminates exposure periods which may be too near the diagnosis time to be etiologically relevant. The analysis of exposures prior to age 35 identifies the time period when the development of female breast cells appears to cease.

The use of textile industry employment (yes/no) or length of time in the textile industry, as appropriate, as a covariate provides some adjustment for chemical exposures. Thus, the increase in the ORs when adjustment was also made for textile industry employment relates to ELF MF exposure.

Finally, controls also had cancer. While many of the excluded cancers may conceivably have ELF MF as a risk factor, some of the non-excluded ones may also. This is especially true if the melatonin hypothesis is correct. Thus, the OR estimates may be biased towards 1.

- Kliukiene *et al.* (1999, 2003, 2004) and Tynes *et al.* (1996) studied occupational ELF MF exposure and breast cancer among Norwegian women in general and radio and telegraph operators in particular. These were follow-up studies. A population-based cohort of 1.1 million women was developed using the 1960, 1970, and 1980 censuses. All women were working at the time of enrollment and had a potential for occupational ELF MF exposure. The follow-up period was from 1961-1992. Date of birth, and census information about occupation and socioeconomic status was obtained. Incidence of breast cancer was obtained from the Cancer Register of Norway. Out-migration information was obtained.

For the countrywide, all occupations study (1999), ELF MF occupational exposure assessment was not optimal, but was as follows. The first method used expert opinion. An expert panel, using written guidelines, decided whether a given occupation had ELF MF exposure above 1 mG for than 4 hours per week, between 4 and 24 hours per week, or more than 24 hours per week. Occupations were identified by a 3-5 digit industry code and a 3-digit occupation code. For cumulative exposure, the mean of each of the three (3) levels of exposure were used: 2 hours; 14 hours, 32 hours (based on a 40 hour week). It was assumed that each subject was in the same occupation from census to census, unless she died, emigrated or turned age 65.

The second method used the Swedish job exposure matrix used in the Forssén *et al.* (2000) study (below), which was constructed from observations of male workers. Cumulative exposure was categorized as below 9 mG-years, between 9 and 14 mG-years, between 14 and 30 mG-years, and above 30 mG-years. Exposure was also classified by number of work hours of exposure above background (1 mG): below 900 hours; 900-999 hours; 1000-1999 hours; 2000 or more hours.

Poisson regression, with adjustment for age, time period, and socioeconomic status, was used to estimate the relative risk (RR) of breast cancer. 22,543 breast cancer cases were diagnosed during the follow-up period. In the total cohort and the two sub-cohorts for those below or at least 50 years of age at inclusion in the cohort (Kliukiene *et al.*, 2004), the RRs were statistically significantly above 1.0 for each category of number of exposed hours, with below 900 hours as the reference category. For each cumulative exposure category above the reference category (below 9 mG-years, the RR for the total was statistically elevated. For the two sub-cohorts, the RRs were significantly elevated for the 9–14 and 14–30 mG-years categories. For the 30+ mG-years category the RRs were elevated, but lower bounds of the 95% CIs were 0.98 and 0.99.

These studies did not have very good occupational data.

For the radio and telegraph operators studies, the same cohort and occupational determination method was used. The Kliukiene *et al.* (2003) study was identical to the Tynes *et al.* (1996) study, except for a longer follow-up. By the end of May 2002, there were 99 breast cancer cases among the 2619 radio and/or telegraph operators in the cohort. The standardized incidence ratio was 1.30, 95% CI = [1.05 – 1.58].

A nested case-control study was also conducted, using the 99 BC cases and 4 controls per case matched on year of birth ± 5 years for cases born prior to 1920 and ± 1 year for cases born in 1920 or later. It was an update of an earlier study by Tynes *et al.* (1996). The reference category consisted of subjects (all radio and/or telegraph operators) who were not registered in the Norwegian Seamen Registry, i.e., had no history of working on merchant ships. ELF MF exposure was not particularly explicit. It seems to have been assumed that that women who had no history of working on merchant ships had lower MF exposure (ELF and radiofrequency) than those with a history of such work. Spot ELF MF and radiofrequency MF measurements in the radio/telegraph rooms of 2 and 3 ships, respectively, were performed. RF magnetic and electric fields were below the detection level of the instruments at the operator's desks. ELF magnetic fields varied from 0.2 mG to 60 mG at the operator's desks. However, the highest exposures were only to the stretched out leg. "Normal" exposure to the body varied from 1 mG to

2 mG. Thus, exposure was certainly not high.

Tertiles of cumulative exposure at sea were used in the statistical analyses, with adjustment for age-at-first birth and parity. Detailed job histories on each ship were available for each 'exposed' subject. For each ship, the amount of time spent in the radio/telegraph room was estimated by an experienced researcher. A rank of 1-3 was assigned: 1 – 'long voyage' for tankers or dry-cargo ships with longer stays as sea; 2 – 'many calls' for trade ships with several loading and discharge ports; 3 – larger passenger ships. Increasing rank implies increasing percentage of time spent in the radio/telegraph room. Exposure was then calculated by summing the product of the number years of service on ships of each rank by the rank of the ships.

Analyses were conducted for total exposure, and for total exposure with lag times of 10 and 20 years prior to BC diagnosis. Analyses were conducted for (1) all cases and controls, for cases and controls below age 50 in the reference year, and for cases and controls at least age 50 in the reference year, and (2) ER+ and ER- cases.

No OR was statistically significant for any analysis without consideration of ER status. However, there was a statistically significant increasing trend in the ORs over cumulative exposure categories in the analyses for all cases, cases younger than 50, and cases at least age 50. There was also a significant upward trend for a 10 year lag time using all cases. The ORs for the highest exposure category were all elevated, but not significant perhaps because of the sample size.

For analyses by ER status, the only significant finding was for ER- cases, age 50+ in the highest exposure category. There were elevated ORs for all exposure categories for all ER- cases, and for the highest exposure category for ER+ cases and for ER+ cases below age 50.

The authors concluded that "occupational exposure to electromagnetic fields increases the risk of (female) breast cancer" (Kliukiene *et al.*, 2003).

- Loomis *et al.* (1994) investigated BC mortality among female electrical utility workers. This study used U.S. national death certificate information, 1985-1989, to identify cases and controls (without leukemia or brain cancer as a cause or contributing cause of death) and occupations. There were 27,814 women with breast cancer and sufficient occupational information, of whom 68 had an "electrical" occupation. There were 110,750 controls, of whom 199 had an "electrical" occupation. The primary factor limiting the sample size was the availability of occupational information. It should be noted that use of occupational data from death certificates is far from optimal. Statistical adjustments were made for age, ethnicity, and social class. Loomis *et al.* found an elevated risk associated with having an electrical occupation recorded on the death certificate: OR=1.38 (p<0.05). The only specific occupation with a statistically significant elevated risk was telephone installers, repairers and line workers: OR=2.17. Electrical engineers and electrical technicians had 'elevated', but not significant risk estimates (OR=1.73 and 1.28). On the other hand, air traffic controllers, telephone operators, data keyers, computer operators, computer programmers did not have 'elevated' risk estimates.

In a letter commenting on the Loomis *et al.* paper, Kantor *et al.* (1995) analyzed essentially the same data set, with the inclusion of data from 1984. They used an industrial hygienist to estimate the probability of occupational ELF MF exposure or video display terminals (0, low, medium or high) among white and black women. The ORs were statistically significant (but not particularly high) for medium or high probability of exposure for both white and black women. When the hygienist actually categorized the level of ELF MF exposure, only medium exposure was associated with a statistically significant OR. High exposure had somewhat lower ORs.

- Forssén *et al.* (2005) published a case-control study of occupational ELF MF exposure and breast cancer. This study may be considered influential, unless reviewed in detail. So considerable detail is provided.

  The Forssén *et al.* (2005) study found no association between occupational ELF MF exposure, as determined by Forssén *et al.* (2005), and breast cancer. The study is singled out because (1) it is essentially well designed, and (2) has a completely inappropriate ELF MF occupational classification scheme based on either non-representative workers in specific occupations or what should be considered quite suspect individual measurements (Forssén *et al.*, 2004). Many occupational groups which are generally considered to contain higher ELF MF exposed occupations have been classified as low or medium-low exposure.

    ** Forssén *et al.* (2005) did find that seamstresses had statistically significantly elevated risk of breast cancer. However, they classified seamstresses as having medium-low ELF MF exposure. **

  Forssén *et al.* (2005) used newly collected exposure data for occupations in which women commonly work (Forssén *et al.*, 2004). The exposure study assessed occupations identified within the Swedish 1980 census. Forty-nine (49) specific occupational titles were identified. Volunteers working in each of these occupations were then ascertained by methods which are not specified. Personal 24-hour ELF MF measurements were obtained on what was presumably supposed to be a typical 24-hour day, using a dosimeter worn at the waist. The volunteers kept a diary so that time periods at work, at home, and elsewhere could be identified. The number of subjects with measurements by occupation ranged from 5 to 24. The total number of subjects measured was 471. There were only 5 observations for Seamstresses, and 5 Radio and Television Assemblers and Repairwomen. The workday measurements were used for classification purposes. In the epidemiologic study of breast cancer, 4 categories of exposure were used: Low (< 1 mG); Medium-Low (1-1.9 mG); Medium-High (2-2.9 mG); and High (≥ 3 mG). The occupations in the categories above 'low' are provided in Table 9. The arithmetic rate of change measure was also calculated. Seamstresses and Radio and Television Assemblers and Repairwomen were both classified as medium-low exposed occupations. The 5 seamstresses measured for exposure had their own small businesses and did not work in apparel manufacturing. They evidently also did not do much sewing. They spent 55% of their workday in fields below 1 mG and only 15% in fields above 3mG. This is only an average of 1 hour and 12 minutes of 'high' exposure during a working day. In the two counties in Sweden in which both the

measurement study and the breast cancer case-control study were performed, there was almost no apparel manufacturing (Forssén *et al.*, 2004; personal communication, M. Feychting, 2007). Still, it is difficult to imagine such low exposures among women who actually work as seamstresses.

The cases and controls were obtained from all women who were employed at any time between 1976 and 1999, based on any of the censuses between 1960 and 1990, in either Stockholm or Gotland counties, Sweden. Subjects entered the study in either 1976 or their 15th birthday, which ever came first, and were followed through 1999 or to the date of their initial breast cancer diagnosis. Cases were identified through the Regional Cancer Registry in Stockholm. The referent year was the year of the case's diagnosis. Controls were selected randomly by age and calendar year, apparently matched to cases. Cases could not also be controls. Both cases and controls had to be living in Stockholm or Gotland counties during the referent year. All information, including occupational history, was obtained from registries. 20,400 cases and 116,227 controls were enrolled in the study. Varying numbers of cases and controls were used in the analyses, depending on the availability of occupational and other data. Statistical adjustment was made for age, referent year, parity, and socioeconomic status.

For statistical analyses, exposure was assessed in various ways: (1) ELF MF exposure for the occupation closest to the time prior to the referent year; (2) ELF MF exposure at the most recent census which was at least 10 years prior to the referent date; (3) ELF MF exposure at the most recent census when the subject was at least age 35. Analyses were also carried out by (4) splitting the study period at 1985, by (5) only using subjects who either always had low exposure or ever having had high exposure, and by (6) defining low exposure as a median less than 1 mG and a third quartile of less than 1.7 mG and high exposure as a median greater than 2.5 mG and a first quartile including 1.7 mG. With these definitions, high exposed occupations were cashiers, working proprietors in retail trade, air stewardesses, dental nurses, cooks, post office clerks, and kitchen maids. No time latency period was used in the analyses related to (3).

There were no significant or elevated adjusted ORs for analysis (1) using the 4 categories of exposure, either for all BC cases, ER positive cases, or ER negative cases, for age below or at least 50. The referent group had ELF MF exposure below 1 mG. There were no significant or elevated adjusted ORs for analysis (1) using low versus high (separated) exposure categories defined by (6), above.

Finally, in a series of analyses based on exposure 10+ years before the referent year, before age 35 for post-menopausal women, referent year before or after 1985, maximum point exposure, rate of change, and proportion of time exposure was above 3 mG, only a single adjusted OR was significant. The significant OR=0.87 and was for medium-high ELF MF exposure among post-menopausal women before age 35.

It is thus fair to say that Forssén *et al.* (2005) found no relationship between their assessment of ELF MF exposure and breast cancer. The authors do recognize that "(t)he major concern in the study is exposure misclassification".

Their job exposure classification is at odds with other classifications. Forssén *et al.* (2004, 2005) have classified Dental Nurses, Cashiers in Retail Stores and Restaurants,

Working Proprietors in Retail Trade, Cooks, and Air Stewardesses as high ELF MF exposure occupations. None of these occupations would be classified as having high ELF MF exposure in any other classification scheme. The common cut-point for high exposure is 10 mG. Cashiers, cooks, and air stewardesses may at times have medium or high exposure, depending on (1) the exposure from scanners, (2) the exposure from microwave ovens, mixers, other motorized kitchen equipment, and (3) the exposure time from sitting near electrical panels on takeoff and landing and in the airplane's kitchen areas.

   ** Forssén *et al.* should conduct a sub-study to determine the actual environment in which the seamstresses in their study worked, the type of machines used (industrial, home; AC or DC operation), and the percent of time spent actually sewing. They also should conduct a study of seamstresses in general in Stockholm and Gotland counties and the in-migration rates. Also, the authors note an occupational category labeled 'textile occupations', which certainly includes seamstresses, but is otherwise undefined in the paper. Textile occupations need to be specified and studied individually, as was done by Hansen *et al.*, 2000. It is important to determine whether the "seamstresses" in the Forssén *et al.* (2005) study have fundamentally different levels of exposure than seamstresses in other studies.**

The only significant occupational finding in this study related to seamstresses. Two analyses were conducted related to seamstresses (Table 10), probably because their exposure assessment was so at odds with every other series of exposure measurements of seamstresses. First, the OR for 'textile occupations', undefined in the paper, versus low ELF MF exposed occupations was 1.37, 95% CI = [1.11 – 1.68]. Second, the OR for 'textile occupations' versus all other occupations, regardless of ELF MF exposure assessment, was 1.33, 95% CI = [1.10 – 1.62]. The authors state that their results "suggest that the increased risk for breast cancer in these occupations might be related to some exposure other than magnetic fields".

'Textile occupations' were not defined, but could certainly have included a multitude of occupations with quite varying chemical exposures, and generally medium or high ELF MF exposures. However, none of the 49 occupational categories, other than seamstress, used in the study appear to relate to textile occupations, if sales and administration are excluded.

The numbers of seamstresses as cases or controls in the study are not provided. However, in the AD studies by Sobel and Davanipour (1995, 1996, 2007), approximately 2% of the controls were seamstresses. Thus, there may have been at least 2000 seamstresses among the controls. Assuming that most, if not all women in "textile occupations" were seamstresses, and based on the OR of "textile occupations" vs ELF MF exposure below 1 mG, the number of seamstresses with BC in the study can be estimated as approximately 475. Rough calculations indicate that if seamstresses are reclassified as having high ELF MF exposure (> 3 mG), the adjusted OR for high occupational ELF MF versus low occupational ELF MF exposure would be about 1.10 and statistically significant. It is worth repeating that the Forssén *et al.* (2004) occupational classification for high ELF MF exposure is (1) not as high as usual and (2) measured workday exposures are unusual for such occupations.

- Forssén *et al.* (2000) conducted an earlier case-control study of occupational and residential ELF MF exposure and breast cancer. The cohort from which the study population was obtained consisted of all Swedish residents who lived within 300 meters of a (high power, 220 or 400 kilovolt) transmission line for at least one year between 1960 and 1985 and were at least age 16 sometime in the period. Subjects in this group living further away from transmission lines essentially had no exposure from such lines. Cases were identified through cancer registries. Controls were randomly selected and matched by age group, residence in the same parish at the time of diagnosis of the case and in the same type of house (single-family/apartment further than 300 meters from the same power line. (The parish/power line criteria were relaxed for 95 cases; a control could not be found for 7 cases.) Residential exposure was calculated from the ELF MF generated by power lines. Occupation information was obtained from census data. An older job- exposure matrix was used to assess occupational ELF MF exposure. Low (< 1.2 mG), medium (1.2 – 1.9 mG), and high (≥ 2.0 mG) exposure categories were selected, based on quartiles. Exposure greater or equal to 2.5 mG was also considered.

  Statistical adjustments were made for the matching variables. Only occupational exposure immediately prior to the diagnosis of BC and only residential exposure at the time of diagnosis was used in the analyses. No information concerning occupations of the subjects was provided. It is unlikely that seamstresses were included in the analyses.

  No significant findings were identified.

  Of 1767 cases and 1766 controls, only 711 and 709, respectively, had residential exposure information, only 744 and 764 had occupational exposure information, and only 197 and 200 had both types of exposure information. For the actual analyses of occupational exposures, with matching variable adjustment, there was complete information for only 440 cases and 439 controls. For analyses using both occupation and residential exposures, and matching variables, there was complete information for only 87 cases and 83 controls.

Partially Positive/Partially Negative Studies

- Coogan et al. (1996, 1998) and McElroy et al. (2007) conducted case-control studies using the same ELF MF exposure classification scheme.
  - The 1996 Coogan et al. study selected breast cancer cases, aged 74 or younger, from the Maine, Wisconsin, Massachusetts, and New Hampshire cancer registries who were diagnosed between April 1988 and December 1991. Controls, aged below 65, were selected from state driver's license lists and were frequency matched to cases by 5-year age intervals. Cases aged below 65 had to have driver's licenses. Controls, aged 65-74, were selected from the Health Care Financing Administration's Medicare beneficiary lists. "Most representative" occupation was obtained via telephone interviews. Occupation duties and industry were obtained if "the occupation was not clear".

    Occupations were coded according to the 1980 Bureau of the Census 3-digit occupational classification. The ELF MF exposure classification scheme identified each of the 3-digit occupation classes as low, medium or high or

background (non-exposed) exposure "potential". It is our opinion that the classification scheme is rather deficient: for example,

1. Welders are classified as having **<u>medium</u>** ELF MF potential exposure;
2. Dressmakers (e.g., seamstress) and tailors are classified as having **<u>low</u>** potential for ELF MF exposure;
3. Shoe repairers are classified as having **<u>low</u>** potential for ELF MF exposure;
4. Electrical/Electronic Engineers are classified as having **<u>high</u>** potential for ELF MF exposure;
5. Statisticians and Scientists are classified as having **<u>medium</u>** potential for ELF MF exposure.

In most classification schemes, including that of Sobel-Davanipour et al., welders, dressmakers (seamstresses) are classified as high ELF MF exposed occupations, shoe repairers, electrical/electronic engineers would be classified as medium exposed occupations, and statisticians and scientists would be classified as low exposed occupations.

Nevertheless, the adjusted OR for breast cancer among subjects having occupations with high potential ELF MF exposure versus background was 1.43, with a 95% CI of (0.99 , 2.09). Among pre-menopausal cases with high exposure potential occupations, the adjusted OR was 1.98, with a 95% CI of (1.04, 3.78).

- Coogan and Aschengrau (1998) essentially replicated the earlier Coogan et al. (1996) study, except for adding non-occupational exposure, e.g., homes close to transmission lines, electric heating, bed-warming device. Cases and controls were obtained from Cape Cod, where elevated rates of breast cancer had been observed. Complete work histories (beginning at age 18) were obtained by interview. Jobs were classified using the methodology in Coogan et al. (1996). There were 259 cases and 738 controls. The crude and adjusted ORs were all below 2.0, except for having a "high" ELF MF job at some point and "other ELF MF exposure". The adjusted OR in this case was 2.3. None of the OR estimates was significant.

- McElroy et al. (2007) replicated the initial Coogan et al. (1996) study with female breast cancer subjects obtained from the Massachusetts, New Hampshire, and Wisconsin cancer registries after the close of recruitment for the Coogan et al. (1996, 1998) studies. Occupational ELF MF exposure using the same methodology as in the Coogan et al. (1996, 1998) studies was estimated for each subject's primary occupation. This was a large study: 6213 cases and 7390 controls. None of the adjusted (or unadjusted) ORs were anywhere near statistical significance. (The largest adjusted OR was 1.21.) However, the trend for increasing adjusted (or unadjusted) ORs for all women and for women who were post-menopausal at diagnosis were statistically significant, with p-values between 0.02 and 0.04.

We emphasize that the ELF MF exposure categories are quite inappropriate.

- Peplonska et al. (2007) conducted a case-control study of 2386 incident BC cases (diagnosed in 2000-2003) and 2502 controls. Lifetime occupational histories and known BC risk factors information were obtained. Occupational information included job title, start and stop dates, work activities and duties, and product(s) made and/or service provided. Occupations were coded to the Standard Industrial Classification Manual (1987) and the Standard Occupational Classification Manual (1980). Occupations were characterized as 'white collar' and 'blue collar'. Analyses are provided by occupation and duration, and by industry and duration. Thus, it is generally not possible to identify subjects with significant ELF MF exposure. For example, the following occupations are combined:
  - ✓ electrical, electronic, agricultural, industrial, mechanical, computer, and other engineers;
  - ✓ engineering and related technologists and technicians;
  - ✓ typists, secretaries, stenographers;
  - ✓ hairdressers and cosmetologists;
  - ✓ machine operators and tenders;
  - ✓ printing machine operators and tenders;
  - ✓ textile apparel and furnishing machine operators and tenders;
  - ✓ textile sewing machine operators and tenders;
  - ✓ welders and solderers.

Analyses by at least somewhat relevant occupational categories for any duration of work are as follows:
1. Engineers (electrical, electronic, agricultural, industrial, mechanical, computer, and others): OR=2.0, 95% CI = (1.05 , 3.8);
2. Health record technologists and technicians: OR=2.4; 95% CI = (1.04 , 5.7);
3. Machine operators and tenders: OR=1.2 95% CI = (1.03 , 1.5);
4. Printing machine operators and tenders: OR=3.1; 95% CI = (1.4 , 7.0);
5. Textile apparel and furnishing machine operators and tenders: OR=1.3; 95% CI = (1.03 , 1.5);
6. Textile sewing machine operators and tenders (a subset of the previous job category): OR=1.2; 95% CI = (0.9 , 1.5);
7. Welders and solderers: OR=1.2; 95% CI = (0.6 , 2.8).

None of these seven occupations showed any trend towards increasing risk with duration of work: ≤ 10 years vs > 10 years.

The analyses by industry are particularly inappropriate.

The authors used a job exposure matrix (JEM) developed by the National Cancer Institute for a brain cancer study (unreferenced) to evaluate ELF MF exposure and the risk of BC. They identified a statistically significant trend with ORs equal to 1.2, 1.2, and 1.5 for low, medium, high ELF MF exposure. (The actual data were not provided in the paper or online supplementary materials. The authors state that the "excesses in the highest exposure category" were almost completely due to textile apparel and furnishing machine operators and tenders. These employees evidently formed "99%" of the entire high ELF MF exposure group.

With respect to considering ELF MF as a risk factor for breast cancer, the authors would have been better served to use the actual job title and descriptions to form categories of ELF MF exposure. Nevertheless, the authors state that "occupations with potential exposure to magnetic fields deserve further evaluation".

- Ray et al. (2007) conducted a large and potentially valuable study of breast cancer among female textile workers in Shanghai, China. The authors took advantage of a randomized trial of breast self-examination efficacy to conduct a case-cohort study of occupational exposures and BC risk. 1709 BC cases and an age-stratified reference sub-cohort of 3155 non-cases were studied. Hazard ratios were estimated for duration in various job categories and exposure duration by Cox proportional hazards methodology.

  A job exposure matrix was developed for ELF MF exposure (Wernil et al., 2006). Admittedly based on a small number of subjects, the proportion of specific processes in the following textile industry areas were found to result in ELF MF exposure: spinning (75%, 8 of 12); weaving (88.9%, 8 of 9); cutting and sewing (60%, 3 of 5); and maintenance (30%, 3 of 10). There was no information about the extent (in instantaneous or cumulative mG) of the exposure.

  Among the weavers, cutters/sewers, and maintenance female personnel, only cutters/sewers and maintenance personnel with 10 – 20 years of experience had hazard ratios exceeding 1.0: HR=1.61, 95% CI = (1.16 , 2.25) and HR=1.83, 95% CI = (1.01 , 3.32), respectively. There were no indications of any trend. (Note: individual simple calculations of odds ratios for having worked primarily as a weaver, as a cutter/sewer, or as a maintenance person showed no increase or decrease in risk of BC.

  Evidently, no information as to what the ELF MF exposures were for various jobs, e.g., sewer, was collected.

F. **Residential Case-Control Studies of ELF MF Exposure as a Risk Factor for Breast Cancer**

Residential ELF MF exposure studies and BC have either used wire configuration coding, proximity to high voltage lines, various protocols of room measurements, or a combination of these methods. These studies have generally not found any increased risk of breast cancer (e.g., Feychting *et al.*, 1998; Davis *et al.*, 2002; London *et al.*, 2003; Schoenfeld *et al.*, 2003). Residential studies have measured actual magnetic fields only in current homes of cases and controls, thus homes which might be etiologically relevant are often or usually without actual measurements. Wire configurations and proximity to high voltage lines were at times used for surrogate measures of exposure related to previous homes. Each of these three methods of assessment of the level of exposure leads to significant classification errors. In addition, residential exposures are, almost always, surely relatively low. Individualized exposure, due for example to home sewing, sitting or sleeping near a panel of circuit breakers, sitting near a water pipe (e.g., in the floor or ceiling), is not identified. For homes near high voltage lines, rooms can have dramatically different ambient levels of ELF MF. For these reasons, these studies are not relevant to the purposes of this review.

G. **Radiofrequency Exposure and Breast Cancer**

There are no epidemiologic studies of radiofrequency MF exposure and breast cancer which do not include ELF MF exposure and which have reasonable data on RF exposure, e.g., Kliukiene *et al.* (2003).

V.   SEAMSTRESSES

Conclusion: *Seamstresses are, in fact, one of the most highly ELF MF exposed occupations, with exposure levels generally above 10 mG over a significant proportion of the workday. They have also been consistently found to be at higher risk of Alzheimer's disease and (female) breast cancer. This occupation deserves specific attention in future studies.*

A.   Sobel-Davanipour et al. Studies

Seamstress was the primary occupation among women with high ELF MF exposure in the Sobel *et al.* (1995, 1996b) and Davanipour *et al.* (2007) studies related to AD. No other published AD study has evidently involved populations in which sewing was a somewhat common occupation. In the 5 independent case-control studies presented in the 3 Sobel & Davanipour papers, most of the high ELF MF exposed women (cases and controls) were seamstresses. (Among women in these case- control studies, the Mantel-Haenszel AD odds ratio for seamstresses is 3.13, p < 0.01). Information about sewing as a hobby, which at least used to be common, was unavailable. Seamstresses have been shown to have very high ELF MF exposures (e.g., Szabó *et al.*, 2006; Kelsey *et al.*, 2003; Deadman and Infante-Rivard, 2002; Hansen *et al.*, 2000). Forssén *et al.* (2004) measured 5 "seamstresses" who owned independent small businesses and found what they classified as medium-low exposure – a mean of 1.7 mG. These 5 individuals used home sewing machines and evidently did not sew very often. Peplonska *et al.* (2007), using a NCI occupational ELF MF classification scheme found that, at least among women, nearly all high exposures occurred among textile machine operators and tenders. Both Forssén *et al.* (2005) and Peplonska *et al.* (2007) found statistically significantly elevated ORs for breast cancer among seamstresses/textile machine operators and tenders.

Sobel and Davanipour (1996c) measured ELF MF exposure from several home sewing machine models, both AC and DC models, to several parts of the body. The results are provided in Table 10. These results show that (1) high ELF MF exposure occurs to many parts of the body, (2) exposures vary by manufacturer, model, and even by machines of the same model, and (3) exposures depend on whether the machine operates by AC or DC current. For Alzheimer's disease and for breast cancer, it is not known where exposures may be most important. The peripheral Abeta hypothesis, if correct, would indicate that exposure to any location is important for AD. To affect pineal production of melatonin, it is not known whether exposure to the pineal gland is what is most important. For example, a majority of breast cancers causally lower pineal melatonin production. Because the melatonin production rebounds after excision of the tumor, the tumor itself must be secreting something that leads to the decline in melatonin production. Thus, it is conceivable that ELF MF exposure may, at least in some individuals, also lead to the peripheral production of something that also causes a lowering of melatonin production. It is also not known whether ELF MF exposure directly to the breast is etiologically important. Note that the right breast receives higher ELF MF exposure from home sewing machines. No studies of right versus left breast cancer and use of home sewing machines have been published.

B. Examples of Studies with 'Questionable' Seamstress Exposure Assessment:

Swedish and German Studies

Most of the Swedish studies on ELF MF and Alzheimer's disease/dementia or breast cancer (e.g., Forssén et al., 2000, 2004, 2005), Andel et al., 2010, Seidler et al., 2007, Feychting et al., 1998a) have relied on an occupational exposure assessment for seamstresses which significantly under-estimates exposure. For example:

- Seidler et al. (2007) uses governmental census categories which lumps seamstresses together with spinners, weavers, knitters, and dyers, all of whom probably have relatively low exposure. Maximum exposure in this occupational category is given as only1.5 mG, which is below the background levels for seamstresses working in factories.
- Forssén et al. (2004) created a job-exposure matrix for occupational ELF MF exposure among women working in the 49 most common or suspected high ELF MF ISCO job categories in Stockholm County using the Swedish 1980 census (Table 14). (ISCO stands for International Standard Classification of Occupations.) Five (5) to 24 subjects were selected in each of these occupations. Each or many of the ISCO job categories include several different occupations. Thus, workers from subgroups were selected. Sampled workers were instructed to wear their dosimeters for 24 hours and to make diary entries if they need to take off the dosimeter. Seamstresses are described as being rather uncommon in Stockholm County, except possibly for repair of clothing. This may account for the very low ELF MF exposure identified. Seamstresses are listed as having a geometric mean occupational exposure of only 1.7 mG. Only about 15% of their time was about 3 mG exposure. Cooks, kitchen maids, air stewardesses, hairdressers/beauticians all are listed as having greater exposure. Housekeeping service work had comparable exposure levels to seamstresses. As discussed in this report, the research by Davanipour, Sobel, and colleagues demonstrates that actual professional seamstresses have a very different exposure experience.

A re-analysis of the data in these studies with the job exposure classification scheme in the Davanipour & Sobel studies (Table 11) would be useful.

Note: The Kliukiene et al. study (2004) from Norway used a rather unique four division scale depending on how many hours of occupational exposure were above 1 mG per week and is thus not related to this discussion.]

Note: Qiu et al., 2004 exposure assessment problems has been discussed in Section D.3.4, above.

ACKNOWLEDGEMENT

Figure 1: Hypothesized Biological Pathway from ELF MF Exposure to AD Development (from Sobel & Davanipour, 1996a)

```
┌──────────────────────┐      ┌──────────────────────────────┐      ┌─────────────────────┐
│ Exposure to ELF MF   │ ───► │ Peripheral or Cerebral        │ ───► │ Increased           │
│                      │      │ Disruption of Cellular        │      │ Intracellular       │
│                      │      │ Calcium Ion Homeostasis       │      │ Calcium Ion         │
│                      │      │                               │      │ Concentration       │
└──────────────────────┘      └──────────────────────────────┘      └─────────────────────┘

┌──────────────────────┐      ┌──────────────────────────────┐
│ Increased Production │ ───► │ Increased Levels of Soluble   │
│ of Soluble Aβ        │      │ Aβ in Blood and/or Brain      │
└──────────────────────┘      └──────────────────────────────┘
                                (Blood Aβ)          (Brain Aβ)

┌──────────────────────────────┐      ┌──────────────────────────────┐
│ If Blood Levels of Soluble Aβ│ ───► │ Increased Brain Level of      │
│ Are Increased, Transportation│      │ Soluble Aβ Participates in    │
│ of Soluble Aβ to and through │      │ Initiation of a Cascade of    │
│ the Blood Brain Barrier      │      │ Events Leading to AD          │
│ perhaps by ApoE or ApoJ      │      │                               │
└──────────────────────────────┘      └──────────────────────────────┘
```
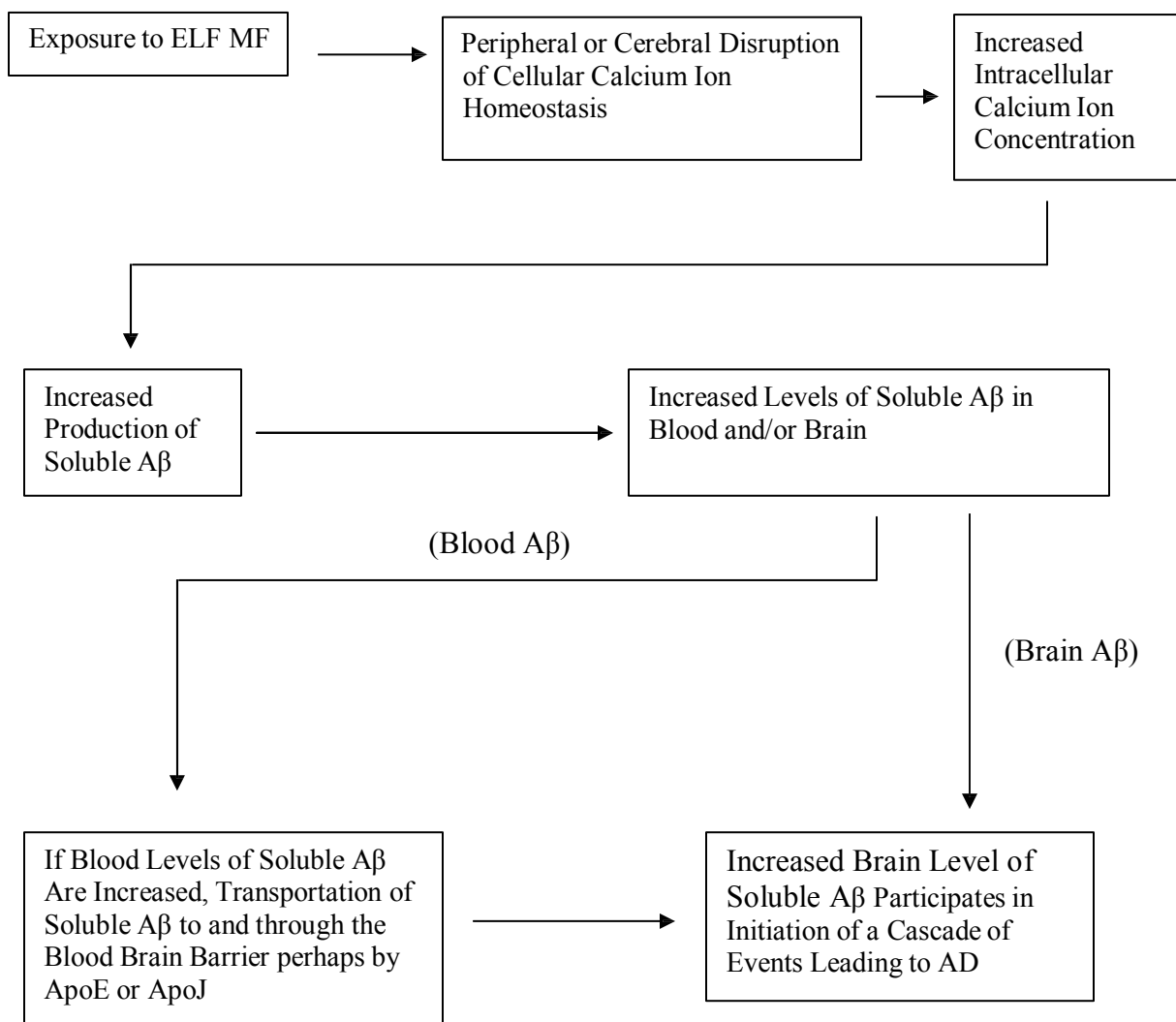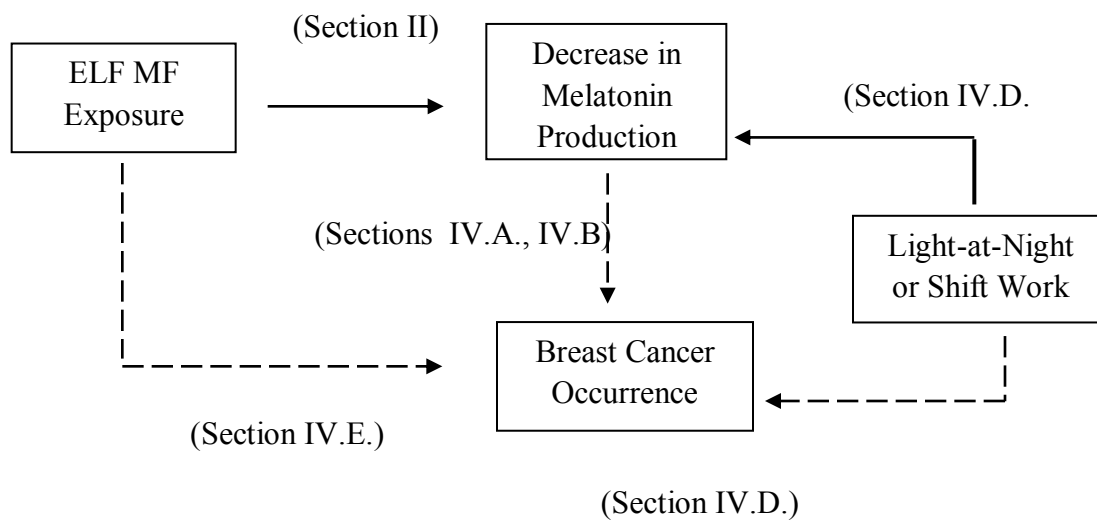
Figure 2: Outline of the Evidence that ELF MF Exposure Causes Breast Cancer through Decreases in Melatonin Production – with Section References

Note: Dashed lines indicate studies directly relating ELF MF exposure, light-at-night, or shift work to breast cancer occurrence.

Table 1: Baseline Data Results from the 1999 Mayeux *et al*. Paper: Means (Standard Deviation)

Variable	Cognitively Normal at Follow-Up	Developed AD (3.6 Year Average Follow-Up)
Sample Size (n)	105	64
Age	73.4 (5.3)	77.4 (5.9)[a]
Education	9.3 (4.6)	7.5 (3.8)[a]
$A\beta_{1-40}$ (pg/ml)	111.8 (44.1)	134.7 (46.4)[a]
$A\beta_{1-42}$ (pg/ml0	51.5 (42.0)	82.4 (68.8)[a]
$A\beta_{1-42}/ A\beta_{1-42}$	0.51 (0.41)	0.67 (0.56)[b]

Notes: Cognitively normal was determined at baseline by the global Cognitive Dementia Rating (CDR) scale with CDR=0 being normal. AD was diagnosed based on a CDR of 0.5 or 1.0, and clinical, functional and neuropsychological assessment as specified by the NINCDS-ADRDA criteria. [a] $p \leq 0.0001$; [b] $p < 0.05$.

Table 2:   Baseline Data Results from the 2003 Mayeux *et al*. Paper: Means (Standard Deviation)

Variable	Cognitively Normal At Follow-Up	Developed AD (Up to 10 Year Follow-Up)
Sample Size (n)	365	86
Age	75.5 (5.9)	79.3 (6.6)[a]
Education	9.0 (4.6)	6.8 (4.5)[a]
$A\beta_{1-40}$ (pg/ml)	133.3 (61.9)	136.2 (46.7)[c]
$A\beta_{1-42}$ (pg/ml)	58.8 (32.9)	76.5 (59.8)[b]
$A\beta_{1-42}/ A\beta_{1-42}$	0.48 (0.3)	0.61 (0.53)[b]

Notes: Cognitively normal was determined at baseline by the global Cognitive Dementia Rating (CDR) scale with CDR=0 being normal. AD was diagnosed based on a CDR of 0.5 or 1.0, and clinical, functional and neuropsychological assessment as specified by the NINCDS-ADRDA criteria. [a] $p \leq 0.001$; [b] $p < 0.05$; [c] Not Significant.

Table 3:   Post-Work Levels of Aβ1-40, Aβ1-42, Aβ1-42/Aβ1-42 by ELF MF exposure among Electrical Workers in the Noonan *et al*. (2002a) Study

ELF MF Exposure	$A\beta_{1-40}$ (pg/ml)	$A\beta_{1-42}$ (pg/ml)	$A\beta_{1-42}/A\beta_{1-42}$	Sample Size
< 0.5 mG	125	136	1.03	20
0.5 – 0.99 mG	137	163	1.11	25
1.0 – 1.99 mG	128	166	1.19	8
≥ 2.0 mG	156	262	1.46	7

Table 4:   Correlation (Corr) between Post-Work Creatinine-Adjusted aMT6s and Amyloid Beta by Number of Minutes between Samples in the Noonan *et al*. (2002a) Study

Number of Minutes	Sample Size	$A\beta_{1-42}$		$A\beta_{1-40}$		$A\beta_{1-42}/A\beta_{1-40}$	
		Corr	p-Value	Corr	p-Value	Corr	p-Value
All Subjects	60	-0.25	0.057	-0.19	0.144	-0.23	0.080
≤ 90	46	-0.30	0.047	-0.22	0.154	-0.27	0.080
≤ 60	37	-0.37	0.027	-0.25	0.150	-0.37	0.029
≤ 30	23	-0.43	0.054	-0.28	0.224	-0.42	0.059

———

Table 5:   Amyloid Beta Levels by Tertile of Post-Shift Creatinine-Adjusted aMT6s Levels in the Noonan *et al.* (2002a) Study

aMT6s/Cr Tertiles* (ng/mg)	$A\beta_{1-42}$		$A\beta_{1-40}$		$A\beta_{1-42}/ A\beta_{1-40}$	
	Mean**	95% CI	Mean**	95% CI	Mean**	95% CI
≤ 1.38	177	[112–258]	133	[111–156]	1.30	[0.86–1.74]
1.39–3.3	214	[120–334]	147	[125–170]	1.33	[0.85–1.90]
> 3.3	123	[58–180]	123	[108–139]	0.82	[0.49–1.26]

*   n=60 subjects in each tertile
** geometric mean averaged over the work shift

Table 6: Percentages of Subjects with Medium to High ELF MF Occupations Exposure

STUDY	CASES	CONTROLS
Sobel *et al.* (1995a)	9.3 %	3.4 %
Sobel *et al.* (1996b)	12.0 %	5.3 %
Davanipour *et al.* (2007)	7.4 %	3.8 %
Harmanci *et al.* (2003)	10.5 %	3.1 %
Feychting *et al.* (1998a)	43.0 %	23.0 % & 19.0 %[#]
Graves *et al.* (1999)	19.1 % & 21.4 %	21.4 % & 22.5 %[^]
Qiu *et al.* (2004)	28.2 %[*]	28.8 %[*]
	34.2 %[**]	42.7 %[**]

Cases & Controls Combined		
Feychting *et al.* (1998)	11.1 %	
Håkansson *et al.* (2003)	80.5 % - likely exposed engineering industry workers	
Johansen *et al.* (2000)	56 % - electrical company workers	
Savitz *et al.* (1998a)	electric utility cohort – percentage not supplied	
Savitz *et al.* (1998b)	23.9 %	

\# Two control groups;
^ Two industrial hygienists
* Based on estimated daily exposure in principal occupation;
** Based on estimated daily exposure in all occupations

Note: The Huss et al. (2009) study was longitudinal and the abstract for the Chang et al. (2004) study did not provide the percentages of cases or controls with high ELF MF exposure.

Table 7: **Odds Ratios for the ELF MF and AD Studies***

Study	Risk Estimate (OR)	95% CI	p-value
Sobel *et al.* (1995) (late-onset; L vs M/H)			
	3.0	1.6 – 5.4	< 0.001
Sobel *et al.* (1996b) (late-onset; L vs M/H)			
	3.9	1.5 – 10.6	0.006
Feychting *et al.* (1998) (mostly late-onset; last occupation; by control group)			
(exposure ≥ 2 mG)	2.4	0.8 – 6.9	--**
	2.7	0.9 – 7.8	--**
(exposure ≥ 5 mG)	4.1	0.7 – 23.5	--**
	8.3	1.1 – 62.7	--**
Graves *et al.* (1999) (late-onset; ever exposed)			
	0.95	0.4 – 2.4	--**
	0.74	0.3 – 2.4	--**
Harmanci *et al.* (2003) (late-onset; exposure as defined in Sobel *et al.* (1995, 1996b)			
	4.0	1.0 – 15.8	--**
Qiu *et al.* (2004) (age ≥ 75; exposure: ≥ 2 mG)			
Men	2.3	1.0 – 5.1	--**
Women	0.8	0.5 – 1.1	--**
Davanipour *et al.* (2007) (exposure as defined in Sobel *et al.* (1995, 1996b)			
M/H vs L	2.2	1.2 – 3.9	< 0.02
H vs L	2.7	0.8 – 9.1	< 0.11
Chang *et al.* (2004) (age: 66-102; exposure: "early exposure to magnetic fields")			
Exp vs No Exp	2.49	0.96 – 6.45	--**

* Studies use various types of controls and definitions of ELF MF exposure. See text.
** p-values were not provided.

Note: the Huss *et al.* (2009) study was longitudinal and is therefore not in this table.

Table 8: Mean ELF MF Exposures (mG) for Home Sewing Machines by Body Location: Continuous 2-Minute Measurements
(Sobel & Davanipour, 1996c)

Sewing Machine	Background	Head	Breast Left	Breast Right	Pelvic Area	Thigh Left	Thigh Right	Knee Left	Knee Right	Lower Right Arm	Right Hand	Foot Pedal
Alternating Current Machines (older machines)												
Bernina 811	0.6	18.6	5.6	12.9	26.9	11.7	90.1	8.9	13.5	251.1	57.0	86.1
Bernina 811	0.9	1.7	2.6	5.4	8.2	4.5	11.6	6.8	36.5	77.1	31.7	102.0
Bernina 817	0.6	8.4	9.6	23.5	41.9	19.1	30.6	9.2	35.4	724.6	135.6	NA
Bernina 817	1.2	12.1	14.2	33.9	51.0	10.3	588.5	8.8	125.7	753.0	132.4	NA
Brother 920D	0.7	2.4	2.1	2.3	1.1	1.3	1.5	1.9	2.3	8.5	16.0	6.2
Necchi Type 525	0.3	5.1	2.0	1.1	2.5	1.1	2.4	2.0	5.1	25.9	22.6	5.9
Sears Kenmore	0.2	1.2	1.9	4.9	5.5	2.2	5.3	2.5	15.8	26.0	17.9	13.8
Singer 625	0.3	4.6	3.6	5.6	5.5	3.9	6.6	6.4	17.2	...	...	...
Singer 5932	0.5	1.2	0.9	2.0	2.7	1.1	2.5	1.0	4.1	8.6	23.0	2.9
Singer 6212C	0.3	7.0	2.8	6.4	2.0	1.4	2.2	1.4	1.9	31.0	26.2	4.4
Viking Husqvarna 6020	0.8	1.5	1.3	1.5	2.7	1.4	2.0	3.1	9.1	5.9	24.9	62.3
White 1410	0.2	2.2	1.6	1.1	1.1	3.2	10.8	4.2	67.5	20.8	18.3	2.8
Direct Current Machines (newer machines)												
Bernina 1000	1.0	1.3	1.6	2.3	2.9	1.9	2.5	2.8	11.2	8.1	41.2	798.0
Bernina 1090S	1.0	1.2	1.6	1.6	1.7	1.2	1.3	1.5	7.7	3.3	22.9	1.0
Elna Diva 900	1.6	5.1	3.9	4.1	4.1	3.0	3.1	3.2	8.4	40.4	57.1	1.8
Singer 3317C	0.7	3.4	1.6	2.9	2.2	2.1	2.2	1.5	11.3	22.1	25.8	5.8
Singer 9015	0.7	2.5	1.9	3.3	4.9	1.7	4.3	2.1	26.2	7.0	28.9	2.3
Viking Husqvarna 500	1.0	3.7	2.7	5.0	3.9	1.8	2.8	2.7	13.8	24.9	39.4	1.1
Percent > 2.0 mG	0%	67%	50%	78%	83%	50%	89%	72%	94%	100%	100%	80%

Note: The Bernina 1000, Bernina 1090S, Elna Diva 900, Singer 3317C, Singer 9015 and Viking Husqvarna 500 were brand new. The Singer 5932, Singer 6212C, and Brother 920D were 3-10 years old. The Bernina 811 and 817 machines, the Sears Kenmore, the Singer 625 the Viking Husqvarna 6020 are probably at least 15 years old. Both the White and the Necchi are fairly old. NA = not applicable, i.e., there was no foot pedal. "..." = no measurements were taken, e.g., because of machine malfunction.

Table 9:   **Classification of Occupations in Forssén *et al.* (2005)**

Classification	Occupation	24-Hour Geometric Mean Average (mG)
High (≥ 3 mG)	Dental Nurse	3.0
	Air Stewardesses	3.0
	Cooks	3.1
	Working Proprietors Retail Trade	3.4 in
	Cashiers in Retail Stores and Restaurants	4.5
Medium-High (2 – 2.9 mG)	Computer Operators	2.0
	Motor Vehicle Drivers	2.0
	Shop Managers	2.1
	Shop Assistants	2.1
	Hairdressers/Beauticians	2.1
	Bank Clerks	2.2
	Kitchen Supervisors	2.4
	Post Office Clerks	2.5
	Waitresses in Restaurants and School Kitchens	2.5
	Kitchen Maids	2.8
Medium-Low (1 – 1.9 mG)	Registered Nurses	1.0
	System Analysts/Programmers	1.2
	Telephone Operators	1.5
	Radio & Television Assemblers and Repairwomen	
	Seamstresses	1.6

Table 10: Odds Ratio Estimates for Textile Occupations in the Forssén *et al.* (2005) Study

Comparison	OR	95% Confidence Interval
Textile Occupations vs Occupations with 24-Hour Exposure Below 1 mG	1.37	[1.11 , 1.68]
Textile Occupations vs All Other Occupations (Regardless of ELF MF Exposure)	1.33	[1.10 , 1.62]

Table 11:   Sobel-Davanipour Occupations Classified as Being Likely to Have Resulted in Medium or High ELF MF Exposure

Medium Exposure	High Exposure
Beautician	Cutter
Carpenter	Power Plant Operator
Clothes Inspector: Manufacturing Company	Repair Sewing Machines
Electric Lineman	Seamstress/Tailor
Electrician	Welder
Electronics Technician	
Electronic Assembler	
Equipment Repair	
Fabric Cutter	
Foam Cutter	
Forklift Operator	
Furniture Maker	
Machine Operator	
Machinery Repair	
Machinist (	
Newspaper Pressman	
Presser: Clothing Manufacturing Company	
Seamstress/Tailor – Part-Time	
Sheet Metal Machine Operator	
Shoemaker/Shoe Repairer	
Typist	
Upholstery; Re-Upholstery	
Welder - Parttime	
Wood Cutter; Machinery Repair - Forestry	
Wood Sander – Furniture	

VI.   REFERENCES

Antolin I, Rodriquez C, Sainz RM, Mayo JC, Uria H, Kotler ML, Rodriguez-Colunga MJ, Tolivia D, Menendez-Pelaez A. Neurohormone melatonin prevents cell damage: Effect on gene expression for antioxidant enzymes. FASEB J 1996;10:882-890.

Antolin I, Obst B, Burkhardt S, Hardeland R. Antioxidative protection in a high-melatonin organism: The dinoflagellate *Gonyaulax polydera* is rescued from lethal oxidative stress by strongly elevated, but physiologically possible, concentrations of melatonin. J Pineal Res 1997;23:182-190.

Arnetz BB, Berg M. Melatonin and adrenocorticotropic hormone levels in video display unit workers during work and leisure. J Occup Environ Med 1996;38:1108-1110.

Barlow-Walden LR, Reiter RJ, Abe A, Pablos M, Menendez-Pelaez A, Chen LD, Poeggeler B. Melatonin stimulated brain glutathione peroxidase activity. Neurochem Int 1995;26:497-502.

Bartsch C, Bartsch H, Karenovics A, Franz H, Peiker G, Mecke D. Nocturnal urinary 6-sulphatoxymelatonin excretion is decreased in primary breast cancer patients compared to age-matched controls and shows negative correlation with tumor-size. J Pineal Res 1997;23:53-58.

Blask DE, Belletier DB, Hill SM, Lemus-Wilson A, Grosso DS, Wilson ST, Wise ME. Pineal melatonin inhibition of tumor promotion in N-nitroso-N-methylurea model of mammary carcinogenesis: Potential involvement of antiestrogenic mechanisms in vivo. J Cancer Research Clinical Oncology 1991;117:526-532.

Blickenstaff RT, Brandstadter SM, Reddy S, Witt R. Potential radioprotective agents. 1: Homologues of melatonin. J Pharm Sci 1994;83:216-218.

Brainard GC, Kavet R, Kheifets LI. The relationship between electromagnetic field and light exposures to melatonin and breast cancer risk: A review of the relevant literature. J Pineal Res 1999;26:65-100.

Bromme HJ, Morke W, Peschke D, Ebelt H, Peschke D. Scavenging effect of melatonin on hydroxyl radicals generated by alloxan. J Pineal Res 2000;29:201-208.

Bubenik GA. Localization, physiological significance and possible clinical implication of gastrointestinal melatonin. Biol Signals Recept 2001;10:350-366.

Burch JB, Reif JSA, Pitrat CA, Keefe TJ, Yost MG. Melatonin levels in electric utility workers (Abstract). 18th Bioelectromagnetics Society Meeting, Victoria, Canada, 1996, pp. 95-96.

Burch JB, Reif JSA, Yost MG, Keefe TJ, Pitrat CA. Nocturnal excretion of a urinary melatonin metabolite among electric utility workers. Scand J Work Environ Health 1998;24:183-189.

Burch JB, Reif JS, Yost MG, Keefe TJ, Pitrat CA. Reduced excretion of a melatonin metabolite in workers exposed to 60 Hz magnetic fields. Am J Epidemiol 1999;150:27-36.

Burch JB, Reif JS, Noonan CW, Yost MG. Melatonin metabolite levels in workers exposed to 60-Hz magnetic fields: Work in substations and with 3-phase conductors. J Occup Environ Med 2000;42:136-142.

Burch JB, Reif JS, Noonan CW, Ichinose T, Bachand AM, Koleber TL, Yost MG. Melatonin metabolite excretion among cellular telephone users. Int J Radiat Biol 2002;78:1029-1036.

Cantor KP, Dosemeci M, Brinton LA, Stewart PA. RE: Breast cancer mortality among female electrical workers in the United States. (Letter). J Natl Cancer Inst 1995;87:227-228.

Cardinali DP, Furio AM, Reyes MP. Clinical perspectives for the use of melatonin as a chronobiotic and cytoprotective agent. Ann N Y Acad Sci 2005;1057:327-336.

Cheng X, van Breemen RB. Mass spectrometry-based screening for inhibitors of β-amyloid protein aggregation. Anal Chem 2005;77:7012-7015.

Cheng Y, Feng Z, Zhang QZ, Zhang JT. Beneficial effects of melatonin in experimental models of Alzheimer disease. Acta Pharmacol Sin 2006;27:129-139.

Clapp-Lilly KL, Smith MA, Perry G, Harris PL, Zhu X, Duffy LK. Melatonin acts as antioxidant and pro-oxidant in an organotypic slice culture model of Alzheimer's disease. Neuroreport 2001a;12:1277-1280.

Clapp-Lilly KL, Smith MA, Perry G, Duffy LK. Melatonin reduces interleukin secretion in amyloid-beta stressed mouse brain slices. Chem Biol Interact 2001b;134:101-107.

Cohen M, Lippman M, Chabner B. Role of pineal gland in aetiology and treatment of breast cancer. Lancet 1978;2:814-816.

Cook MR, Graham C, Kavet R, Stevens RG, Davis S, Kheifets L. Morning urinary assessment of nocturnal melatonin secretion in older women. J Pineal Res 2000;28:41-47.

Davanipour Z, Tseng C-C, Lee P-J, Sobel E. A Case-Control Study of Occupational Magnetic Field Exposure and Alzheimer's Disease: Results from the California Alzheimer's Disease Diagnosis and Treatment Centers. BMC Neurol 2007;7:13 (URL: http://www.biomedcentral.com/1471-2377/7/13).

Davis S, Kaune WT, Mirick DK, Chen C, Stevens RG. Residential magnetic fields, light-at-night, and nocturnal urinary 6-sulfatoxymelatonin concentration in women. Am J Epidemiol 2001a;154:591-600.

Davis S, Mirick DK, Stevens RG. Night shift work, light at night, and risk of breast cancer. J Natl Cancer Inst 2001b;93:1557-1562.

Davis S, Mirick DK, Stevens RG Residential magnetic fields and the risk of breast cancer. <u>Am J Epidemiol</u> 2002;155:446-454.

Davis S, Mirick DK, Chen C, Stanczyk FZ. Effects of 60-Hz magnetic field exposure on nocturnal 6-sulfatoxymelatonin, estrogens, luteinizing hormone, and follicle-stimulating hormone in healthy reproductive-age women: results of a crossover trial. <u>Ann Epidemiol</u> 2006;16:622-631.

Deadman JE, Infante-Rivard C. Individual estimation of exposures to extremely low frequency magnetic fields in jobs commonly held by women. <u>Am J Epidemiol</u> 2002;155:368-378.

Del Giudice E, Facchinetti F, Nofrate V, Boccaccio P, Minelli T, Dam M, Leon A, Moschini G. Fifty Hertz electromagnetic field exposure stimulates secretion of beta-amyloid peptide in cultured human neuroglioma. <u>Neurosci Lett</u> 2007 Mar 1; [Epub ahead of print]

Eck-Enriquez K, Kiefer TL, Spriggs LL, Hill SM. Pathways through which a regimen of melatonin and retinoic acid induces apoptosis in MCF-7 human breast cancer cells. <u>Breast Cancer Res Treat</u> 2000;61:229-239.

Feng Z, Chang Y, Cheng Y, Zhang BL, Qu ZW, Qin C, Zhang JT. Melatonin alleviates behavioral deficits associated with apoptosis and cholinergic system dysfunction in the APP 695 transgenic mouse model of Alzheimer's disease. <u>J Pineal Res</u> 2004;37:129-136.

Feng Z, Qin C, Chang Y, Zhang JT. Early melatonin supplementation alleviates oxidative stress in a transgenic mouse model of Alzheimer's disease. <u>Free Radic Biol Med</u> 2006;40:101-109.

Feychting M, Pedersen NL, Svedberg P, Floderus B, Gatz M. Dementia and occupational exposure to magnetic fields. <u>Scand J Work Environ Health</u> 1998a;24:46-53.

Feychting M, Forssén U, Rutqvist LE, Ahlbom A. Magnetic fields and breast cancer in Swedish adults residing near high-voltage power lines. <u>Epidemiology</u> 1998b;9:392-397.

Feychting M, Jonsson F, Pedersen NL, Ahlbom A. Occupational magnetic field exposure and neurodegenerative disease. <u>Epidemiology</u> 2003;14:413-419.

Feychting M, Forssén U. Electromagnetic fields and female breast cancer. <u>Cancer Causes Control</u> 2006;17:553-558.

Forssén UM, Feychting M, Rutqvist LE, Floderus B, Ahlbom A. Occupational and residential magnetic field exposure and breast cancer in females. <u>Epidemiology</u> 2000;11:24-29.

Forssén UM, Mezei G, Nise G, Feychting M. Occupational magnetic field exposure among women in Stockholm County, Sweden. <u>Occup Environ Med</u> 2004;61:594-602.

Forssén UM, Rutqvist LE, Ahlbom A, Feychting M. Occupational magnetic fields and female breast cancer: a case-control study using Swedish population registers and new exposure data. Am J Epidemiol 2005;161:250-259.

Gobba F, Bravo G, Scaringi M, Roccatto L. No association between occupational exposure to ELF magnetic field and urinary 6-sulfatoxymelatonin in workers. Bioelectromagnetics 2006;27:667-673.

Graham C, Cook MR, Riffle DW, Gerkovich MM, Cohen. Nocturnal melatonin levels in human volunteers exposed to intermittent 60 Hz magnetic fields. Bioelectromagnetics 1996;17:263-273.

Graham C, Cook MR, Riffle DW. Human melatonin during continuous magnetic field exposure. Bioelectromagnetics 1997;18:166-171.

Graham C, Cook MR, Kavet R, Sastre A, Smith DK. Prediction of nocturnal plasma melatonin from morning urinary measures. J Pineal Res 1998;24:230-238. Erratum in: J Pineal Res 1999;26:128.

Graves AB, Rosner D, Echeverria D, Mortimer JA, Larson EB. Occupational exposure to electromagnetic fields and Alzheimer disease. Alzheimer Dis Assoc Disord 1999;13:165-170.

Håkansson N, Gustavsson P, Johansen C. Floderus B. Neurodegenerative disease in welders and other workers exposed to high levels of magnetic fields. Epidemiology 2003;14:420-426.

Halliwell B, Gutteridge JM. Oxygen free radicals and iron in relation to biology and medicine: Some problems and concepts. Arch Biochem Biophys 1986;246:501-514.

Halliwell B. Can oxidative DNA damage be used as a biomarker of cancer risk in humans? Problems, resolutions and preliminary results from nutritional supplementation studies. Free Rad Res 1998;29:469-486.

Hansen NH, Sobel E, Davanipour Z, Gillette LM, Wilson BW, Niiranen J.   EMF exposure assessment in the Finnish garment industry: Evaluation of proposed EMF exposure metrics. Bioelectromagnetics 2000;21:57-67.

Hansen J. Increased breast cancer risk among women who work predominantly at night. Epidemiology 2001;12:74-77.

Hardeland R, Blazer I, Poeggeler B, Fuhrberg B, Uria H, Behrmann G, Wolf R, Meyer TJ, Reiter RJ. On the primary functions of melatonin in evolution: Mediation of photoperiodic signals in a unicell, photooxidation and scavenging of free radicals. J Pineal Res 1995;18:104-111.

Harmanci H, Emre M, Gurvit H, Bilgic B, Hanagasi H, Gurol E, Sahin H, Tinaz S. Risk Factors for Alzheimer Disease: A Population-Based Case-Control Study in Istanbul, Turkey. Alzheimer Dis Assoc Disord 2003;17:139-145.

Hrushesky WJM, Blask DE. Re: Melatonin and breast cancer: A prospective study. (Letter) J Natl Cancer Inst 2004;96: 888-889.

Jang MH, Jung SB, Lee MH, Kim CJ, Oh YT, Kang I, Kim J, Kim EH. Melatonin attenuates amyloid beta25-35-induced apoptosis in mouse microglial BV2 cells. Neurosci Lett 2005;380:26-31.

Johansen C. Exposure to elecromagnetic fields and risk of central nervous system disease in utility workers. Epidemiology 2000;11:539-543.

Juutilainen J, Stevens RG, Anderson LE, Hansen NH, Kilpelainen M, Kumlin T, Laitinen JT, Sobel E, Wilson BW. Nocturnal 6-hydroxymelatonin sulfate excretion in female workers exposed to magnetic fields. J Pineal Res 2000;28:97-104.

Juutilainen J, Kumlin T. Occupational magnetic field exposure and melatonin: Interaction with light-at-night. Bioelectromagnetics 2006;27:423-426.

Karbownik M, Reiter RJ, Qi W, Garcia JJ, Tan DX, Manchester LC, Vijayalaxmi. Protective effects of melatonin against oxidation of guanine bases in DNA and decreased microsomal membrane fluidity in rat liver induced by whole body ionizing radiation. Mol Cell Biochem 2000;211:137-144.

Karbownik M, Reiter RJ, Cabrera J, Garcia JJ. Comparison of the protective effect of melatonin with other antioxidants in the hamster kidney model of estradiol-induced DNA damage. Mutat Res 2001;474:87-92.

Kaune WT, Davis S, Stevens RG. Relation between residential magnetic fields, light at night, and nocturnal urine melatonin levels in women. Vol. 1: Background and purpose, methods, results, discussion. Report TR-107242-V1. Electric Power Research Institute, Palo Alto, 1997a.

Kaune WT, Davis S, Stevens RG. Relation between residential magnetic fields, light at night, and nocturnal urine melatonin levels in women. Vol. 2: Magnetic Field Exposure Analysis. Final Report TR-107242-V2. Electric Power Research Institute, Palo Alto, 1997b.

Kelsh MA, Bracken TD, Sahl JD, Shum M, Ebi KL. Occupational magnetic field exposures of garment workers: results of personal and survey measurements. Bioelectromagnetics 2003;24:316-326.

Kliukiene J, Tynes T, Martinsen JI, Blaasaas KG, Andersen A. Incidence of breast cancer in a Norwegian cohort of women with potential workplace exposure to 50 Hz magnetic fields. Am J Ind Med 1999;36:147-154.

Kliukiene J, Tynes T, Andersen A. Follow-up of radio and telegraph operators with exposure to electromagnetic fields and risk of breast cancer. Eur J Cancer Prev 2003;12:301-307.

Kliukiene J, Tynes T, Andersen A. Residential and occupational exposures to 50-Hz magnetic fields and breast cancer in women: a population-based study. Am J Epidemiol 2004;159:852-861.

Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, Bergstrom M, Savitcheva I, Huang GF, Estrada S, Ausen B, Debnath ML, Barletta J, Price JC, Sandell J, Lopresti BJ, Wall A, Koivisto P, Antoni G, Mathis CA, Langstrom B. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. Ann Neurol 2004;55:306-319.

Kothari LS, Shah PN, Mhatre MC. Pineal ablation in varying photoperiods and the incidence of 9,10-dimethyl-1,2-benzathracene induced mammary cancer in rats. Cancer Lett 1984;22:99-102.

Kotler M, Rodriguez C, Sainz RM, Antolin I, Menendez-Pelaez A. Melatonin increases gene expression for antioxidant enzymes in rat brain cortex. J Pineal Res 1998;24:83-89.

Labréche F, Goldberg MS. Exposure to organic solvents and breast cancer in women. Am J Ind Med 1997;32:1-14.

Labréche F, Goldberg MS, Valois MF, Nadon L, Richardson L, Lakhani R, Latreille B. Occupational exposures to extremely low frequency magnetic fields and postmenopausal breast cancer. Am J Ind Med 2003;44:643-652.

Lahiri DK, Chen D, Ge YW, Bondy SC, Sharman EH. Dietary supplementation with melatonin reduces levels of amyloid beta-peptides in the murine cerebral cortex. J Pineal Res 2004;36:224-231.

Levallois P, Dumont M, Touitou Y, Gingras S, Mâsse B, Gauvin D, Kröger E, Bourdages M, Douville P. Effects of electric and magnetic fields from high-power lines on female urinary excretion of 6-sulfatoxymelatonin. Am J Epidemiol 2001;154:601-609.

Lewy AJ, Wehr TA, Goodwin FK, Newsome DA, Markey SP. Light suppresses melatonin secretion in humans. Science 1980;210:1267-1269.

Lie JA, Roessink J, Kjaerheim K. Breast cancer and night work among Norwegian nurses. Cancer Causes Control 2006;17:39-44.

London SJ, Pogoda JM, Hwang KL, Langholz B, Monroe KR, Kolonel LN, Kaune WT, Peters JM, Henderson BE. Residential magnetic field exposure and breast cancer risk: a nested case-control study from a multiethnic cohort in Los Angeles County, California. Am J Epidemiol 2003;158:969-980.

Loomis DP, Savitz DA, Ananth CV. Breast cancer mortality among female electrical workers in the United States. J Natl Cancer Inst 1994;86:921-925.

Lowden A, Akerstedt T, Wibom R. Suppression of sleepiness and melatonin by bright light exposure during breaks in night work. J Sleep Res 2004;13:37-43.

Maestroni GJM. Therapeutic potential of melatonin in immunodeficiency states, viral diseases, and cancer. In: (eds.) Heuther G, Kochen W, Simat TJ, Steinhart H (eds),   Tryptophan, Serotonin, and Melatonin: Basic Aspects and Applications, Kluwer Academic/Plenum Publishers, New York, 217-226, 1999.

Matsubara E, Bryant-Thomas T, Pacheco Quinto J, Henry TL, Poeggeler B, Herbert D, Cruz-Sanchez F, Chyan YJ, Smith MA, Perry G, Shoji M, Abe K, Leone A, Grundke-Ikbal I, Wilson GL, Ghiso J, Williams C, Refolo LM, Pappolla MA, Chain DG, Neria E. Melatonin increases survival and inhibits oxidative and amyloid pathology in a transgenic model of Alzheimer's disease. J Neurochem 2003;85:1101-1108. Erratum in: J Neurochem 2003;86:1312.

Mayeux R, Tang M-X, Jacobs DM, Manly J, Bell K, Merchant C, Small SA, Stern Y, Wisniewski HM, Mehta PD: Plasma amyloid beta-peptide 1-42 and incipient Alzheimer's disease. Ann Neurol 1999;46;412-416.

Mayeux R, Honig LS, Tang M-X, Manly J, Stern Y, Schupf N, Mehta PD. Plasma A[beta]40 and A[beta]42 and Alzheimer's disease: relation to age, mortality, and risk. Neurology 2003;61:1185-1190.

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939-944.

Melchiorri D, Reiter RJ, Attia AM, Hara M, Burgos A, Nistico G. Potent protective effect of melatonin on in vivo paraquat-induced oxidative damage in rats. Life Sci 1995;56:83-89.

Milham S, Hatfield JB, Tell R. Magnetic fields from steel-belted radial tires: implications for epidemiologic studies. Bioelectromagnetics 1999;20:440-445.

Montilla P, Tunez I, Munoz MC, Soria JV, Lopez A. Antioxidative effect of melatonin in rat brain oxidative stress induced by Adriamycin. Rev Esp Fisiol 1997;53:301-305.

Noonan C, Reif J, Burch J, Ichinose TY, Yost MG, Magnusson K: Relationship between amyloid β protein and melatonin metabolite in a study of electric utility workers. J Occup Environ Med 2002a;44:769-775.

Noonan CW, Reif JS, Yost M, Touchstone J. Occupational exposure to magnetic fields in case-referent studies of neurodegenerative diseases. Scand J Work Environ Health 2002b;28:42-48.

O'Leary ES, Schoenfeld ER, Stevens RG, Kabat GC, Henderson K, Grimson R, Gammon MD, Leske MC; Electromagnetic Fields and Breast Cancer on Long Island Study Group. Shift work, light at night, and breast cancer on Long Island, New York. Am J Epidemiol 2006;164:358-366.

Pablos MI, Chuang JI, Reiter JR, Ortiz GG,, Daniels WM, Sewerynek E, Melchiorri D, Poeggeler B. Time course of melatonin-induced increase in glutathione peroxidase activity in chick tissues. Biol Signals 1995;4:325-330.

Pappolla MA, Sos M, Omar RA, Bick RJ, Hickson-Bick DL, Reiter RJ, Efthimiopoulos S, Robakis NK. Melatonin prevents death of neuroblastoma cells exposed to the Alzheimer amyloid peptide. J Neurosci 1997;**17**:1683-1690.

Pappolla M, Bozner P, Soto C, Shao H, Robakis NK, Zagorski M, Frangione B, Ghiso J. Inhibition of Alzheimer beta-fibrillogenesis by melatonin. J Biol Chem 1998;273:7185-7188.

Pappolla MA, Chyan YJ, Poeggeler B, Bozner P, Ghiso J, LeDoux SP, Wilson GL. Alzheimer beta protein mediated oxidative damage of mitochondrial DNA: prevention by melatonin. J Pineal Res 1999;27:226-229.

Pappolla MA, Chyan YJ, Poeggeler B, Frangione B, Wilson G, Ghiso J, Reiter RJ. An assessment of the antioxidant and the antiamyloidogenic properties of melatonin: implications for Alzheimer's disease. J Neural Transm 2000;107:203-231.

Pappolla MA, Simovich MJ, Bryant-Thomas T, Chyan YJ, Poeggeler B, Dubocovich M, Bick R, Perry G, Cruz-Sanchez F, Smith MA. The neuroprotective activities of melatonin against the Alzheimer beta-protein are not mediated by melatonin membrane receptors. J Pineal Res 2002;32:135-142.

Park RM, Schulte PA, Bowman JD, Walker JT, Bondy SC, Yost MG, Touchstone JA, Dosemeci M. Potential occupational risks for neurodegenerative diseases. Am J Ind Med 2005;48:63-77.

Peplonska B, Stewart P, Szeszenia-Dabrowska N, Rusiecki J, Garcia-Closas M, Lissowska J, Bardin-Mikolajczak A, Zatonski W, Gromiec J, Brzeznicki S, Brinton LA, Blair A. Occupation and breast cancer risk in Polish women: a population-based case-control study. Am J Ind Med 2007;50:97-111.

Pfluger DH, Minder CE. Effects of exposure to 16.7 Hz magnetic fields on urinary 6-hydroxymelatonin sulfate excretion of Swiss railway workers. J Pineal Res 1996;21:91-100.

Pieri C, Marra M, Moroni F, Recchioni R, Marcheselli F. Melatonin: A peroxyl radical scavenger more effective than vitamin E. Life Sci 1994;55:PL271-276.

Poeggeler B, Miravalle L, Zagorski MG, Wisniewski T, Chyan YJ, Zhang Y, Shao H, Bryant-Thomas T, Vidal R, Frangione B, Ghiso J, Pappolla MA. Melatonin reverses the profibrillogenic activity of apolipoprotein E4 on the Alzheimer Amyloid Aβ peptide. Biochemistry 2001;40:14995-15001.

Qiu C, Fratiglioni L, Karp A, Winblad B, Bellander T. Occupational exposure to electromagnetic fields and risk of Alzheimer's disease. Epidemiology 2004;15:687-694.

Quinn J, Kulhanek D, Nowlin J, Jones R, Pratico D, Rokach J, Stackman R. Chronic melatonin therapy fails to alter amyloid burden or oxidative damage in old Tg2576 mice: implications for clinical trials. Brain Res 2005;1037:209-213.

Reiter RJ. Pineal melatonin: Cell biology of its synthesis and of its physiological interactions. Endocr Rev 1991;12:151-158.

Reiter RJ, Melchiorri D, Sewerynek E, Poeggeler B, Barlow-Walden L, Chuang J, Ortiz GG, Acuna-Castroviejo D.. A review of the evidence supporting melatonin's role as an antioxidant. J Pineal Res 1995;18:1-11.

Reiter RJ, Tang L, Garcia JJ, Munoz-Hoyos A. Pharmacological actions of melatonin in oxygen radical pathophysiology. Life Sci 1997;60:2255-2271.

Reiter RJ, Tan D-X, Qi W, Manchester LC, Karbownik M, Calvo JR. Pharmacology and physiology of melatonin in the reduction of oxidative stress in vivo. Biol Signals Recept 2000;9:160-171.

Reiter RJ, Tan DX, Manchester LC, Qi W. Biochemical reactivity of melatonin with reactive oxygen and nitrogen species: A review of the evidence. Cell Biochem Biophys 2001a;34:237-256.

Reiter RJ, Acuna-Castroviejo D, Tan DX, Burkhardt S. Free radical-mediated molecular damage: Mechanisms for the protective actions of melatonin in the central nervous system. Ann NY Acad Sci 2001b;939:200-215.

Reiter RJ, Tan DX, Burkhardt S. Reactive oxygen and nitrogen species and cellular and organismal decline; Amelioration with melatonin. Mech Ageing Dev 2002:123:1007-1019.

Rosales-Corral S, Tan DX, Reiter RJ, Valdivia-Velazquez M, Martinez-Barboza G, Acosta-Martinez JP, Ortiz GG. Orally administered melatonin reduces oxidative stress and proinflammatory cytokines induced by amyloid-beta peptide in rat brain: a comparative, in vivo study versus vitamin C and E. J Pineal Res 2003;35:80-84.

Santini R, Messagier R, Claustrat B, Fillion-Robin M, Youbicier-Simo BJ. Video screen exposure and 6-sulfatoxymelatonin urinary excretion in women. Pathol Biol (Paris) 2003;51:143-146 (French).

Savitz DA, Checkoway H, Loomis DP. Magnetic field exposure and neurodegenerative disease mortality among electric utility workers. Epidemiology 1998a;9:398-404.

Savitz DA, Loomis DP, Tse CK. Electrical occupations and neurodegenerative disease: Analysis of U.S. mortality data. Arch Environ Health 1998b;53:71-74.

Schernhammer ES, Laden F, Speizer FE, Willett WC, Hunter DJ, Kawachi I, Colditz GA. Rotating night shifts and risk of breast cancer in women participating in the nurses' health study. J Natl Cancer Inst 2001;93:1563-1568.

Schernhammer ES, Laden F, Speizer FE, Willett WC, Hunter DJ, Kawachi I, Fuchs CS, Colditz GA. Night-shift work and risk of colorectal cancer in the nurses' health study. J Natl Cancer Inst 2003;95:825-828.

Schernhammer ES, Rosner B, Willett WC, Laden F, Colditz GA, Hankinson SE. Epidemiology of urinary melatonin in women and its relation to other hormones and night work. *Cancer Epidemiol Biomarkers Prev* 2004;13:936-943.

Schernhammer ES, Hankinson SE. Urinary melatonin levels and breast cancer risk. J Natl Cancer Inst 2005;97:1084-1087.

Schoenfeld ER, O'Leary ES, Henderson K, Grimson R, Kabat GC, Ahnn S, Kaune WT, Gammon MD, Leske MC; EBCLIS Group. Electromagnetic fields and breast cancer on Long Island: a case-control study. Am J Epidemiol 2003;158:47-58.

Shah PN, Mhatre MC, Kothari LS. Effect of melatonin on mammary carcinogenesis in intact and pinealectomized rates in varying photoperiods. Cancer Res 1984;44:3403-3407.

Shen YX, Wei W, Yang J, Liu C, Dong C, Xu SY. Improvement of melatonin to the learning and memory impairment induced by amyloid beta-peptide 25 - 35 in elder rats. Acta Pharmacol Sin 2001;22:797-803.

Shen YX, Wei W, Xu SY. Protective effects of melatonin on cortico-hippocampal neurotoxicity induced by amyloid beta-peptide 25-35. Acta Pharmacol Sin 2002a;23:71-76.

Shen YX, Xu SY, Wei W, Sun XX, Liu LH, Yang J, Dong C. The protective effects of melatonin from oxidative damage induced by amyloid beta-peptide 25-35 in middle-aged rats. J Pineal Res 2002b;32:85-89.

Siemiatycki J, Nadon L, Lakhani R, Bégin D, Gérin M. Exposure assessment. In: Siemiatycki J, editor. Risk Factors for Cancer in the Workplace. Boca Raton, FL: CRC Press, pp. 45-114, 1991.

Siemiatycki J, Fritschi, L, Nadon L, Gérin M. Reliability of an expert rating procedure retrospective assessment of occupational exposures for community-based case-control studies. Am J Ind Med 1997;31:280-286.

Sizonenko PC. Normal sexual maturation. Pediatrician. 1987;14:191-201.

Skribanek Z, Balaspiri L, Mak M. Interaction between synthetic amyloid-beta-peptide (1-40) and its aggregation inhibitors studied by electrospray ionization mass spectrometry. J Mass Spectrom 2001;36:1226-1229.

Small GW, Kepe V, Ercoli LM, Siddarth P, Bookheimer SY, Miller KJ, Lavretsky H, Burggren AC, Cole GM, Vinters HV, Thompson PM, Huang SC, Satyamurthy N, Phelps ME, Barrio JR. PET of brain amyloid and tau in mild cognitive impairment. N Engl J Med 2006;355:2652-2663.

Sobel E, Davanipour Z, Sulkava R, Erkinjuntti T, Wikstrom J, Henderson VW, Buckwalter G, Bowman JD, Lee PJ. Occupations with exposure to electromagnetic fields: a possible risk factor for Alzheimer's disease. Am J Epidemiol 1995;142:515-524.

Sobel E, Davanipour Z. Electromagnetic field exposure may cause increased production of amyloid beta and eventually lead to Alzheimer's disease. Neurology 1996a;47:1594-1600.

Sobel E, Dunn M, Davanipour Z, Qian Z, Chui HC. Elevated risk of Alzheimer's disease among workers with likely electromagnetic field exposure. Neurology 1996b;47:1477-1481.

Sobel E, Davanipour Z. Preliminary Investigation into E*MF* Exposures Resulting from the Use of Home Sewing Machines: Final Report. Funded by the RAPID Program, Department of Energy, 1996c.

Srinivasan V, Pandi-Perumal S, Cardinali D, Poeggeler B, Hardeland R. Melatonin in Alzheimer's disease and other neurodegenerative disorders. Behav Brain Funct 2006;2:15 (doi:10.1186/1744-9081-2-15).

Stasica P, Ulanski P, Rosiak JM. Melatonin as a hydroxyl radical scavenger. J Radioanal Nucl Chem 1998;232:107-113.

Subramanian A, Kothari L. Suppressive effect by melatonin on different phases of 9,10-dimethyl-1,2-benzanthracene (DMBA)-induced rat mammary gland carcinogenesis. Anticancer Drugs 1991a;2:297-303.

Subramanian A, Kothari L. Melatonin, a suppressor of spontaneous murine mammary tumors. J Pineal Res 1991b;10:136-140.

Szabó J, Mezai K, Thuróczy G, Mezei G. Occupational 50 Hz magnetic field exposure measurements among female sewing machine operators in Hungary. Bioelectromagnetics 2006;27:451-457.

Tamarkin L, Cohen M, Roselle D, Reichert C, Lippman M, Chabner B. Melatonin inhibition and pinealectomy enhancement of 7,12-dimethylbenz(a)anthracene-induced mammary tumors in the rat. Cancer Res 1981;41:4432-4436.

Tan DX, Chen LD, Poeggeler B, Manchester LC, Reiter RJ. Melatonin: A potent, endogenous hydroxyl radical scavenger. Endocrine J 1993a;1:57-60.

Tan DX, Poeggeler B, Reiter RJ, Chen LD, Chen S, Manchester LC, Barlow-Walden LR. The pineal hormone melatonin inhibits DNA-adduct formation induced by the chemical carcinogen safrole in vivo. Cancer Lett 1993b;70:65-71.

Tan DX, Reiter RJ, Chen LD, Poeggeler B, Manchester LC, Barlow-Walden LR. Both physiological and pharmacological levels of melatonin reduce DNA adduct formation induced by the carcinogen safrole. Carcinogenesis 1994;15:215-218.

Tan DX, Manchester LC, Reiter RJ, Plummer BF. Cyclic 3-hydroxymelatonin: A melatonin metabolite generated as a result of hydroxyl radical scavenging. Biol Signals Recept 1999;8:70-74.

Tan DX, Manchester LC, Reiter RJ, Qi WB, Karbownik M, Calvo JR. Significance of melatonin in antioxidant defense system: Reactions and products. Biol Signals Recept 2000;9:137-159.

Tan DX, Reiter RJ, Manchester LC, Yan MT, El-Sawi M, Sainz RM, Mayo JC, Kohen R, Allegra M, Hardeland R. Chemical and physical properties and potential mechanisms: Melatonin as a broad spectrum antioxidant and free radical scavenger. Curr Top Med Chem 2002;2:181-197.

Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ. One molecule, many derivatives: A never-ending interaction of melatonin with reactive oxygen and nitrogen species? (Mini Review) J Pineal Res 2007;42:28-42.

Tang F, Nag S, Shiu SY, Pang SF. The effects of melatonin and Ginkgo biloba extract on memory loss and choline acetyltransferase activities in the brain of rats infused intracerebroventricularly with beta-amyloid 1-40. Life Sci 2002;71:2625-2631.

Teplitzky SR, Kiefer TL, Cheng Q, Dwivedi PD, Moroz K, Myers L, Anderson MB, Collins A, Dai J, Yuan L, Spriggs LL, Blask DE, Hill SM. Chemoprevention of NMU-induced rat mammary carcinoma with the combination of melatonin and 9-cis-retinoic acid. Cancer Lett 2001;26:155-163.

Touitou Y, Lambrozo J, Camus F, Charbuy H. Magnetic fields and the melatonin hypothesis: a study of workers chronically exposed to 50-Hz magnetic fields. Am J Physiol Regul Integr Comp Physiol 2003;284:R1529-1535.

Travis RC, Allen DS, Fentiman IS, Key TJ. Melatonin and breast cancer: A prospective study. J Natl Cancer Inst 2004;96:375-482.

Tynes T, Hannevik M, Andersen A, Vistnes AI, Haldorsen T. Incidence of breast cancer in Norwegian female radio and telegraph operators. Cancer Causes Control 1996;7:194-204.

Vijayalaxmi, Reiter RJ, Meltz ML. Melatonin protects human blood lymphocytes from radiation-induced chromosome damage. Mutat Res 1995;346:23-31.

Vijayalaxmi, Reiter RJ, Herman TS, Meltz ML. Melatonin and radioprotection from genetic damage: DNA damage in human blood lymphocytes. Mutat Res 1996;371:221-228.

Vijayalaxmi, Reiter RJ, Meltz ML, Herman TS. Melatonin: Possible mechanisms involved in its "radioprotective" effect. Mutat Res 1998;404:187-189.

Vijayalaxmi, Meltz ML, Reiter RJ, Herman TS, Kumar KS. Melatonin and protection from whole-body irradiation: Survival studies in mice. Mutat Res 1999;425:21-27.

Vijayalaxmi, Meltz ML, Reiter RJ, Herman TS, Kumar KS. Melatonin and protection from whole-body irradiation: Survival studies in mice. Mutat Res 1999;425:21-27.

Vijayalaxmi, Thomas CR, Reiter RJ, Herman TS. Melatonin: From basic research to cancer treatment clinics. <u>J Clin Oncol</u> 2002;20:2575-2601.

Wang JZ, Wang ZF. Role of melatonin in Alzheimer-like neurodegeneration. <u>Acta Pharmacol Sin</u> 2006;27:41-49.

Weinstock M, Shoham S. Rat models of dementia based on reductions in regional glucose metabolism, cerebral blood flow and cytochrome oxidase activity. <u>J Neural Transm</u> 2004;111:347-366.

Wertheimer N, Leeper E. RE: Are electric or magnetic fields affecting mortality from breast cancer in women? (Letter) <u>J Natl Cancer Inst</u> 1994;86:1797.

Zatta P, Tognon G, Carampin P. Melatonin prevents free radical formation due to the interaction between beta-amyloid peptides and metal ions [Al(III), Zn(II), Cu(II), Mn(II), Fe(II)]. <u>J Pineal Res</u> 2003;35:98-103.

Ziolko SK, Weissfeld LA, Klunk WE, Mathis CA, Hoge JA, Lopresti BJ, DeKosky ST, Price JC. Evaluation of voxel-based methods for the statistical analysis of PIB PET amyloid imaging studies in Alzheimer's disease. <u>Neuroimage</u> 2006;33:94-102.

VII.   UPDATE REFERENCES

Chang Q, He Y, Ni B, Feng K, Jiang Y, Jiang B. A case-control study on the risk factors of Alzheimer's disease in military elderly men. Zhonghua Liu Xing Bing Xue Za Xhi 2004;25:890-893.

Chen C, Ma X, Zhong M, Yu Z. Extremely low-frequency electromagnetic fields exposure and female breast cancer risk: A meta-analysis based on 24,338 cases and 60,628 controls. Breast Cancer Res Treat 2010;123:569-576.

Choi SH, Leight SN, Lee VM-Y, Li T, Wong PC, Johnson JA, Saraiva MJ, Sisodia SS. Accelerated Aβ deposition in APPswe/PSIΔE9 mice with hemizygous deletions of TTR (transthyretin). J Neurosci 2007;27:7006-7010.

Coogan PF, Clapp RW, Mewcomb PA, Wenzl TB, Bogdan G, Mettendorf R, Baron JA, Longnecker MP. Occupational exposure to 60-Hertz magnetic fields and risk of breast cancer in women. Epidemiology 1996;7:459-464.

Coogan PF, Aschengrau A. Exposure to power frequency magnetic fields and risk of breast cancer in the Upper Cape Cod Cancer Incidence Study. Arch Environ Health 1998;53:359-467.

Cosentino S, Stern Y, Sokolov E, Scarmeas N, Manly J, Tang M-X, Schupf N, Mayeux R. Plasma amyloid β predicts cognitive decline. Arch Neurol 2010;67:1485-1490.

Coskun PE, Wyrembak J, Derbereva O, Melkonian G, Doran E, Lott IT, Head E, Cotman CW, Wallace DC. Systemic mitochondrial dysfunction and the etiology of Alzheimer's disease and down syndrome dementia. J Alzheimers Dis 2010;20 Suppl 2:S293-S310.

Davanipour Z, Poulsen HE, Weimann A, Sobel E. Endogenous melatonin and oxidatively damaged guanine in DNA. BMC Endocrine Disorders 2009;9:22.

Devanand DP, Schupf N, Stern Y, Parsey R, Pelton GH, Mehta P, Mayeux R. Plasma Aβ and PET PiB binding are inversely related to mild cognitive impairment. Neurology 2011;77:125-131.

Erren TC. A meta-analysis of epidemiologic studies of electric and magnetic fields and breast cancer in women and men. Bioelectromagnetics 2001;Suppl 5:S105-S119.

Floderus B, Persson T, Stenlund C. Magnetic-field exposures in the workplace: reference distribution and exposures in occupational groups. Int J Occup Environ Health 1996;2:226-238.

Ganguli M, Dodge HH, Shen C, Pandav RS, DeKosky ST, Alzheimer disease and mortality: a 15-year epidemiological study. Arch Neurol 2005;62:779-784.

Garcia AM, Sisternas A, Hoyos SP. Occupational exposure to extremely low frequency electric and magnetic fields and Alzheimer disease: A meta-analysis. Int J Epidemiol 2008;37:329-340.

Girgert R, Hanf V, Emons G, Gründker C. Signal transduction of the melatonin receptor MT1 is disrupted in breast cancer cells by electromagnetic fields. Bioelectromagnetics 2010;31:237-245.

Giunta S, Valli MB, Galeazzi R, Fattoretti P, Corder EH, Galeazzi L. Transthyretin inhibition of amyloid beta aggregation and toxicity. Clin Biochem. 2005;38:1112-1119.

Huss A, Spoerri A, Egger M, Röösli M (for the Swiss National Cohort Study). Residence near power lines and mortality from neurodegenerative diseases: Longitudinal Study of the Swiss population. Am J Epidemiol 2009;169:2000-2005.

Jin YP, Gatz M, Johansson B, Pedersen NL. Sensitivity and specificity of dementia coding in two Swedish disease registries. Neurology 2004;63:739-741.

Johansen C, Olsen JH. Risk of cancer among Danish utility workers – a nationwide cohort study. Am J Epidemiol1998;147:548-555.

Johansen C, Raaschou-Nielsen O, Skotte J, Thomsen BL, Olsen JH. Validation of a job-exposure matrix for assessment of utility worker exposure to magnetic fields. Appl Occup Environ Hyg 2002;17:304-310.

Johansen C, Nielsen OR, Olsen JH, Schüz. Risk for leukaemia and brain and breast cancer among Danish utility workers: A second follow-up. Occup Environ Med 2007;64:782-784.

Link CD. Expression of human beta-amyloid peptide in transgenic Caenorhabditis elegans. Proc Natl Acad Sci USA 1995;92:9368-9372.

Maes A, Verschaeve L. Can cytogenetics explain the possible association between exposure to extreme low-frequency magnetic fields and Alzheimer's disease? J Appl Toxicol 2012;32:81-87.

Mayeux R, Schupf N. Blood-based biomarkers for Alzheimer's disease: plasma Aβ40 and Aβ42, and genetic variants. Neurobiol Aging 2011;32 (Suppl 1):S10-S19.

McElroy JA, Egan KM, Titus-Ernstoff L, Anderson HA, Trentham-Dietz A, Hampton JM, Newcomb PA. Occupational exposure to electromagnetic field and breast cancer risk in a large, population-based, case-control study in the United States. J Occup Environ Med 2007;49:266-274.

Peplonska B, Stewart P, Szeszenia-Dabrowska N, Rusiecki J, Garcis-Closas M, Lissowska J, Bardin-Mikolajczal A, Zatonski W, Gromiec J, Brzeznicki S, Brinton LA, Blair A. Occupation and breast cancer risk in Polish women: A population-based case-control study. Am J Ind Med 2007;50:97-111.

Ray RM, Gao DL, Li W, Wernli KJ, Astrakianakis G, Seixas NS, Camp JE, Fitzgibbons ED, Feng Z, Thomas DB, Checkoway H. Occupational exposures and breast cancer among women textile workers in Shanghai. Epidemiology 2007;18:383-392.

Röösli M, Lörtscher M, Egger M, Pfluger D, Schreier N, Lörtscher E, LocherP spoerri A, Minder C. Mortality from neurodegenerative disease and exposure to extremely low-frequency magnetic fields: 31 years of observations on Swiss railway employees. Neuroepidemiology 2007;28:197-206.

Schernhammer ES, Berrino F, Krogh V, Secreto G, Micheli A, Venturelli E, Sieri S, Sempos CT, Cavalleri A, Schünemann HJ, Strano S, Muti P. Urinary 6-sulfatoxymelatonin levels and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 2008;100:898-905.

Schernhammer ES, Hankinson SE. Urinary melatonin levels and postmenopausal breast cancer risk in the Nurses' Health Study cohort. Cancer Epidemiol Biomarkers Prev 2009;18:74-79.

Schernhammer ES, Berrino F, Krogh V, Secreto G, Micheli A, Venturelli E, Grioni S, Sempos CT, Cavalleri A, Schünemann HJ, Strano S, Muti P. Urinary 6-sulfatoxymelatonin levels and risk of breast cancer in premenopausal women: the Ordet cohort. Cancer Epidemiol Biomarkers Prev 2010;19:729-737.

Schupf N, Patel B, Pang D, Zigman WB, Silverman W, Mehta PD, Mayeux R. Elevated plasma beta-amyloid peptide Abeta(42) levels, incident dementia, and mortality in Down syndrome. Arch Neurol 2007;64:1007-1013.

Schupf N, Tang M-X, Fukuyama H, Manly J, Andrews H, Mehta P, Ravetch J, Mayeux R. Peripheral Aβ subspecies as risk biomarkers of Alzheimer's disease. Proc Natl Acad Sci USA 2008;105:14052-14057.

Schupf N, Zigman WB, Tang M-X, Pang D, Mayeux R, Mehta P, Silverman W. Change in plasma Aβ peptides and onset of dementia in adults with Down Syndrome. Neurology 2010;75:1639-1644.

Schwarzman AL, Gregori L, Vitek MP, Lyubski S, Strittmatter WJ, Enghilde JJ, Bhasin R, Silverman J, Weisbraber KH, Coyle PK, Zagorski MG, Talafous J, Eisenberg M, Saunders AM, Roses AD, Goldgaber D. Transthyretin sequesters amyloid β protein and prevents amyloid formation. Proc Natl Acad Sci USA 1994;91:8368-8372.

Schwarzman AL, Goldgaber D. Interaction of transthyretin with amyloid beta-protein: Binding and inhibition of amyloid formation. Ciba Found Symp 1996;199:146-160.

Seidler a, Geller P, Nienhaus A, Bernhardt T, Ruppe I, Eggert S, Hietanen M, Kauppinen T, Frölich L. Occupational exposure to low frequency magnetic fields and dementia: A case-control study. Occup Environ Med 2007;64:108-114.

Serot J-M, Christmann D, Dubost T, Couturier M. Cerebrospinal fluid transthyretin: Aging and lat onset Alzheimer's disease. J Neurol Neurosurg Psychiatry 1997;63:506-508.

Söderqvist F, Carlberg M, Mild KH, Hardell L. Exposure to an 890-MHz mobile phone-like signal and serum levels of S100B and transthyretin in volunteers. Toxicol Letters 2009;189;63-66.

Söderqvist F, Carlberg M, Hardell L. Mobile and cordless telephones, serum transthyretin and the blood-cerebrospinal fluid barrier: A cross-sectional study. Environmental Health 2009;8:19.

Söderqvist F, Carlberg M, Hardell L, Mild KH. Radiofrequency fields, transthyretin, and Alzheimer's disease. J Alzheimers Dis 2010;20:599-606.

Song F, Poljak A, Valenzuela M, Mayeux R, Smythe GA, Sachdey PS. Meta-analysis of plasma amyloid-β levels in Alzheimer's disease. J Alzheimers Dis 2011;26:365-375.

Stein TD, Johnson JA. Lack of neurodegeneration in transgenic mice overexpressing mutant amyloid precursor protein is associated with increased levels of transthyretin and the activation of cell survival pathways. J Neurosci 2002;22:7380-7388.

Wati H, Kawarabayashi T, Matsubara E, Kasai A, Hirasawa T, Kubota T, Harigaya Y, Shoji M, Maeda S. Transthyretin accelerates vascular Aβ deposition in a mouse model of Alzheimer's disease. Brain Pathology 2009;19:48-57.

Wernli KJ, Astrakianakis G, Camp JE, Ray RM, Chang C-K, Li GD, Thomas DB, Checkoway H, Seixas NS. Development of a Job Exposure Matrix (JEM) for the Textile Industry in Shanghai, China. J Occup Environ Hyg 2006;3:521-529.

Xu S, Zhou Z, Zhang L, Yu Z, Zhang W, Want Y, Wang X, Li M, Chen Y, Chen C, He M, Zhang G, Zhong M. Exposure to 1800 MHz radiofrequency radiation induces oxidative damage to mitochondrial DNA in primary cultured neurons. Brain Res 2010;1311:189-196.

_____

Evidence for Breast Cancer Promotion

(Melatonin Studies in Cells and Animals)

CL Sage, MA, Sage Associates,

Santa Barbara, CA   USA

TABLE OF CONTENTS

Introduction

The subject of breast cancer and studies of melatonin has a long and rich history replete with destroyed scientific reputations and career-ending charges of misconduct of scientists who have contributed stellar scientific work that has proved extremely inconvenient for governmental agencies and military and industrial interests (Liburdy). References are given in each section below to facilitate locating the pertinent references for each section.

II. Melatonin and ELF-EMF

Evidence which supports a possible mechanism for ELF-EMF and breast cancer is the consistent finding (in five separate labs) that environmental levels of ELF-EMF can act at the cellular level to enhance breast cancer proliferation by blocking melatonin's natural oncostatic action in MCF-7 cells (Liburdy, 1993; Luben et al, 1996; Morris et al, 1998; Blackman et al, 2001; Ishido, et al, 2001). ELF-EMF levels between 0.6 and 1.2 µT have been shown to consistently block the protective effects of melatonin.

The series of papers reporting increased breast cancer cell proliferation when ELF-EMF at environmental levels negatively affects the oncostatic actions of melatonin in MCF-7 cells should warrant new public exposure guidelines or planning target limits for the public, and for various susceptible segments of the population.

References

Liburdy, R. P., T. R. Sloma, et al, 1993. ELF magnetic fields, breast cancer, and melatonin: 60 Hz fields block melatonin's oncostatic action on ER+ breast cancer cell proliferation. J of Pineal Research. 14: 89-97.

Luben et al, 1996. Replication of 12 mG EMF effects on melatonin responses of MCF-7 breast cancer cells in vitro. Abstract A-1 of the 1996 Annual review of research on biological effects of electric and magnetic fields from the generation, delivery and use of electricity, November 17-21, 1996. San Antonio, Texas, p.1

Luben et al, 1998. Independent replication of 60-Hz 1.2 µT EMF effects on melatonin and tamoxifen responses of MCF-7 cells in vitro. Abstract A-3.4, Bioelectromagnetics Society Annual Meeting, St. Pete Beach, FL. June 7-11, p 17-18.

Morris et al, 1998. In vitro exposure of MCF-7 human breast cancer cells to 60-Hz magnetic fields. Abstract p-125A, Bioelectromagnetics Society Annual Meeting, St. Pete Beach, FL. June 7-11, p 204-205.

Ishido et al, 2001. Magnetic fields (MF) of 50 Hz at 1.2 µT as well as 100 µT cause uncoupling of inhibitory pathways of adenylyl cyclase mediated by melatonin 1a receptor in MF-sensitive MCF-7 cells.

D.E. Blask, S.M. Hill, Effects of melatonin on cancer: studies on MCF-7 human breast cancer cells in culture, J. Neural Transm. Suppl. 21 (1986) 433–449.

Loberg LI et al 1999. Gene expression in human breast epithelial cells exposed to 60 Hz magnetic fields, Carcinogenesis 20 1633–1636.

III. Tamoxifen and ELF-EMF

Girgert et al (2005) reported that *"the anti-estrogenic activity of tamoxifen is reduced in two subclones of MCF-7 cells under the influence of ELF/EMF to different extent. Dose-response curves of the growth-inhibitory effect of tamoxifen are shifted towards higher concentrations leading to a reduced growth inhibition at a given concentration. Our observations confirm results from a previous report describing a reduced inhibitory effect of tamoxifen at 1^{-7} M from 40% to only 17% by exposure to an EMF of 1.2 µT"* (Harland t al, 1997). Further, Girgert et al conclude that *"From a medical point of view, it is disturbing that maximal induction of cell proliferation by tamoxifen at a field strength of 1.2 µT is observed at concentration of 10^{-6} M. This is exactly the serum concentration achieved in BC patients under standard oral therapy."* (De Cupis et al, 1997).

The Girgert et al paper confirms prior findings that environmental level ELF-EMF inhibits the antiproliferative action of tamoxifen in MCF-7 human breast cancer cells. Four other papers reporting this effect include Liburdy et al, 1997; Harland et al, 1997; Harland et al, 1999; and Blackman et al, 2001).

References

Liburdy et al, 1997. Magnetic Fields, Melatonin, Tamoxifen, and Human
Breast Cancer Cell Growth. In: Stevens R. G., Wilson B. W., Anderson L.E. (Eds). The Melatonin Hypothesis - Breast Cancer and Use of Electric Power. Battelle Press, Columbus, Richland 1997: 669- 700.

Harland et al, 1997. Environmental magnetic fields inhibit the antiproliferative action of tamoxifen and melatonin in a human breast cancer cell line. Bioelectromagnetics, 18, 555-562.

Harland et al, 1999. Evidence for a slow time-scale of interaction for magnetic fields inhibiting tamoxifen's antiproliferative action in human breast cancer cells. Cell
Biochemistry & Biophysics, 31(3), 295-306.

Blackman et al, 2001. The influence of 1.2 µT, 60 Hz magnetic fields on melatonin and tamoxifen-induced inhibition of MCF-7 cell growth. Bioelectromagnetics, 22(2), 122-128.

Girgert et al, 2005. Induction of tamoxifen resistance in breast cancer cells by ELF electromagnetic fields. Biochemical & Biophysics Research Communications, 336, 1144-1149.

A. De Cupis et al, 1997. Oestrogen/growth factor cross-talk in breast carcinoma: a specific target for novel antioestrogens, TIPS 18 245–251.

IV. Animal Studies and ELF-EMF

Anderson, L. E., G. A. Boorman, et al. (1999). Effect of 13 week magnetic field exposures on DBMA-initiated mammary gland carcinomas in female Sprague-Dawley Rats. Carcinogenesis. 20: 1615-1620.

Beniashvili, D. S., V. Bilanishvili, et al. (1991). Low-frequency electromagnetic radiation enhances the induction of rat mammary tumors by nitrosomethyl urea. Cancer Letters. 61: 75-79.

Ekstrom, T., K. H. Mild, et al. (1998). Mammary tumours in sprague-dawley rats after initiation with dmba followed by exposure to 50 Hz electromagnetic fields in a promotional scheme. Cancer Letters. 123: 107-111.

Loscher, W., M. Mevissen, et al. (1993). Tumor promotion in a breast cancer model by exposure to a weak alternating magnetic field. Cancer Letters. 71: 75-81.

Loscher, W., U. Wahnschaffe, et al. (1994). Effects of weak alternating magnetic fields on nocturnal melatonin production and mammary carcinogenesis in rats. Oncology. 51: 288-295.

Mevissen, M., A. Stamm, et al. (1993). Effects of magnetic fields on mammary tumor development induced by 7, 12-dimethylbenz(a)anthracene in rats. Bioelectromagnetics. 14: 131-143.

Mevissen M et al, 1995. In vivo exposure of rats to a weak alternating magnetic field increases ornithine decarboxylase activity in the mammary gland by a similar extent as the carcinogen DMBA, Cancer Lett. 90 (1995) 207–214.

Mevissen, M., A. Lerchl, et al. (1996). Exposure of DMBA-treated female rats in a 50 Hz, 50 microtesla magnetic field: effects on mammary tumor growth. Carcinogenesis. 17: 903-910.

Mevissen, M., A. Lerchl, et al. (1996). Study on pineal function and DMBA-induced breast cancer formation in rats during exposure to a 100-mg, 50 Hz magnetic field. J of Toxicology & Environmental Health. 48: 169-185.

Mevissen, M., M. Haussler, et al. (1998). Complex effects of long-term 50 Hz magnetic field exposure in vivo on immune functions in female Sprague-Dawley rats depend on duration of exposure. Bioelectromagnetics. 19: 259-270.

Thun-Battersby, S., M. Mevissen, et al. (1999). Exposure of Sprague-Dawley rats to a 50 Hz, 100 uTesla magnetic field for 27 weeks facillitates mammary tumorigenisis in the

V. Epidemiological Studies on Breast Cancer and ELF-EMF
Female Breast Cancer Studies

References

Milham S. (in press) 2006. Electric typewriter exposure and increased female breast cancer mortality in typists. Medical Hypotheses. Elsevier Ltd.

Cantor, K. P., M. Dosemeci, et al. (1995). Re: 'Breast cancer mortality among female electrical workers in the United States' (letter). J of the National Cancer Institute. 87: 227-228.

Cantor, K. P., P. A. Stewart, et al. (1995). Occupational exposures and female breast cancer mortality in the United States. J of Occupational & Environmental Medicine. 37: 336-348.

Demers, P. and e. al. (1991). Occupational Exposure to Electromagnetic fields and breast cancer in men. Amer J of Epidemiology. 134: 340-347.

Coogan, P. F., R. W. Clapp, et al. (1996). Occupational exposure to 60Hz Magnetic Fields and risk of breast cancer in women. Epidemiology. 7: 459-464.

Erren, T. (2001). "A meta-analysis of epidemiologic studies of electric and magnetic fields and breast cancer in women and men." Bioelectromagnetics(Supplement 5, 2001): S105-S119.

Floderus, B., C. Stenlund, et al. (1999). Occupational magnetic field exposure and site-specific cancer incidence: a Swedish cohort study. Cancer Causes & Control. 10: 323-332.

Feychting, M., Forssen, U, L. E. Rutqvist, et al. (1998). Magnetic fields and breast cancer in Swedish adults residing near high-voltage power lines. Epidemiology.

Forssen, U. M., M. Feychting, et al. (2000). Occupational and Residential magnetic field exposure and breast cancer in females. Epidemiology. 11: 24-29.

Loomis, D. P., D. A. Savitz, et al. (1994). Breast cancer mortality among female electrical workers in the United States. J of the National Cancer Institute. 86: 921- 925.

Petralia, S. A., W.-H. Chow, et al. (1998). Occuational risk factors for breast cancer among women in Shanghai. Amer J Industrial Med. 34: 477-483.

Rosenbaum, P. F., J. E. Vena, et al. (1994). Occupational exposures associated with male breast cancer. Amer J of Epidemiology. 139: 30-36.

Stenlund, C. and B. Floderus (1997). Occupational exposure to magnetic fields in relation to male breast cancer and testicular cancer: a Swedish case-control study. Cancer Causes & Control. 8: 184-191.

Tynes, T. H., M; Andersen, A; Vistnes, AL; Haldorsen, T (1996). "Incidence of breast cancer in Norwegian female radio and telegraph operators." Cancer Causes Control 7: 197-204.

Tynes et al, 1992. Incidence of cancer in Norwegian workers potentially exposed to electromagnetic fields. American Journal of Epidemiology, 136, 81-88.

Vena, J. E., J. L. Freudenheim, et al. (1994). Risk of premenopausal breast cancer and use of electric blankets. Amer J of Epidemiology. 140: 974-979.

Verkasalo et al, 1996. Magnetic fields of high voltage power lines and risk of cancer in Finnish adults: nationwide cohort study. British Medical Journal, 313(7064), 1047–1051.

VI. Male Breast Cancer Studies

References

Demers et al, 1991. Occupational exposure to electromagnetic fields and breast cancer in men. American Journal of Epidemiology, 134, 340-347.

Feychting, M., Forssen, U, L. E. Rutqvist, et al. (1998). Magnetic fields and breast cancer in Swedish adults residing near high-voltage power lines. Epidemiology.

Floderus et al, 1999. Occupational magnetic field exposure and site-specific cancer incidence: a Swedish cohort study. Cancer Causes and Control, 10, 323-332.

Floderus et al, 1994. Incidence of selected cancers in Swedish railway workers, 1961-1979. Cancer Causes and Control, 5, 189-194.

Guenel et al, 1993. Incidence of cancer in persons with occupational exposure to electromagnetic fields in Denmark. British Journal of Industrial Medicine, 50, 758-764.

Johansen et al, 1998. Risk of Cancer Among Danish Utility Workers – A Nationwide Cohort Study. American Journal of Epidemiology, 147, 548-555.

Loomis et al, 1992. Cancer of breast among men in electrical occupations. Lancet, 339, 1482-1483.

Matanowski, G. M., P. N. Breysse, et al. (1991). Electromagnetic field exposure and male breast cancer. Lancet. 337: 737.

Stendtlund et al, 1997, Occupational exposure to magnetic fields in relation to male breast cancer and testicular cancer: A Swedish case-control study. Cancer Causes & Control, 8, 184-191.

Theriault et al, 1994. . Cancer risks associated with occupational exposure to magnetic fields among electric utility workers in Ontario and Quebec, Canada and France. 1970-1989. American Journal of Epidemiology, 139, 550-572.

Tynes et al, 1994. Incidence of cancer among workers in Norwegian hydroelectric power companies. Scand. J. Work Environ. Health, 20, 339-344.

VII. Conclusions

Conclusion: The constellation of relevant scientific papers providing mutually-reinforcing evidence for an association between power-frequency electromagnetic fields (ELF-EMF) and breast cancer is strongly supported in the scientific literature.

Conclusion: ELF at environmental levels negatively affects the oncostatic effects of both melatonin and tamoxifen on human breast cancer cells. Numerous epidemiological studies over the last two decades have reported increased risk of male and female breast cancer with exposures to residential and occupational levels of ELF. Animal studies have reported increased mammary tumor size and incidence in association with ELF exposure.

Conclusion: ELF limits for public exposure should be revised to reflect increased risk of breast cancer at environmental levels possibly as low as 2 mG or 3 mG; certainly as low as 4 mG.

Evidence for Disruption by the Modulating Signal

Carl F. Blackman,* PhD
Founder, Former President and
Full Member of the Bioelectromagnetics Society
Raleigh, NC USA

*opinions expressed are not necessarily those of his employer,
the US Environmental Protection Agency

Table of Contents

I.   Introduction

Modulation signals are one important component in the delivery of EMF signals to which cells, tissues, organs and individuals can respond biologically. At the most basic level, modulation can be considered a pattern of pulses or repeating signals which have specific meaning in defining that signal apart from all others. Modulated signals have a specific 'beat' defined by how the signal varies periodically over time. Pulsed signals occur in an on-off pattern, which can either be smooth and rhythmic, or sharply pulsed in quick bursts. Amplitude and frequency modulation involves two very different processes where the high-frequency signal, called the carrier wave, has a low-frequency signal that is superimposed on or 'rides' on the carrier frequency. In amplitude modulation, the lower-frequency signal is embedded on the carrier wave as changes in its amplitude as a function of time, whereas in frequency modulation, the lower-frequency signal is embedded as slight changes in the frequency of the carrier wave. Each type of low-frequency modulation conveys specific 'information', and some modulation patterns are more effective (more bioactive) than others depending on the biological reactivity of the exposed material. This enhanced interaction can be a good thing for therapeutic purposes in medicine, but can be deleterious to health where such signals could stimulate disease-related processes, such as increased cell proliferation in precancerous lesions. Modulation signals may interfere with normal, non-linear biological functions. More recent studies of modulated RF signals report changes in human cognition, reaction time, brainwave activity, sleep disruption and immune function. These studies have tested the RF and ELF-modulated RF signals from emerging wireless technologies (cell phones) that rely on pulse modulated RF to transmit signals. Thus modulation can be considered as information content embedded in the higher frequency carrier wave that may have health consequences beyond any effect from the carrier wave directly.

In mobile telephony, for example, modulation is one of the underlying ways to categorize the radiofrequency signal of one telecom carrier from another (TDMA from CDMA from GSM). Modulation is likely a key factor in determining whether and when biological reactivity might be occurring, for example in the new technologies which make use of modulated signals, some modulation (the packaging for delivery for an EMF 'message') may be bioactive, for example, frequencies are similar to those found in brain wave patterns. If a new technology happens to use brain wave frequencies, the chances are higher that it will have effects, in comparison, for

example, to choosing some lower or higher modulation frequency to carry the same EMF information to its target. This chapter will show that other EMF factors may also be involved in determining if a given low-frequency signal directly or as a modulation of a radiofrequency wave can be bioactive. Such is the evolving nature of information about modulation. It argues for great care in defining standards that are intended to be protective of public health and well-being. This section describes some features of exposure and physiological conditions that are required in general for non-thermal effects to be produced, and specifically *to illustrate how modulation is a fundamental factor which should be taken into account in public safety standards*.

II.   The Old Standards (Based on Heating and Electric Current Flow in Tissues)

It is universally accepted that radiofrequency radiation (RFR) can cause tissue heating and that extremely low frequency (ELF) fields, e.g., 50 and 60 Hz, can cause electrical current flows that shock and even damage or destroy tissues. These factors alone are the underlying bases for present exposure standards. EMF exposures that cause biological effects at intensities that do not cause obvious thermal changes, that is, effects via non-thermal mechanisms, have been widely reported in the scientific literature over the last several decades. The current public safety limits do not take modulation into account and thus are no longer sufficiently protective of public health where chronic exposure to pulsed or pulse-modulated signal is involved, and where sub-populations of more susceptible individuals may be at risk from such exposures.

III.   Laboratory Studies

Published laboratory studies have provided evidence for more than 40 years on bioeffects at much lower intensities than cited in the various widely publicized guidelines for limits to prevent harmful effects. Many of these reports show EMF-caused changes in processes associated with cell growth control, differentiation and proliferation which are biological processes of considerable interest to scientists who study the molecular and cellular basis of cancer. EMF effects have been reported in gene induction, transmembrane signaling cascades, gap junction communication, immune system action, rates of cell transformation, and breast cancer cell

4

growth. These reports have cell growth control as a common theme. Other more recent studies on brainwave activity, cognition and human reaction time lend credence to modulation (pulsed RF and ELF-modulated RF) as a concern for wireless technologies, most prominently from cell phone use.

Experimental results are described below to illustrate the influence of each EMF parameter, while also demonstrating that it is highly unlikely the effects are due to EMF-caused current flow or heating.

Several papers in the 1960s and early 1970s reported that ELF fields could alter circadian rhythms in laboratory animals and humans. In the latter 1960s, a paper reported that the EMF environment in planned space capsules could cause human response time changes, i.e., the interval between a signal and the human response (Hamer, 1968). Subsequent experiments by that research group were conducted with monkeys, and showed similar response time changes and also EEG pattern changes (Gavalas, 1970; Gavalas-Medici, 1976). The investigators shifted the research subject to cats and observed EEG pattern changes, ability to sense and behaviorally respond to the ELF component of RFR, and the ability of minor electric current to stimulate the release of an inhibitory neurotransmitter, GABA, and simultaneous release of a surrogate measure, calcium ions, from the cortex (Kaczmarek, 1973, 1974). At this time the investigators adopted newly hatch chickens as sources of brain tissue and observed changes in the release of calcium ions from in vitro specimens as a function of ELF frequency directly or as amplitude modulation ('am') of RFR (RFRam) (Bawin, 1975, 1976, 1978a, 1978b; Sheppard, 1979). Tests of both EMF frequency and intensity dependences demonstrated a single sensitive region (termed 'window') over the range of frequency and intensity examined. This series of papers showed that EMF-induced changes could occur in several species (human, monkey, cat and chicken), that calcium ions could be used as surrogate measures for a neurotransmitter, that ELF fields could produce effects similar to RFRam (note: without the 'am', there was no effect although the RFR intensity was the same), and that the dose and frequency response consisted of a single sensitivity window.

An independent research group published a series of papers replicating and extending this earlier

work (Blackman et al., 1979, 1980a, 1980b, 1981, 1982, 1985, 1988a, 1988b, 1989, 1990; Joines and Blackman et al., 1981a, 1981b, 1986). These papers reported multiple windows in intensity and in frequency within which calcium changes were observed in the chick brain experimental systems under EMF exposure. Three other independent groups reported intensity and frequency windows for calcium, neurotransmitter or enolase release under EMF exposure of human and animal nervous system-derived cells in vitro (Dutta et al., 1984, 1989, 1992, 1994), of rat pancreatic tissue slices (Albert et al., 1980), and of frog heart (Schwartz et al., 1990) but not atrial strips in vitro (Schwartz et al., 1993). This series of papers showed that multiple frequency and intensity windows were a common phenomenon that required the development of new theoretical concepts to provide a mechanism of action paradigm.

Additional aspects of the EMF experiments with the chick brain described by Blackman and colleagues, above, also revealed critical co-factors that influenced the action of EMF to cause changes in calcium, including the influence of the local static magnetic field, and the influence of physico-chemical parameters, pH, temperature and ionic strength of the bathing solution surrounding the brain tissue during exposure. This information provides clues for and constraints on any theoretical mechanism that is to be developed to explain the phenomenon. These factors demonstrate that the current risk assessment paradigms, which ignore them, are incomplete and thus may not provide the level of protection currently assumed.

The detailed set of frequency and intensity combinations under which effects were observed, were all obtained from chickens incubated for 21 days in an electrically heated chamber containing 60-Hz fields. Tests were performed to determine if the 60-Hz frequency of ELF fields (10 volts per meter in air) during incubation, i.e., during embryogenesis and organogenesis, would alter the subsequent calcium change responses of the brain tissue to EMF exposure. The published papers (Blackman et al., 1988b; Joines et al., 1986) showed that the brain tissue response was changed when the field during the incubation period was 50 Hz rather than 60 Hz. This result is consistent with an anecdotal report of adult humans, who were institutionalized because of chemical sensitivities, were also responsive to EMF fields that were present in the countries where they were born and raised (Blackman, 2006). This information indicates there may be animal and human exposure situations where EMF imprinting could be an

important factor in laboratory and epidemiological situations. EMF imprinting, which may only become manifest when a human is subjected to chemical or biological stresses, could reduce ability to fight disease and toxic insult from environmental pollution, resulting in a population in need of more medical services, with resulting lost days at work.

Fundamental exposure parameters that must be considered when establishing a mode (or mechanism) of action for non-thermal EMF-induced biological effects.

A. Intensity

There are numerous reports of biological effects that show intensity "windows", that is, regions of intensity that cause changes surrounded by higher and lower intensities that show no effects from exposure. One very clear effect is 16-Hz, sine wave-induced changes in calcium efflux from brain tissue in a test tube because it shows two very distinct and clearly separated intensity windows of effects surrounded by regions of intensities that caused no effects (Blackman et al., 1982). There are other reports for similar multiple windows of intensity in the radiofrequency range (Blackman et al., 1989; Dutta et al., 1989, 1992; Schwartz et al., 1990). Note that calcium ions are a secondary signal transduction agent active in many cellular pathways. These results show that intensity windows exist, they display an unusual and unanticipated "non linear" (non-linear and non-monotonic) phenomenon that has been mostly ignored in all risk assessment and standard setting exercises, save the National Council for Radiation Protection and Measurements. (NCRP) 1986 publication. Protection from multiple intensity windows has never been incorporated into any risk assessment; to do so would call for a major change in thinking. These results mean that lower intensity is not necessarily less bioactive, or less harmful.

Multiple intensity windows appeared as an unexpected phenomenon in the late 1970s and 1980s. There has been one limited attempt to model the phenomenon (Thompson et al., 2000). However, there are publications from two independent research groups showing multiple intensity windows for 50 MHz, 147 MHz, and 450 MHz fields when amplitude-modulated at 16 Hz using the calcium ion release endpoint in chicken brains, in vitro. The incident intensities (measured in air) for the windows at the different carrier frequencies do not align at the same values. However, Joines et al., (1981a, 1981b) and Blackman et al. (1981) noted the windows of

intensity align across different carrier frequencies if one converts the incident intensity to the intensity expected within the sample at the brain surface, but correcting for the different dielectric constants in the samples at the different carrier frequencies. The uniqueness of this response provides a substantial clue to theoreticians but it is interesting that no publications have appeared attempting to address this relationship. It is obvious that this phenomenon is one that needs further study.

B. Frequency

Frequency-dependent phenomena are common occurrences in nature. For example, the human ear only hears a portion of the sound that is in the environment, typically from 20 to 20000 Hz, which is a frequency "window." Another biological frequency window can be observed for plants grown indoors. Given normal indoor lighting the plants may grow to produce lush vegetation but not produce flowers unless illuminated with a lamp that emits a different spectrum of light. Similarly, there are examples of EMF-caused biological effects that occur as a result of EMF of concern to us in a frequency-dependent manner that cannot be explained by current flow or heating. The examples include reports of calcium ion efflux from brain tissue in vitro at low frequency (Blackman et al., 1988a, 1988b) and at high frequency (Blackman et al., 1981; Joines and Blackman, 1981). The bioactive frequency regions observed in these studies have never been explicitly considered for use in any EMF risk assessments, thus demonstrating the incomplete nature of current exposure limits.

There are also EMF frequency-dependent alterations in the action of nerve growth factor (NGF) to stimulate neurite outgrowth (growth of primitive axons or dendrites) from a peripheral-nerve-derived cell (PC-12) in culture (Blackman et al., 1995, 1999; Trillo et al., 1996). The combined effect of frequency and intensity is also a common occurrence in both the sound and the light examples given above. Too much or too little of either frequency or intensity show either no or undesirable effects. Similarly, in low intensity EMF work, "islands" of effective combinations of intensity and frequency are surrounded by a "sea" of null effects (Blackman et al., 1988a). Although the mechanisms responsible for these effects have not been establish, the effects represent a heretofore unknown phenomenon that may have ramifications for risk assessment and standard setting. Nerve growth and neurotransmitter release that can be altered by different

combinations of EMF frequencies and intensities, especially in developing organisms like children, could conceivably produce over time a subsequent altered ability to successfully or fully respond behaviorally to natural stressors in the adult environment; research is urgently need to test this possibility in animal systems.

Nevertheless, this phenomenon is ignored in the development of present exposure standards that rely primarily on biological responses to intensities within a relatively narrow band of frequencies, based on an energy deposition endpoint.

C. Static Magnetic Field

The magnetic field of the earth at any given location has a relatively constant intensity as a function of time. However, the intensity value, and the inclination of the field with respect to the gravity vector, varies considerable over the face of the earth. More locally, these features of the earth's magnetic field can also vary by more than 20% inside man-made structures, particularly those with steel support structures. There are many reports of EMF-caused effects being dependent on the static magnetic field intensity (cf. Blackman et al., 1985) and of its orientation, with respect to an oscillating magnetic field (Blackman et al., 1990; Blackman et al., 1996). One aspect common to many of these reports is that the location in the active frequency band is determined by the intensity of the static magnetic field. There have been many attempts to explain this phenomenon but none has been universally accepted. However, it is clear that if a biological response depends on the static magnetic field intensity, and even its orientation with respect to an oscillating field, then the conditions necessary to reproduce the phenomenon are very specific and might easily escape detection (cf. Blackman and Most, 1993). The consequences of these results are that there may be exposure situations that are truly detrimental (or beneficial) to organisms but that are insufficiently common on a large scale that they would not be observed in epidemiological studies; they need to be studied under controlled laboratory conditions to determine impact on health and wellbeing.

D. Electric & Magnetic Components

Both the electric and the magnetic components have been shown to directly and independently cause biological changes. There is one report that clearly distinguishes the distinct biological

responses caused by the electric field and by the magnetic field. Marron et al. (1988) show that electric field exposure can increase the negative surface charge density of an amoeba, Physarum polycephalum, and that magnetic field exposure of the same organism causes changes in the surface of the organism to reduce its hydrophobic character. Other scientists have used concentric growth surfaces of different radii and vertical magnetic fields to determine if the magnetic or the induced electric component is the agent causing biological change. Liburdy (1992), examining calcium influx in lymphocytes, and Greene et al. (1991), monitoring ornithine decarboxylase (ODC) activity in cell culture, showed that the induced electric component was responsible for their results. In contrast, Blackman et al. (1993a, 1993b) monitoring neurite outgrowth from two different clones of PC-12 cells and using the same exposure technique used by Liburdy and by Greene showed the magnetic component was the critical agent in their experiments. EMF-induced changes on the cell surface, where it interacts with its environment, can dramatically alter the homeostatic mechanisms in tissues, whereas changes in ODC activity are associated with the induction of cell proliferation, a desirable outcome if one is concerned about wound healing, but undesirable if the concern is tumor cell growth. This information demonstrates the multiple, different ways that EMF can affect biological systems. Current analyses for risk assessment and standard setting have ignored this information, thus making their conclusions of limited value.

E. Sine and Pulsed Waves

Important characteristics of pulsed waves that influenced the number and characteristics of the sine wave representations include the following: 1) frequency, 2) pulse width, 3) intensity, 4) rise and fall time, and 5) the frequency, if any, within the pulse ON time. Chiabrera et al. (1979) showed that pulsed fields caused de-differentiation of amphibian red blood cells. Scarfi et al. (1997) showed enhanced micronuclei formation in lymphocytes of patients with Turner's syndrome (only one X chromosome) but no change in micronuclei formation when the lymphocytes were exposed to sine waves (Scarfi et al., 1996). Takahashi et al. (1986) monitored thymidine incorporation in Chinese hamster cells and explored the influence of pulse frequency (two windows of enhancement seen), pulse width (one window of enhancement seen) and intensity (two windows of enhancement seen followed by a reduction in incorporation). Ubeda et al. (1983) showed the influence of difference rise and fall times of pulsed waves on chick

embryo development.

It is important to note that the frequency spectrum of pulsed waves can be represented by a sum of sine waves which, to borrow a chemical analogy, would represent a mixture or a soup of chemicals, anyone of which could be biologically active. Risk assessment and exposure limits have been established for specific chemicals or chemical classes of compounds that have been shown to cause undesirable biological effects. Risk assessors and the general public are sophisticated enough to recognize that it is impossible to declare all chemicals safe or hazardous; consider the difference between food and poisons, both of which are chemicals. A similar situation occurs for EMF; it is critical to determine which combinations of EMF conditions have the potential to cause biological harm and which do not.

Obviously, pulse wave exposures represent an entire genre of exposure conditions, with additional difficulty for exact independent replication of exposures, and thus of results, but with increased opportunities for the production of biological effects. Current standards were not developed with explicit knowledge of these additional consequences for biological responses.

F. Mechanisms

Two recent papers have the possibility of advancing understanding in this research area. Chiabrera et al. (2000) created a theoretical model for EMF effects on an ion's interaction with protein that includes the influence of thermal energy and of metabolism. Before this publication, theoreticians assumed that biological effects in living systems could not occur if the electric signal is below the signal caused by thermal noise, in spite of experimental evidence to the contrary. In this paper, the authors show that this limitation is not absolute, and that different amounts of metabolic energy can influence the amount and parametric response of biological systems to EMF. The second paper, by Marino et al. (2000), presents a new analytical approach to examine endpoints in systems exposed to EMF. The authors, focusing on exposure-induced lyphoid phenotypes, report that EMF may not cause changes in mean values of endpoints, but rather in variances in those same endpoints. They provide further evidence using immunological endpoints from exposed and sham treated mice (Marino et al., 2001a, 2001b, 2001c). Additional research has emerged from this laboratory on EMF-induced animal and human brain activity

11

changes that provides more evidence for the value of their research approach (Marino et al., 2002, 2003, 2004; Carrubba et al., 2006, 2007a, 2007b). *It is apparent that much remains to be examined and explained in EMF biological effects research through more creative methods of analysis than have been used before. The models described above need to be incorporated into risk assessment determinations.*

IV. Problems with Segregation of Effects by Artificial Frequency Bands that Ignore Modulation

One fundamental limitation of most reviews of EMF biological effects is that exposures are segregated by the physical (engineering/technical) concept of frequency bands favored by the engineering community. This is a default approach that follows the historical context established in the past by the incremental addition of newer technologies that generate increasingly higher frequencies. However, this approach fails to consider unique responses from biological systems that are widely reported at various combinations of frequencies, modulations and intensities.

When common biological responses are observed without regard for the particular, engineering-defined EMF frequency band in which the effects occur, this reorganization of the results can highlight the commonalities in biological responses caused by exposures to EMF across the different frequently bands. An attempt to introduce this concept to escape the limitations of the engineering-defined structure occurred with the development of the 1986 NCRP radiofrequency exposure guidelines because published papers from the early 1970s to the mid 1980s (to be discussed below) demonstrated the need to include amplitude modulation as a factor in setting of maximum exposure limits. The 1986 NCRP guideline was the one and only risk evaluation that included an exception for modulated fields.

The current situation argues strongly for a change in the way risk assessment is conducted,

especially for the last 15 to 20 years. Unfortunately, subsequent risk evaluations did not follow the NCRP example, but returned to the former engineering-defined analysis conditions, in part because scientists who reported non-thermal effects were not placed on the review committees, and in the terms of Slovic (1999) "Risk assessment is inherently subjective and represent a blend of science and judgment with important psychological, social, cultural, and political factors. … Whoever controls the definition of risk controls the rational solution to the problem at hand. … Defining risk is thus an exercise in power." It appears that by excluding scientists experienced with producing non-thermal biological effects, the usually sound judgment by the selected committees was severely limited in its breadth-of-experience, thereby causing the members to retreat to their own limited areas of expertise when forced to make judgments, as described by Slovic (1999), "Public views are also influenced by worldviews, ideologies, and values; so are scientists' views, particularly when they are working at limits of their expertise." The current practice of segregating scientific investigations (and resulting public health limits) by artificial divisions of frequency dramatically dilutes the impact of the basic science results, thereby reducing and distorting the weight of evidence in any evaluation process (see evaluations of bias by Havas 2000, referring to NRC 1997 compared to NIEHS 1998 and NIEHS 1999).

A. Suggested Research

Are there substitute approaches that would improve on the health-effects evaluation situation? As mentioned above, it may be useful in certain cases to develop a biologically based clustering of the data to focus on and enrich understanding of certain aspects of biological responses. Some examples to consider for biological clustering include: 1) EMF features, such as frequency and intensity inter-dependencies, 2) common cofactors, such as the earth's magnetic field or co-incident application of chemical agents to perturb and perhaps sensitize the biological system to EMF, or 3) physiological state of the biological specimen, such as age or, sensitive sub-populations, including genetic predisposition (Fedrowitz et al., 2004, 2005).

To determine if this approach has merit, one could combine reports of biological effects found in the ELF (including sub-ELF) band with effects found in the RF band when the RF exposures are amplitude modulated (AM) using frequencies in the ELF band. The following data should be used: 1) human response time changes under ELF exposure (Hamer, 1968), 2) monkey response

13

time and EEG changes under ELF exposure (Gavalas et al., 1970; Gavales-Medici & Day-Magdaleno, 1976), 3) cat brain EEG, GABA and calcium ion changes induced by ELF and AM-RF (Kaczmarek and Adey, 1973, 1974; Bawin et al. 1973), 4) calcium ion changes in chick brain tissue under ELF and AM-RF (Bawin et al., 1975, 1976, 1978a, 1978b; Sheppard et al., 1979; Joines and Blackman et al., , 1981a, 1981b, 1986; Blackman et al., 1979, 1980a, 1980b, 1981, 1982, 1985, 1988a, 1988b, 1989, 1990), and 5) calcium changes under AM-RF in brain cells in culture (Dutta et al., 1984, 1989, 1992) and in frog heart under AM-RF (Schwartz et al., 1990). The potential usefulness of applying biological clustering in the example given above even though AM is used, is that the results may have relevance to assist in the examination of some of the effects reportedly caused by cellular phone exposures which include more complex types of modulation of RF. This suggestion is reasonable because three groups have recently reported human responses to cell phone emissions that include changes in reaction times (Preece et al., 1998, 1999; Koivisto et al. 2000a, 2000b; Krause et al., 2000a, 2000b) or to brain wave potentials that may be associated with reaction time changes (Freude et al., 1998, 2000).

The papers described above, published in the 1960s through 1991, foreshadowed the more recent publications in 1999 and 2000 showing response time changes, or associated measures, in human subjects during exposure to cell phone-generated radiation (although none of the earlier studies was acknowledged in these recent reports on cognition and reaction time). Without guidance from this extensive earlier work, the development of the mechanistic bases for non-thermal effects from EMF exposures will be substantially delayed.

V. Conclusions

• There is substantial scientific evidence that some modulated fields (pulsed or repeated signals) are bioactive, which increases the likelihood that they could have health impacts with chronic exposure even at very low exposure levels. Modulation signals may interfere with normal, non-linear biological processes.

• Modulation is a fundamental factor that should be taken into account in new public safety standards; at present it is not even a contributing factor.

• To properly evaluate the biological and health impacts of exposure to modulated RFR (carrier waves), it is also essential to study the impact of the modulating signal (lower frequency fields or ELF-modulated RF).

• Current standards have ignored modulation as a factor in human health impacts, and thus are inadequate in the protection of the public in terms of chronic exposure to some forms of ELF-modulated RF signals.

• The current IEEE and ICNIRP standards are not sufficiently protective of public health with respect to chronic exposure to modulated fields (particularly new technologies that are pulse-modulated and heavily used in cellular telephony).

• The collective papers on modulation appear to be omitted from consideration in the recent WHO and IEEE science reviews. This body of research has been ignored by current standard setting bodies that rely only on traditional energy-based (thermal) concepts.

• More research is needed to determine which modulation factors, and combinations are bioactive and deleterious at low intensities, and are likely to result in disease-related processes and/or health risks; however this should not delay preventative actions supporting public health and wellness.

• If signals need to be modulated in the development of new wireless technologies, for example, it makes sense to use what existing scientific information is available to avoid the most obviously deleterious exposure parameters and select others that may be less likely to interfere with normal biological processes in life.

• The current membership on Risk Assessment committees needs to be made more inclusive, by adding scientists experienced with producing non-thermal biological effects.

• The current practice of segregating scientific investigations (and resulting public health limits) by artificial divisions of frequency needs to be changed because this approach dramatically dilutes the impact of the basic science results and eliminates consideration of modulation signals, thereby reducing and distorting the weight of evidence in any evaluation process.

Disclaimer: the opinions expressed in this text are those of its author, and are not necessarily those of his employer.

VI. References

Abelson, P.H. 1989. Science 245: 241.

Albert, E., Blackman, C, Slaby, F. 1980. In Berteaud and Servantie (eds): URSI Ondes Electromagnetique et Biologie, p325-329.

Bawin, S.M., Gavalas-Medici, R.J., Adey, W.R. 1973. Brain Res., 58: 365-384.

Bawin, S.M., Kaczmarek, L.K., Adey, W.R. 1975. Ann. N. Y. Acad. Sci. 247:74-81.

Bawin, S.M., Adey, W.R. 1976. Proc. Natl. Acad. Sci. USA 73:1999-2003.

Bawin, S.M., Adey, W.R., Sabbot, I.M. 1978a. Proc. Natl. Acad. Sci. USA 75:6314-6318.

Bawin, S.M., Sheppard, A., Adey, W.R. 1978b. Bioelectrochemistry and Bioenergetics 5:67-76.

Blackman, C.F., Elder, J.A., Weil, C.M., Benane, S.G., Eichinger, D.C., House, D.E. 1979. Radio Sci. 14(6S):93-98.

Blackman, C.F., Benane, S.G., Elder, J.A., House, D.E., Lampe, J.A., Faulk, J.M. 1980a. Bioelectromagnetics 1:35-43.

Blackman, C.F., Benane, S.G., Joines, W.T., Hollis, M.A., House, D.E. 1980b. Bioelectromagnetics 1:277-283.

Blackman, C.F., Joines, W.T., and Elder, J.A. In Illinger KH (ed): 1981. "Biological Effects of Nonionizing Radiation, "Symposium Series Proceedings Vol. 157. Washington, DC,American Chemical Society, pp. 299-314.

Blackman, C.F., Benane, S.G., Kinney, L.S., Joines, W.T., and House, D.E. 1982. Radiation Research 92: 510-520.

Blackman, C.F., Benane, S.G., Rabinowitz, J.R., House, D.E., and Joines, W.T. 1985. Bioelectromagnetics 6(4): 327-337.

Blackman, C.F., Benane, S.G., Elliott, D.J., Wood, A.R., House, D.E., and Pollock, M.M. 1988a. Bioelectromagnetics 9:215-227.

Blackman, C.F., House, D.E., Benane, S.G., Joines, W.T., and Spiegel, R.J. 1988b. Bioelectromagnetics 9(2):129-140.

Blackman, C.F., Kinney, L.S., House, D.E., Joines, W.T. 1989. Bioelectromagnetics 10(2):115-128.

Blackman, C.F., Benane, S.G., House, D.E., Elliott, D.J. 1990. Bioelectromagnetics, 11:159-167.

Blackman, C.F., Most, B. 1993. Bioelectromagnetics, 14:413-431.

Blackman, C.F., Benane, S.G., House, D.E. and Pollock, M.M. 1993a. Bioelectromagnetics 14:273-286.

Blackman, C.F., Benane, S.G., House, D.E. 1993a, 1993b. FASEB J., 7:801-806.

Blackman, C.F., Benane, S.G., House, D.E. 1995. Bioelectromagnetics, 16:387-395.
Blackman, C.F., Blanchard, J.P., Benane, S.G., House, D.E. 1996. Biochemical and Biophysical Research Communications 220: 807-811.

Blackman, C.F., Blanchard, J.P., Benane, S.G., House, D.E. 1999. Bioelectromagnetics, 20:5-12.

Blackman, C.F. 2006. Electromagn. Biol. Med. 25: 217-225.

Carrubba S, Frilot C, Chesson A, Marino AA. 2006. J Neurosci Methods. 157:39-47.

Carrubba S, Frilot II C, Chesson AL Jr, Marino AA. 2007a. Neuroscience. 144:356-67.

Carrubba S, Frilot C, Chesson AL, Marino AA. Neurosci Lett. 417:212-6, 2007b.
Chiabrera, A., Bianco, B., Moggia, E., Kaufman, J.J. 2000. Bioelectromagnetics. 21(4):312-24.

Chiabrera, A., Hinsenkamp, M., Pilla, A.A., Ryaby, J., Ponta, D., Belmont, A., Beltrame, F., Grattarola, M., Nicolini, C. J. Histochem. 1979. Cytochem. 27: 375-381.

Chiabrera, A., Bianco, B., Moggia, E., Kaufman, J.J. 2000. Bioelectromagnetics 21(4):312-24.

Dutta, S.K., Subramoniam, A.,Ghosh, B., Parshad, R. 1984. Bioelectromagnetics 5:71-78.

Dutta, S.K., Ghosh, B., Blackman, C.F. 1989. Bioelectromagnetics 10(2):197-202.

Dutta, S.K., Das, K., Ghosh, B., and Blackman, C.F. 1992. Bioelectromagnetics, 13: 317-322.

Dutta, S.K., Verma, M., Blackman, C.F. 1994. Bioelectromagnetics 15:377-384.

Fedrowitz, M., Kamino, K., Loscher, W. 2004. Cancer Res. 64:243-251.

Fedrowitz, M., Loscher, W. 2005. Oncology 69:486-498.

Freude, G, Ullsperger, P, Eggert, S, Ruppe, I. 1998. Bioelectromagnetics 19(6): 384-387.

Freude, G, Ullsperger, P, Eggert, S, Ruppe, I. 2000. Eur. J Appl. Physiol. 81(1-2): 18-27.

Greene, J.J., Skowronski, W.J., Mullins, J.M., Nardone, R.M., Penafiel, M., Meister, R. 1991. Biochem. Biophys. Res. Comm. 174:742-749.

Gavalas, R.J., Walter, D.O., Hamer, J., Adey, W.R. 1970. Brain Res. 18: 491-501.
Gavalas-Medici, R, Day-Magdaleno, S.R. 1976. Nature 261:256-258.

Hamer, J. 1968. Commun. Behav. Biol., 2(5) part A: 217-222.

Havas, M. 2000. Environ. Rev. 8:173-253.

Joines, W.T. Blackman, C.F. 1981a. Bioelectromagnetics 2: 411-413.

Joines, W.T., Blackman, C.F., Hollis, M.A. 1981b. IEEE Transactions on Bio-Medical Engineering, BME-28:563-573.

Joines, W.T., Blackman, C.F., Spiegel, R.J. 1986. Bioelectromagnetics 7(2): 163-176.

Kaczmarek, L.K., Adey, W.R. 1973. Brain Res. 63: 331-342.

Kaczmarek, L.K., Adey, W.R. 1974. Brain Res. 66: 537-540.

Koivisto M, Revonsuo A, Krause C, Haarala C, Sillanmaki L, Laine M, Hamalainen H. 2000a. Neuroreport. 11(2):413-415.

Koivisto M, Krause CM, Revonsuo A, Laine M, Hamalainen H. 2000b. Neuroreport. 11(8):1641-1643.

Krause CM, Sillanmaki L, Koivisto M, Haggqvist A, Saarela C, Revonsuo A, Laine M, Hamalainen H. 2000a. Int. J Radiat. Biol. 76(12):1659-1667.

Krause CM, Sillanmaki L, Koivisto M, Haggqvist A, Saarela C, Revonsuo A, Laine M, Hamalainen H.. 2000b. Neuroreport. 11(4):761-764.

Liburdy, R.P. 1992. FEBS Lett. 301(1):53-59.

Marino, A., Wolcott, R.M., Chervenak, R., Jourd'heuil, F., Nilsen, E., Frilot II, C. Am J 2000. Physiol Regulatory Integrative Comp Physiol 279:R761-R768.

Marino AA, Wolcott RM, Chervenak R, Jourd'heuil F, Nilsen E, Frilot II C., Pruett SB. 2001a. Neuroimmunomodulation. 9:65-77.

Marino AA, Wolcott RM, Chervenak R, Jourd'heuil F, Nilsen E, Frilot LL C. 2001b. Bioelectromagnetics. 22:529-46.

Marino AA, Wolcott RM, Chervenak R, Jourd'heuil F, Nilsen E, Frilot II C. 2001c, Immunol Invest. 30:313-34.

Marino AA, Nilsen E, Frilot C. 2003. Brain Res. 964:317-26.

Marino AA, Nilsen E, Chesson AL Jr, Frilot C. 2004. Clin Neurophysiol. 115:1195-201.

Marron, M.T., Goodman, E.M., Sharpe, P.T., Greenebaum, B. 1988. FEBS Letters 230:13-16.

NIEHS Working Group Report, 1998. Assessment of health effects from exposure to

power-line frequency electric and magnetic fields. NIH Pub No. 98-3981, page 402.

NIEHS Report, 1999. Health effects from exposure to power-line frequency electric and magnetic fields. NIH Pub No. 99-4493, p. iii, NRC.

National Research Council, 1997. (U.S.) Committee on the possible effects of electromagnetic fields on biologic systems. National Academy Press, Washington, D.C. 356 pp.

National Council for Radiation Protection and Measurements. 1986. NCRP Report 86, Biological effects and exposure criteria for radiofrequency electromagnetic fields.

Ossenkopp, K.-P., Kavaliers, M, Hirst, M. 1983. Neurosci. Lett. 40: 321-325.

Preece, A.W., Wesnes, K.A., Iwi, G.R. 1998. Int. J Radiat. Biol. 74 (4):463-70.

Preece, A.W., Iwi, G., Davies-Smith, A., Wesnes, K., Butler, S, Lim, E., Varey, A. 1999. Int J Radiat Biol. 75(4):447-56.

Scarfi, M.R., Prisco, F., Lioi, M.B., Zeni, O., Dela Noce, M., Di Petro, R. Bersani, F. 1996. European Bioelectromagnetics Association 3rd International Congress, 29 Feb - 3 March, Nancy, France.

Scarfi, M.R., Prisco, F., Lioi, M.B., Zeni, O., Della Noce, M., Di Pietro, R., Franceschi, C., Iafusco, D., Motta, M., Bersani, F. 1997. Bioelectrochemistry and Bioenergetics 43:221-226.

Schwartz, J.-L., House, D.E., Mealing, G.A.R. 1990. Bioelectromagnetics 11:349-358.

Schwartz, J.-L., Mealing, G.A.R. 1993. Bioelectromagnetics. 14:521-33.

Sheppard, A.R., Bawin, S.M., Adey, W.R. 1999. Radio Sci 14(6S):141-145.

Slovic, P. 1999. Risk Anal. 19(4):689-701.

Takahashi, K., Kaneko, I., Date, M., Fukada, E. 1986. Experientia 42:185-186.
Thompson, C.J., Yang, Y.S., Anderson, V., Wood, A.W. 2000. Bioelectromagnetics. 21(6):455-64.

Trillo, M.A., Ubeda, A., Blanchard, J.P., House, D.E., Blackman, C.F. 1996. Bioelectromagnetics, 17:10-20.

Ubeda, A., Leal J., Trillo, M.A., Jimenez, M.A., Delgado, J.M.R. 1983. J Anat 137:513-536.

Evidence for Disruption by Modulation
Role of Physical and Biological Variables in Bioeffects of Non-Thermal Microwaves for Reproducibility, Cancer Risk and Safety Standards
2012 Supplement

Prof. Igor Belyaev, Ph D, Dr Sc

Associate Professor, Faculty of Natural Sciences

Stockholm University, Stockholm, Sweden

Head Research Scientist, Laboratory of Molecular Genetics, Cancer Research Institute

Slovak Academy of Science, Bratislava, Slovakia

Head Research Scientist, Laboratory of Radiobiology

General Physics Institute, Russian Academy of Science

Moscow, Russia

ABSTRACT

Diverse biological responses to non-thermal (NT) microwaves (MW), including adverse health effects related to increased cancer risk, have been studied by multiple research groups all over the world. In approximately half of these studies, no any effects were found (negative studies), while the other half reported the NT MW effects (positive studies). This fact is often referred to as non-reproducibility of the NT MW effects. In most cases, such a conclusion is based on comparing studies, which significantly differ in important biological and physical variables/parameters. The aim of this chapter is to provide an overview of the complex dependence of the NT MW effects on various physical and biological parameters, which must be controlled in replication studies. To the aim of this paper, all studies available to the author, which included analysis of different variables/parameters and reported some positive NT MW response to be a reference for analyzing its dependence on physical and biological parameters, were included. Selection criteria included relevant experimental design, methodological quality and statistical analysis. Besides dependencies on carrier frequency, modulation, genotype, physiological traits, presence of radical scavengers and antioxidants, reported by many research groups, the emerging data suggest dependencies of the NT MW effects on polarization, intermittence and coherence time of exposure, static magnetic field, electromagnetic stray fields, sex, age, individual traits, cell density during exposure. This overview provides clear evidence that in most cases, the references to non-reproducibility of the NT MW effects are not correct. Unfortunately, most reviews and panels in the field do not include analysis of various biological variables and physical parameters when comparing the data on the NT MW effects from different studies. As result, misleading conclusion is often made that MW at NT levels produce no "reproducible" effects. Our analysis suggests that different (bandwidth, frequency, modulation, polarization) NT MW signals should be considered as separate agents in setting the safety standards. The data also indicate that duration of exposure may be as important as power density (PD) and specific absorption rate (SAR), and, therefore, the "dose" and duration of exposure should also be considered in safety standards along with PD/SAR. Further evaluation of the dependencies of NT MW effects on biological and physical variables/parameters are needed for understanding the mechanisms by which NT MW affect biological systems, planning *in vivo* and epidemiological studies, setting the safety standards, and minimizing the adverse effects of MW from mobile communication.

Keywords: non-thermal effects of microwaves, mobile (cellular) phones, safety standards.

I.   THERMAL VERSUS NON-THERMAL EFFECTS

Exposures to electromagnetic fields vary in many parameters: power (specific absorption rate, incident power density), wavelength/frequency, near field/far field, polarization (linear, circular), continues wave (CW) and pulsed fields (that include variables such as pulse repetition rate, pulse width or duty cycle, pulse shape, pulse to average power, etc.), modulation (amplitude, frequency, phase, complex), static magnetic field (SMF) and electromagnetic stray fields at the place of exposure, overall duration and intermittence of exposure (continuous, interrupted), acute and chronic exposures. With increased absorption of energy, so-called thermal effects of microwaves (MW) are usually observed that deal with MW-induced heating. Specific absorption rate (SAR) or power density (PD) is a main determinate for thermal MW effects. Several other physical parameters of exposure have been reported to be of importance for so-called non-thermal (NT) biological effects, which are induced by MW at intensities well below any measurable heating (Grundler, Jentzsch et al. 1988; Iskin 1990; Devyatkov, Golant et al. 1994; Pakhomov, Akyel et al. 1998; Adey 1999; Belyaev, Shcheglov et al. 2000; Betskii, Devyatkov et al. 2000; Banik, Bandyopadhyay et al. 2003; Grigoriev, Stepanov et al. 2003; Grigoriev 2004; Lai 2005; Belyaev 2010; Cifra, Fields et al. 2011) (Pakhomov and Murphy 2000).

Most often, current safety standards are based on thermal MW effects observed in short-term (acute) exposures. On the other hand, NT MW effects, especially those induced during prolonged (chronic) exposures, are accepted and taken into account for setting the national safety standards in some countries such as Russia (Grigoriev, Stepanov et al. 2003; Grigoriev 2004; Grigoriev, Nikitina et al. 2005). It should be noted that, in contrast to the ICNIRP (International Commission for Non-Ionizing Radiation Protection) safety standards (ICNIRP 1998) which are based on the acute thermal effects of MW, the standards adopted by the Russian National Committee on Non-Ionizing Radiation Protection (RNCNIRP) are based on experimental data from chronic (up to 4 month) exposures of animals to MW at various physical parameters including intensity, frequency and modulation, obtained from research performed in the former Soviet Union (Grigoriev, Stepanov et al. 2003; Grigoriev 2004; Grigoriev, Nikitina et al. 2005).

Since setting the current safety standards, the situation with exposure of the general population to MW has changed significantly. Nowadays, most of the human population is chronically exposed to MW signals from various sources including mobile phones and base stations. These exposures are characterized by low intensities, varieties and complexities of signals, and long-term durations of exposure that are comparable with a lifespan. So far, the "dose" (accumulated absorbed energy that is measured in radiobiology as the dose rate multiplied by exposure time) is not adopted for the MW exposures and SAR or PD is usually used for guidelines. To what degree SAR/PD can be applied to the nowadays NT MW chronic exposures is not known and the current state of research demands reevaluation of the safety standards (Grigoriev, Nikitina et al. 2005).

The literature on the NT MW effects is very broad. About half of available experimental studies report non-thermal biological effects of microwaves (Huss, Egger et al. 2007). There are four lines of evidence for the NT MW effects: (1) altered cellular responses in laboratory *in vitro* studies and results of chronic exposures *in vivo* studies (Grigoriev, Stepanov et al. 2003; Lai 2005; Cook, Saucier et al. 2006); (2) results of medical application of NT MW in the former Soviet Union countries (Sit'ko 1989; Devyatkov, Golant et al. 1994; Betskii, Devyatkov et al. 2000; Pakhomov and Murphy 2000; Pakhomov and Murphy 2000); (3) hypersensitivity to electromagnetic fields (EMF) ; (4) epidemiological studies suggesting increased cancer risks from using mobile phones longer than 10 years (Kundi, Mild et al. 2004; Lonn, Ahlbom et al. 2004; Hardell, Eriksson et al. 2005).

The first data on the NT effects of MW in so-called millimeter range (wavelength 1-10 mm in vacuum) was obtained by Vilenskaya and co-authors (Vilenskaya, Smolyanskaya et al. 1972) and Devyatkov (Devyatkov 1973). Highly resonant effects of ultra-weak MW (near 70 GHz) on the

induction of λ-phage were first established by Webb (Webb 1979), and subsequently corroborated (Lukashevsky and Belyaev 1990). In these and subsequent studies the observed spectra of MW action were found to have the following common properties: (*1*) the MW effects were strongly dependent on the frequency (frequency windows), (*2*) there was an associated power (intensity) threshold below which no effect was observed, and above which the effects of exposure depended only weakly on power over several orders of magnitude (so-called S-shaped or sigmoid dependence), (*3*) the occurrence of MW effects depended on the duration of exposure, a certain minimum duration of exposure was necessary for an effect to manifest itself. These important regularities of the NT MW effects have previously been reviewed (Postow and Swicord 1986; Grundler, Jentzsch et al. 1988; Golant 1989; Iskin 1990; Belyaev 1992; Devyatkov, Golant et al. 1994; Pakhomov, Akyel et al. 1998; Hyland 2000; Pakhomov and Murphy 2000).

The first investigations of the NT MW effects at lower frequency ranges were performed by several research groups in USSR (Presman, IuI et al. 1961; Presman 1963) and in USA by Frey (Frey 1967; Frey 1974), Blackman and colleagues (Blackman, Benane et al. 1980; Blackman, Benane et al. 1980; Joines and Blackman 1980) and Adey and colleagues (Adey, Bawin et al. 1982; Lin-Liu and Adey 1982). These groups found dependence of the NT MW effects on modulation. The effect of pulse-modulated MW was related to peak power, whereas average power was found to be relatively unimportant (Frey 1974). Frequency dependence of the MW effects have been reported (Frey 1974).

Since that time, other groups have confirmed and extended the main findings of these pioneering studies. Below, survey of recent studies, which evaluate dependence of the NT MW effects on physical parameters and biological variables, is provided.

II.   FREQUENCY DEPENDENCE AND FREQUENCY WINDOWS

The effects of NT MW on DNA repair in *E. coli* K12 AB1157 were studied by the method of anomalous viscosity time dependence (AVTD) (Belyaev, Alipov et al. 1992; Belyaev, Alipov et al. 1992). The AVTD method is a sensitive technique to detect changes in conformation of nucleoids/chromatin induced by either genotoxic or stress factors (Belyaev and Harms-Ringdahl 1996; Belyaev, Shcheglov et al. 1996; Belyaev, Alipov et al. 1997; Sarimov, Malmgren et al. 2004; Belyaev, Hillert et al. 2005; Markova, Hillert et al. 2005). Significant inhibition of DNA repair was found when X-ray-irradiated cells were exposed to MW within the frequency ranges of 51.62-51.84 GHz and 41.25-41.50 GHz. The effects were observed within two "frequency windows", both

displaying a pronounced resonance character with the resonance frequencies of 51.755 GHz and 41.32 GHz, respectively (Belyaev, Alipov et al. 1992; Belyaev, Alipov et al. 1992). Of note, these MW effects were observed at PD well below any thermal effects and could not be accounted for by heating. The frequency windows of resonance type have often been termed "resonances" as also will be used below.

The resonance frequency of 51.755 GHz was stable within the error of measurements, ± 1 MHz with decreasing the PD from $3 \cdot 10^{-3}$ to 10^{-19} W/cm^2 (Belyaev, Alipov et al. 1992; Belyaev, Shcheglov et al. 1996). At the same time, the half-width of the resonance decreased from 100 MHz to 3 MHz revealing an extremely sharp dependence on frequency (Q ~ 10^4). This sharp narrowing of the 51.755 GHz resonance with decreasing the PD from $3 \cdot 10^{-3}$ to 10^{-7} W/cm^2 followed by an emergence of new resonances, 51.675 ± 0.001, 51.805 ± 0.002, and 51.835 ± 0.005 GHz (Belyaev, Shcheglov et al. 1996; Shcheglov, Belyaev et al. 1997). The half-widths of all these resonances including the main one, 51.755 ± 0.001 GHz, were about 10 MHz at the PD of 10^{-10} W/cm^2. These data were interpreted in the framework of the model of electron-conformational interactions as a splitting of the main resonance 51.755 GHz by the MW field (Belyaev, Shcheglov et al. 1996).

The MW effects were studied at different PD and several frequencies around the resonance frequency of 51.675 GHz (Shcheglov, Belyaev et al. 1997). This resonance frequency was found to be stable, ± 1 MHz, within the PD range of 10^{-18} - 10^{-8} W/cm^2. Along with disappearance of the 51.675 GHz resonance response at the sub-thermal PD of 10^{-6} - 10^{-3} W/cm^2, a new resonance effect arose at 51.688 ± 0.002 GHz (Shcheglov, Belyaev et al. 1997). This resonance frequency was also stable within the PD range studied.

Taken together, the data on NT MW effects on chromatin (Belyaev, Alipov et al. 1992; Belyaev, Shcheglov et al. 1996; Shcheglov, Belyaev et al. 1997) suggested a sharp rearrangement of the frequency spectra of MW action, which was induced by the sub-thermal MW (Belyaev, Alipov et al. 1992; Belyaev, Shcheglov et al. 1996; Shcheglov, Belyaev et al. 1997). The half-widths of all three resonances depended on PD, changing either from 2-3 MHz to 16-17 MHz (51.675 GHz and 51.668 GHz resonances) or from 2-3 MHz to 100 MHz (51.755 GHz resonance) (Belyaev, Shcheglov et al. 1996; Shcheglov, Belyaev et al. 1997). The data indicated also that dependencies of half-width on PD might vary for different resonance frequencies.

Significant narrowing in resonance response with decreasing PD has been found when studying the growth rate in yeast cells (Grundler 1992) and chromatin conformation in thymocytes of rats (Belyaev and Kravchenko 1994). In the Gründler's study, the half-width of the resonance (near 41 GHz) decreased from 16 MHz to 4 MHz as PD decreased from 10^{-2} W/cm^2 to 5 pW/cm^2 (Grundler 1992).

Thus, the results of studies with different cell types indicate that narrowing of the resonance window upon decrease in PD is one of the general regularities in cell response to NT MW. This regularity suggests that many coupled oscillators are involved non-linearly in the response of living cells to NT MW as has previously been predicted by Fröhlich (Frohlich 1968).

Gapeev et al. studied effects of MW exposure (frequency range 41.75-42.1 GHz, frequency increment 50 MHz, PD 240 μW/cm^2) on the respiratory burst induced by calcium ionophore A23187 and phorbol ester 12-myristate 13-acetate (PMA) in the peritoneal neutrophils of mice (Gapeev, Safronova et al. 1996; Gapeyev, Safronova et al. 1997). MW inhibited the respiratory burst. MW effect displayed resonance-like dependence on frequency, the resonance frequency and half-width of the resonance being 41.95 GHz and 160 MHz, respectively (Q= 260) (Gapeev, Safronova et al. 1996; Gapeyev, Safronova et al. 1997). In other studies, Gapeev et al. analyzed acute zymosan-induced paw edema in mice (Gapeyev, Mikhailik et al. 2008; Gapeyev, Mikhailik et al. 2009). MW exposure of animals at the PD of 0.1 mW/cm^2 resulted in decrease of the paw edema that was frequency-dependent in the range of 42-43 GHz.

Based on the extrapolation from the data obtained in the extremely high frequency range (30-300 GHz), the values for half-width of resonances at the frequency range of mobile phones (0.9–2 GHz) were estimated to be 1-10 MHz (Sarimov, Malmgren et al. 2004). Effects of GSM (Global System for Mobile Communication) MW on chromatin conformation and 53BP1 (tumor suppressor p53 binding protein 1)/γ-H2AX (phosphorylated H2AX histone) DNA repair foci in human lymphocytes were studied in this frequency range (Sarimov, Malmgren et al. 2004; Belyaev, Hillert et al. 2005; Markova, Hillert et al. 2005; Belyaev, Markova et al. 2009). These MW effects depended on carrier frequency (Sarimov, Malmgren et al. 2004; Markova, Hillert et al. 2005; Belyaev, Markova et al. 2009). This dependence was replicated in independent experiments with lymphocytes from twenty six healthy and hypersensitive persons (Belyaev, Hillert et al. 2005; Markova, Hillert et al. 2005; Belyaev, Markova et al. 2009).

Tkalec and colleagues exposed duckweed (*Lemna minor L.*) to MW at the frequencies of 400, 900, and 1900 MHz (Tkalec, Malaric et al. 2005). The growth of plants exposed for 2 h to a 23 V/m electric field of 900 MHz significantly decreased in comparison with the control, while an electric field of the same strength but at 400 MHz did not have such effect. A modulated field at 900 MHz strongly inhibited the growth, while at 400 MHz modulation did not influence the growth significantly. At both frequencies, a longer exposure mostly decreased the growth and the highest electric field (390 V/m) strongly inhibited the growth. Exposure of plants to lower field strength (10 V/m) for 14 h caused a significant decrease at 400 and 1900 MHz while 900 MHz did not influence the growth. Peroxidase activity in exposed plants varied, depending on the exposure characteristics.

Observed changes were mostly small, except in plants exposed for 2 h to 41 V/m at 900 MHz where a significant increase (41%) was found. The authors concluded that MW might influence plant growth and, to some extent, peroxidase activity. However, the effects of MW strongly depended on the characteristics of the field exposure such as frequency and modulation. These dependences were replicated in further studies (Tkalec, Malaric et al. 2007; Tkalec, Malaric et al. 2009).

Remondini et al. analyzed changes in gene expression in human EA.hy926 endothelial cells using gene microarrays (Remondini, Nylund et al. 2006). Cells were exposed to MW (SAR 1.8-2.5 W/kg, 1 h exposure) either at 900-MHz GSM Basic mode or 1800-MHz GSM Basic mode. Exposure to 900 MHz resulted in up-regulation in 22 genes and down-regulation in 10 genes. No significant change in gene expression was observed after exposure to 1800 MHz.

III. NON-LINEARITY: SIGMOID INTENSITY DEPENDENCES AND POWER WINDOWS

Devyatkov with colleagues have found and published in Russian that wide variety of NT MW effects *in vitro* and *in vivo* display sigmoid dependence on intensity above certain intensity thresholds (Devyatkov 1973).

In English literature, one of the earliest observation of threshold in response to NT MW was published by Frey (Frey 1967). In this study, the threshold of 30 µW/cm2 was found in the study by Frey on Brain stem evoked responses to RF in cats (Frey 1967). This value was 4 orders of magnitude lower then intensities needed to cause internal body temperature increase.

In their pioneering study on blood-brain barrier (BBB) permeability, Oscar and Hawkins exposed rats to MW at 1.3 GHz and analyzed BBB permeability by measuring uptake of several neutral polar substances in certain areas of the brain (Oscar and Hawkins 1977). A single, 20 min exposure, to continuous wave (CW) MW increased the uptake of D-mannitol at average power densities of less than 3 mW/ cm^2. Increased permeability was observed both immediately and 4 h after exposure, but not 24 h after exposure. After an initial rise at 0.01 mW/ cm^2, the permeability of cerebral vessels to saccharides decreased with increasing microwave power at 1 mW/cm^2. Thus, the effects of MW were observed within the power window of 0.01- 0.4 mW/cm^2. The findings on "power windows" for BBB permeability have been subsequently corroborated by the group of Persson and Salford (Salford, Brun et al. 1994; Persson, Salford et al. 1997). In their recent study, the effects of GSM MW on the permeability of the BBB and signs of neuronal damage in rats were investigated using a real GSM programmable mobile phone in the 900 MHz band (Eberhardt, Persson et al. 2008). The rats were exposed for 2 h at an SAR of 0.12, 1.2, 12, or 120 mW/kg.

Albumin extravazation and also its uptake into neurons increased after 14 d. The occurrence of dark neurons in the rat brains increased later, after 28 d. Both effects were seen already at 0.12 mW/kg with only slight increase, if any, at higher SAR values.

Sigmoid intensity dependences and power windows for the NT MW effects were observed in many other studies as previously reviewed (Postow and Swicord 1986; Grundler, Jentzsch et al. 1988; Golant 1989; Iskin 1990; Devyatkov, Golant et al. 1994; Blackman 2009).

Since 1980, there have been numerous reports of biological effects that show intensity "windows", that is, regions of intensity that cause changes surrounded by higher and lower intensities that show no effects from exposure, see for review (Blackman 2009). These results mean that lower intensity is not necessarily less bioactive, or less harmful.

Olcerst at al have reported that MW-induced increase in rubidium passive efflux did not increase monotonically with absorbed power (Olcerst, Belman et al. 1980). In fact, the highest exposure (SAR 390 mW/g) resulted in an increase, not statistically different from the lowest exposure level (SAR 100 mW/g) For sodium ions, at the greatest SAR of 390 mW/g, the effect was the smallest (Olcerst, Belman et al. 1980).

The data obtained in experiments with *E coli* cells and rat thymocytes provided new evidence for sigmoid type of PD dependence and suggested that, similar to ELF effects, MW effects may be observed within specific "intensity windows" (Belyaev, Shcheglov et al. 1992; Belyaev and Kravchenko 1994; Belyaev, Shcheglov et al. 1996; Shcheglov, Belyaev et al. 1997). The most striking example of the sigmoid PD dependence was found at the resonance frequency of 51.755 GHz (Belyaev, Shcheglov et al. 1996). When exposing *E. coli* cells at the cell density of $4\cdot10^8$ cell/ml, the effect reached saturation at the PD of 10^{-18}-10^{-17} W/cm^2 and did not change up to PD of 10^{-3} W/cm^2. In these experiments, the direct measurements of PD below 10^{-7} W/cm^2 were not available and lower PD was obtained using calibrated attenuators. Therefore, some uncertainty in the evaluation of the lowest PD was possible. The background MW radiation in this frequency range has been estimated to be 10^{-21}-10^{-19} W/m^2/Hz (Kolbun and Lobarev 1988). Based on the experimentally determined half-width of the 51.755 GHz resonance, 1 MHz (Belyaev, Shcheglov et al. 1996), the background PD was estimated as 10^{-19}-10^{-17} W/cm^2 within the 51.755 GHz resonance. The resonance MW effects on *E. coli* cells were observed at the PD very close to the estimated background value (Belyaev, Shcheglov et al. 1993; Belyaev, Alipov et al. 1994; Belyaev, Shcheglov et al. 1996; Shcheglov, Belyaev et al. 1997; Shcheglov, Alipov et al. 2002). These data suggested that the PD dependence of MW effect at the specific resonance frequencies might have intensity threshold just slightly above the background level. Dependence of the MW effect on PD at one of the resonance frequencies, 51.675 GHz, had the shape of "intensity window" in the PD range

from 10^{-18} to 10^{-8} W/cm^2 (Shcheglov, Belyaev et al. 1997). It is interesting, that no MW effect at this resonance frequency was observed at sub-thermal and thermal PD. This type of PD dependence has supported hypothesis about possible rearrangement of the frequency MW spectra action by the MW field (Belyaev, Shcheglov et al. 1996). The position of the PD window varied between different resonance frequencies and depended on cell density during exposure of cells (Shcheglov, Belyaev et al. 1997). Despite some uncertainty in the evaluation of PD at the levels below 10^{-7} W/cm^2 in the referred studies the data indicated that NT MW at the resonance frequencies may result in biological effects at very low intensities comparable with intensities from base stations and other MW sources used in mobile communication.

Gapeev et al. have studied dependence of the MW effects at the resonance frequency of 41.95 GHz on the respiratory burst induced by calcium ionophore A23187 and PMA in the peritoneal neutrophils of mice (Gapeev, Safronova et al. 1996; Gapeyev, Safronova et al. 1997). Inhibitory effects of MW exposure has been observed at the PD of 0.001 mW/cm^2 and displayed sigmoid dependence on PD at higher power densities (Gapeev, Safronova et al. 1996; Gapeyev, Safronova et al. 1997). In other study, Gapeev et al. analyzed acute zymosan-induced paw edema in mice (Gapeyev, Mikhailik et al. 2009). MW exposure of animals at the frequency of 42.2GHz and exposure duration of 20 min decreased the paw edema. Sigmoid dependence of this effect on PD has been obtained with a maximum at the PD of 0.1 mW/cm^2.

French et al. exposed human astrocytoma cells to EMR at 835 MHz at a power density of either 40 mWcm^2 or 8.1 mWcm^2 (French, Donnellan et al. 1997). Lower power signal was more potent than high power signal. At the lower power density, it was observed that the rate of DNA synthesis decreased, and that the cells flattened and spread out in comparison to unexposed cultures. At higher power density there were no effects seen on cell proliferation, but alteration in cell morphology included increased cell spreading and also the appearance of actin-containing blebs at localized sites on the membrane. It was hypothesized that 835 MHz radiation at low power density may be affecting a signal transduction pathway involved in cell proliferation.

Sigmoid dependence of the negative impact of mobile phone usage on semen quality in human males was found in recent study analyzing motility, vitality, ROS generation by the whole cell, ROS generation by the mitochondria, oxidative DNA damage and DNA fragmentation (De Iuliis, Newey et al. 2009). Specifically, all of the responses examined showed an extremely rapid change at low SAR exposures that then reached a plateau at a point where around 30% of the sperm population was affected.

Hintzsche et al. have recently reported sigmoid dependence on PD in the range up to 4.3 mW/cm^2 for non-thermal effects of MW on mitotic spindle in human-hamster hybrid cells (Hintzsche, Jastrow et al. 2011).

Sun et al. have investigated the effects of exposure to a 1.8-GHz radiofrequency radiation (RFR) at different intensities on epidermal growth factor (EGF) receptor clustering and phosphorylation in human amniotic (FL) cells (Sun, Shen et al. 2012). The results showed that exposure to RFR at specific absorption rate (SAR) of 0.5, 1.0, 2.0, or 4.0 W/kg for 15 min significantly induced EGF receptor clustering and enhanced phosphorylation of the tyrosine-1173 residue in FL cells. The RFR effect displayed a sigmoid-dependence on SAR with a prominent plateau in the range of 0.5-4 W/kg and a threshold below 0.5 W/kg.

It should be mentioned that almost all biophysical mechanisms, which have previously been proposed to account for NT MW effects, predict thresholds in dependence of these effects in intensity (Grundler, Jentzsch et al. 1988; Golant 1989; Iskin 1990; Devyatkov, Golant et al. 1994; Golo 2005; Matronchik and Belyaev 2008).

To conclude, since 1970, there have been numerous reports of biological effects that show thresholds, sigmoid dependence of the NT MW effects on intensity and also "power windows", that is, regions of intensity that cause changes surrounded by higher and lower intensities that show no effects from exposure. These results mean that: (i) lower intensity is not necessarily less bioactive, or less harmful;(ii)the NT effects may be observed at intensities above thresholds which are very close to background levels and similar to intensities from base stations.

IV. DOSE AND DURATION OF EXPOSURE

So far, the "dose" (accumulated absorbed energy that is measured in radiobiology as the dose rate multiplied by exposure time) is not adopted for the MW exposures and PD or SAR (dose rate analog in radiobiology) is usually used for guidelines. To what degree SAR/PD can be applied to the nowadays NT MW chronic exposures is not exactly known and the current state of research demands reevaluation of the safety standards (Grigoriev, Nikitina et al. 2005).

Based on mechanistic consideration of the NT MW effects, Frey has suggested that the toxicology model used by investigators was not the appropriate model on which to design MW experiments (Frey 1993). With chemical substance in a toxicology model, a dose-response relationship is usually observed: the greater the dose, the greater the effect. In analogy with toxicology, MW experiments tended to be designed with high doses and with little regard for other parameters such

as modulation and frequency. This might be one reason why many MW studies yielded so little useful information (Frey 1993).

The role of exposure duration in combination with dose rate/SAR for appearance and persistence of the NT MW effects have been analyzed by many research groups using various end-points.

Koveshnikova et al. exposed rats to pulsed MW (carrier frequency 3 GHz, pulse repetition 400 Hz, rectangular pulses of 2 μs, power flux density , PD, of 100, 500 and 2500 μW/cm2), during 60 days, 12 h/daily (Koveshnikova and Antipenko 1991) (is a determining factor 1991b). Chromosomal abreactions (CA) were analyzed in hepatocytes. Exposure was performed at three arrays of pulses so that 16, 29 or 48 arrays of pulses per 1 min were generated. The ratio of the obtained doses per animal was 1 : 1.8 : 3, correspondingly. Increased level of CA was generally observed at PD > 100 $\mu W/cm^2$. Importantly, the differences between PD disappeared when the dose per animal increased. In particular, even the PD of 100 μW/cm2 induced CA at higher absorbed doses. These data support the notion that the absorbed dose may be an important parameter for estimation of risks.

Bozhanova with co-authors reported that the effect of cellular synchronization induced by NT MW depended on duration of exposure and PD (Bozhanova, Bryukhova et al. 1987). The dependence on duration of exposure fitted to exponential function. The important observation was that in order to achieve the same synchronization of cells, the decrease in PD could be compensated by the increase in the duration of exposure.

MW exposure of *E. coli* cells and rat thymocytes at PDs of 10^{-5}-10^{-3} W/cm^2 resulted in significant changes in chromatin conformation if exposure was performed at resonance frequencies during 5-10 min (Belyaev, Alipov et al. 1992; Belyaev, Shcheglov et al. 1992; Belyaev and Kravchenko 1994). Decrease in the MW effects due to lowering the PD by orders of magnitude down to 10^{-14}-10^{-17} W/cm^2 could be compensated by several-fold increase of exposure time to 20-40 min (Belyaev, Alipov et al. 1994). At the relatively longer duration of exposure, more then 1 h, and the lowest PD of 10^{-19} W/cm^2, the same effect was induced as at highest PDs and shorter durations (Belyaev, Alipov et al. 1994).

Kwee and Raskmark analyzed effects of MW at 960 MHz and various SARs, 0.021, 0.21, and 2.1 mW/kg on proliferation of human epithelial amnion cells (Kwee and Raskmark 1998). These authors found linear correlations between exposure time to MW at 0.021 and 2.1 mW/kg and the MW-induced changes in cell proliferation albeit no such clear correlation was seen at 0.21 mW/kg.

Peinnequin et al. have studied effects of 24 or 48 h MW 2.45 GHz exposure at non-thermal level, 5 mW/cm^2, on apoptosis in human T-cell line Jurkat clone E6-1 (Peinnequin, Piriou et al. 2000). MW affected Fas -, but neither butyrate- nor ceramide - induced apoptosis. This effect depended on exposure time and was observed only upon 48 h exposure.

Croft et al. have tested twenty-four subjects participated in a single-blind fully counterbalanced cross-over design, where both resting EEG and phase-locked neural responses to auditory stimuli were measured while a mobile phone (MP) was either operating or turned off (Croft, Chandler et al. 2002). MP exposure altered resting EEG, decreasing 1-4 Hz activity (right hemisphere sites), and increasing 8-12 Hz activity as a function of exposure duration. MP exposure also altered early phase-locked neural responses, attenuating the normal response decrement over time in the 4-8 Hz band, decreasing the response in the 1230 Hz band globally and as a function of time, and increasing midline frontal and lateral posterior responses in the 30-45 Hz band. The data have shown that active MPs affect neural function in humans and do so as a function of exposure duration.

Caraglia et al. have evaluated the in vivo effect of MW-EMF in human epidermoid cancer KB cells (Caraglia, Marra et al. 2005). It was found that MW-EMF induced time-dependent apoptosis (45% after 3 h) that was paralleled by an about 2.5-fold decrease of the expression of ras and Raf-1 and of the activity of ras and Erk-1/2.

Gapeyev et al. studied anti-inflammatory effect of low-intensity MW exposure (0.1 mW/cm^2) using the model of acute zymosan-induced footpad edema in mice (Gapeyev, Mikhailik et al. 2008). Single whole-body MW exposure of mice at the frequencies of 42.2, 51.8, and 65 GHz after zymosan injection reduced both the footpad edema and local hyperthermia. At the frequency of 42.2 GHz the effect had sigmoid dependence on exposure duration with a maximum at 20-80 min. A linear dependence on the exposure duration with significantly lower increment was observed at a 10-fold less intensity (0.01 mW/cm^2). However, this decrease in the effect was compensated by a slight increase in duration of exposure from 80 min to 120 min.

Recently, the negative impact of mobile phone usage on semen quality in human males was repeatedly found to correlate with the duration of exposure (Agarwal, Deepinder et al. 2008; Agarwal, Desai et al. 2009).

Gerner et al. exposed human fibroblats to modulated GSM 1800 MHz at 2 W/kg (Gerner, Haudek et al. 2010). While short-term exposure within 2 hours did not significantly alter the proteome, an 8-h exposure caused a significant and reproducible increase in protein synthesis. Most of the proteins found to be induced were chaperones, which are mediators of protein folding. Heat-induced proteome alterations detectable with used proteome methodology would require heating

greater than 1°C. Because GSM-induced heating was less than 0.15°C, a heat-related response was excluded. These data further supported the notion that the exposure time seems to be a critical factor.

Differentiated astroglial cell cultures were exposed for 5, 10, or 20 min to either 900 MHz continuous waves or 900 MHz waves modulated in amplitude at 50 Hz (Campisi, Gulino et al. 2010). The strength of the electric field at the sample position was 10 V/m (rms). The irradiation conditions allowed the exclusion of any possible thermal effect. A significant increase in ROS levels and DNA fragmentation was found only after exposure of the astrocytes to modulated MW for 20 min. No evident effects were detected when shorter time intervals were used.

Adang et al. exposed Wistar albino rats to low-level RF during 21 months to two different microwave frequencies and exposure modes, 2 h a day, seven days a week (Adang, Remacle et al. 2009). After 14 and 18 months of exposure, the authors observed a significant increase in white blood cells and neutrophils of about 15% and 25%, respectively. Lymphocytes fell down after 18 months of exposure with about 15% compared to the sham-exposed group. No effects were observed at shorter duration of exposure. Exposure may probably have worked as a trigger and influenced the immune system, which reacted to this stressor by increasing the percentage of monocytes in the peripheral blood circulation.

Schrader et al. analysed production of spindle disturbances in FC2 cells, a human-hamster hybrid (A(L)) cell line, by MW with a field strength of 90 V/m at a frequency of 835 MHz (Schrader, Munter et al. 2008). Sigmoid dependence on time of exposure was observed with linear increase up to 30 min of exposure and saturation at longer exposures up to 2 h.

Markova et al. have found that inhibitory effect of MW on the 53BP1 foci leveled off at 1h-exposure (Markova, Malmgren et al. 2010). Human mesenchymal stem cells (MSC) and fibroblasts were expsosed to MW at GSM 915 MHz/UMTS 1947 MHz and SAR of 37/39 mW/kg. No further increase in effects was observed both in MSC and fibroblasts at prolongation of exposure to 3 h. This data are in agreement with previous results obtained in human peripheral blood lymphocytes that MW effects were the same at 1-h and 2-h exposures (Belyaev, Hillert et al. 2005; Markova, Hillert et al. 2005).

Panagopoulos and Margaritis have studied the effects of different durations of a single (continuous), daily exposure, ranging from 1 min up to 21 min, to EMF from GSM 900 MHz (Global System for Mobile telecommunications) and DCS 1800 MHz (Digital Cellular System-referred to also as GSM 1800 MHz), on the reproductive capacity of Drosophila melanogaster (Panagopoulos and Margaritis 2010). The insects were exposed to each type of radiation at intensity of about 10 μW/cm^2, corresponding to a distance of 20 or 30 cm from the antenna of a DCS 1800 or

a GSM 900 mobile phone handset, respectively. The results show that the reproductive capacity decreases almost linearly with increasing exposure duration to both GSM 900 and DCS 1800 radiation, suggesting that short-term exposures to these radiations have cumulative effects. Additionally, the results show that GSM 900 MHz radiation is slightly more bioactive than DCS 1800 MHz radiation, at the same exposure durations and under equal radiation intensities.

In some studies, the prolonged MW exposures were associated with less prominent effects than shorter exposures (Nikolova, Czyz et al. 2005; Tkalec, Malaric et al. 2007; Markova, Malmgren et al. 2010). This type of dependence on exposure duration was explained by adaptation of the exposed biosystems to the MW exposure (Markova, Malmgren et al. 2010).

Esmekaya et al. exposed human peripheral blood lymphocyte to GSM modulated MW radiation at 1.8 GHz and SAR of 0.21 W/kg for 6, 8, 24 and 48 h (Esmekaya, Aytekin et al. 2011). The authors reported morphological changes in exposed lymphocytes. Longer exposure periods led to destruction of organelle and nucleus structures. Chromatin change and the loss of mitochondrial crista occurred in cells exposed to RF for 8 h and 24 h and were more pronounced in cells exposed for 48 h. RF exposure did not increase the temperature. The authors concluded that the greater damage occurred after longer periods of exposure to NT MW.

Tepe Çam and Seyhan have analyzed DNA damage in hair root cells of volunteers before and after they have used 900-MHz GSM mobile phone for 15 or 30 min. The 900-MHz GSM exposure significantly increased single-strand DNA breaks in cells of hair roots close to the position of phone at the heads of volunteers. 30 min talking by mobile phone induced more DNA damage than 15 min talking (Cam and Seyhan 2012).

Nazıroğlu et all have measured cytosolic free Ca^{2+} in human leukemia cells during 1-24 h exposure to 2.45 GHz electromagnetic radiation at the average SAR of 1.63 W/kg (Naziroglu, Cig et al. 2012). Radiation induced increase of cytosolic free Ca^{2+} concentration was time-dependent and was highest at 24-h exposure.

In some studies, prolonged MW exposures were associated with less prominent effects than shorter exposures (Nikolova, Czyz et al. 2005; Tkalec, Malaric et al. 2007; Markova, Malmgren et al. 2010). This type of dependence on exposure duration was accounted for adaptation of the exposed systems to the MW exposure. The magnitude of adaptation depends on a number of biological variables that will be considered elsewhere.

In recent German study, 24 out of 60 participants were exposed to MW from base station at a power density of < 60 µW/m², 20 participants to 60 - 100 µW/m², and 16 participants to more than 100 µW/m² (Buchner and Eger 2011). The values of the stress hormones adrenaline and noradrenaline grew significantly during the first 6 months after starting the GSM base station; the

values of the precursor substance dopamine substantially decreased in this time period. The initial condition was not restored even after 1.5 years. Due to the not regulable chronic difficulties of the stress balance, the phenylethylamine levels dropped until the end of the investigation period. These effects show a dose-effect relationship.

Recently reported general indications of a dose–response relationship between chronic exposure to cellular phone MW and parotid gland malignancy indicate necessity of the dose approach at the epidemiological level (Duan, Zhang et al. 2011). For the first time in epidemiology of RF-induced tumors, Cardis et al. have used estimates of radio frequency energy deposition at the centre of tumors in the brain as a measure of MW dose (Cardis, Armstrong et al. 2011). An increased risk of glioma was seen in individuals at the highest quintile of radio frequency dose, though reduced risks were seen in the four lower quintiles. When risk was examined as a function of dose received in different time windows before diagnosis, an increasing trend was observed with increasing MW dose (for exposures 7 years or more in the past.

In conclusion, the data from different groups suggest that duration of exposure and dose may have significant role for the NT MW effects. In specially designed studies, reduction in dose rate/SAR could be compensated by prolongation of exposure time in order to achieve the same MW effect. The temporal nature of the MW effects contributes to the apparent lack of consistent results reported in the literature. Emerging epidemiology data indicate that the dose of MW exposure may correlate with the increased brain tumor risk.

V. TIME AFTER EXPOSURE

The MW effects on *E. coli* cells significantly depended on the post-exposure time (Belyaev, Shcheglov et al. 1993; Belyaev, Alipov et al. 1994; Shcheglov, Alipov et al. 2002). This dependence had an initial phase of increase about 100 min post-exposure followed by a phase, which was close to a plateau, around 100 min. A trend to decrease in effect was observed at longer times up to 300 min (Belyaev, Shcheglov et al. 1993; Shcheglov, Alipov et al. 2002).

Significant MW-induced changes in chromatin conformation were observed when rat thymocytes were analyzed in-between 30-60 min after exposure to MW (Belyaev and Kravchenko 1994). This effect nearly disappeared if the cells were incubated more than 80 min between exposure and analysis.

Gapeev et al. have studied dependence of the MW effect on the function of the mouse peritoneal neutrophils in dependence on duration of exposure at the frequency of 41.95 GHz and

the PD of 240 μW/cm² (Gapeev, Safronova et al. 1996; Gapeyev, Safronova et al. 1997). This dependence had a bell-shaped form with the maximal effects at 20 - 40 min of exposure.

In recent studies, human lymphocytes from peripheral blood of healthy and hypersensitive to EMF persons were exposed to NT MW from the GSM mobile phones (Belyaev, Hillert et al. 2005; Markova, Hillert et al. 2005). NT MW induced changes in chromatin conformation similar to those induced by heat shock, which remained up to 24 h after exposure. It was found in the same and following studies that GSM MW at the carrier frequency of 915 MHz and UMTS (Universal Mobile Telecommunications System) MW at 1947.4 MHz inhibited formation of 53BP1/γ-H2AX DNA repair foci and these adverse effects remained during 72 h after an 1-h exposure (Belyaev, Hillert et al. 2005; Markova, Hillert et al. 2005; Belyaev, Markova et al. 2009). The same group has reported that contrary to human fibroblast, which were able to adapt during chronic exposure to GSM/UMTS non-thermal MW, human stem cells did not adapt (Markova, Malmgren et al. 2010). Jorge-Mora et al. investigated the effects of MW 2.45 GHz radiation on the paraventricular nucleus (PVN) of the hypothalamus, extracted from brains of exposed rats (Jorge-Mora, Misa-Agustino et al. 2011). Expression of c-Fos was analyzed in rats exposed once or repeatedly (ten times in 2 weeks) to MW at non-thermal SAR of 0.0776 and 0.301 W/kg. High SAR triggered an increase of the c-Fos marker 90 min or 24 h after radiation, and low SAR resulted in c-Fos counts higher than in control rats after 24 h. Repeated irradiation at 0.0776 W/kg increased cellular activation of PVN by more than 100% compared to animals subjected to acute irradiation and to repeated non-radiated repeated session control animals. The results suggest that the time of exposure to single or repeated doses of NT MW is a determining factor, though possibly not the only factor, in establishing the power levels that may produce a response.

Lu et al. have demonstrated that reactive oxygen species (ROS) plays an important role in the process of apoptosis in human peripheral blood mononuclear cell (PBMC), which is induced by the exposure to 900 MHz radiofrequency electromagnetic at the SAR of 0.4W/kg when the exposure lasts longer than two hours (Lu, Huang et al. 2012).

The data indicate that there is a time window for observation of the NT MW effects, which may be dependent on endpoint measured, cell type, duration and PD of exposure.

VI. COHERENCE TIME

MW exposure of L929 fibroblasts was performed by the group of Litovitz (Litovitz, Krause et al. 1993). MW at 915 MHz modulated at 55, 60, or 65 Hz approximately doubled ornithine

decarboxylase (ODC) activity after 8 h. Switching the modulation frequency from 55 to 65 Hz at coherence times of 1.0 s or less abolished enhancement, while times of 10 s or longer provided full enhancement. These results suggested that the microwave coherence effects are remarkably similar to those observed previously with extremely low frequency (ELF) magnetic fields by the same authors.

VII. INTERMITTENCE

Diem and colleagues exposed cultured human diploid fibroblasts and cultured rat granulosa cells to intermittent and continuous MW (1800 MHz; SAR 1.2 or 2 W/kg; different modulations; during 4, 16 and 24 h; intermittent 5 min on/10 min off or continuous exposure) (Diem, Schwarz et al. 2005). Comet assay was applied to analyze DNA single- and double-strand breaks. MW-induced effects occurred after 16 h exposure in both cell types and after different mobile-phone modulations. The intermittent exposure showed a stronger effect than continuous exposure.

Remondini et al. analyzed changes in gene expression in human HL-60 leukemia cells using gene microarrays (Remondini, Nylund et al. 2006). Cells were exposed to MW (SAR 1.0-1.3 W/kg, 1800 MHz DTX mode, 24 h exposure) either continuously or intermittently, 5 min ON/5 min OFF. Gene expression was affected by intermittent exposure but not continuous exposure.

Elhag et al. investigated effect of near field EMR from GSM mobile phones on the oxidant and antioxidant status in rats (Elhag, Nabil et al. 2007). Rats were subjected to either intermittent exposure (15 min/day for four days) or acute exposure for 1 h. Significant drop in the plasma concentration of vitamin C, vitamin E, vitamin A and reduced glutathione (GSH) was observed in both exposed groups as compared to controls. EMR exposure of rats produced a significant decrease in catalase (CAT) and superoxide dismutase (SOD) activities, with the values of these activities for acute-exposure group is significantly lower than those of intermittent exposure. The authors concluded that the effects of acute exposure to mobile phones on the rat's antioxidant status is significantly higher than those of intermittent exposure of the same type of radiation.

Chavdoula et al used a 6-min daily exposure of dipteran flies, Drosophila melanogaster, to GSM-900MHz (Global System for Mobile Telecommunications) mobile phone electromagnetic radiation (EMR), to compare the effects between the continuous and four different intermittent exposures of 6 min total duration on the insect's reproductive capacity as well as on the induction of apoptosis (Chavdoula, Panagopoulos et al. 2010). It was found that intermittent exposure, similar to continuous exposure, decreases the reproductive capacity and alters the actin-cytoskeleton network

of the egg chambers, another known aspect of cell death, and that this effect is due to DNA fragmentation. Intermittent exposures with 10-min intervals between exposure sessions proved to be almost equally effective as continuous exposure of the same total duration, whereas longer intervals between the exposures seemed to allow the organism the time required to recover and partly overcome the above-mentioned effects of the GSM exposure.

VIII. MODULATION

Several types of modulations used in mobile communication have previously been reviewed (Foster and Repacholi 2004; Blackman 2009; Juutilainen, Hoyto et al. 2011). In particular, the 2G signals use the Gaussian Minimum Shift Keying (GMSK) modulation, have a high coherence, extremely low frequency amplitude modulation spectra, high crest factor (pulsed signal) and a power regulation with an update in the order of seconds. In contrast, the 3G Wideband Code-Division Multiple Access (WCDMA) uses essentially Quadrature Phase Shift Keying (QPSK) modulation, has a low coherence and a broad-band extremely low frequency amplitude modulation spectrum.

While considering effect of modulation, all other parameters, which are important for appearance of biological effects induced by NT MW, should be taken into account. In particular it is useless to include in analysis the papers where no effects of NT MW were detected at all because usually these studies do not scan the parameters of exposure in wide range to enable detecting the NT MW effects. Even more importantly is to analyze separately different types of modulations because each type may result in its own specific effect. When such approach is used, clear evidence is emerging for the effects of specific modulations. For example, among three studies on cancer-relevant non-genotoxic endpoints, biological effects (apoptosis, altered cell proliferation, lipid peroxidation) were induced by GSM modulated signal but not by a CW signal (Juutilainen, Hoyto et al. 2011). All these studies involved combined exposure to RF fields and other agents, and found GSM-modulation-specific effects on apoptosis. Another example is increased power in the alpha band (8–12 Hz) of EEG, which has been consistently seen in several studies most of which have used GSM-type modulation and have found that signals with pulse modulation are more biologically active than CW fields, or that signals with higher degree of modulation (e.g., handset-like signals) are more biologically active than signals with lower degree of modulation (e.g., base station-like signals). Studies that have used only GSM-type signals have provided additional evidence for effects of modulated RF signals on human brain functions (van Rongen, Croft et al.

2009). Overall, the consistency of the positive findings indicates that there may be reproducible modulation-specific effects on the human central nervous system (Juutilainen, Hoyto et al. 2011). This result is consistent with the well-known notion that properly modulated RF may be a useful tool in experiments directed at understanding nervous system function (Frey 1967).

Using aforementioned approach, it became clear that significant body of papers where NT MW effects were observed and modulated and unmodlatlated signals were carefully compared revealed the differences. There is strong experimental evidence for the role of modulation in the diverse biological effects of NT MW both in vitro and in vivo (Lin-Liu and Adey 1982; Byus, Lundak et al. 1984; Dutta, Subramoniam et al. 1984; Byus, Kartun et al. 1988; Dutta, Ghosh et al. 1989; Veyret, Bouthet et al. 1991; Gapeev, Iakushina et al. 1997; Litovitz, Penafiel et al. 1997; Penafiel, Litovitz et al. 1997; Persson, Salford et al. 1997; d'Ambrosio, Massa et al. 2002; Huber, Treyer et al. 2002; Markkanen, Penttinen et al. 2004; Huber, Treyer et al. 2005). Examples include different types of modulation such as amplitude-, speech and phase modulations: (i) Amplitude modulation at 16 Hz, but not 60 Hz or 100 Hz, of a 450-MHz MW increased activity of ODC (Byus, Kartun et al. 1988). (ii) Speech-modulated 835-MHz MW produced no effect on ODC as compared to the typical signal from a TDMA (Time Division Multiple Access) digital cellular phone (Penafiel, Litovitz et al. 1997). (iii) Phase-modulated GSM-1800 MW (Gaussian Minimum Shift Keying, GMSK) at 1.748 GHz induced micronuclei in human lymphocytes while CW MW did not (d'Ambrosio, Massa et al. 2002).

Normal human lymphocytes were exposed for 5 days to continuous wave (CW) or pulsed wave (PW) 2450-MHz radiation at non-heating (37 degrees C) and various heating levels (temperature increases of 0.5, 1.0, 1.5, and 2 degrees C) (Czerska, Elson et al. 1992). The pulsed exposures involved 1-microsecond pulses at pulse repetition frequencies from 100 to 1,000 pulses per second at the same average SAR levels as the CW exposures. At non-heating levels, CW exposure did not affect lymphoblastoid transformation. At heating levels both conventional and CW heating enhanced transformation to the same extent and correlate with the increases in incubation temperature. PW exposure enhanced significantly transformation at non-heating levels. At heating levels PW exposure enhanced transformation to a greater extent than did conventional or CW heating. Authors concluded that PW 2450-MHz radiation acts differently on the process of lymphoblastoid transformation in vitro compared with CW 2450-MHz radiation at the same average SARs.

Bolshakov and Alexeeev used microelectrode and voltage-clamp techniques to record spontaneous electrical activity and ionic currents of Lymnea stagnalis neurons during exposure to a 900-MHz field in a waveguide-based apparatus (Bolshakov and Alekseev 1992). The field was

pulse-modulated at repetition rates ranging from 0.5 to 110 pps, or it was applied as a continuous wave (CW). When subjected to pulsed waves (PW), rapid, burst-like changes in the firing rate of neurons occurred at SARs of a few W/kg. If the burst-like irregularity was present in the firing rate under control conditions, irradiation enhanced its probability of occurrence. The effect had a threshold SAR near 0.5 W/kg. CW radiation had no effect on the firing rate pattern at the same SAR. Thus, the effect was dependent on modulation. Mediator-induced, current activation of acetylcholine, dopamine, serotonin, or gamma-aminobutyric-acid receptors of the neuronal soma was not altered during CW or PW exposures and, hence, could not have been responsible for the bursting effect.

Gapeev and co-authors studied production of reactive oxygen species (ROS) in isolated peritoneal neutrophils of mice using a model of synergistic reaction of calcium ionophore A23187 and phorbol ester PMA (Gapeev, Iakushina et al. 1997; Gapeyev, Yakushina et al. 1998). MW exposure at 41.95 GHz, continuous wave mode and 50 μW/cm^2, inhibited ROS production. MW modulated with the frequency of 1 Hz resulted in stimulation of the synergistic reaction. Modulation frequencies of 0.5, 2, 4, and 8 Hz did not cause significant effects, and modulation frequencies of 0.1, 16, and 50 Hz inhibited the synergistic reaction.

In other study, Gapeev et al. analyzed acute zymosan-induced paw edema in mice (Gapeyev, Mikhailik et al. 2009). MW exposure of animals at the PD of 0.1- 0.7 mW/cm^2 and some "effective" frequencies in the range of 42-43 GHz decreased the paw edema. Application of different modulation frequencies from the range of 0.03–100 Hz to MW exposure at the effective carrier frequency of 42.2 GHz did not lead to considerable changes in the effect. In contrast, modulation of MW at the "ineffective" carrier frequencies of 43.0 and 61.22 GHz by frequencies from the ranges of 0.07–0.1 and 20–30 Hz resulted in a maximal anti-inflammatory effects. The results suggested a complex dependence of the anti-inflammatory action of low-intensity MW on carrier and modulation frequencies.

Capri et al. evaluated the nonthermal effects of both a 900 MHz GSM signal and a 900 MHz CW RF field at low SARs (70–76 mW/kg average) on human peripheral blood mononuclear cells (PBMCs) *in vitro* (Capri, Scarcella et al. 2004). Data obtained from cells exposed to a GSM-modulated RF field showed a slight decrease in cell proliferation when PBMCs were stimulated with the lowest mitogen concentration and a slight increase in the number of cells with altered distribution of phosphatidylserine across the membrane. Data obtained from CW-exposed cultures showed no difference with respect to sham-exposed cultures in any of the end points studied.

Huber with coauthors investigated effects of MW similar to those used in mobile communication, a "base-station-like" and a "handset-like" signal (10 g tissue-averaged spatial peak-

SAR of 1 W/kg for both conditions), on waking regional cerebral blood flow (rCBF) in 12 healthy young men (Huber, Treyer et al. 2005). The effect depended on the spectral power in the amplitude modulation of the carrier frequency such that only "handset-like" MW exposure with its stronger low-frequency components but not the "base-station-like" MW exposure affected rCBF. This finding supported previous observations of these authors (Huber, Treyer et al. 2002) that pulse modulation of MW is of importance for changes in the waking and sleep EEG, and substantiated the notion that pulse modulation is crucial for MW-induced alterations in brain physiology.

Markkanen et al. exposed cdc48-mutated *Saccharomyces cerevisiae* yeast cells to 900 or 872 MHz MW, with or without exposure to ultraviolet (UV) radiation, and analyzed apoptosis (Markkanen, Penttinen et al. 2004). Amplitude modulated (217 pulses per second) MW significantly enhanced UV induced apoptosis in cells, but no effect was observed in cells exposed to unmodulated fields at the identical time-average SAR of 0.4 W/kg that was lower than the ICNIRP safety standards.

Persson and colleagues studied effects of MW of 915 MHz as CW and pulse-modulated with different pulse power and at various time intervals on permeability of the blood-brain barrier (BBB) in Fischer 344 rats (Persson, Salford et al. 1997). Albumin and fibrinogen were demonstrated immunochemically and classified as normal versus pathological leakage. The CW-pulse power varied from 0.001 W to 10 W and the exposure time from 2 min to 960 min. The frequency of pathological rats significantly increased in all exposed rats. Grouping the exposed animals according to the level or specific absorption energy (J/kg) gave significant difference in all levels above 1.5 J/kg. The exposure was 915 MHz MW either pulse modulated at 217 Hz with 0.57 ms pulse width, at 50 Hz with 6.6 ms pulse width, or CW. The frequency of pathological rats was significantly higher in MW-exposed groups than in controls and the frequency of pathological rats after exposure to pulsed radiation was significantly less than after exposure to CW.

In a study by Lypez-Martin et al. (Lopez-Martin, Brogains et al. 2009), GSM-exposed picrotoxin-pretreated rats showed differences in clinical and EEG signs, and in c-Fos expression in the brain, in comparison to picrotoxin-treated rats exposed to an equivalent dose of unmodulated radiation. Neither MW exposure caused tissue heating, so thermal effects could be ruled out. The most marked effects of GSM MW on c-Fos expression in picrotoxin-treated rats were observed in limbic structures, olfactory cortex areas and subcortical areas, the dentate gyrus, and the central lateral nucleus of the thalamic intralaminar nucleus group. Nonpicrotoxin-treated animals exposed to unmodulated radiation showed the highest levels of neuronal c-Fos expression in cortical areas. These results suggested a specific effect of the pulse GSM modulation on brain activity of a picrotoxin-induced seizure-proneness rat model.

Luukkonen et al. investigated effects of MW at 872 MHz and relatively high SAR value (5 W/kg) on intracellular reactive oxygen species (ROS) production and DNA damage in human SH-SY5Y neuroblastoma cells. The experiments also involved combined exposure to MW and menadione, a chemical inducing intracellular ROS production and DNA damage. Both CW and a pulsed signal similar to that used in GSM mobile phones were used. Exposure to the CW radiation increased DNA breakage in comparison to the cells exposed only to menadione. Comparison of the same groups also showed that ROS level was higher in cells exposed to CW RF radiation at 30 and 60 min after the end of exposure. No effects of the GSM-like modulated signal were seen on either ROS production or DNA damage.

Hinrikus et al. (Hinrikus, Bachmann et al. 2008) evaluated the effects of MW (450 MHz) pulse-modulated at the frequencies of 7, 14 and 21 Hz on human electroencephalographic (EEG) rhythms. The field power density at the scalp was 0.16 m W/cm^2. Modulated microwaves caused an increase in the average EEG alpha (17%) and beta (7%) power but the theta rhythm remained unaffected. Increases in the EEG alpha and beta power were statistically significant during the first half-period of the exposure interval (30 s) at the modulation frequencies of 14 and 21 Hz. The authors concluded that the effect of the 450-MHz MW modulated at 7, 14 and 21 Hz varies depending on the modulation frequency.

Hoyto et al. exposed human SH-SY5Y neuroblastoma and mouse L929 fibroblast cells to MW (SAR of 5 W/kg) at 872 MHz using continuous-waves (CW) or a modulated GSM-like signal under isothermal conditions (Hoyto, Luukkonen et al. 2008). Menadione was used to induce reactive oxygen species, and tert-butylhydroperoxide (t-BOOH) was used to induce lipid peroxidation. Two statistically significant differences related to MW exposure were observed: Lipid peroxidation induced by t-BOOH was increased in SH-SY5Y (but not in L929) cells, and menadione-induced caspase 3 activity was increased in L929 (but not in SH-SY5Y) cells. Both differences were statistically significant only for the GSM-modulated signal.

Franzellitti et al. exposed human trophoblast HTR-8/SVneo cells to MW at 1.8 GHz CW and differently modulated GSM signals (GSM-217Hz, (speaking only): and GSM-Talk (34% of speaking and 66% of hearing):) during 4 - 24 h (Franzellitti, Valbonesi et al. 2008). The inducible HSP70C transcript was significantly enhanced after 24 h exposure to GSM-217 Hz signals while being reduced after 4 and 16 h exposure to GSM-Talk signal. In another study of the same group , HTR-8/SVneo cells were exposed for 4, 16 or 24 h to 1.8 GHz continuous wave (CW) and different GSM signals, namely GSM-217 Hz and GSM-Talk (intermittent exposure: 5 min field on, 10 min field off). The alkaline comet assay was used to evaluate primary DNA damages and/or strand breaks due to uncompleted repair processes in HF-EMF exposed samples. The amplitude-

modulated signals GSM-217 Hz and GSM-Talk induced a significant increase in comet parameters in trophoblast cells after 16 and 24 h of exposure, while the un-modulated CW was ineffective (Franzellitti, Valbonesi et al. 2010).

Only CW RF resulted in statistically significant effect on immune system of the exposed rats (Campisi, Gulino et al. 2010). In this study, primary rat neocortical astroglial cell cultures were exposed to MW for 5, 10, or 20 min to either 900 MHz continuous waves or 900 MHz waves modulated MW in amplitude at 50 Hz using a sinusoidal waveform and 100% modulation index. The strength of the electric field (rms value) at the sample position was 10 V/m. A significant increase in ROS levels and DNA fragmentation was found only after exposure of the astrocytes to modulated EMF for 20 min. No evident effects were detected when shorter time intervals or continuous waves were used. The irradiation conditions allowed the exclusion of any possible thermal effect. The results show the importance of the amplitude modulation in the interaction between EMF and neocortical astrocytes (Campisi, Gulino et al. 2010).

There are studies where similar effects of modulated and CW MW were observed. Adang et al. exposed Wistar albino rats to low-level CW and pulse-amplitude modulated RF during 21 months at 970 MHz (Adang, Remacle et al. 2009). Similar effects on immune system were observed in both groups.

Significant amount of *in vivo* studies under varying parameters of exposure (intensity, frequency, exposure time, modulation, intermittence) have been performed in Russia/Soviet Union and published in Russian. Retrospective analysis of 52 Russian/Soviet *in vivo* studies with animals (mice, rats, rabbits, guinea pigs) on chronic exposure to MW has recently been published (Grigoriev, Stepanov et al. 2003). In these studies, various endpoints were measured up to 4 month of chronic exposure including analysis of: weight of animal body, histological analysis and weight of tissues, central nervous system, arterial pressure, blood and hormonal status, immune system, metabolism and enzymatic activity, reproductive system, teratogenic and genetic effects. Based on their analysis, the authors concluded that: "exposure to modulated MW resulted in bioeffects, which can be different from the bioeffects induced by CW MW; exposure to modulated MW at low intensities (non-thermal levels) could result in development of unfavorable effects; direction and amplitude of the biological response to non-thermal MW, both *in vitro* and *in vivo*, depended on type of modulation; often, but not always, modulated MW resulted in more pronounced bioeffects than CW MW; the role of modulation was more pronounced at lower intensity levels".

One review of the Russian/Soviet studies on the role of modulation on MW effects is available in English (Pakhomov and Murphy 2000). The authors conclude that "a number of good-quality studies have convincingly demonstrated significant bioeffects of pulsed MW. Modulation

often was the factor that determined the biological response to irradiation, and reactions to pulsed and CW emissions at equal time-averaged intensities in many cases were substantially different". Since that time, more studies have been published in Russian which show the role of modulation in experiemnts with animals (Dolgacheva, Semenova et al. 2000; Pashovkina and Akoev 2000; Pashovkina and Akoev 2001; Pashovkina and Akoev 2001; Akoev, Pashovkina et al. 2002).

In conclusion, significant amount of in vitro and in vivo studies from different research groups, although not universally reported, clearly indicated dependence of the NT MW effects on modulation.

IX. POLARIZATION

Polarization is a property of electromagnetic <u>waves</u> that describes the orientation of their <u>oscillations</u> versus direction of propagation. In most cases, electromagnetic wave propagates in free space as a <u>transverse wave</u> - the polarization is perpendicular to the wave's direction of propagation. The electric field may be oriented in a single direction (<u>linear polarization</u>), or it may rotate as the wave propagates (<u>circular</u> or <u>elliptical polarization</u>). In the latter cases, the oscillations can rotate either towards the right (right-handed polarization) or towards the left (left-handed polarization) in the direction of propagation.

The effects of circularly polarized (CP) MW were studied in *E. coli* cells at the frequencies from two frequency windows (resonances) that were identified using linearly polarized (LP) MW, within the frequency ranges of 51.62-51.84 GHz and 41.25-41.50 GHz (Belyaev, Alipov et al. 1992; Belyaev, Shcheglov et al. 1992). At the resonance frequency of 51.76 GHz, right-handed CP MW inhibited repair of X-ray-induced DNA damages (Belyaev, Alipov et al. 1992; Belyaev, Shcheglov et al. 1992). In contrast to right-handed polarization, left-handed CP MW had virtually no effect on the DNA repair, while the efficiency of LP MW was in-between of two circular polarizations. Inversion in effectiveness of circular polarizations was observed at another resonance frequency, 41.32 GHz. In contrast to the frequency of 51.76 GHz, left-handed CP MW at 41.32 GHz significantly inhibited DNA repair, while right polarization was almost ineffective. MW of the same CP affected cells at several frequencies tested within each resonance, alternative CP being almost ineffective (Belyaev, Alipov et al. 1992; Belyaev, Shcheglov et al. 1992; Belyaev, Shcheglov et al. 1992). Therefore, specific sign of effective CP, either left- or right-, was the attribute of each resonance. Two different types of installations, based on either spiral waveguides (Belyaev, Shcheglov et al. 1992) or quarter-wave mica plates (Belyaev, Alipov et al. 1992; Belyaev,

Shcheglov et al. 1992; Shcheglov, Belyaev et al. 1997; Ushakov, Shcheglov et al. 1999; Ushakov, Alipov et al. 2005), were used to produce CP MW. Similar results were observed regardless the way of producing the MW of different polarizations.

Pre-irradiation of *E. coli* cells to X-rays inverted the sign of effective polarization (Belyaev, Alipov et al. 1992; Belyaev, Shcheglov et al. 1992). This inversion was observed for two different resonances, 41.32 and 51.76 GHz. Neither resonance frequencies, nor half-widths of the resonance changed during the inversions in effective CPs. The effects of left- and right-handed CP MW become the same at 50 cGy (Belyaev, Alipov et al. 1992). At this dose, about one single stranded DNA break per haploid genome was induced. X-ray-induced DNA breaks result in relaxation of the supercoiled DNA-domains. It is known that the majority of DNA in living cells has a right-handed helicity (B-form) but a minor part, in order of 1 %, may alternate from the B-form with the form of left-handed helix (Z-form). Supercoiling is connected with transitions between right B-form to left Z-form in these DNA sequences. Therefore, the data suggested that difference in biological effects of polarized MW might be connected with DNA helicity and supercoiling of DNA-domains.

Supercoiling of DNA-domains is changed during cell cycle because of transcription, replication, repair, and recombination. It can also be changed by means of DNA-specific intercalators such as ethidium bromide (EtBr). EtBr changes supercoiling and facilitates the transition of DNA sequences from Z-form to B-form. Preincubation of *E. coli* AB1157 cells with EtBr inverted the effective polarization at the resonance frequency of 51.755 GHz and right-handed MW became more effective than left polarization (Ushakov, Shcheglov et al. 1999). EtBr changed the supercoiling of DNA-domains starting at a concentration of 1 μg/ml as measured with the AVTD in different cell types including *E. coli* (Belyaev, Shcheglov et al. 1996; Belyaev, Alipov et al. 1997; Belyaev, Eriksson et al. 1999). These data provided further evidence that DNA may be a target for the NT MW effects.

The effects of MW on conformation of nucleoids in *E. coli* cells have recently been studied at the power flux density of 100 μW/cm^2 (Ushakov, Alipov et al. 2006). Linearly polarized MW resulted in significant effects within specific frequency windows of resonance type in the range of 51-52 GHz. The distances between frequency windows were about 55-180 MHz. Only one of the two possible circular polarizations, left-handed or right-handed, was effective at each frequency window. The sign of effective circular polarization alternated between frequency windows.

While most data on the role of polarization in MW effects on chromatin have been obtained by the same research group (Belyaev, Alipov et al. 1992; Belyaev, Shcheglov et al. 1992; Belyaev, Shcheglov et al. 1992; Alipov, Belyaev et al. 1993; Belyaev, Alipov et al. 1993; Belyaev, Shcheglov et al. 1993; Belyaev and Kravchenko 1994; Shcheglov, Belyaev et al. 1997; Ushakov,

Shcheglov et al. 1999; Ushakov, Alipov et al. 2005; Ushakov, Alipov et al. 2006), recent data of others corroborated our findings at least partially (Shckorbatov, Pasiuga et al. 2009). These authors analyzed the condensation of chromatin in human buccal epithelium cells and human fibroblasts by the method of vital indigo carmine staining. MW induced chromatin condensation in dependence on polarization (Shckorbatov, Pasiuga et al. 2009). The same research group investigated the effects influence of linear and left-handed and right-handed elliptically polarized MW at 36.65 GHz on chromatin in human fibroblast nuclei (Shckorbatov, Pasiuga et al. 2010). Microwave irradiation at 10 and 100 $\mu W/cm^2$ induced chromatin condensation. The right-handed elliptically polarized radiation was more active than the left-handed polarization.

Obviously, the difference in effects of right- and left polarizations could not be explained by the heating or by the mechanism dealing with "hot-spots" due to unequal SAR distribution. The data about the difference in effects of differently polarized MW, the inversion of effective circular polarization between resonances and after irradiation of cells with X-rays and incubation with EtBr provided strong evidence for the non-thermal mechanisms of MW effects. These data suggested chiral asymmetry in the target for the NT MW effects, one of which is presumably chromosomal DNA (Belyaev, Alipov et al. 1992), and selection rules on helicity if quantum-mechanical approach is applied (Belyaev, Shcheglov et al. 1992).

Lai and Singh have consistently reported that circularly polarized MW exposure at 2450 MHz induced DNA damage in brain cells of the exposed rats (Lai and Singh 1995; Lai and Singh 1996; Lai and Singh 1997). Replication studies have also tested circularly polarized MW exposure at 2450 MHz and no induced DNA damage was reported (Malyapa, Ahern et al. 1997; Malyapa, Ahern et al. 1998; Lagroye, Anane et al. 2004). All these replication studies have used another exposure system. However, handedness of circular polarization has not been described neither in original study, no in replications. If the handedness was different between studies it could reasonably account for inconsistency.

In some studies, MW of circular polarization with undefined handedness were used, but the obtained effects were not compared with alternative circular polarization or linear polarization (Bartsch, Kupper et al. 2010).

XI. ELECTROMAGNETIC ENVIRONMENT

It is very likely that background EMF might be of importance for the MW effects. This hypothesis is based on the experimental observations that SMF, ELF magnetic fields, and MW at

low intensities induced similar effects in cells under specific conditions of exposure (Belyaev, Alipov et al. 1999; Belyaev, Shcheglov et al. 2000; Belyaev and Alipov 2001; Binhi, Alipov et al. 2001; Belyaev, Hillert et al. 2005). Despite very little has been achieved for mechanistic explanation of such effects, there are attempts to consider the effects of EMF in a wide frequency range in the frames of the same physical models (Chiabrera, Bianco et al. 1991; Matronchik, Alipov et al. 1996; Chiabrera, Bianco et al. 2000; Binhi 2002; Panagopoulos, Karabarbounis et al. 2002; Matronchik and Belyaev 2005; Matronchik and Belyaev 2008).

Litovitz and colleagues found that the ELF magnetic noise inhibited the effects of MW on ODC in L929 cells (Litovitz, Penafiel et al. 1997). The ODC enhancement was found to decrease exponentially as a function of the noise root mean square amplitude. With 60 Hz amplitude-modulated MW, complete inhibition was obtained with noise levels at or above 2 μT. With the DAMPS (Digital Advanced Mobile Phone System) cellular phone MW, complete inhibition occurred with noise levels at or above 5 μT. Further studies by the same group revealed that the superposition of ELF noise inhibited hypoxia de-protection caused by long term repeated exposures of chick embryos to MW (Di Carlo, White et al. 2002).

The effect of a magnetic noise on microwave-induced spatial learning deficit in the rat was investigated by Lai (Lai 2004). Rats were exposed to MW (2450 MHz CW, PD 2 mW/cm^2, average whole-body SAR 1.2 W/kg) alone or in combination with noise exposure (60 mG). Microwave-exposed rats had significant deficit in learning. Exposure to noise alone did not significantly affect the performance of the animals. However, simultaneous exposure to noise significantly attenuated the microwave-induced spatial learning deficit. The author concluded that simultaneous exposure to a temporally incoherent magnetic field blocks MW-induced spatial learning and memory deficits in the rat (Lai 2004).

Lai and Singh studied combined effects of a temporally incoherent magnetic noise (45 mG) and MW (CW 2450 MHz, PD 1 mW/cm^2, average whole-body SAR of 0.6 W/kg) in rat brain cells (Lai and Singh 2005). MW exposure induced significant DNA breakages as measured with both neutral and alkaline comet assays. Exposure to noise alone did not significantly affect cells. However, simultaneous noise exposure blocked the MW-induced effects.

Burch et al. have analyzed the relationship between cellular telephone use and excretion of the melatonin metabolite 6-hydroxymelatonin sulfate (6-OHMS) in two populations of male electric utility workers (Study 1, $n=149$; Study 2, $n=77$) (Burch, Reif et al. 2002). Participants collected urine samples and recorded cellular telephone use over 3 consecutive workdays. Personal 60-Hz magnetic field (MF) and ambient light exposures were characterized on the same days. A repeated measures analysis was used to assess the effects of cellular telephone use, alone and combined with

MF exposures, after adjustment for age, participation month and light exposure. No change in 6-OHMS excretion was observed among those with daily cellular telephone use >25 min in Study 1 (5 worker-days). Study 2 workers with >25 min cellular telephone use per day (13 worker-days) had lower creatinine-adjusted mean nocturnal 6-OHMS concentrations ($p=0.05$) and overnight 6-OHMS excretion ($p=0.03$) compared with those without cellular telephone use. There was also a linear trend of decreasing mean nocturnal 6-OHMS/creatinine concentrations ($p=0.02$) and overnight 6-OHMS excretion ($p=0.08$) across categories of increasing cellular telephone use. A combined effect of cellular telephone use and occupational 60-Hz MF exposure in reducing 6-OHMS excretion was also observed in Study 2. The authors concluded that exposure-related reductions in 6-OHMS excretion were observed in Study 2, where daily cellular telephone use of >25min was more prevalent. Prolonged use of cellular telephones may lead to reduced melatonin production, and elevated 60-Hz MF exposures may potentiate the effect.

Yao and colleagues investigated the influence of the GSM-like MW at 1.8 GHz on DNA damage and intracellular reactive oxygen species (ROS) formation in human lens epithelial cells (hLECs) (Yao, Wu et al. 2008). DNA damage examined by alkaline comet assay was significantly increased after 3 W/kg and 4 W/kg radiation, whereas the double-strand breaks (DSB) evaluated by γ-H2AX foci were significantly increased only after 4 W/kg radiation. Significantly elevated intracellular ROS levels were detected in the 3-W/kg and 4-W/kg groups. After exposure to 4 W/kg for 24 hours, hLECs exhibited significant G_0/G_1 arrest. All the effects were blocked when the MW exposure was superposed with a 2 μT electromagnetic noise. The authors concluded that superposed electromagnetic noise blocks MW-induced DNA damage, ROS formation, and cell cycle arrest.

It has previously been reported that resonance effects of MW on *E. coli* cell depend on the magnitude of static magnetic field at the place of MW exposure (Belyaev, Alipov et al. 1994). This dependence was explained by the model of electron-conformational interactions that also predicted possible shift of resonance frequencies in dependence on SMF (Belyaev, Shcheglov et al. 1996).

More recently, Ushakov with co-authors exposed *E. coli* cells to MW at the PD of 10^{-10} W/cm^2 and the frequencies of 51.675, 51.755 and 51.835 GHz (Ushakov, Alipov et al. 2005). In this study, cells were exposed to MW at various values of SMF within the range of geomagnetic filed: 22, 49, 61, or 90 μT. The authors observed that the effects of MW exposure on the conformation of nucleoids depended on the SMF during exposure.

Gapeev at al. analyzed effects of MW (41.85-42.1 GHz, frequency increment 50 MHz, PD 50 μВт/cm^2, 20 min exposure) on synergistic reaction of calcium ionophore A23187 and phorbol ester PMA in activation of the respiratory burst of the peritoneal neutrophils of mice (Gapeev,

Iakushina et al. 1997). The MW exposure was performed at various SMF. At a SMF of 50 µT, the authors observed frequency-dependent inhibition of the synergetic reaction with maximal effect at the frequency of 41.95 GHz. In the same frequency range, frequency-dependent activation of the synergetic reaction with a maximal effect at the frequency of 42.0 GHz was found at a SMF of 95 µT. The authors concluded that increasing the SMF from 50 to 95 µT resulted in the inversion of ten MW effects and the shift of the resonance frequency by 50 MHz (Gapeev, Iakushina et al. 1997; Gapeev, Iakushina et al. 1999). Moreover, these effects of MW at the 41.95 GHz and 42.0 GHz were not found at the SMF of ±1, 28.3, 75.5 or 117.3 µT suggesting that the NT MMW effects may appear only at specific values of SMF (Gapeev, Iakushina et al. 1997; Gapeev, Iakushina et al. 1999).

During 1997–2008, Bartsch et al. have performed two long-term (I and II) and two life-long (III and IV) experiments analyzing the effect of chronic exposure to a low-intensity GSM-like signal (900 MHz pulsed with 217 Hz, 100 µW/cm² average power flux density, 38–80 mW/kg SAR for whole body) on health and survival of unrestrained female Sprague-Dawley rats kept under identical conditions (Bartsch, Kupper et al. 2010). Radiofrequency continued up to 37 months. In experiment I no adverse health effects of chronic RF-exposure were detectable, neither by macroscopic nor detailed microscopic pathological examinations. Also in experiment II no apparent macroscopic pathological changes due to treatment were apparent. In the course of two complete survival experiments (2002–2005; 2005–2008) median survival was significantly shortened under RF-exposure in both experiments by 9.06% (95% CI 2.7 to 15.0%) (p=0.0064); i.e by 72 days in experiment III and 77 days in experiment IV (Bartsch, Kupper et al. 2010). Based on their thorough analysis of possible reasons for variability in RF effects from year to year, the authors assumed that theses variations follow the course of solar activity within the 11-years' sunspot cycle which, according to theirs reported observations, seems to affect pineal melatonin secretion which is an integral part of endogenous defense against cancer. The activity of the sun may influence laboratory animals via changes in the geomagnetic field, which is omnipresent and perceived by specific receptors, e.g. retinal melanopsin, also involved in the light-mediated synchronization of the SCN (central circadian clock of the brain) and controlling the circadian secretion of pineal melatonin.

The observations indicating dependence of the NT MW effects on SMF and EMF stray field may be of significant interest for further development of physical theory for the NT MW effects and development of safe mobile communication.

XII. CELL-TO-CELL INTERACTION IN RESPONSE TO MICROWAVES

The effects of NT MW at the resonance frequency of 51.755 GHz on conformation of nucleoids in *E. coli* cells were analyzed with respect to cell density during exposure (Belyaev, Alipov et al. 1994). The per-cell-normalized effect of MW increased by a factor of 4.7 ± 0.5 on average if cell density increased by one order of magnitude, from $4\cdot10^7$ to $4\cdot10^8$ cell/ml. These data suggested a co-operative nature of cell response to MW, which is based on cell-to-cell interaction during exposure. This suggestion was in line with the observed partial synchronization of cells after exposure to MW.

The co-operative nature of cell response to MW at the resonance frequency of 51.755 GHz was confirmed in further studies with *E. coli* cells (Belyaev, Shcheglov et al. 1996; Shcheglov, Belyaev et al. 1997; Shcheglov, Alipov et al. 2002). In addition, dependence of the per-cell-normalized effect on cell density was found for two other resonances, 51.675 GHz and 51.688 GHz. These data suggested that dependence on cell density during exposure is a general attribute of the resonance response of *E. coli* cells to NT MW. At the cell density of $4\cdot10^8$ cells/ml, the average intercellular distance was approximately 13 µm that is 10 times larger than the linear dimensions of *E. coli* cells (Belyaev, Alipov et al. 1994; Shcheglov, Alipov et al. 2002). Therefore, no direct physical contact seemed to be involved in the cell-to-cell interaction. Two mechanisms, biochemical and electromagnetic, were considered to account for the co-operative nature in the resonance response to weak EMF in wide frequency range including ELF, MW and ionizing radiation (Belyaev 1993; Belyaev, Alipov et al. 1994; Alipov, Shcheglov et al. 2003). The first one, biochemical, is based on release of secondary chemical messengers (ions, radicals, or molecules) by those cells, which were directly targeted. Via diffusion, these messengers can induce response in other cells. The second mechanism, electromagnetic, is based on reemission of secondary photons. According to this mechanism, reemitted photons can induce response in other cells if the intercellular distance is shorter than the length of photon absorption. The experimental data on MW effects fitted better to the electromagnetic mechanism but a combination of two mechanisms was also possible (Belyaev, Alipov et al. 1994; Shcheglov, Alipov et al. 2002). In particular, radicals with prolonged lifetimes might be involved in the observed cell-to-cell communication during response to EMF (Belyaev, Alipov et al. 1998).

The absorption length of photons with the frequencies of 10^{12}-10^{13} Hz corresponds to the intracellular distance at the cell density of $5\cdot10^8$ cell/ml, at which saturation in the dependences of EMF effects on cell density was observed (Belyaev, Alipov et al. 1994; Belyaev, Alipov et al. 1995; Belyaev, Alipov et al. 1998; Shcheglov, Alipov et al. 2002). Such photons may be involved in cell-

to-cell communication according to the electromagnetic mechanism and in agreement with the prediction of Fröhlich that biosystems support coherent excitations within frequency range of 10^{11}-10^{12} Hz (Frohlich 1968). From this point of view, cell suspension may respond to NT MW as a whole. In this case, the number of the exposed cells should be large enough to facilitate cell-to-cell communication during the responses to MW at specific parameters of exposure such as frequency, modulation, and polarization. Interestingly, the cell density for saturation of both MW and ELF effects was about $5 \cdot 10^8$ cell/ml that is close to cell densities in soft tissues of eukaryotes (Belyaev, Alipov et al. 1998; Shcheglov, Alipov et al. 2002). Such density of cells in the tissues may be important for regulation of living systems by electromagnetic cell-to-cell communication. Cellular membranes and DNA have been considered as possible sources of coherent excitations and photons, which may be involved in electromagnetic cell-to-cell communication (Frohlich 1968; Belyaev, Shcheglov et al. 1996; Belyaev, Alipov et al. 1998).

PD dependences of the MW effect at the 51.755 GHz resonance frequency were considerably different between two cell densities, $4 \cdot 10^7$ cells/ml and $4 \cdot 10^8$ cells/ml (Belyaev, Shcheglov et al. 1996). However, the resonance frequency of 51.755 GHz did not shift with the changes in cell density. The half-width of the 51.755 GHz resonance did not depend on cell density either. Contrary to the 51.755 GHz resonance response, the half-width of the 51.675 GHz resonance depended on cell density (Shcheglov, Belyaev et al. 1997). The data suggested that intracellular interaction during the NT MW exposures at some specific frequencies might affect sub-cellular targets for NT MW. This target is presumably chromosomal DNA that is organized in the DNA-domains (Belyaev, Alipov et al. 1992; Belyaev, Alipov et al. 1993; Matronchik and Belyaev 2005).

In all studies concerning dependence of the MW effects on cell density, the cells occupied a negligible part of the exposed volume and could not change the absorption of MW even at the highest cell densities (Belyaev, Alipov et al. 1994; Belyaev, Shcheglov et al. 1996; Shcheglov, Belyaev et al. 1997; Shcheglov, Alipov et al. 2002). Striking difference in the cell responses at various cell densities provided further evidence for non-thermal mechanism of the observed MW effects.

Significant MW effect on synchronization of *Saccharomyces carlsbergensis* yeast cells were observed by Golant and co-authors (Golant, Kuznetsov et al. 1994). Exposure to MW at 30 μW/cm^2 and 46 GHz induced synchronization as measured by cell density and bud formation. The authors assumed that MW induced cell-to-cell interaction resulting in the observed synchronization.

Possible role of intrinsic electromagnetic fields in cell-to-cell communication and mechanisms of their generation have recently been reviewed (Cifra, Fields et al. 2011).

XIII. GENETIC BACKGROUND AND CELL TYPE

Belyaev et al. have studied effects of MW on *E. coli* cells of three isogenic strains with different length of chromosomal DNA (Belyaev, Alipov et al. 1993). Bacterial chromosomal DNA in the cells of N99 wild type stain was lengthened by inserting DNA from λ and λ*imm*^{434}*bio*^{10} phages. Two strains were obtained with increased length of chromosomal DNA, N99(λ) and N99(λ,λ*imm*^{434}*bio*^{10}). The cells of these 3 strains were exposed to MW 10^{-10} at W/cm^2 and 10-17 frequencies within the ranges of 41.24-41.37 GHz and 51.69-51.795 GHz. The changes in chromatin conformation were analyzed before and after exposure. Clear resonance responses to MW were observed for each strain in both frequency ranges. However, each strain had its own resonance frequency, which were statistically significantly different between strains. All resonances had the same amplitude and half-width (Belyaev, Alipov et al. 1993). In each frequency band, all 3 resonances had the same effective circular polarization: right-handed in the 41.24-41.37 GHz band and left-handed within 51.69-51.795 GHz. All these data have led to conclusion that lengthening of chromosomal DNA resulted in shifting the resonance MW spectra of action. Importantly, these shifts in resonance frequencies could not be explained by the genetic activity of the inserted DNA. On the other hand, theoretical consideration based on oscillations of the DNA-domains regarding a whole nucleoid provided a good correlation between the increasing in the DNA length and the shifts in resonances (Belyaev, Alipov et al. 1993). A detailed analysis of MW effects on the cells of another *E. coli* strain, AB1157, at 10^{-10} W/cm^2 and various frequencies within 51.69-51.795 GHz, revealed the resonance frequency of 51.755±0.001 GHz (Belyaev, Shcheglov et al. 1996). This value was statistically significantly different from the resonance frequency of 51.765±0.002 in response of *E. coli* N99 cells to MW in the same frequency range (Belyaev, Shcheglov et al. 1996). It should be noted that both strains, AB1157 and N99, are considered as wild type strains. Nevertheless, these strains are different in their genotypes by several gene markers (Lukashevsky and Belyaev 1990; Belyaev, Alipov et al. 1992). These data provided evidence that cells of different origin, even being considered as wild type cells, might have different resonance responses to NT MW because of differences in their genotypes.

Stagg with colleagues exposed tissue cultures of transformed and normal rat glial cells to modulated MW (TDMA that conforms to the North American digital cellular telephone standard) at 836.55 MHz (Stagg, Thomas et al. 1997). Results from DNA synthesis assays differed for these two cell types. Sham-exposed and MW-exposed cultures of primary rat glial cells showed no significant differences for either log-phase or serum-starved condition. C6 glioma cells exposed to MW at 5.9

$\mu W/g$ SAR (0.9 mW/cm^2) exhibited small (20-40 %) but significant increases in 38 % of [^{3}H]-thymidine incorporation experiments.

Repacholi with co-authors chronically exposed wild-type mice and E mu-Pim1 transgenic mice, which are moderately predisposed to develop lymphoma spontaneously, to plane-wave pulse-modulated MW at 900 MHz with a pulse repetition frequency of 217 Hz and a pulse width of 0.6 ms (Repacholi, Basten et al. 1997). Incident power densities were 2.6-13 W/m^2 and SARs were 0.008-4.2 W/kg, averaging 0.13-1.4 W/kg. The lymphoma risk was found to be significantly higher in the exposed transgenic mice. No effects were seen in the wild type mice.

Markkanen with colleagues found that MW affected the UV-induced apoptosis in *Saccharomyces cerevisiae* yeast cells KFy437 (cdc48-mutant) but did not modify apoptosis in KFy417 (wild-type) cells (Markkanen, Penttinen et al. 2004).

Czyz with colleagues exposed pluripotent embryonic stem (ES) cells of wild-type and deficient for the tumor suppressor p53 to pulse modulated GSM MW at 1.71 GHz (Czyz, Guan et al. 2004). Two dominant GSM modulation schemes (GSM-217 and GSM-Talk), which generate temporal changes between GSM-Basic (active during talking phases) and GSM-DTX (discontinuous transmission, which is active during listening phases thus simulating a typical conversation), were applied to the cells at and below the ICNIRP safety standards, 2 and 1.5 W/kg . GSM-217 MW induced a significant upregulation of mRNA levels of the heat shock protein hsp70 of p53-deficient ES cells differentiating in vitro, paralleled by a low and transient increase of c-jun, c-myc, and p21 levels in p53-deficient, but not in wild-type cells. Theses data further substantiated the notion that the genetic background determines cellular responses to GSM MW.

Nylund and Leszczynski have examined cell response to MW (900 MHz GSM-like signal, average SAR of 2.8 W/kg) using two human endothelial cell lines: EA.hy926 and EA.hy926v1 (Nylund and Leszczynski 2006). Gene expression changes were examined using cDNA Expression Arrays and protein expression changes were examined using 2-DE and PDQuest software. The same genes and proteins were differently affected by exposure in each of the cell lines.

Remondini et al. analyzed changes in gene expression in six human cell lines by gene microarrays (Remondini, Nylund et al. 2006). Cells were exposed to MW at 900 MHz GSM Basic mode, SAR 1.8-2.5 W/kg, 1 h exposure. Most cell lines responded to GSM-900 MHz, except for the CHME5 human microglial cells.

Rat1 and HeLa human cells were subjected to RF exposure at a frequency of 875 MHz with an intensity of 0.07 mW/cm2 (Friedman, Kraus et al. 2007). In Rat1 cells, phosphorylation peaked at 15 min after irradiation and returned to basal level within 30 min, whereas, in HeLa cells, peak phosphorylation was at 5 min after stimulation and decreased thereafter. Increases in Hb-

EGF release upon mobile phone irradiation were detected in both Rat1 and HeLa cell lines, although the amount released from irradiated HeLa cells was much higher than that released from Rat1 cells.

Zhao et al. studied whether expression of genes related to cell death pathways are dysregulated in primary cultured neurons and astrocytes by exposure to MW from GSM cell phone at the frequency of 1900 MHz for 2 h (Zhao, Zou et al. 2007). Microarray analysis and real-time RT-PCR have shown up-regulation of caspase-2, caspase-6 and Asc (apoptosis associated speck-like protein containing a card) gene expression in neurons and astrocytes. Up-regulation occurred in both "on" and "stand-by" modes in neurons, but only in "on" mode in astrocytes. Additionally, astrocytes showed up-regulation of the Bax gene. The authors concluded that even relatively short-term exposure to the cell phone radiation can up-regulate elements of apoptotic pathways in cells derived from the brain, and that neurons appear to be more sensitive to this effect than astrocytes.

Hoyto et al. analyzed the effects of MW exposure on cellular ornithine decarboxylase (ODC) activity in fibroblasts, two neural cell lines and primary astrocytes (Hoyto, Juutilainen et al. 2007). Several exposure times and exposure levels were used, and the fields were either unmodulated or GSM-like-modulated. Murine L929 fibroblasts, rat C6 glioblastoma cells, human SH-SY5Y neuroblastoma cells, and rat primary astrocytes were exposed to RF radiation at 872 MHz in a waveguide exposure chamber equipped with water cooling. Cells were exposed for 2, 8, or 24 hours to CW MW or to a GSM type signal pulse modulated at 217 Hz. ODC activity in rat primary astrocytes was decreased statistically significantly and consistently in all experiments performed at two exposure levels (1.5 and 6.0 W/kg) and using GSM modulated or CW radiation. In the secondary cell lines, ODC activity was generally not affected. The authors concluded that ODC activity was affected by MW exposure in rat primary neural cells, but the secondary cells used in this study showed essentially no response. In further studies by the same group, the difference in response of human SH-SY5Y neuroblastoma and mouse L929 fibroblast cells to a GSM-modulated MW at 872 MHz was replicated (Hoyto, Luukkonen et al. 2008).

Human cultured fibroblasts of three different donors and three different short-term human lymphocyte cultures were exposed to UMTS-like MW at 1950 MHz and the SAR below safety limit of 2 W/kg by Schwarz et al. (Schwarz, Kratochvil et al. 2008). The alkaline comet assay and the micronucleus assay were used to analyze genotoxic effects. UMTS exposure increased the comet tail factor (CTF) and induced centromere-negative micronuclei in human cultured fibroblasts in a dose and time-dependent way. No UMTS effect was obtained with lymphocytes, either unstimulated or stimulated with phytohemagglutinin. The authors concluded that UMTS exposure may cause genetic alterations in some but not in all human cells in vitro.

Del Vecchio et al. have tested viability, proliferation, and vulnerability of neural cells, after continuous radiofrequency (RF) electromagnetic fields exposure (global system for mobile telecommunications (GSM) modulated 900 MHz signal at a specific absorption rate (SAR) of 1 W/kg and maximum duration 144 h) generated by transverse electromagnetic cells. Two cellular systems, SN56 cholinergic cell line and rat primary cortical neurons were used (Del Vecchio, Giuliani et al. 2009). Exposure to RF did not change viability/proliferation rate of the SN56 cholinergic cells or viability of cortical neurons. Co-exposure to RF exacerbated neurotoxic effect of hydrogen peroxide in SN56, but not in primary cortical neurons, whereas no cooperative effects of RF with glutamate and 25-35AA beta-amyloid were found. These data suggest that only under particular circumstances (cell type and type of co-exposure) exposure to GSM modulated, 900MHz signal act as a co-stressor for oxidative damage of neural cells.

Gerner et al. exposed four different human cell types exposed to modulated GSM 1800 MHz at 2 W/kg (Gerner, Haudek et al. 2010). While short-term exposure did not significantly alter the proteome, an 8-h exposure caused a significant increase in protein synthesis in Jurkat T-cells and human fibroblasts, and to a lesser extent in activated primary human mononuclear cells (Gerner, Haudek et al. 2010). Quiescent (metabolically inactive) mononuclear white blood cells, did not detectably respond to GSM 1800 MHz. Most of the proteins found to be induced were chaperones, which are mediators of protein folding. Heat-induced proteome alterations detectable with used proteome methodology would require heating greater than 1°C. Because GSM-induced heating was less than 0.15°C, a heat-related response was excluded.

Dragicevic et al. evaluated brain mitochondrial function in aged Tg mice and non-transgenic (NT) littermates following 1 month of daily exposure to EMF at 918 MHz frequency, involved modulation with Gaussian minimal-shift keying (GMSK) signal, and SAR levels that varied between 0.25 and 1.05 W/kg (Dragicevic, Bradshaw et al. 2011).The cognitively-important brain areas of cerebral cortex and hippocampus in EMF-exposed mice exhibited clear increases in maximum mitochondrial respiration, while the striatum and amgydala were unaffected. For Tg mice, long-term EMF treatment induced a dramatic reduction in mitochondrial ROS levels in both cerebral cortex and hippocampus, but not in striatum or amygdala. By contrast, NT mice given EMF treatment did not show significant changes in ROS levels within any of the four brain areas analyzed. Therefore, EMF treatment reduced ROS levels selectively in Tg mice and selectively in cognitively-important brain areas.

Finally, it follows from the emerging data that MW effects are dependent on genotype and cell-type. These dependences may explain, at least partly, the discrepancies among studies from

different laboratories and demand careful selection of biological objects in designing the replication studies.

XIV. SEX-AND AGE-RELATED DIFFERENCES

There are few studies consistently indicating that MW may exert a sex-related influence on brain activity.

Papageorgiou and co-authors investigated the sex-related influence of MW similar to that emitted by GSM900 mobile phones on brain activity (Papageorgiou, Nanou et al. 2004). Baseline EEG energy of males was greater than that of females, and exposure to MW decreased EEG energy of males and increased that of females. Memory performance was invariant to MW exposure and sex influences.

Smythe and Costall reported the effects of mobile phone exposure on short- and long-term memory in male and female subjects (Smythe and Costall 2003). The results showed that males exposed to an active phone made fewer spatial errors than those exposed to an inactive phone condition, while females were largely unaffected. These results further indicated that mobile phone exposure has functional consequences for human subjects, and these effects appear to be sex-dependent.

Nam and colleagues exposed volunteers of both sex to MW emitted by a CDMA cellular phone for half an hour (Nam, Kim et al. 2006). Physiological parameters such as systolic and diastolic blood pressures, heart rate, respiration rate, and skin resistance were simultaneously measured. All the parameters for both groups were unaffected during the exposure except for decreased skin resistance of the male subjects (Nam, Kim et al. 2006).

Güler et al. exposed infant female and male white rabbits to 1800 MHz GSM like RF signal at SAR of 1.8 W/kg for 15 min/day during 7-14 days (Guler, Tomruk et al. 2012). Lipid peroxidation levels in the liver tissues of female and male infant rabbits increased under RF radiation exposure. Liver 8-hydroxy-2 '-deoxyguanosine (8-OHdG) levels of female rabbits exposed to RF radiation were also found to increase when compared with the levels of non-exposed infants. However, there were no changes in liver 8-OHdG levels of male rabbits under RF exposure.

Santini et al. have performed a survey study on symptoms experienced during use of digital cellular phones using questionnaire of 161 students and workers in a French engineering school (Santini, Seigne et al. 2001). A significant increase in concentration difficult ($p < 0.05$) was reported by users of 1800-MHz (DCS) cellular phones compared to 900-MHz (GSM) phone users.

In users of cellular phones, women significantly (p < 0.05) complained more often of sleep disturbance than men. This sex difference for sleep complaint was not observed between women and men non-users of cellular phone. The use of both cellular phones and VDT significantly increased concentration difficulty. Digital cellular phone users also significantly (p < 0.05) more often complained of discomfort, warmth, and picking on the ear during phone conversation in relation with calling duration per day and number of calls per day. The complaint warmth on the ear might be a signal to users for stopping the call.

Prevalence of women (usually around 70%) among subjects, which report hypersensitivity to electromagnetic fields of wide frequency range including MW, may also provide indirect evidence for the gender-dependent effects of MW.

In his pioneering study concerning age in cancer risk from MW exposure, Hardell and colleagues found that the highest risks were associated with >5-year latency period in the youngest age group studied, 20-29-year, for analog phones (OR = 8.17, 95% CI = 0.94-71), and cordless phones (OR = 4.30, 95% CI = 1.22-15) (Hardell, Mild et al. 2004). Of note, no participants of age less 20 years were involved on this study. In further studies from the Hardell's group, highest risk was found in the age group <20 years at time of first use of wireless phones (Hardell and Carlberg 2009; Hardell, Carlberg et al. 2009).

Nam with co-authors reported that skin resistance in teenagers decreased by exposure to CDMA MW from cellular phones whereas no effects were seen in adults (Nam, Kim et al. 2006).

Capri et al. analyzed CD25, CD95, CD28 molecules in unstimulated and stimulated CD4+ e CD8+ T cells in vitro (Capri, Salvioli et al. 2006). Peripheral blood mononuclear cells (PBMCs) from young and elderly donors were exposed or sham-exposed to RF (1,800 MHz, SAR 2 W/kg) with or without mitogenic stimulation. No significant changes in the percentage of these cell subsets were found between exposed and sham-exposed lymphocytes in both young and elderly donors. Nevertheless, RF exposure induced a slight, but significant, downregulation of CD95 expression in stimulated CD4+ T lymphocytes from elderly, but not from young donors. This age-related result is noteworthy given the importance of such molecule in regulation of the immune response.

XV. INDIVIDUAL TRAITS

Shckorbatov et al. investigated electrokinetic properties of cell nuclei and condensation of heterochromatin in human buccal epithelium cells in response to MW at 42.2 GHz (Shckorbatov,

Grigoryeva et al. 1998). MW exposure decreased electric charge of cell nuclei and an increased chromatin condensation in dependence on individual traits of donors.

Individual variability in effects of GSM and UMTS MW on chromatin conformation and 53BP1/γ-H2AX DNA repair foci was observed in studies with lymphocytes from hypersensitive to EMF subjects and healthy persons (Sarimov, Malmgren et al. 2004; Belyaev, Hillert et al. 2005; Markova, Hillert et al. 2005; Belyaev, Markova et al. 2009). The same individual variability was reported for response of chromatin condensation human lymphocytes to ELF magnetic fields (Sarimov, Alipov et al. 2011). This variability correlated with initial state of chromatin in the exposed cells (Sarimov, Alipov et al. 2011). Thus, the data from two different research groups have indicated that the NT MW effects on human cells depended on initial sate of chromatin that individually varied between subjects.

Zotti-Martelli with colleagues exposed peripheral blood lymphocytes from nine different healthy donors for 60, 120 and 180 min to CW MW with a frequency of 1800 MHz and PD of 5, 10, and 20 mW/cm^2 and analyzed DNA damage using micronucleus (MN) assay (Zotti-Martelli, Peccatori et al. 2005). Both spontaneous and induced MN frequencies varied in a highly significant way among donors, and a statistically significant increase of MN, although rather low, was observed dependent on exposure time and PD. The data analysis highlighted a wide inter-individual and reproducible variability in the response.

Hinrikus et al. (Hinrikus, Bachmann et al. 2008) evaluated the effects of pulse-modulated MW (450 MHz) on human EEG rhythms. Thirteen healthy volunteers were exposed to MW; the field power density at the scalp was 0.16 m W/cm^2. Differences were found in individual sensitivity to exposure. Increases in the EEG beta power appeared statistically significant in the case of four subjects. In other study, the same authors confirmed and extended their observations on individual sensitivity to exposure with pulse-modulated MW. The experiments were carried out on four different groups of healthy volunteers. A 450-MHz MW modulated at 7 Hz (first group), 14 and 21 Hz (second group), 40 and 70 Hz (third group), 217 and 1000 Hz (fourth group) frequencies was applied. MW exposure, SAR 0.303 W/kg, increased the EEG energy. The proportion of subjects significantly affected was similar in all groups except for the 1000 Hz group: in the first group 16% at 7 Hz modulation; in the second group 31% at 14 Hz modulation and 23% at 21 Hz modulation; in the third group 20% at 40 Hz and 13% at 70 Hz modulation; in the fourth group 16% at 217 Hz and 0% at 1000 Hz modulation frequency.

Sannino et al. evaluated the induction of micronuclei in response to MW (900 MHz, average SAR of 1.25 W/kg) exposure and subsequent treatment with mitomycin C in peripheral blood lymphocytes from five human volunteers (Sannino, Sarti et al. 2009). MW exposure reduced the

level of mitomycin C –induced micronuclei in cells collected from four donors (i.e., responders). However, the effect of MW was not observed in the remaining donor (i.e., non-responder). The overall data indicated the existence of heterogeneity in the MW response among individuals.

Human sensitivity to radio frequency (RF) standing waves was tested using a movable reflecting wall (Huttunen, Hanninen et al. 2009). When the reflector was moved, the position of the maximums of the standing waves changed and the electromagnetic intensity changed in the body of the standing test subject. The computer with an AD-converter registered the signals of the hand movement transducer and the RF-meter with 100MHz dipole antennas. A total of 29 adults of different ages were tested. There were 9 persons whose hand movement graphs included features like the RF-meter. Six showed responses that did not correlate with the RF-meter. There were also 14 persons who did not react at all. Sensitive persons seem to react to crossing standing waves of the RF signals.

To conclude, while only few studies were performed, to evaluate individual sensitivity, the obtained results indicate dependence of response to MW exposure on individual traits.

XVI. PHYSIOLOGICAL VARIABLES: STAGE OF CELL GROWTH, TEMPERATURE, OXYGEN, DIVALENT METALS

The importance of physiological variables, which may include all conditions of cell culture growth such as aeration, the composition of the growth and exposure media, on NT MW effects has previously been reviewed (Grundler, Jentzsch et al. 1988). Since that time, significant body of new data has been accumulated unequivocally supporting the role of physiological variables for the NT MW effects, which should be carefully taken into account when replicating the original studies.

Belyaev et al. have reported that both value and direction of the MW effects strongly depended on the phase of culture growth, at which *E. coli* cells were exposed to CP or LP MW (100 $\mu W/cm^2$) at the resonance frequencies of 41.32 GHz and 51.76 GHz (Belyaev, Shcheglov et al. 1993; Belyaev, Alipov et al. 1994). At logarithmic phase of growth, MW resulted in condensation of nucleoids. In contrast, MW exposure decondensed nucleoids in cells if exposure was performed at the stationary phase of growth. It is known, that the state of nucleoid condensation depends on cell activity. In stationary cells nucleoids are more condensed compared to logarithmic cells that divide actively. It was concluded that MW are able to either stimulate or inhibit activity of the cells in dependence on stage of growth, stationary or logarithmic, respectively. Higher variability in effects was observed for logarithmic phase and effects were more stable for the stationary phase

that is characterized by partial synchronization of cells (Belyaev, Shcheglov et al. 1993; Belyaev, Alipov et al. 1994). There was no effect at all if cells were exposed at the end of the logarithmic phase where the MW effects changed their direction from inhibition to stimulation (Belyaev, Alipov et al. 1994). Another peculiarity was observed at the very beginning of the logarithmic stage, where the condensation of chromatin induced by MW was relatively weak. The AVTD data were confirmed by the electrophoretic analysis of proteins bound to DNA (Belyaev, Shcheglov et al. 1993). The effect in the stationary phase was characterized by a decrease in the quantity of several DNA-bound proteins with molecular weights of 61, 59, 56, 26, and 15 kDa. In contrast, abundance of some DNA-bound proteins, 61, 56, 51 and 43 kDa increased after exposure at the logarithmic phase. The decrease or increase in the abundance of DNA-bound proteins correlated with the observed changes in the state of nucleoids, decondensation or condensation, respectively.

Shcheglov et al. have studied effects of MW at the PD range of 10^{-18} to $3 \cdot 10^{-3}$ W/cm^2 stationary on logarithmic and stationary cells at various cell densities (Shcheglov, Alipov et al. 2002). Relatively weak response to MW was observed in exponentially growing cells. Partially synchronized stationary cells were more sensitive, especially at the cell densities above 10^8 cell/ml. The data suggested that the co-operative responses of cells to MW vary in dependence on phase of growth.

Recent data by Ushakov and colleagues indicated that the MW effects on *E. coli* cells depended on concentration of oxygen in the cell suspension during exposure (Ushakov, Alipov et al. 2005). This dependence might suggest that oxygen concentration should be indicated in order to improve reproducibility in replication studies.

Biological systems have been shown to be very sensitive to perturbations at conditions where critical components are at phase transition points, governed by local temperature, ionic strength and pH. This phenomenon was demonstrated by independent laboratories using 2.45-GHz MW radiation associated with a phase transition in lipid-protein complexes around 20-25 ^{0}C (Olcerst, Belman et al. 1980; Fisher, Poznansky et al. 1982; Liburdy and Vanek 1985; Allis and Sinha-Robinson 1987; Liburdy and Vanek 1987).

Fisher et al. have reported an effect of low-level 2450-MHz MW on total and ouabain-sensitive 24Na$^+$ flux from human erythrocytes. Erythrocytes washed and loaded with 24Na$^+$ were exposed at an absorption rate of 2.0-3.0 mW/ml suspension in a waveguide system under temperature- controlled conditions for 1 or 2 hr. Experiments were run in parallel, with exposed and sham- irradiated (control) samples, at various temperatures between 7 and 35^{0}C. Continuous-wave electromagnetic radiation at 2450 MHz had a significant effect on 24Na$^+$ efflux, but only in the temperature range 22-25^{0}C. Total efflux increased an average of 23%; this was the result of an

increase in the ouabain-insensitive component (mean, 33%) and a decrease in the ouabain-sensitive portion (mean, 18%). These results indicated increased passive Na+ efflux and decreased ATPase-mediated Na+ efflux in erythrocytes exposed to low-level microwaves at 22-25^{0}C (Fisher, Poznansky et al. 1982).

Liburdy and Vanek have shown that MW-induced protein shedding is oxygen and temperature dependent (Liburdy and Vanek 1987). Microwaves (2450 MHz, 60 mW/g) resulted in the release or shedding of at least 11 low-molecular-weight proteins (<31,000 Da) from rabbit erythrocytes maintained in physiological buffer. This release was oxygen dependent and occured in 30 min for exposures conducted within the special temperature region of 17-21^{0}C, which is linked to a structural or conformational transition in the cell membrane. Shedding of 26,000 and 24,000 Da proteins was unique to MW treatment, with enhanced release of 28,000 and < 15,000 Da species upon MW exposure. Two-dimensional isoelectric focusing revealed that proteins of< 14,000 Da shed during microwave treatment exhibited a pI of 6.8-7.3 not seen in sham-treated cells. When erythrocytes were maintained at 17-21^{0}C in the absence of divalent cations, release of 28,000-31,000 and < 14,000 Da components was detected. This indicated that cation-bridge stability may be important for release of these proteins. The results provided evidence that MW alter erythrocyte protein composition at temperatures linked to a transition in the cell membrane and that destabilization of salt bridges may play a role in an interaction mechanism for protein release (Liburdy and Vanek 1987).

The ATPase activity in human red blood cell membranes was investigated in vitro as a function of temperature and exposure to 2,450-MHz continuous wave microwave radiation to confirm and extend a report of Na$^+$ transport inhibition under certain conditions of temperature and exposure (Allis and Sinha-Robinson 1987). Assays were conducted spectrophotometrically during microwave exposure with a custom-made spectrophotometer-waveguide apparatus. Temperature profiles of total ATPase and Ca^{+2} ATPase (ouabain-inhibited) activity between 17 and 31 degrees C were graphed as an Arrhenius plot. Each data set was fitted to two straight lines which intersect between 23 and 24 degrees C. The difference between the total and Ca^{+2} ATPase activities, which represented the Na$^+$/K$^+$ ATPase activity, was also plotted and treated similarly to yield an intersection near 25 degrees C. Exposure of membrane suspensions to electromagnetic radiation, at a dose rate of 6 W/kg and at five temperatures between 23 and 27 degrees C, resulted in an activity change only for the Na$^+$/K$^+$ ATPase at 25 degrees C. The activity decreased by approximately 35% compared to sham-irradiated samples. A possible explanation for the unusual temperature/microwave interaction was proposed (Allis and Sinha-Robinson 1987).

Therefore, temperature may be an important variable, which should be taken into account while analyzing response of cells to MW.

Similar to the effects of ELF (Belyaev, Alipov et al. 1999), the MW effects were reported to be dependent on concentration of divalent ions (Gapeev, Iakushina et al. 1997).

In conclusion, physiological parameters such as stage of cell growth, temperature, oxygen an divalent ions temperature may be an important variable, which should be taken into account while analyzing response of cells to MW.

XVII. ANTIOXIDANTS AND RADICAL SCAVENGERS

Oxidative stress caused by biological, chemical and physical factors has been associated with increased risk of human cancer at various sites. Human cells induce and/or activate several oxidant generating enzymes that produce high concentrations of diverse free radicals and oxidants. These reactive species can damage DNA, RNA, lipids and proteins, leading to increased mutations and altered function of enzymes and proteins, thus contributing to the multistage carcinogenesis process. Control of oxidative stress is being explored as an approach to chemoprevention of human cancers (IARC 2002).

It is well known that endogenous (intracellular) free radicals, which are collectively called reactive oxygen species (ROS), arise from mitochondrial oxidative metabolism and other reactions in cells (Pollycove and Feinendegen 2003). The estimated average generation rate is $\sim 10^9$ ROS per cell per day (Beckman and Ames 1998), which results in 10^6 oxidative DNA damage, 10^5 SSBs and 0.1 DSBs per cell per day (Pollycove and Feinendegen 2003).

In their pioneering study, Lai and Singh described the effects of MW on the rat brain cells as measured using a microgel electrophoresis assay (Lai and Singh 1996). These effects were significantly blocked by treatment of rats either with the spin-trap compound N-tert-butyl-α-phenylnitrone or with melatonin, both agents being free radical scavengers and antioxidants (Lai and Singh 1997). These data suggested that free radicals might be involved in the effects of MW. The ability of scavengers and antioxidants has been tested by many other research groups an in all cases, this treatment inhibiter the reported TN MW effects.

Oktem andcolleagues exposed rats to MW from GSM900 mobile phone with and without melatonin treatment (Oktem, Ozguner et al. 2005). Malondialdehyde (MDA), an index of lipid peroxidation, and urine N-acetyl-beta-d-glucosaminidase (NAG), a marker of renal tubular damage, were used as markers of oxidative stress-induced renal impairment. Superoxide dismutase (SOD),

catalase (CAT), and glutathione peroxidase (GSH-Px) activities were studied to evaluate changes in antioxidant status. In the MW-exposed group, while tissue MDA and urine NAG levels increased, SOD, CAT, and GSH-Px activities were reduced. Melatonin treatment inhibited these effects. The authors concluded that melatonin might exhibit a protective effect on mobile phone-induced renal impairment in rats.

Ozguner and colleagues exposed Wistar-Albino rats to MW from GSM900 mobile phone with and without melatonin and analyzed histopathologic changes in skin (Ozguner, Aydin et al. 2004). MW induced increase in thickness of stratum corneum, atrophy of epidermis, papillamatosis, basal cell proliferation, granular cell layer (hypergranulosis) in epidermis and capillary proliferation. Impairment in collagen tissue distribution and separation of collagen bundles in dermis were all observed in exposed animals as compared to the control group. Most of these changes, except hypergranulosis, were prevented with melatonin treatment. The authors concluded that exposure to GSM900 MW caused mild skin changes and melatonin treatment could reduce these changes. In other studies of the same group, the ability of melatonin to reduce various MW-induced effects was confirmed and inhibitory potential of the antioxidant caffeic acid phenethyl ester (CAPE) was reported (Ozguner, Altinbas et al. 2005; Ozguner, Oktem et al. 2005; Ozguner, Oktem et al. 2005; Ozguner, Bardak et al. 2006).

Ayata et al. analyzed the effects of 900 MHz MW with and without melatonin on fibrosis, lipid peroxidation, and anti-oxidant enzymes in rat skin (Ayata, Mollaoglu et al. 2004). The levels of MDA and hydroxypyroline and the activities of SOD, GSH-Px, and CAT were studied. MDA and hydroxyproline levels and activities of CAT and GSH-Px were increased significantly in the exposed group without melatonin and decreased significantly in the exposed group with melatonin. SOD activity was decreased significantly in the exposed group and this decrease was not prevented by the melatonin treatment. The authors assumed that the rats irradiated with MW suffer from increased fibrosis and lipid peroxidation and that melatonin can reduce the fibrosis and lipid peroxidation caused by MW.

Ilhan with co-authors investigated oxidative damage in brain tissue of rats exposed to GSM900 MW with and without pretreatment with Ginkgo biloba (Gb) (Ilhan, Gurel et al. 2004). MW induced oxidative damage measured as: (i) increase in MDA and nitric oxide (NO) levels in brain tissue, (ii) decrease in brain SOD and GSH-Px activities, and (iii) increase in brain xanthine oxidase and adenosine deaminase activities. These MW effects were prevented by the Gb treatment. Furthermore, Gb prevented the MW-induced cellular injury in brain tissue revealed histopathologically. The authors concluded that reactive oxygen species may play a role in the

adverse effects of GSM900 MW and Gb prevents the MW-induced oxidative stress by affecting antioxidant enzymes activity in brain tissue.

Guney et al.examined 900 MHz mobile phone-induced oxidative stress that promotes production of ROS and investigated the role of vitamins E and C, which have antioxidant properties, on endometrial tissue against possible 900 MHz mobile phone-induced endometrial impairment in rats (Guney, Ozguner et al. 2007). The animals were randomly grouped (eight each) as follows: 1) Control group (without stress and EMR, Group I), 2) sham-operated rats stayed without exposure to EMR (exposure device off, Group II), 3) rats exposed to 900 MHz EMR (EMR group, Group III) and 4) a 900 MHz EMR exposed + vitamin-treated group (EMR + Vit group, Group IV). A 900 MHz EMR was applied to EMR and EMR + Vit group 30 min/day, for 30 days. Endometrial levels of nitric oxide (NO, an oxidant product) and malondialdehyde (MDA, an index of lipid peroxidation), increased in EMR exposed rats while the combined vitamins E and C caused a significant reduction in the levels of NO and MDA. Likewise, endometrial superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities decreased in EMR exposed animals while vitamins E and C caused a significant increase in the activities of these antioxidant enzymes. In the EMR group histopathologic changes in endometrium, diffuse and severe apoptosis was present in the endometrial surface epithelial and glandular cells and the stromal cells. Diffuse eosinophilic leucocyte and lymphocyte infiltration were observed in the endometrial stroma whereas the combination of vitamins E and C caused a significant decrease in these effects of EMR. It is concluded that oxidative endometrial damage plays an important role in the 900 MHz mobile phone-induced endometrial impairment and the modulation of oxidative stress with vitamins E and C reduces the 900 MHz mobile phone-induced endometrial damage both at biochemical and histological levels.

Koylu et al. studied the effects of MW on the brain lipid peroxidation in rats, and the possible protective effects of melatonin on brain degeneration induced by MW (Koylu, Mollaoglu et al. 2006). The levels of lipid peroxidation in the brain cortex and hippocampus increased in the MW group compared with the control group, although the levels in the hippocampus were decreased by combined administration of MW and melatonin. Brain cortex lipid peroxidation levels were unaffected by melatonin treatment. The authors concluded that melatonin may prevent MW-induced oxidative stress in the hippocampus by strengthening the antioxidant defense system.

Balci et all exposed albino Wistar rats to mobile-phone-emitted radiation and analyzed oxidant/antioxidant balance in corneal and lens tissues. The results of this study suggest that mobile telephone radiation leads to oxidative stress in corneal and lens tissues and that antioxidants such as vitamin C can help to prevent these effects (Balci, Devrim et al. 2007).

Sokolovic et al. evaluated the intensity of oxidative stress in the brain of Wistar rats chronically exposed to MW from mobile phones (SAR = 0.043-0.135 W/kg) during 20, 40 and 60 days (Sokolovic, Djindjic et al. 2008). A significant increase in brain tissue malondialdehyde (MDA) and carbonyl group concentration was found. Decreased activity of catalase (CAT) and increased activity of xanthine oxidase (XO) remained after 40 and 60 days of MW exposure. Melatonin treatment significantly prevented the increases in MDA content and XO activity in the brain tissue after 40 days of exposure while it was unable to prevent the decrease of CAT activity and increase of carbonyl group contents. The authors concluded that exposure to the mobile phone MW caused oxidative damage in the brain and that treatment with melatonin significantly prevented this oxidative damage.

Gajski and Garaj-Vrhovac investigated the radioprotective effect of bee venom against DNA damage induced by 915-MHz microwave radiation (SAR of 0.6 W/kg) (Gajski and Garaj-Vrhovac 2009). Whole blood lymphocytes of Wistar rats are treated with 1 mg/mL bee venom 4 hours prior to and immediately before irradiation. Standard and formamidopyrimidine-DNA glycosylase (Fpg)–modified comet assays were used to assess basal and oxidative DNA damage produced by ROS. Bee venom decreased basal and oxidative DNA damage induced by microwave radiation. The difference between the comet assay results in the presence and in the absence of Fpg-enzyme suggested that oxidative stress is responsible for the DNA damage induced by microwave radiation. Among other possible mechanisms, antioxidant activity of bee venom may likely account for the radioporotective effect.

Esmekaya et al. analyzed effects of 1.8 GHz GSM alone and in combination with Ginkgo biloba (EGb 761) pre-treatment in human peripheral blood lymphocytes (Esmekaya, Aytekin et al. 2011). RF exposure significantly increased frequency of sister chromatid exchanges (SCE) and inhibited cell viability. No temperature difference was observed between sham control and RF exposed cells, so the observed effects may be considered as non-thermal. EGb 761 pre-treatment significantly reduced both RF effects. The authors concluded that EGb 761 had a protective role against RF induced mutagenesis.

Ozgur et al investigated oxidative damage and antioxidant enzyme status in the liver of guinea pigs exposed to mobile phone-like radiofrequency radiation (RFR) and the potential protective effects of N-acetyl cysteine (NAC) and epigallocatechin-gallate (EGCG) on the oxidative damage (Ozgur, Gler et al. 2010). Nine groups of guinea pigs were used to study the effects of exposure to an 1800-MHz Global System for Mobile Communications (GSM)-modulated signal (average whole body Specific Absorption Rate (SAR) of 0.38W/kg, 10 or 20 min per day for seven days) and treatment with antioxidants. Significant increases in malondialdehyde (MDA) and total

nitric oxide (NO) levels and decreases in activities of superoxide dismutase (SOD), myeloperoxidase (MPO) and glutathione peroxidase (GSH-Px) were observed in the liver of guinea pigs after RFR exposure. NAC treatment induced increase in hepatic GSH-Px activities, whereas EGCG treatment alone attenuated MDA level. Extent of oxidative damage was found to be proportional to the duration of exposure. Authors concluded that the adverse effect of RFR may be related to the duration of mobile phone use. NAC and EGCG may protect the liver tissue against the RFR-induced oxidative damage and enhance antioxidant enzyme activities.

Female rats were exposed to a mobile phone signal (900 MHz), the mobile phone plus vitamin C group was exposed to a mobile phone signal (900 MHz) and treated orally with vitamin C (Imge, Kilicoglu et al. 2010). Malondialdehyde (MDA), antioxidant potential (AOP), superoxide dismutase, catalase (CAT), glutathione peroxidase (GSH-Px), xanthine oxidase, adenosine deaminase (ADA) and 5'nucleotidase (5'-NT) were analyzed in brain tissues. MW exposure caused an inhibition in 5'-NT and CAT activities. GSH-Px activity and the MDA level were also found to be reduced in the mobile phone group but not significantly. Vitamin C caused a significant increase in the activity of GSH-Px and non-significant increase in the activities of 5'-NT, ADA and CAT enzymes. The results suggest that vitamin C may play a protective role against detrimental effects of mobile phone radiation in brain tissue.

To conclude this section, several studies consistently show that supplementation with antioxidants and radical scavengers can reduce MW effects. In other words, the level of radicals should be considered as an important parameter for the NT MW effects. Moreover, these studies indicate that induction of radicals is one of the key events in bioeffeds of NT MW.

XVIII. CO-EXPOSURE

Zmyslony et al have studied effects of 930 MHz continuous wave (CW) electromagnetic field, 1.5 W/kg, on the reactive oxygen species (ROS) level in rat lymphocytes (Zmyslony, Politanski et al. 2004). Acute (5 and 15 min) exposure did not induce ROS. However, this exposure increased effect of $FeCl_2$, 10 µg/ml.

Co-exposure to RF (global system for mobile telecommunications (GSM) modulated 900MHz signal at a specific absorption rate (SAR) of 1 W/kg and maximum duration 144 h) exacerbated neurotoxic effect of hydrogen peroxide in SN56, but not in primary cortical neurons (Del Vecchio, Giuliani et al. 2009). These data suggest that only under particular circumstances

(cell type and type of co-exposure) exposure to GSM modulated, 900MHz signal act as a co-stressor for oxidative damage of neural cells.

XIX. REPLICATION STUDIES

Obviously, not taking into account the dependences of NT MW effects on a number of physical parameters and biological variables may result in misleading conclusions regarding the reproducibility of these effects. Especially important might be the observations that NT MW could inhibit or stimulate the same functions dependent on conditions of exposure (Pakhomov, Akyel et al. 1998). Under different conditions of exposure, MW either increased or decreased the growth rate of yeast cells (Grundler, Jentzsch et al. 1988), the radiation-induced damages in mice (Sevast'yanova 1981), the respiratory burst in neutrophils of mice (Gapeev, Iakushina et al. 1997), the condensation of nucleoids in *E coli* cells (Belyaev, Shcheglov et al. 1993; Belyaev, Alipov et al. 1994) and human lymphocytes (Sarimov, Malmgren et al. 2004). Potentially bi-directional effects of MW should be taken into account in replication studies.

In some cases when the conditions were kept in strict control, the effects we reproduced. Highly resonant effects of ultra-weak MW (near 70 GHz) on the induction of λ-phage were first established by Webb (Webb 1979), and subsequently corroborated (Lukashevsky and Belyaev 1990).

Despite of considerable body of studies with NT MW in biology, only a few studies were performed to independently replicate the original data on the NT MW effects. It should be noted, that these replications are usually not completely comparable with the original studies because of either missing description of important parameters of exposure or significant differences in these parameters between original study and replication. One well-known attempt to replicate the results of Gründler was the study by Gos and co-authors (Gos, Eicher et al. 1997). No MW effects were observed in this replication study. However, the deviations from the Gründler's protocol might be a simple reason for poor reproducibility. For example, synchronized cells were used in studies of Gründler. Contrary to the Gründler's original protocol, Gos used exponentially growing cells. If the MW effects in yeast cells are dependent on stage of growth, cell density and intercellular interactions as it has been described for *E. coli* cells (Belyaev, Shcheglov et al. 1993; Belyaev, Alipov et al. 1994; Belyaev, Shcheglov et al. 1996; Shcheglov, Belyaev et al. 1997), no response should be expected in the logarithmic phase of growth. Gos and colleagues used *S. cerevisiae* strain with the auxotrophy mutations for leucine and uracil. Gründler used the wild type strain. It might

suggest another cause for the deviations between the data of Gründler and Gos. Despite orientation of SMF in respect to electric and magnetic components of MW was the same, the values of SMF were different. The stray ELF field was 120 nT in the study by Gos, that is higher than usually observed background fields, < 50 nT. The spectral characteristics of the background fields, which were described only in the study by Gos, might be also different. In addition, the conditions of cell cultivation might vary between studies; for example, the data on oxygen concentration in media used in both studies are not available.

Lai and Singh have consistently reported that circularly polarized MW exposure at 2450 MHz induced DNA damage in brain cells of the exposed rats (Lai and Singh 1995; Lai and Singh 1996; Lai and Singh 1997). Replication studies have also tested circularly polarized MW exposure at 2450 MHz and no induced DNA damage was reported (Malyapa, Ahern et al. 1997; Malyapa, Ahern et al. 1998; Lagroye, Anane et al. 2004). All these replication studies have used another exposure system. However, handedness of circular polarization has not been given neither in original study, no in replications. If the handedness was different between studies it could reasonably account for inconsistency.

Most reviews of the experimental studies do not include analysis of various biological variables and physical parameters when comparing the data on the NT MW effects from different studies. As result, misleading conclusion is often made that MW at NT levels produce no "reproducible" effects.

XX. SIMILARITY OF MICROWAVE AND ELF EFFECTS

Mobile phones not only expose the user to RF EMF but also to ELF EMF (Linde and Mild 1997; Heath, Jenvey et al. 1998; Jokela, Puranen et al. 2004; Ilvonen, Sihvonen et al. 2005; Cook, Saucier et al. 2006; Perentos, Iskra et al. 2007). Perentos et al. have recently measured and characterized the ELF magnetic field from several commercial GSM handsets (the RF characteristics being already well understood) using different probes which covered frequency range from static magnetic fields ("0 Hz") to 2 GHz. Peak ELF fields at the front sides of 5 commercial GSM phones were assessed and a maximum of 22.4 µT was reported (Perentos, Iskra et al. 2008). The main ELF component at the 217 Hz was about 1 µT at the distance of 3 cm from the handset front side. The overall pulse peak was 4.2 times greater than the 217 Hz component. 217 Hz magnetic field decreased with distance and reached 0.3 µT approximately at 5 cm from the front handset side. The overall ELF pulse peak produced by all ELF components was 4.2 times greater

than the 217 Hz component. The ELF fields higher 0.3 μT have consistently been shown to correlate with increased risk of children leukemia in several studies covering European countries, USA and Japan (Kabuto, Nitta et al. 2006; Yang, Jin et al. 2008). Similar to RF, ELF has been classified by the IARC as possible carcinogen "2B". It has been known for long time that weak ELF fields and NT MW result to similar effects with significant overplaying of molecular biological pathways for their appearance (Adey 1981; Blank and Goodman 2009; Davanipour and Sobel 2009). Multiple data on ELF biological effects at intensities below the ICNIRP standards are available showing their complex dependence of the ELF effects on biological and physical variables (Belyaev, Alipov et al. 1999; Blank and Goodman 2009; Phillips, Singh et al. 2009; Sarimov, Alipov et al. 2011). In particular, stress response, molecular pathways for generation of reactive oxygen species (ROS), increased sensitivity of stem cells, and inhibition of melatonin production (Burch, Reif et al. 2000) were suggested as mechanisms which link observed increase in cancer risks and effects of exposure at the cellular level. EMF effects in a wide frequency range from ELF to MW have been considered in the frames of the same physical models (Chiabrera, Bianco et al. 1991; Matronchik, Alipov et al. 1996; Chiabrera, Bianco et al. 2000; Binhi 2002; Panagopoulos, Karabarbounis et al. 2002; Matronchik and Belyaev 2005; Matronchik and Belyaev 2008).

In many cases, because of ELF modulation and additional ELF fields created by the MW sources, for example by mobile phones, it is difficult to distinguish the effects of exposures to ELF and MW. Therefore, these combined exposures and their possible cancer risks should be considered in combination.

XXI. CANCER RISK ASSESSMENT FROM MECHANISTIC POINT OF VIEW

At present, a new situation has arisen when a significant part of the general population is exposed chronically (much longer than previously investigated durations of exposures) to NT MW from different types of mobile communication including GSM and UMTS/3G phones and base stations, WLAN (Wireless Local Area Networks), WPAN (Wireless Personal Area Networks such as Bluetooth), DECT (Digital Enhanced (former European) Cordless Telecommunications) wireless phones (Joseph, Frei et al. 2010). Multiple sources of mobile communication result in chronic exposure of general population to MW at the non-thermal levels. These exposures are characterized by low intensities, varieties and complexities of signals, and long-term durations of exposure that are comparable with a lifespan.

Most of the real signals that are in use in mobile communication have not been tested so far. Very little research has been done with real signals and for durations and intermittences of exposure that are relevant to chronic exposures from mobile communication. In some studies, so-called "mobile communication-like" signals were investigated that in fact were different from the real exposures in such important aspects as intensity, carrier frequency, modulation, polarization, duration and intermittence.

Emerging evidence suggests that the SAR concept, which has been widely adopted for safety standards, is not useful alone for the evaluation of health risks from NT MW of mobile communication. The role of other exposure parameters such as frequency, modulation, polarization, duration, and intermittence of exposure should be taken into account.

IARC has recently classified RF as a 'Possible Human Carcinogen' (Class 2B) (Baan, Grosse et al. 2011). Contrary to other panels, such as ICNIRP, whose members dismiss the NT MW effects based on their "non-reproducibility" and lack of comprehensive mechanisms, the IARC working group included scientists, which argued for existence of non-thermal effects and their complex dependence on variety of biological and physical parameters which should be included in consideration. By its classification, IARC has justified implementation of the Precautionary Principle, confirmed the existence of non-thermal effects that can cause health risks, and indicated that the current safety standards are insufficient to protect health.

The data about the effects of MW at super low intensities and significant role of duration of exposure in these effects along with the data showing that adverse effects of NT MW from GSM/UMTS mobile phones depend on carrier frequency and type of the MW signal suggest that MW from base-stations/masts, wireless routers, WI-FI and other wireless devices and exposures in common use today can also produce adverse effects at prolonged durations of exposure.

So far, most laboratory and epidemiological studies did not control important features of the NT MW effects and therefore, only limited conclusion regarding health effects of MW from mobile communication can be drawn from these studies. The group of Hardell was the first epidemiologic studying separately the MW signals from cordless phones, analogue phones and digital phones (Hardell, Hansson Mild et al. 2001; Hardell, Hansson Mild et al. 2003; Hardell, Eriksson et al. 2005; Hardell and Hansson Mild 2005). This approach is valid from the mechanistic point of view.

Nowadays, it is almost impossible to select control unexposed groups because the whole population in many countries is exposed to wide range of MW signals from various sources such as mobile phones, base stations/masts, WLAN, WPAN, DECT wireless phones and given that duration of exposure (at least 10 years for cancer latency period) is also important for the effects of NT MW along PD/SAR. Exposure from downlink sources (base stations *etc.*) may contribute up to

90% of total environmental outdoor-urban exposure in European countries while exposure to DECT phone is comparable to exposure to mobile phones (Frei, Mohler et al. 2009; Frei, Mohler et al. 2010; Joseph, Frei et al. 2010). In other words, there are no unexposed control groups available for epidemiologic studies in the developed countries. Substantial variation in relative ratio of downlink and uplink signals between countries (Joseph, Frei et al. 2010) can at least partially account for differences in epidemiologic data because of variation in exposure of control groups to downlink signals.

While several national registers (Norway, Australia, Finland, Denmark) report increased incidence of brain cancer, US and Swedish ones do not. This inconsistence may be accounted by deficit in reporting of tumors to the Swedish Cancer Registry (Hardell and Carlberg 2009).

Importantly, because the signals are completely replaced by other signals faster then once per 10 years, duration comparable with latent period, epidemiologic studies can not provide basement for assessment of upcoming new signals.

As far as different types of MW signals (carrier frequency, modulation, polarization, far and near field, intermittence, coherence, *etc.*) may produce different effects, cancer risks should ideally be estimated for each MW signal separately. In other words, one type of MW signal would correspond to one chemical compound. That means, for example, that each from 124 signals involved in GSM uplink mobile communication should be separately evaluated to fit situation accepted for estimation of cancer risks from chemical compounds.

It now appears that most, if not all, adult tissues and organs including blood and brain contain stem cells (Metcalfe and Ferguson 2008). Almost all hematopoietic and solid neoplasms arise from cancer stem cells that are dysfunctional versions of a normal stem cells. Current models for radiation carcinogenesis have paid much attention to the stochastic process of energy deposition in cells, but accumulating evidences have shown that the nature of the target cells, i.e. tissue stem cells and progenitor cells, needs to be taken into consideration (Niwa 2010; Richardson 2011). Stem cell self-renewal and progenitor differentiation is regulated by the specialized microenvironment— or "niche"—in which these cells reside (Alvarez-Buylla and Lim 2004) and which regulate stem cells (Morrison and Spradling 2008; Johansson, Cappello et al. 2010; Kim and Shivdasani 2012; Sugiyama and Nagasawa 2012)..Importance of stem cells for carcinogenesis, challenges the definition of volume for SAR determination in safety standards. Instead of random distribution of targets for carcinogenesis, localized distribution of SAR in stem cells and niches is needed. Because very small size of the niches in different tissues including the brain (Kazanis 2012), the SAR averaging should be performed at volumes much less then currently accepted 10 g. Decreasing the sensitive volume to the stem cell niches with sizes down to 10 μm (Richardson 2011) may likely

put almost all mobile phones out of the current safety standards, even given that they are only based on thermal effects and do not consider any other parameters except for SAR. From point view of stem cell organization, the volume of SAR determination may be especially important for setting the safety standards for children. During brain development, most stem cells and their niches are spatially ephemeral and temporally transient as the cellular and molecular "puzzle" behind neurogenesis and morphogenesis is "assembled" and "disassembled" at a dazzling pace. In contrast, in the adult, neural stem cells and their niches are retained in restricted regions with their local developmental processes occurring for the life (Alvarez-Buylla and Lim 2004).

It should be anticipated that some part of the human population, such as children, pregnant women and groups of hypersensitive persons could be especially sensitive to the NT MW exposures.

XXII. CONCLUSIONS

Non-thermal effects of microwaves depend on variety of biological and physical parameters that should be taken into account in setting the safety standards. These exposures can cause health risk. The current safety standards are insufficient to protect from non-thermal microwave effects. Emerging evidence suggests that the SAR concept, which has been widely adopted for safety standards, is not useful alone for the evaluation of health risks from NT MW of mobile communication. Other parameters of exposure, such as frequency, modulation, duration, dose should be taken into account. New standards should be developed based on knowledge of mechanisms of non-thermal effects. Importantly, because the signals of mobile communication are completely replaced by other signals faster then once per 10 years, duration comparable with latent period, epidemiologic studies cannot provide basement for cancer risk assessment from upcoming new signals. Precautionary Principle should be implemented while new standards are in progress. In many cases, because of ELF modulation and additional ELF fields created by the MW sources, for example by mobile phones, it is difficult to distinguish the effects of exposures to ELF and MW. Therefore, these combined exposures and their possible cancer risks should be considered in combination. It should be anticipated that some part of the human population, such as children, pregnant women and groups of hypersensitive persons could be especially sensitive to the non-thermal microwave exposures.

REFERENCES

Adang D, Remacle C, Vorst AV. 2009. Results of a long-term low-level microwave exposure of rats. IEEE Transactions on Microwave Theory and Techniques 57(10):2488-2497.

Adey WR. 1981. Tissue interactions with nonionizing electromagnetic fields. Physiological Reviews 61:435-514.

Adey WR. 1999. Cell and molecular biology associated with radiation fields of mobile telephones. Review of Radio Science 1996-1999, W R Stone and S Ueno Oxford, Oxford University Press:845-872.

Adey WR, Bawin SM, Lawrence AF. 1982. Effects of weak amplitude-modulated microwave fields on calcium efflux from awake cat cerebral cortex Bioelectromagnetics 3:295-307.

Agarwal A, Deepinder F, Sharma RK, Ranga G, Li J. 2008. Effect of cell phone usage on semen analysis in men attending infertility clinic:An observational study Fertility and Sterility 89:124-128.

Agarwal A, Desai NR, Makker K, Varghese A, Mouradi R, Sabanegh E, et al. 2009. Effects of radiofrequency electromagnetic waves .RF-EMW. from cellular phones on human ejaculated semen:an in vitro pilot study Fertility & Sterility 92:1318-1325.

Akoev IG, Pashovkina MS, Dolgacheva LP, Semenova TP, Kalmykov VL. 2002. [Enzymatic activity of some tissues and blood serum from animals and humans exposed to microwaves and hypothesis on the possible role of free radical processes in the nonlinear effects and modification of emotional behavior of animals] Radiatsionnaia Biologiia Radioecologiia 42:322-330.

Alipov ED, Shcheglov VS, Sarimov RM, Belyaev IY. 2003. Cell-density dependent effects of low-dose ionizing radiation on E coli cells. Radiatsionnaia Biologiia Radioecologiia 43:167-171.

Alipov YD, Belyaev IY, Kravchenko VG, Polunin VA, Shcheglov VS. 1993. Experimental justification for generality of resonant response of prokaryotic and eukaryotic cells to MM waves of super-low intensity. Physics of the Alive 1:72-80.

Allis JW, Sinha-Robinson BL. 1987. Temperature-specific inhibition of human red cell Na+/K+ ATPase by 2,450-MHz microwave radiation Bioelectromagnetics 8:203-212.

Alvarez-Buylla A, Lim DA. 2004. For the long run:maintaining germinal niches in the adult brain. Neuron 41:683-686.

Ayata A, Mollaoglu H, Yilmaz HR, Akturk O, Ozguner F, Altuntas I. 2004. Oxidative stress-mediated skin damage in an experimental mobile phone model can be prevented by melatonin. Journal of Dermatology 31:878-883.

Baan R, Grosse y, Lauby-Secretan b, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, et al. 2011. Carcinogenicity of radiofrequency electromagnetic fields Lancet Oncology 12:624-626.

Balci M, Devrim E, Durak I. 2007. Effects of mobile phones on oxidant/antioxidant balance in cornea and lens of rats. Current Eye Research 32:21-25.

Banik S, Bandyopadhyay s, Ganguly S. 2003. Bioeffects of microwave - a brief review. Bioresource Technology 87:155-159.

Bartsch H, Kupper H, Scheurlen U, Deerberg F, Seebald E, Dietz K, et al. 2010. Effect of chronic exposure to a GSM-like signal .mobile phone. on survival of female Sprague-Dawley rats:modulatory effects by month of birth and possibly stage of the solar cycle Neuro Endocrinology Letters 31:457-473.

Beckman KB, Ames BN. 1998. The free radical theory of aging matur. Physiological Reviews 78:547-581.

Belyaev I. 2010. Dependence of non-thermal biological effects of microwaves on physical and biological variables:implications for reproducibility and safety standards European Journal of Oncology - Library NON-THERMAL EFFECTS AND MECHANISMS OF INTERACTION BETWEEN ELECTROMAGNETIC FIELDS AND LIVING MATTER An ICEMS Monograph L Giuliani and M Soffritti Bologna, Italy, RAMAZZINI INSTITUTE, Vol 5:187-218. Available at:http://wwwicemseu/papershtm?f=/c/a/2009/12/15/MNHJ1B49KHDTL

Belyaev IY .1992. Some biophysical aspects of the genetic effects of low intensity millimeter waves. Bioelectrochemistry Bioenergetics 27:11-18.

Belyaev IY. 1993. Biological effects of low dose ionizing radiation and weak electromagnetic fields 7th Workshop on Microdosimetry S G Andreev Suzdal, MIFI Publisher:128-146.

Belyaev IY, Alipov ED. 2001. Frequency-dependent effects of ELF magnetic field on chromatin conformation in Escherichia coli cells and human lymphocytes. Biochimica et Biophysica Acta 1526:269-276.

Belyaev IY, Alipov ED, Harms-Ringdahl M. 1999. Effects of weak ELF on E coli cells and human lymphocytes:role of genetic, physiological and physical parameters. Electricity and Magnetism in Biology and Medicine F Bersani NY, Kluwer Academic:481-484.

Belyaev IY, Alipov YD, Harms-Ringdahl M. 1997. Effects of zero magnetic field on the conformation of chromatin in human cells. Biochimica et Biophysica Acta 1336:465-473.

Belyaev IY, Alipov YD, Matronchik AY. 1998. Cell density dependent response of E coli cells to weak ELF magnetic fields. Bioelectromagnetics 19:300-309.

Belyaev IY, Alipov YD, Matronchik AY, Radko SP. 1995. Cooperativity in E coli cell response to resonance effect of weak extremely low frequency electromagnetic field. Bioelectrochemistry and Bioenergetics 37:85-90.

Belyaev IY, Alipov YD, Polunin VA, Shcheglov VS. 1993. Evidence for dependence of resonant frequency of millimeter wave interaction with Escherichia coli Kl2 cells on haploid genome length Electro- & Magnetobiology 12:39-49.

Belyaev IY, Alipov YD, Shcheglov VS. 1992. Chromosome DNA as a target of resonant interaction between Escherichia coli cells and low-intensity millimeter waves. Electro- & Magnetobiology 11:97-108.

Belyaev IY, Alipov YD, Shcheglov VS, Lystsov VN. 1992. Resonance effect of microwaves on the genome conformational state of E coli cells. Z Naturforsch [C] 47:621-627.

Belyaev IY, Alipov YD, Shcheglov VS, Polunin VA, Aizenberg OA. 1994. Cooperative response of Escherichia coli cells to the resonance effect of millimeter waves at super low intensity. Electro- & Magnetobiology 13:53-66.

Belyaev IY, Eriksson S, Nygren J, Torudd J, Harms-Ringdahl M. 1999. Effects of ethidium bromide on DNA loop organisation in human lymphocytes measured by anomalous viscosity time dependence and single cell gel electrophoresis. Biochimica et Biophysica Acta .BBA. - General Subjects 1428:348-356.

Belyaev IY, Harms-Ringdahl M. 1996. Effects of gamma rays in the 05-50-cGy range on the conformation of chromatin in mammalian cells0 Radiation Research 145:687-693.

Belyaev IY, Hillert L, Protopopova M, Tamm C, Malmgren LOG, Persson BRR, et al. 2005. 915 MHz microwaves and 50 Hz magnetic field affect chromatin conformation and 53BP1 foci in human lymphocytes from hypersensitive and healthy persons. Bioelectromagnetics 26:173-184.

Belyaev IY, Markova E, Hillert L, Malmgren LOG, Persson BRR. 2009. Microwaves from UMTS/GSM mobile phones induce long-lasting inhibition of 53BP1/g-H2AX DNA repair foci in human lymphocytes. Bioelectromagnetics 30:129-141.

Belyaev IY, Shcheglov VS, Alipov ED, Ushakov VL. 2000. Non-thermal effects of extremely high frequency microwaves on chromatin conformation in cells in vitro:dependence on physical, physiological and genetic factors IEEE Transactions on Microwave Theory and Techniques 48.11.:2172-2179.

Belyaev IY, Shcheglov VS and Alipov YD .1992. Existence of selection rules on helicity during discrete transitions of the genome conformational state of Ecoli cells exposed to low-level millimeter radiation. Bioelectrochemistry and Bioenergetics 27:405-411.

Belyaev IY, Shcheglov VS, Alipov YD. 1992. Selection rules on helicity during discrete transitions of the genome conformational state in intact and X-rayed cells of Ecoli in millimeter range of electromagnetic field. In:Charge and Field Effects in Biosystems M Journal of Allen, S F Cleary, A E Sowers and D D Shillady Basel, Switzerland, Birkhauser 3:115-126.

Belyaev IY, Shcheglov VS, Alipov YD, Polunin VA. 1996. Resonance effect of millimeter waves in the power range from 10.-19. to 3 x 10.-3. W/cm^2 on Escherichia coli cells at different concentrations. Bioelectromagnetics 17:312-321.

Belyaev IY, Shcheglov VS, Alipov YD, Radko SP. 1993. Regularities of separate and combined effects of circularly polarized millimeter waves on E coli cells at different phases of culture growth. Bioelectrochemistry and Bioenergetics 31:49-63.

Belyaev SY, Kravchenko VG. 1994. Resonance effect of low-intensity millimeter waves on the chromatin conformational state of rat thymocytes. Zeitschrift für Naturforschung [C] Journal of biosciences 49:352-358.

Betskii OV, Devyatkov ND, Kislov VV. 2000. Low intensity millimeter waves in medicine and biology. Critical Reviews in Biomedical Engineering 28:247-268.

Binhi VN. 2002. Magnetobiology: Underlying physical problems. San Diego, Academic Press.

Binhi VN, Alipov YD, Belyaev IY. 2001. Effect of static magnetic field on E coli cells and individual rotations of ion-protein complexes. Bioelectromagnetics 22:79-86.

Blackman C. 2009. Cell phone radiation:Evidence from ELF and RF studies supporting more inclusive risk identification and assessment. Pathophysiology 16:205-216.

Blackman CF, Benane SG, Elder JA, House DE, Lampe JA, Faulk JM. 1980. Induction of calcium-ion efflux from brain tissue by radiofrequency radiation:effect of sample number and modulation frequency on the power-density window. Bioelectromagnetics 1:35-43.

Blackman CF, Benane SG, Joines WT, Hollis MA, House DE. 1980. Calcium-ion efflux from brain tissue:power-density versus internal field-intensity dependencies at 50-MHz RF radiation. Bioelectromagnetics 1:277-283.

Blank M, Goodman R. 2009. Electromagnetic fields stress living cells. Pathophysiology 16:71-78.

Bolshakov MA, Alekseev SI. 1992. Bursting responses of Lymnea neurons to microwave radiation. Bioelectromagnetics 13:119-129.

Bozhanova TP, Bryukhova AK, Golant MB. 1987. About possibility to use coherent radiation of extremely high frequency for searching differences in the state of living cells Medical and biological aspects of millimeter wave radiation of low intensity. Devyatkov ND , Fryazino IRE. Academy of Science, USSR 280 p:90-97.

Buchner K, Eger H. 2011. Changes of clinically important neurotransmitters under the influence of modulated RF fields - A long-term study under real-life conditions. Original study in German Umwelt - Medizin - Gesellschaft 24:44-57.

Burch JB, Reif JS, Noonan CW, Ichinose T, Bachand AM, KoleberTL, et al. 2002. Melatonin metabolite excretion among cellular telephone users. International Journal of Radiation Biology 78:1029-1036.

Burch JB, Reif JS, Noonan CW, Yost MG. 2000. Melatonin metabolite levels in workers exposed to 60-hz magnetic fields:Work in substations and with 3-phase conductors. Journal of Occupational and Environmental Medicine 42:136-142.

Byus CV, Kartun K, Pieper S, Adey WR. 1988. Increased ornithine decarboxylase activity in cultured cells exposed to low energy modulated microwave fields and phorbol ester tumor promoters. Cancer Research 48:4222-4226.

Byus CV, Lundak RL, Fletcher RM, Adey WR. 1984. Alterations in protein kinase activity following exposure of cultured human lymphocytes to modulated microwave fields. Bioelectromagnetics 5:341-351.

Cam ST, Seyhan N. 2012. Single-strand DNA breaks in human hair root cells exposed to mobile phone radiation. International Journal of Radiation Biology 88:420-424.

Campisi A, Gulino M, Acquaviva R, Bellia P, Raciti G, Grasso R, et al. 2010. Reactive oxygen species levels and DNA fragmentation on astrocytes in primary culture after acute exposure to low intensity microwave electromagnetic field. Neuroscience Letters 473:52-55.

Capri M, Salvioli S, Altilia S, Sevini F, Remondini D, Mesirca P, et al. 2006. Age-dependent effects of in vitro radiofrequency exposure .mobile phone. on CD95+ T helper human lymphocytes. Annals od the New York Academy of Sciences 1067:493-499.

Capri M, Scarcella E, Fumelli C, Bianchi E, Salvioli S, Mesirca P, et al. 2004. In vitro exposure of human lymphocytes to 900 MHz CW and GSM modulated radiofrequency:studies of proliferation, apoptosis and mitochondrial membrane potential. Radiation Research 16:211-218.

Caraglia M, Marra M, Mancinelli F, D'Ambrosio G, Massa R, Giordano A, et al. 2005. Electromagnetic fields at mobile phone frequency induce apoptosis and inactivation of the multi-chaperone complex in human epidermoid cancer cells. Journal of Cell Physiology 204:539-548.

Cardis E, Armstrong BK, Bowman JD, Giles GG, Hours M, Krewski D, et al. 2011. Risk of brain tumours in relation to estimated RF dose from mobile phones:results from five Interphone countries. Occupational & Environmental Medicine 68:631-640.

Chavdoula ED, Panagopoulos DJ, Margaritis LH. 2010. Comparison of biological effects between continuous and intermittent exposure to GSM-900-MHz mobile phone radiation:Detection of apoptotic cell-death features. Mutation Research 700:51-61.

Chiabrera A, Bianco B, Caufman JJ, Pilla AA. 1991. Quantum dynamics of ions in molecular crevices under electromagnetic exposure. In:Electromagnetics in Medicine and Biology, Brighton ct & Pollack SR. San Francisco, San Francisco Press:21-26.

Chiabrera A, Bianco B, Moggia E, Kaufman JJ. 2000. Zeeman-Stark modeling of the RF EMF interaction with ligand binding. Bioelectromagnetics 21:312-324.

Cifra M, Fields JZ, Farhadi A. 2011. Electromagnetic cellular interactions. Progress in Biophysics and Molecular Biology 105:223-246.

Cook CM, Saucier DM, Thomas AW, Prato FS. 2006. Exposure to ELF magnetic and ELF-modulated radiofrequency fields:the time course of physiological and cognitive effects observed in recent studies. 2001-2005. Bioelectromagnetics 27:613-627.

Croft RJ, Chandler JS, Burgess AP, Barry RJ, Williams JD, Clarke AR. 2002. Acute mobile phone operation affects neural function in humans. Clinical Neurophysiology 113:1623-1632.

Czerska EM, Elson EC, Davis CC, Swicord ML, Czerski P. 1992. Effects of continuous and pulsed 2450-MHz radiation on spontaneous lymphoblastoid transformation of human lymphocytes in vitro. Bioelectromagnetics 13:247-259.

Czyz J, Guan K, Zeng Q, Nikolova T, Meister A, Schönborn F, et al. 2004. High frequency electromagnetic fields .GSM signals. affect gene expression levels in tumor suppressor p53-deficient embryonic stem cells Bioelectromagnetics 25:296-307.

d'Ambrosio G, Massa R, Scarfi MR, Zeni O.Schuderer J, Kuster N, Wobus AM. 2002. Cytogenetic damage in human lymphocytes following GMSK phase modulated microwave exposure. Bioelectromagnetics 23:7-13.

Davanipour Z, Sobel E .2009. Long-term exposure to magnetic fields and the risks of Alzheimer's disease and breast cancer: Further biological research. Pathophysiology 16:149-156.

De Iuliis GN, Newey RJ, King BV, Aitken RJ. 2009. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. PLoS One 4:e6446.

Del Vecchio G, Giuliani A, Fernandez M, Mesirca P, Bersani F, Pinto R, et al. 2009. Effect of radiofrequency electromagnetic field exposure on in vitro models of neurodegenerative disease. Bioelectromagnetics 30:564-572.

Devyatkov N.D. 1973. Influence of electromagnetic radiation of millimeter range on biological objects. In Russian. Usp Fiz Nauk 116:453-454.

Devyatkov ND, Golant MB, Betskij OV.1994. Peculiarities of usage of millimeter waves in biology and medicine. In Russian. Moscow, IRE RAN.

Di Carlo A, White N, Guo F, Garrett P, Litovitz T. 2002. Chronic electromagnetic field exposure decreases HSP70 levels and lowers cytoprotection. Journal of Cell Biochem 84:447-454.

Diem E, Schwarz C, Adlkofer F, Jahn O, Rüdiger H. 2005. Non-thermal DNA breakage by mobile-phone radiation .1800 MHz. in human fibroblasts and in transformed GFSH-R17 rat granulosa cells in vitro. Mutation Research 583:178-183.

Dolgacheva LP, Semenova TP, Abzhalelov BB, Akoev IG. 2000. [The effect of electromagnetic radiation on the monoamine oxidase A activity in the rat brain.] Radiatsionnaia Biologiia, Radioecologiia 40:429-432.

Dragicevic N, Bradshaw PC, Mamcarz M, Lin X, Wang L, Cao C, et al. 2011. Long-term electromagnetic field treatment enhances brain mitochondrial function of both Alzheimer's transgenic mice and normal mice:a mechanism for electromagnetic field-induced cognitive benefit? Neuroscience 185:135-49..

Duan Y, Zhang HZ, Bu RF. 2011. Correlation between cellular phone use and epithelial parotid gland malignancies. International Journal of Oral Maxillofacial Surgery 40:966-972.

Dutta SK, Ghosh B, Blackman CF. 1989. Radiofrequency radiation-induced calcium ion efflux enhancement from human and other neuroblastoma cells in culture. Bioelectromagnetics 10:197-202.

Dutta SK, Subramoniam A, Ghosh B, Parshad R. 1984. Microwave radiation-induced calcium ion efflux from human neuroblastoma cells in culture. Bioelectromagnetics 5:71-78.

Eberhardt JL, Persson BR, Brun AE, Salford LG, Malmgren LO. 2008. Blood-brain barrier permeability and nerve cell damage in rat brain 14 and 28 days after exposure to microwaves from GSM mobile phones. Electromagnetic Biology & Medicine 27:215-229.

Elhag MA, Nabil GM, Attia AM. 2007. Effects of electromagnetic field produced by mobile phones on the oxidant and antioxidant status of rats. Pakistan Journal of Biological Sciences 10:4271-4274.

Esmekaya MA, Aytekin E, Ozgur E, Güler G, Ergun MA, Omeroğlu S, et al. 2011. Mutagenic and morphologic impacts of 18GHz radiofrequency radiation on human peripheral blood lymphocytes .hPBLs. and possible protective role of pre-treatment with Ginkgo biloba (EGb 761). Science of the Total Environment 410-411:59-64.

Fisher PD, Poznansky MJ, Voss VA. 1982. Effect of microwave radiation .2450 MHz. on the active and passive components of 24Na+ efflux from human erythrocytes. Radiation Research 92:411-422.

Foster KR, Repacholi MH. 2004. Biological effects of radiofrequency fields:does modulation matter? Radiation Research 162:219-225.

Franzellitti S, Valbonesi P, Ciancaglini N, Biondi C, Contin A, Bersani F, et aL. 2010. Transient DNA damage induced by high-frequency electromagnetic fields .GSM 18 GHz. in the human trophoblast HTR-8/SVneo cell line evaluated with the alkaline comet assay. Mutation Research 683:35-42.

Franzellitti S, Valbonesi P, Biondi C, Contin A, Fabbri E. 2008. HSP70 expression in human trophoblast cells exposed to different 18 Ghz mobile phone signals. Radiation Research 170:488-497.

Frei P, Mohler E, Bürgi A, Fröhlich J, Neubauer G, Braun-Fahrländer C, et al. 2010. Classification of personal exposure to radio frequency electromagnetic fields .RF-EMF. for epidemiological research: Evaluation of different exposure assessment methods. Environment International 36:714-720.

Frei P, Mohler E, Neubauer G, Theis G, Bürgi A, Fröhlich J, et al. 2009. Temporal and spatial variability of personal exposure to radio frequency electromagnetic fields. Environmental Research 109:779-785.

French PW, Donnellan M, McKenzie DR. 1997. Electromagnetic radiation at 835 MHz changes the morphology and inhibits proliferation of a human astrocytoma cell line. Bioelectrochemistry & Bioenergetics 43:13-18.

Frey AH. 1967. Brain stem evoked responses associated with low-intensity pulsed UHF energy. Journal of Applied Physiology 23:984-988.

Frey AH. 1974. Differential biologic effects of pulsed and continuous electromagnetic fields and mechanisms of effect. Annals of the New York Academy of Sciences 238:273-279.

Frey AH. 1993. Electromagnetic field interactions with biological systems. FASEB Journal 7:272-281.

Friedman J, Kraus S, Hauptman Y, Schiff Y, Seger R. 2007. Mechanism of short-term ERK activation by electromagnetic fields at mobile phone frequencies. The Biochemical Journal 405:559-568.

Frohlich H. 1968. Long-range coherence and energy storage in biological systems. International Journal of Quantum Chemistry 2:641-652.

Gajski G, Garaj-Vrhovac V. 2009. Radioprotective effects of honeybee venom .Apis mellifera. against 915-MHz microwave radiation-induced DNA damage in Wistar rat lymphocytes: in vitro study. International Journal of Toxicology 28:88-98.

Gapeev AB, IakushinaVS, Chemeris NK, Fesenko EE. 1997. Modulated extremely high frequency electromagnetic radiation of low intensity activates or inhibits respiratory burst in neutrophils depending on modulation frequency. In Russian. Biofizika 425:1125-1134.

Gapeev AB, IakushinaVS, Chemeris NK, Fesenko EE. 1999. Dependence of EHF EMF effects on the value of the static magnetic field. Dokl Akad Nauk 369.404-407.

Gapeev AB, IakushinaVS, Chemeris NK, Fesenko EE. 1996. [Modification of the activity of murine peritoneal neutrophils upon exposure to millimeter waves at close and far distances from the emitter.] Biofizika 41:205-219.

Gapeev AB, Mikhailik EN, Chemeris NK. 2008. Anti-inflammatory effects of low-intensity extremely high-frequency electromagnetic radiation: Frequency and power dependence. Bioelectromagnetics 29:197-206.

Gapeev AB, Mikhailik EN, Chemeris NK. 2009. Features of anti-inflammatory effects of modulated extremely high-frequency electromagnetic radiation. Bioelectromagnetics 30:454-61.

Gapeev AB, SafronovaVG, Chemeris NK, Fesenko EE. 1997. Inhibition of the production of reactive oxygen species in mouse peritoneal neutrophils by millimeter wave radiation in the near and far field zones of the radiator. Bioelectrochemistry & Bioenergetics 43:217-220.

Gapeev AB, Yakushina VS, Chemeris NK, Fesenko EE. 1998. Modification of production of reactive oxygen species in mouse peritoneal neutrophils on exposure to low-intensity modulated millimeter wave radiation. Bioelectrochemistry & Bioenergetics 46:267-272.

Gerner C, Haudek V, Schandl U, Bayer E, Gundacker N, Hutter HP, et al. 2010. Increased protein synthesis by cells exposed to a 1,800-MHz radio-frequency mobile phone electromagnetic field, detected by proteome profiling. International Archives of Occupational and Environmental Health 83:691-702.

Golant MB. 1989. Resonance effect of coherent millimeter-band electromagnetic waves on living organisms. In Russian. Biofizika 34:1004-1014.

Golant MB, Kuznetsov AP, Bozhanova TP. 1994. The mechanism of synchronizing yeast cell cultuResearch with EHF-radiation. In Russian. Biofizika 39:490-495.

Golo VL. 2005. Three-wave interaction between interstrand modes of the DNA. Journal of Experimental and Theoretical Physics 101:372-379.

Gos P, Eicher B. Kohli J, Heyer WD. 1997. Extremely high frequency electromagnetic fields at low power density do not affect the division of exponential phase Saccharomyces cerevisiae cells. Bioelectromagnetics 18:142-155.

Grigoriev Y, Nikitina V, Rubtcova N, Pokhodzey L, Grigoriev O, Belyaev I, et al. 2005. The Russian National Committee on Non-Ionizing Radiation Protection .RNCNIRP. and the radiation guidelines Transparency Forum for Mobile Telephone Systems, Stockholm, Available at:http://memberschellose/igorbelyaev/guidelinespdf

Grigoriev YG. 2004. Bioeffects of modulated electromagnetic fields in the acute experiments .results of Russian researches. Annual of Russian National Committee on Non-Ionisng Radiation Protection Moscow, ALLANA:16-73

Grigoriev YG, StepanovVS, Nikitina VN, Rubtcova NB, Shafirkin AV, Vasin VL. 2003. ISTC Report Biological effects of radiofrequency electromagnetic fields and the radiation guidelines. Results of experiments performed in Russia/Soviet Union Moscow, Institute of Biophysics, Ministry of Health, Russian Federation.

Grundler W. 1992. Intensity- and frequency-dependent effects of microwaves on cell growth rates. Bioelectrochemistry & Bioenergetics 27:361-365.

Grundler W, Jentzsch V, Keilmann F, Putterlik V. 1988. Resonant cellular effects of low intensity microwaves. Biological Coherence and Response to External Stimuli H Frцlich Berlin, Springer-Verlag:65-85.

Guler G, Tomruk A, Ozgur E, Sahin D, Sepici A, Altan N, et al. 2012. The effect of radiofrequency radiation on DNA and lipid damage in female and male infant rabbits. International Journal of Radiation Biology 88:367-373.

Guney M, Ozguner F, Oral B, Karahan N, Mungan T. 2007. 900 MHz radiofrequency-induced histopathologic changes and oxidative stress in rat endometrium:protection by vitamins E and C. Toxicology & Industrial Health 23:411-420.

Hardell L, Carlberg M. 2009. Mobile phones, cordless phones and the risk for brain tumours. International Journal of Oncology 35:5-17.

Hardell L , Carlberg M, Hansson Mild K. 2009. Epidemiological evidence for an association between use of wireless phones and tumor diseases. Pathophysiology 16:113-22.

Hardell L, Eriksson M, Carlberg M, Sundström C, Mild KH. 2005. Use of cellular or cordless telephones and the risk for non-Hodgkin's lymphoma. International Archives of Occupational and Environmental Health DOI 101007/s00420-005-0003-5

Hardell L, Mild KH. 2005. Mobile phone use and acoustic neuromas Epidemiology 16:415; author reply 417-418.

Hardell L, Mild KH, Carlberg M. 2003. Further aspects on cellular and cordless telephones and brain tumours. International Journal of Oncology 22:399-407.

Hardell L, Mild KH, Pahlson A, Hallquist A. 2001. Ionizing radiation, cellular telephones and the risk for brain tumours. European Journal of Cancer Prevention 10:523-529.

Hardell L, Mild KH, Carlberg M, Hallquist A. 2004. Cellular and cordless telephone use and the association with brain tumors in different age groups. Archives of Environmental Health 59:132-137.

Heath B, Jenvey S, Cosic I. 1998. Investigation of analogue and digital mobile phone low frequency radiation spectrum characteristics. Proceedings of the 2nd International Conference on Bioelectromagnetism 83-84.

Hinrikus H, Bachmann M, Lass J, Tomson R, Tuulik V. 2008. Effect of 7, 14 and 21 Hz modulated 450 MHz microwave radiation on human electroencephalographic rhythms. International Journal of Radiation Biology 84:69-79.

Hintzsche H, Jastrow C, Kleine-Ostmann T, Stopper H, Schmid E, Schrader T. 2011. Terahertz radiation induces spindle disturbances in human-hamster hybrid cells. Radiation Research 175:569-574.

Hoyto A, Naarala JJ, 2007. Ornithine decarboxylase activity is affected in primary astrocytes but not in secondary cell lines exposed to 872 MHz RF radiation. International Journal of Radiation Biology 83:367-374.

Höytö A, Luukkonen J, Juutilainen J, Naarala J. 2008. Proliferation, oxidative stress and cell death in cells exposed to 872 MHz radiofrequency radiation and oxidants. Radiation Research 170:235-243.

Huber R, Treyer V, Borbély AA, Schuderer J, Gottselig JM, Landolt HP, et al. 2002. Electromagnetic fields, such as those from mobile phones, alter regional cerebral blood flow and sleep and waking. EEG Journal of Sleep Research 11:289-295.

Huber, R, Treyer V, Schuderer J, Berthold T, Buck A, Kuster N, et al. 2005. Exposure to pulse-modulated radio frequency electromagnetic fields affects regional cerebral blood flow. European Journal of Neuroscience 21:1000-1006.

Huss A, Egger M, Hug K, Huwiler-Müntener K, Röösli M. 2007. Source of funding and results of studies of health effects of mobile phone use:systematic review of experimental studies. Environmental Health Perspectives 115:1-4.

Huttunen P, Hanninen O , Myllyla R. 2009. FM-radio and TV tower signals can cause spontaneous hand movements near moving RF reflector. Pathophysiology 16:201-204.

Hyland GJ. 2000. Physics and biology of mobile telephony. Lancet 356(9244):1833-1836.

IARC. 2002. Biennial Report 2002-2003. Lyon, France, IARC Press 80:183.

ICNIRP. 1998. ICNIRP Guidelines Guidelines for limiting exposure to time-varying electric, magnetic, and electromagnetic fields .up to 300 GHz. Health Physics 74:494-522.

Ilhan A, Gurel A, Armutcu F, Kamisli S, Iraz M, Akyol O, et al. 2004. Ginkgo biloba prevents mobile phone-induced oxidative stress in rat brain. Clinical Chim Acta 340:153-162.

Ilvonen S, Sihvonen AP, Karkkainen K, Sarvas K. 2005. Numerical assessment of induced ELF currents in the human head due to the battery current of a digital mobile phone. Bioelectromagnetics 26:648-656.

Imge EB, Kilicoglu B, Devrim E, Cetin R, Durak I. 2010. Effects of mobile phone use on brain tissue from the rat and a possible protective role of vitamin C - a preliminary study. International Journal of Radiation Biology 86:1044-1049.

Iskin VD. 1990. Biological effects of millimeter waves and correlation method of their detection. In Russian. Kharkov, Osnova.

Johansson PA, Cappello S, Gotz M. 2010. Stem cells niches during development--lessons from the cerebral cortex. Current Opinions in Neurobiology 20:400-407.

Joines WT, Blackman CF. 1980. Power density, field intensity, and carrier frequency determinants of RF-energy-induced calcium-ion efflux from brain tissue. Bioelectromagnetics 1:271-275.

Jokela K, Puranen L, Sihvonen AP. 2004. Assessment of the magnetic field exposure due to the battery current of digital mobile phones. Health Physics 86:56-66.

Jorge-Mora T, Misa-Agustiño MJ, Rodríguez-González JA, Jorge-Barreiro FJ, Ares-Pena FJ, López-Martín E. 2011. The effects of single and repeated exposure to 245 GHz radiofrequency fields on c-fos protein expression in the paraventricular nucleus of rat hypothalamus. Neurochemical Research 36:2322-2332.

Joseph W, Frei P, Roösli M, Thuróczy G, Gajsek P, Trcek T, et al. 2010. Comparison of personal radio frequency electromagnetic field exposure in different urban areas across Europe. Environmental Research 110:658-663.

Juutilainen J, Hoyto A, Kumlin T, Naarala J. 2011. Review of possible modulation-dependent biological effects of radiofrequency fields. Bioelectromagnetics 32:511-34.

Kabuto M, Nitta H, Yamamoto S, Yamaguchi N, Akiba S, Honda Y, et al. 2006. Childhood leukemia and magnetic fields in Japan:a case-control study of childhood leukemia and residential power-frequency magnetic fields in Japan. International Journal of Cancer 119:643-650.

Kazanis I. 2012. Can adult neural stem cells create new brains? Plasticity in the adult mammalian neurogenic niches:realities and expectations in the era of regenerative biology. Neuroscientist 18:15-27.

Kim TH, Shivdasani RA. 2012. Stem cell niches: famished paneth cells, gluttonous stem cells. Current Biology 22:R579-580.

Kolbun ND, Lobarev VE. 1988. Problems of bioinformational interaction in millimeter range. In Russian. Kibernet Vychislitelnaya Tekhnika 78:94-99.

Koveshnikova IV, Antipenko EN. 1991. [On the quantitative regularities of the cytogenic effect of microwaves] Radiobiologiia 31:149-151.

Köylü H, Mollaoglu H, Ozguner F, Naziroglu M, Delibas N. 2006. Melatonin modulates 900 Mhz microwave-induced lipid peroxidation changes in rat brain. Journal of Toxicology and Industrial Health 22:211-216.

Kundi M, Mild K, Hardell L, Mattsson MO. 2004. Mobile telephones and cancer - a review of epidemiological evidence. Journal of Toxicology & Environmental Health B. Critical Reviews 7:351-384.

Kwee S, Raskmark P. 1998. Changes in cell proliferation due to environmental non-ionizing radiation 2 Microwave radiation. Bioelectrochemistry & Bioenergetics 44:251-255.

Lagroye I, Anane R, Wettring BA, Moros EG, Straube WL, Laregina M, et al. 2004. Measurement of DNA damage after acute exposure to pulsed-wave 2450 MHz microwaves in rat brain cells by two alkaline comet assay methods. International Journal of Radiation Biology 80:11-20.

Lai H. 2004. Interaction of microwaves and a temporally incoherent magnetic field on spatial learning in the rat. Physiology Behavior 82:785-789.

Lai H. 2005. Biological effects of radiofrequency electromagnetic field. Encyclopedia of Biomaterials and Biomedical Engineering. Wnek GE & Bowlin GI, New York, NY, Marcel Decker:1-8.

Lai H, Singh NP. 1995. Acute low-intensity microwave exposure increases DNA single-strand breaks in rat brain cells. Bioelectromagnetics 16:207-210.

Lai H, Singh NP. 1996. Single- and double-strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radiation. International Journal of Radiation Biology 69:513-521.

Lai H, Singh NP. 1997. Melatonin and a spin-trap compound block radiofrequency electromagnetic radiation-induced DNA strand breaks in rat brain cells. Bioelectromagnetics 18:446-454.

Lai H, Singh NP. 2005. Interaction of microwaves and a temporally incoherent magnetic field on single and double DNA strand breaks in rat brain cells. Electromagnetic Biology & Medicine 24:23-29.

Liburdy RP, Vanek, PF Jr. 1985. Microwaves and the cell membrane II Temperature, plasma, and oxygen mediate microwave-induced membrane permeability in the erythrocyte. Radiation Research 102:190-205.

Liburdy RP, Vanek, PF Jr. 1987. Microwaves and the cell membrane III Protein shedding is oxygen and temperature dependent:evidence for cation bridge involvement. Radiation Research 109:382-395.

Lin-Liu S, Adey WR. 1982. Low frequency amplitude modulated microwave fields change calcium efflux rates from synaptosomes. Bioelectromagnetics 3:309-322.

Linde T, Mild KH. 1997. Measurement of low frequency magnetic fields from digital cellular telephones. Bioelectromagnetics 18:184-186.

Litovitz TA, Krause D, Penafiel M, Elson EC, Mullins JM. 1993. The role of coherence time in the effect of microwaves on ornithine decarboxylase activity. Bioelectromagnetics 14:395-403.

Litovitz TA, Penafiel M, Farrel JM, Krause D, Meister R, and Mullins JM. 1997. Bioeffects induced by exposure to microwaves are mitigated by superposition of ELF noise. Bioelectromagnetics 18:422-430.

Lonn S, Ahlbom A, Hall P, Feychting M. 2004. Mobile phone use and the risk of acoustic neuroma. Epidemiology 15:653-659.

López-Martín E, Bregains J, Relova-Quinteiro JL, Cadarso-Suárez C, Jorge-Barreiro FJ, Ares-Pena FJ. 2009. The action of pulse-modulated GSM radiation increases regional changes in brain activity and c-Fos expression in cortical and subcortical areas in a rat model of picrotoxin-induced seizure proneness. Journal of Neuroscience Research 87:1484-1499.

Lu YS, Huang BT, Huang YX. 2012. Reactive oxygen species formation and apoptosis in human peripheral blood mononuclear cell induced by 900 MHz mobile phone radiation. Oxidative Medicine & Cellular Longevity 2012:740280.

Lukashevsky KV, Belyaev IY. 1990. Switching of prophage lambda genes in Escherichia coli by millimeter waves. Medical Science Research I8:955-957.

Malyapa RS, Ahern EW, Bi C, Straube WL, LaRegina M, Pickard WF, et al. 1998. DNA damage in rat brain cells after in vivo exposure to 2450 MHz electromagnetic radiation and various methods of euthanasia. Radiation Research 149:637-645.

Malyapa RS, Ahern EW, Straube WL, Moros EG, Pickard WF, Roti Roti JL. 1997. Measurement of DNA damage after exposure to 2450 MHz electromagnetic radiation. Radiation Research 148:608-617.

Markkanen A, Penttinen P, Naarala J, Pelkonen J, Sihvonen AP, Juutilainen J. 2004. Apoptosis induced by ultraviolet radiation is enhanced by amplitude modulated radiofrequency radiation in mutant yeast cells. Bioelectromagnetics 25:127-133.

Markovà E, Hillert L, Malmgren L, Persson BR, Belyaev IY. 2005. Microwaves from GSM mobile telephones affect 53BP1 and gamma-H2AX foci in human lymphocytes from hypersensitive and healthy persons. Environmental Health Perspectives 113:1172-1177.

Markovà E, Malmgren LOG, Belyaev IY. 2010. Microwaves from mobile phones inhibit 53BP1 focus formation in human stem cells more strongly than in differentiated cells:possible mechanistic link to cancer risk. Environmental Health Perspectives 118:394-399.

Matronchik AI, Alipov ED, Beliaev IY. 1996. A model of phase modulation of high frequency nucleoid oscillations in reactions of E coli cells to weak static and low-frequency magnetic fields. In Russian. Biofizika 41:642-649.

Matronchik AI, Beliaev IY. 2005. Model of slow nonuniform rotation of the charged DNA domain for effects of microwaves, static and alternating magnetic fields on conformation of nucleoid in living cells. Fröhlich Centenary International Symposium Coherence and Electromagnetic Fields in Biological Systems .CEFBIOS-2005. Journal of Pokorny Prague, Czech Republic, Institute of Radio Engineering and Electronics, Academy of Sciences of the Czech Republic:63-64.

Matronchik AI, Beliaev IY. 2008. Mechanism for combined action of microwaves and static magnetic field: slow non uniform rotation of charged nucleoid. Electromagnetic Biology & Medicine 27:340-354.

Metcalfe AD, Ferguson MW. 2008. Skin stem and progenitor cells:using regeneration as a tissue-engineering strategy. Cellular & Molecular Life Sciences 65:24-32.

Morrison SJ, Spradling AC. 2008. Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell 132:598-611.

Nam KC, Kim SW, Kim SC, Kim DW. 2006. Effects of RF exposure of teenagers and adults by CDMA cellular phones. Bioelectromagnetics 27:509-514.

Nazıroğlu M, Ciğ B, Doğan S, Uğuz AC, Dilek S, Faouzi D. 2012. 245-Gz wireless devices induce oxidative stress and proliferation through cytosolic Ca^{2+} influx in human leukemia cancer cells. International Journal of Radiation Biology 88:449-456.

Nikolova T, Czyz J, Rolletschek A, Blyszczuk P, Fuchs J, Jovtchev G, et al. 2005. Electromagnetic fields affect transcript levels of apoptosis-related genes in embryonic stem cell-derived neural progenitor cells. FASEB Journal 19:1686-1688.

Niwa O. 2010. Roles of stem cells in tissue turnover and radiation carcinogenesis. Radiation Research 174:833-839.

Nylund R, Leszczynski D. 2006. Mobile phone radiation causes changes in gene and protein expression in human endothelial cell lines and the response seems to be genome- and proteome-dependent. Proteomics 6:4769-4780.

Oktem F, Ozguner F, Mollaoglu H, Koyu A, Uz E. 2005. Oxidative damage in the kidney induced by 900-MHz-emitted mobile phone:protection by melatonin. Archives of Medical Research 36:350-355.

Olcerst RB, Belman S, Eisenbud M, Mumford WW, Rabinowitz JR. 1980. The increased passive efflux of sodium and rubidium from rabbit erythrocytes by microwave radiation. Radiation Research 82:244-256.

Oscar KJ, Hawkins TD. 1977. Microwave alteration of the blood-brain barrier system of rats. Brain Research 126:281-293.

Ozguner F, Altinbas A, Ozaydin M, Dogan A, Vural H, Kisioglu AN, et al. 2005. Mobile phone-induced myocardial oxidative stress:protection by a novel antioxidant agent caffeic acid phenethyl ester. Toxicology & Industrial Health 21:223-230.

Ozguner F, Aydin G, Mollaoglu H, Gökalp O, Koyu A, Cesur G. 2004. Prevention of mobile phone induced skin tissue changes by melatonin in rat: an experimental study. Toxicology & Industrial Health 20:133-139.

Ozguner F, Bardak Y, Comlekci C. 2006. Protective effects of melatonin and caffeic acid phenethyl ester against retinal oxidative stress in long-term use of mobile phone: a comparative study. Molecular & Cellular Biochemistry 282:83-88.

Ozguner F, Oktem F, Armagan A, Yilmaz R, Koyu A, Demirel R, et al. 2005. Comparative analysis of the protective effects of melatonin and caffeic acid phenethyl ester .CAPE. on mobile phone-induced renal impairment in rat. Molecular & Cellular Biochemistry 276:31-37.

Ozguner F, Oktem F, Ayata A, Koyu A, Yilmaz HR. 2005. A novel antioxidant agent caffeic acid phenethyl ester prevents long-term mobile phone exposure-induced renal impairment in rat. Prognostic value of malondialdehyde, N-acetyl-beta-D-glucosaminidase and nitric oxide determination. Molecular & Cellular Biochemistry 277:73-80.

Ozgur E, Gler G, Seyhan N. 2010. Mobile phone radiation-induced free radical damage in the liver is inhibited by the antioxidants n-acetyl cysteine and epigallocatechin-gallate. International Journal of Radiation Biology 86:935-945.

Pakhomov AG, Akyel Y, Pakhomova ON, Stuck BE, Murphy MR. 1998. Current state and implications of research on biological effects of millimeter waves: a review of the literature. Bioelectromagnetics 19:393-413.

Pakhomov AG, and Murphy MR. 2000. Comprehensive review of the research on biological effects of pulsed radiofrequency radiation in Russia and the former Soviet Union. Advances in Electromagnetic Fields in Living System JC Lin New York, Kluwer Academic/Plenum Publishers 3:265-290.

Pakhomov AG, Murthy PR. 2000. Low-intensity millimeter waves as a novel therapeutic modality. IEEE Transactions on Plasma Science 28:34-40.

Panagopoulos DJ, Karabarbounis A, Margaritis LH. 2002. Mechanism for action of electromagnetic fields on cells. Biochemical & Biophysical Research Communications 298:95-102.

Panagopoulos DJ, Margaritis LH. 2010. The effect of exposure duration on the biological activity of mobile telephony radiation. Mutation Research 699:17-22.

Papageorgiou CC, Nanou ED, Tsiafakis VG, Capsalis CN, Rabavilas AD. 2004. Gender related differences on the EEG during a simulated mobile phone signal. Neuroreport 15:2557-2560.

Pashovkina MS, Akoev IG. 2000a. [Changes in serum alkaline phosphatase activity during in vitro exposure to amplitude-modulated electromagnetic field of ultrahigh frequency .2375 MHz. in guinea pigs] Biofizika 45:130-136.

Pashovkina MS, Akoev IG. 2001b. [Effect of low-intensity pulse-modulated microwave on human blood aspartate aminotransferase activity] Radiatsionnaia biologiia, radioecologiia 41:59-61.

Pashovkina MS, Akoev IG. 2001c. [Effect of low intensity pulse-modulated electromagnetic radiation on activity of alkaline phosphatase in blood serum] Radiatsionnaia Biologiia, Radioecologiia 41:62-66.

Peinnequin A, Piriou A, Mathieu J, Dabouis V, Sebbah C, Malabiau R, et al. 2000. Non-thermal effects of continuous 245 GHz microwaves on Fas-induced apoptosis in human Jurkat T-cell line. Bioelectrochemistry 51:157-161.

Penafiel LM, Litovitz T, Krause D, Desta A, Mullins JM. 1997. Role of modulation on the effect of microwaves on ornithine decarboxylase activity in L929 cells. Bioelectromagnetics 182:132-141.

Perentos N, Iskra S, McKenzie RJ, Cosi I. 2007. Characterization of pulsed ELF magnetic fields generated by GSM mobile phone handsets. World Congress on Medical Physics and Biomedical Engineering 2006, Vol 14, Pts 1-6 14:2706-2709.

Perentos N, Iskra S, McKenzie RJ, Cosi I. 2008. Simulation of pulsed ELF magnetic fields generated by GSM mobile phone handsets for human electromagnetic bioeffects research. Australasian Physical & Engineering Sciences in Medicine 31:235-242.

Persson BRR, Salford KG, Brun A. 1997. Blood-Brain Barrier permeability in rats exposed to electromagnetic fields used in wireless communication. Wireless Networks 3:455-461.

Phillips JL, Singh NP, Lai H. 2009. Electromagnetic fields and DNA damage. Pathophysiology 16:79-88.

Pollycove M, Feinendegen LE. 2003. Radiation-induced versus endogenous DNA damage: possible effect of inducible protective responses in mitigating endogenous damage. Human & Experimental Toxicology 22:290-306.

Postow E, Swicord ML. 1986. Modulated fields and window effects. CRC Handbook of Biological Effects of Electromagnetic Fields C Polk and E Postow Boca Raton, FL, CRC Press:425-460.

Presman AS. 1963. [Problems in the Biological Action of Microwaves] Usp Sovrem Biol 56:161-179.

Presman AS, IuI L, Levitina MA. 1961. [Biological effect of microwaves] Usp Sovrem Biol 51:84-103.

Remondini D, Nylund R, Reivinen J, Poulletier de Gannes F, Veyret B, et al. 2006. Gene expression changes in human cells after exposure to mobile phone microwaves. Proteomics 6:4745-4754.

Repacholi MH, Basten A, Gebski V, Noonan D, Finnie J, Harris AW. 1997. Lymphomas in E mu-Pim1 transgenic mice exposed to pulsed 900 MHZ electromagnetic fields. Radiation Research 1475:631-640.

Richardson RB. 2011. Stem cell niches and other factors that influence the sensitivity of bone marrow to radiation-induced bone cancer and leukaemia in children and adults. International Journal of Radiation Biology 87:343-359.

Salford LG, Brun A, Sturesson K, Eberhardt JL, Persson BR. 1994. Permeability of the blood-brain barrier induced by 915 MHz electromagnetic radiation, continuous wave and modulated at 8, 16, 50, and 200 Hz. Microscopy Research & Technique. 27:535-542.

Sannino A, Sarti M, Reddy SB, Prihoda TJ, Vijayalaxmi, Scarfi MR. 2009. Induction of adaptive response in human blood lymphocytes exposed to radiofrequency radiation. Radiation Research 171:735-742.

Santini R, Seigne M, Bonhomme-Faivre L, Bouffet S, Defrasne E, Sage M. 2001. [Symptoms reported by mobile cellular telephone users] Pathologie-biologie. Paris. 49:222-226.

Sarimov R, Alipov ED, Belyaev IY. 2011. Fifty hertz magnetic fields individually affect chromatin conformation in human lymphocytes:dependence on amplitude, temperature, and initial chromatin state. Bioelectromagnetics 32:570-579.

Sarimov R, Malmgren L, Markova E, Persson B, Belyaev IY. 2004. Non-thermal GSM microwaves affect chromatin conformation in human lymphocytes similar to heat shock. IEEE Transactions on Plasma Science 32:1600-1608.

Schrader T, Münter K, Kleine-Ostmann T, Schmid E. 2008. Spindle disturbances in human-hamster hybrid .AL. cells induced by mobile communication frequency range signals. Bioelectromagnetics 29:626-639.

Schwarz C, Kratochvil E, Pilger A, Kuster N, Adlkofer F, Rüdiger HW. 2008. Radiofrequency electromagnetic fields .UMTS, 1,950 MHz. induce genotoxic effects in vitro in human fibroblasts but not in lymphocytes. International Archives of Occupational Environmental Health 81:755-767.

Sevast'yanova, L A .1981. Nonthermal effects of millimeter radiation. In Russian. Devyatkov ND. Moscow, Institute of Radioelctronics of USSR Academy of Science:86-109.

Shcheglov VS, Alipov ED, Belyaev IY. 2002. Cell-to-cell communication in response of E coli cells at different phases of growth to low-intensity microwaves. Biochimica et Biophysica Acta 1572:101-106.

Shcheglov VS, Belyaev IY, Ushakov VL, Alipov YD. 1997. Power-dependent rearrangement in the spectrum of resonance effect of millimeter waves on the genome conformational state of E coli cells. Electro- & Magnetobiology 16:69-82.

Shckorbatov YG, Grigoryeva NN, Shakhbazov VG, Grabina VA, Bogoslavsky AM. 1998. Microwave irradiation influences on the state of human cell nuclei. Bioelectromagnetics 19:414-419.

Shckorbatov YG, Pasiuga VN, Goncharuk EI, Petrenko TP, Grabina VA, Kolchigin NN, et al. 2010. Effects of differently polarized microwave radiation on the microscopic structure of the nuclei in human fibroblasts. Journal of Zhejiang University Science B 11:801-805.

Shckorbatov YG, Pasiuga VN, Kolchigin NN, Grabina VA, Batrakov DO, Kalashnikov VV, et al. 2009. The influence of differently polarised microwave radiation on chromatin in human cells. International Journal of Radiation Biology 85:322-329.

Sit'ko SP, Ed .1989. The 1st All-Union Symposium with International Participation Use of Millimeter Electromagnetic Radiation in Medicine. Kiev, Ukraine, USSR, TRC Otklik.

Smythe JW, Costall B. 2003. Mobile phone use facilitates memory in male, but not female, subjects. Neuroreport 14:243-246.

Sokolovic D, Djindjic B, Nikolic J, Bjelakovic G, Pavlovic D, Kocic G, et al. 2008. Melatonin reduces oxidative stress induced by chronic exposure of microwave radiation from mobile phones in rat brain. Journal of Radiation Research 49:579-586.

Stagg RB, Thomas WJ, Jones RA, Adey WR. 1997. DNA synthesis and cell proliferation in C6 glioma and primary glial cells exposed to a 83655 MHz modulated radiofrequency field. Bioelectromagnetics 18:230-236.

Sugiyama T, Nagasawa T. 2012. Bone marrow niches for hematopoietic stem cells and immune cells. Inflammation & Allergy Drug Targets 11:201-206.

Sun W, Shen X, Lu D, Fu Y, Lu D, Chiang H. 2012. A 18-GHz radiofrequency radiation induces EGF receptor clustering and phosphorylation in cultured human amniotic (FL) cells. International Journal of Radiation Biology 88:239-244.

Tkalec M, Malarić K, Pavlica M, Pevalek-Kozlina B, Vidaković-Cifrek Z. 2009. Effects of radiofrequency electromagnetic fields on seed germination and root meristematic cells of Allium cepa L. Mutation Research - Genetic Toxicology & Environmental Mutagenesis 672:76-81.

Tkalec M, Malarić K, Pevalek-Kozlina B. 2005. Influence of 400, 900, and 1900 MHz electromagnetic fields on Lemna minor growth and peroxidase activity. Bioelectromagnetics 26:185-193.

Tkalec M, Malarić K, Pevalek-Kozlina B. 2007. Exposure to radiofrequency radiation induces oxidative stress in duckweed Lemna minor L. Science of the Total Environment 388:78-89.

Ushakov VL, Alipov ED, Shcheglov VS, Beliaev IY. 2006. Peculiarities of non-thermal effects of microwaves in the frequency range of 51-52 GHz on E coli cells. Radiatsionnaia Biologiia, Radioecologiia 46:719-728.

Ushakov VL, Shcheglov VS, Beliaev IY, Harms-Ringdahl M. 1999. Combined effects of circularly polarized microwaves and ethidium bromide on E coli cells. Electro- & Magnetobiology 18:233-242.

van Rongen E, Croft R, Juutilainen J, Lagroye I, Miyakoshi J, Saunders R, et al. 2009. Effects of radiofrequency electromagnetic fields on the human nervous system. Journal of Toxicology Environmental Health B Crit Rev 12:572-597.

Veyret B, Bouthet C, Deschaux P, de Seze R, Geffard M, Joussot-Dubien J, et al. 1991. Antibody responses of mice exposed to low-power microwaves under combined, pulse-and-amplitude modulation. Bioelectromagnetics 12:47-56.

Vilenskaya RL, Smolyanskaya AZ, Adamenko VG, Buldasheva ZN, Gelvitch EA, Golant MB, et al. 1972. Induction of the lethal colicin synthesis in E coli K12 C600 .E1. by means the millimeter radiation. In Russian. Bull Eksperim Biol Med 4:52-54.

Webb SJ. 1979. Factors affecting the induction of Lambda prophages by millimetre waves. Physics Letters 73A:145-148.

Yang Y, Jin X, Yan C, Tian Y, Tang J, Shen X. 2008. Case-only study of interactions between DNA repair genes .hMLH1, APEX1, MGMT, XRCC1 and XPD. and low-frequency electromagnetic fields in childhood acute leukemia. Leukemia & Lymphoma 49:2344-2350.

Yao K, Wu W, Yu Y, Zeng Q, He J, Lu D, et al. 2008. Effect of superposed electromagnetic noise on DNA damage of lens epithelial cells induced by microwave radiation. Investigative Ophthalmology & Visual Science 49:2009-2015.

Yao K, Wu W, Yu Y, Zeng Q, He J, Lu D, et al. 2009. Retraction. Effect of superposed electromagnetic noise on DNA damage of lens epithelial cells induced by microwave radiation. Investigative Ophthalmology & Visual Science 50:4530.

Zhao TY, Zou SP, Knapp PE. 2007. Exposure to cell phone radiation up-regulates apoptosis genes in primary culturese of neurons and astrocytes. Neuroscience Letters 412:34-38.

Zmyślony M, Politanski P, Rajkowska E, Szymczak W, Jajte J. 2004. Acute exposure to 930 MHz CW electromagnetic radiation in vitro affects reactive oxygen species level in rat lymphocytes treated by iron ions. Bioelectromagnetics 25:324-328.

Zotti-Martelli L, Peccatori M, Maggini V, Ballardin M, Barale R. 2005. Individual responsiveness to induction of micronuclei in human lymphocytes after exposure in vitro to 18

Plausible Genetic and Metabolic Mechanisms for the Bioeffects of Very Weak ELF Magnetic Fields on Living Tissues

Paul Héroux, PhD, Professor
Department of Epidemiology, Biostatistics and Occupational Health
McGill University Faculty of Medicine, and
Department of Surgery, InVitroPlus Laboratory
McGill University Health Center
Montreal, Quebec, Canada

Ying Li, PhD
Department of Surgery, InVitroPlus Laboratory
McGill University Health Center
Montreal, Quebec, Canada

1

I. INTRODUCTION

A. The "kT Problem"

The biological effects of weak extremely-low frequency (ELF) magnetic fields (MFs) have long been a subject of controversy, with many expressing skepticism as to their very existence: ELF-MFs have lacked a credible mechanism of interaction between MFs and living material.

A prominent conceptual objection has been the "kT problem" (Binhi, 2007). This "problem" can be summarized by the very large ratio between the energy available from a quantum of ELF radiation (2.47×10^{-13} eV) and the thresholds for ionization of atoms (4.34 eV for potassium), chemical activation (~ 0.7 eV), or even the 0.156 eV able to transfer protons across gA channels (Chernyshev, 2002).

What these numbers show is that ELF MFs are certainly not able to have effects through these particular mechanisms, but a detailed theoretical analysis (Binhi, 2007) does not preclude that ELF-MF effects could occur in other ways. MFs can alter the shape of the orbitals of particles without substantially altering their energies, possibly leading to very low thresholds for MF biological effects. Rather than a pure energy problem, as stated above, the true "problem" is to determine if biological structures exist that can be disturbed by very low-amplitude ELF MFs.

II. KEY SCIENTIFIC EVIDENCE

B. Magnetic Sensors

Modern electronics provides interesting examples, such as the MOSFET, where tiny signals can control large energies: a voltage applied to a gate with nominally zero current allows control of substantial drain currents. Biological systems have their own sources of energy, and the MF need only contribute a perturbing influence.

In the context of ELF MF effects, it is useful to examine the transducers of MF-measuring instruments. Induction coils have long been the item of choice for many such instruments, but they suffer from a lack of analogy with possible biological equivalents, in that they gather signal from

substantial surfaces (the coil core), and then concentrate the action of the magnetic flux variations gathered over that considerable area at a single point, the contact of the winding.

Hall-effect probes are closer to the mark, in that they detect the potential difference created by a MF on a current flowing in a semi-conductor. Here, the MF acts to deflect a current flow that is powered by an extraneous source. This device dissociates the energy available from the MF itself from the energy it controls.

Another electronic device even closer to the biological transducer we seek is the Spin Tunnel Junction (Micromagnetics, 2012). Such a junction is made of two ferromagnetic metal layers separated by an insulating barrier of a few nanometers (Fig. 6). If a small voltage is applied across the junction, electrons will tunnel through the barrier, according to the ambient MF. The device's MF sensitivity is based on spin-coherent tunneling: the probability of an electron tunneling across the barrier is dependent on its spin, because an electron of a given spin must tunnel to an unfilled state of the same spin. Even the simplest free-electron descriptions of Spin Polarization and Tunneling MagnetoResistance confirm that junction characteristics are determined not only by the ferromagnetic layers, but depend as well on the properties of the barrier (Tsymbal, 2003). Solid-state Spin Tunnel Junctions can detect MFs as low as 0.26 nT at 60-Hz. What these solid-state devices demonstrate is that very small MFs can have effects within the bulk of materials, and that changes in the properties of insulating materials can affect electron tunneling.

C. Magnetic Fields and Incubators

MF experiments with living cells are immediately faced with a practical problem. Cell culture incubators have within them relatively large MFs, due to their relatively weak attenuation of environmental MFs, and to the necessity of implementing controlled heating, humidity and CO_2 concentration conditions. The first control simulates body temperature, the second avoids osmotic imbalance through evaporation, and the third stabilizes pH values within cell culture media. Table 1 was compiled in a survey of 46 incubators used in research (Su, 2012), and showed that average MFs in water-jacketed CO_2 incubators range from 0.9 to 13 μT.

The reaction of many investigators to this situation has been to compensate for the high backgrounds by using even larger MFs in their experiments. According to the conventional dose-responses expected in Toxicology, the effect of an agent can be detected even in the presence of a background exposure, since the biological response is expected to rise smoothly with dose. Many

3

investigators must also have felt that more robust data would be obtained using larger exposures, and that background MFs in incubators could be tolerated.

Table 1. Summary MF Table of 46 Surveyed Incubators (in µT).

Brand	Model	Type	Mean	Min	Max	Max Background
New Brunswick	G-25	Shaker	**0.39**	0.2	0.81	2.06*
Chicago Surgical Ele.	N.A.	General	**0.61**	0.25	1.21	3.32*
Forma Scientific	3956	General	**0.76**	0.2	2.64	0.22
Fisher Sci.	Isotemp	General	**0.76**	0.05	1.85	0.32
Fisher Sci.	637D	General	**0.84**	0.22	2.49	0.23
Forma Scientific	3157	CO_2 W	**0.91**	0.11	2.66	1.77*
Thermo Electron	N.A.	Shaker	**0.98**	0.57	1.58	5.86*
Nuaire	US auto flow	CO_2 W	**0.99**	0.4	2.28	1.34*
Thermo Forma	3310	CO_2 W	**1.04**	0.32	3.75	0.68*
Innova New Brunswick	4200	Shaker	**1.17**	0.31	2.97	0.4
Fisher Isotemp	281	General	**1.86**	1.2	2.22	0.47
Baxter	WJ501	CO_2 W	**1.87**	0.77	5.27	1.6*
Sanyo	N.A.	CO_2	**2.77**	0.85	6.72	0.3
New Brunswick	G-25	Shaker	**2.79**	0.42	16.13	0.31
Sanyo O_2/ CO_2	MCO-18M	CO_2	**2.8**	1.48	4.14	0.81*
Sanyo	MCO_19AIC	CO_2	**2.94**	1.63	5.17	3.31*
Sanyo	MCO-20AIC	CO_2	**3.12**	1.22	6.64	6.68*
Hera Cell	240	CO_2	**3.28**	2.36	4.62	1.48*
Baxter	Tempcon	General	**3.36**	0.61	7.43	1*
Innova New Brunswick	4000	Shaker	**3.47**	1.27	9.53	0.36
Hera Cell	N.A.	CO_2	**3.65**	2.68	4.49	0.26*
Thermo Scientific	370	CO_2	**3.84**	1.9	7.01	0.64*
New Brunswick	C25	Shaker	**3.88**	0.33	17.74	0.96*
Thermo Electron	3110	CO_2 W	**3.91**	1.19	8.56	0.92*
Nuaire	Nu4750	CO_2 W	**3.95**	0.77	10.38	0.64*
Thermo Scientific	370	CO_2	**3.99**	2.03	6.25	0.96*
Forma Scientific	3130	CO_2 W	**4.67**	1.53	11.14	1.37*
Forma Scientific	3110	CO_2 W	**5.44**	1.77	12.59	2.42*
Fisher Sci.	546	CO_2 W	**6.58**	2.36	16.88	0.38
Forma Scientific	N.A.(Old)	CO_2	**6.71**	2.32	16.83	1.36*
Thermo Electron	3130	CO_2 W	**6.79**	1.73	16.97	18.9***
Thermo Electron	3110	CO_2	**7.55**	1.83	18.28	3.92*
Revco	N.A.(Old)	CO_2	**7.67**	3.57	17.76	1.27*
Napco	3550	CO_2	**7.8**	3.52	13.42	2.84*
Thermo Electron	Napco 3550	CO2	**7.83**	3.81	12.13	1.63*
Fisher Sci.	Isotemp 546	CO_2 W	**9.61**	2.34	37.58	0.76*
Thermo Forma	3110	CO_2 W	**9.73**	2.73	24.14	0.47*
N.A.	N.A.	General	**10.46**	3.57	19.51	0.2

4

Thermo Forma	3110	CO_2 W	**11.89**	3.3	30.41	0.49*
Gallenkamp	N.A.	General	**11.96**	3.06	37.17	2.3*
Fisher Sci.	610	CO_2	**12.3**	5.15	35.52	1.59*
Forma Scientific	3158	CO_2 W	**13.08**	2.62	50.64	1.61*
Labline	3527	Shaker	**14.04**	3.62	42.74	11.87**
WWR international	2005	General	**15.48**	4.92	47.37	1.28
Forma Scientific	546	CO_2	**16.5**	2.61	74.47	3.45*
Sanyo	MIR152	CO_2	**26.98**	5.67	120	0.34*

Type "CO_2 W" means CO_2 incubator with water jacket. "Max Background" refers to measurements outside the incubators. * measured at 50 cm or halfway between the incubator and other electric equipment. ** 5 cm to another incubator. *** 10 cm to a power outlet panel. For more details, refer to Dong and Héroux, 2012.

D. Magnetic Shielding

If it is desired to eliminate the background MFs of incubators to low levels, shielding must be implemented within the incubators. We achieved this in our own experiments using structural steel cylinders 6.3 mm in thickness. As shown in Fig. 1, culture vessels are centered in concentric rectangular structural steel pipes 5.1 x 7.6 x 20 cm, 7.6 x 10.2 x 20 cm and 15.2 x 24.5 x 36 cm. This configuration reduces 60-Hz MFs by a factor of 144, providing "unexposed" cells with a MF environment of 3 nT, slightly below the measurement floor (5 nT at 60-Hz) of our Narda EFA-300 MF instrument (Li, 2012a). The shielding weighs about 20 kg, and is subject to corrosion, if used in the incubator for long periods of time. Fig. 2 shows the change along the axis of the shielding in the triaxially integrated MF. Static MFs within the shields are slightly lower than 50 µT, as structural steel is de-magnetized during production, but of random direction.
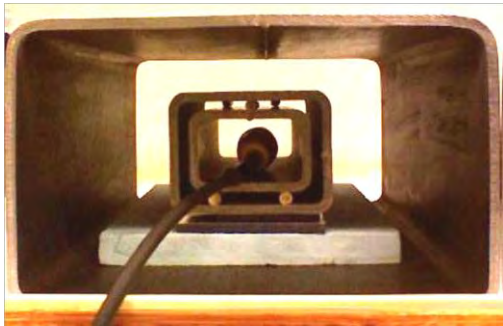

Fig. 1. The three layers of magnetic shielding. The Narda EFA-300's MF probe is in place of the culture vessel. MF coils for exposure are below, but not in contact with the two smaller shields, insulated from the outer shield by a layer of rigid foam.
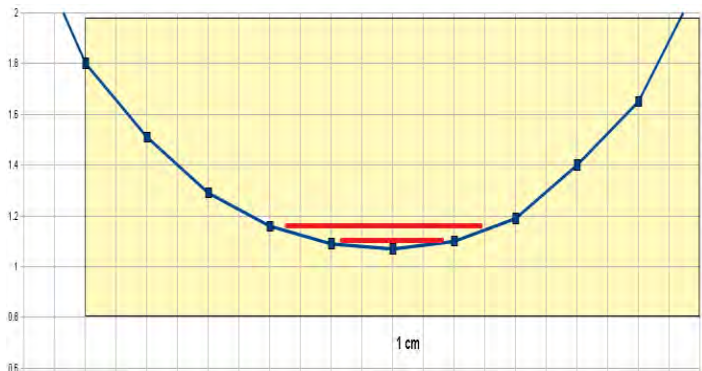

5

Fig. 2. MF density (µT) generated by an exposure coil vs longitudinal distance inside a magnetic shield pair. The two red lines show the extent of T-25 and T-12 culture vessels, and the yellow rectangle is the smaller shield outline.

E. Experiments on Cells

We conducted experiments on 5 cancer cell lines, with the objective of bringing high precision to our *in vitro* determinations. This objective was reached using automated data acquisition and real-time computer vision, which allowed automated recognition of cells, apobodies and decay particles in cell cultures (Héroux, 2004). In order to reduce deviations related to changing cell culture media, our work used a single synthetic medium (rather than Fetal Bovine Serum) for all 5 cancer models investigated (Li, 2012b).

We first focused our work on changes in the behavior of our cell models under various levels of oxygen. Somewhat surprisingly, all 5 models survived even under anoxic (0 % oxygen) conditions, confirming the exceptional flexibility of cancers cells, able to thrive under anoxia, presumably by finding glycolysis-based sources of cellular energy even in the absence of oxygen.
Low oxygen conditions are actually quite representative of the normal environment of many cells in the body, and are certainly a better *in vitro* representation of the environment of tumor cells, which grow in oxygen and nutrient-restricted environments.

Withdrawal of oxygen suppresses metabolism, as a major combustible of mitochondrial ATP synthesis, oxygen, is eliminated. Metabolism can also be suppressed by a number of chemicals such as oligomycin, imatinib and melatonin-vitamin C, which we collectively designated as "metabolic restrictors".

F. Karyotype Contraction

When grown under *anoxia* (as opposed to *atmoxia* which is 21 % oxygen, and the commonly used cell culture condition) our 5 cancer cell models lost 6 to 8 chromosomes from their normal

number (Table 3). Further, in the presence of strong doses of antioxidant metabolic restrictors, the cell lines quickly reverted to almost normal chromosome numbers (47 – 49). The anoxic cells showed increases in proliferation rate, and the acquisition of a stable, stem phenotype.

Using our 5 hyperploid (54 – 69 chromosomes) cancer cell models, we found that our cells adjusted their chromosome numbers up or down, to match their micro-environment, through rapid mechanisms of endo-reduplication (unscheduled, extra-mitotic chromosome duplication) or chromosome loss. We called this reversible loss of chromosomes under suppressed metabolism "Karyotype Contraction" (KC).

Anoxic K562 displays a very stable karyotype, with 75 % of the cells having either 61 or 62 chromosomes. With the knowledge that metabolic changes would change these chromosome counts, we then set out to investigate the effects of ELF MFs on this model, while we carefully controlled MFs using the shielding techniques described above. We were then using KC as a metabolic scale.

Starting from cell cultures maintained in a pre-industrial environment (less than 4 nT 60-Hz MF), our 5 cancer cell lines were exposed to constant ELF-MFs within the range of 0.025 to 5 μT, and the cells were examined for karyotype changes after 6 days.
As shown in Table 2, all cancer cells lines lost chromosomes from MF exposures, with a mostly flat dose-response. It seemed that the number of chromosomes lost was more specifically connected to the particular cell type than to the MF level, although the two erythro-leukemia cell types both showed a dose-response between 25 and 400 nT.

Surprisingly, constant MF exposures for three weeks allowed a rising return to the baseline, unperturbed karyotypes. From this point, small MF increases or decreases (10 %) were then again capable of inducing karyotype contractions (Li, 2012a).

Table 2. Karyotype Contraction (mean number of chromosomes lost over 6 days)

Magnetic Field (nT)	Anoxic K562 Erythroleukemia	Atmoxic HEL Erythroleukemia	Atmoxic NCI-H460 Lung cancer	Anoxic MCF-7 Breast cancer	Atmoxic COLO-320DM Colon cancer
25	2.21				
50	4.92	10.22	7.52	11	5.36
100	8.18	11.55			
200	11.04				

400	10.4	12.79	7.55	10.64	5.85
700	9.52				
1000	7.69			10.68	
1500	9.94				
5000	12.1	13.03	7.46	10.95	5.78

Table 3. Karyotype Contraction (mean number of chromosomes lost over 6 days)

Cell	Atmoxic Modal Chromosome Number	Anoxic KC	Anoxic to MF Saturation KC	Atmoxic to MF Saturation KC	Atmoxic to Anti-Oxidant Suppression KC*
K-562 Erythroleukemia	69	7	10.12		21.34
HEL Erythroleukemia	66	7		12.91	18
MCF-7 Breast cancer	82	8	10.82		18
NCI-H460 Lung cancer	57	6		7.51	10
COLO-320DM Colon cancer	54	6		5.66	7.7
Average	*65.6*	*6.8*	*10.47*	*8.69*	*15.01*
Condition	+ O_2	- O_2	- O_2 + MFs	O_2 + MFs	O_2 + Oxidative Inhibition

The conclusion from these observations was that MFs act as a metabolic inhibitor, even at very low levels commonly encountered in the normal environment.

G. ATP Synthase

Supplementary tests carried out by comparing MF-exposed cell cultures to cultures exposed to various metabolic suppressors showed that the MF-exposed cultures were remarkably similar to those exposed to oligomycin A, a specific inhibitor of the Fo segment of the enzyme ATP Synthase (ATPS).

But how could MFs as low as 25 nT alter the activity of ATPS? ATPS has the structure of a motor-generator than normally produces ATP using the energy of a flow of protons through a turbine-like structure, Fo. MFs apparently impaired the flow of protons through ATPS Fo.
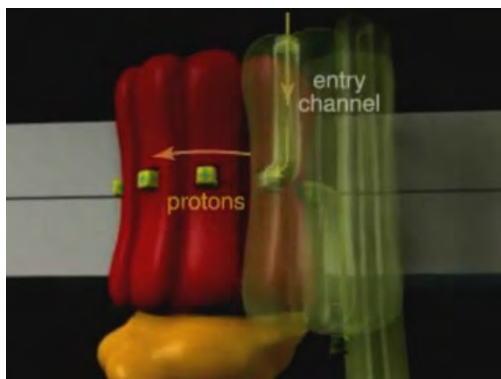
Fig. 3. The structure of ATPS Fo: entry and exit channels for the movement of protons (Yoshida, Tokyo Institute of Technology).

Russian physicists (Semikhina 1981; Semikhina 1988) have reported that very low levels of ELF MFs (25 nT) can alter the structure of water, and that the effects of the altered water structure would be particularly important under high concentrations of protons and water molecules. An interesting aspect of these changes in water structure is that the transition between states takes several hours.

As it turns out, the entry and exit channels of ATPS Fo (Fig. 3) are hydrophilic channels, which means that they are expected to be filled with water molecules, and the intermembrane potential of mitochondria maintains a large electric field (180 kV/cm) which concentrates protons within them. These locations seem ideal to embody the low level effects documented by Semikhina and Kiselev.
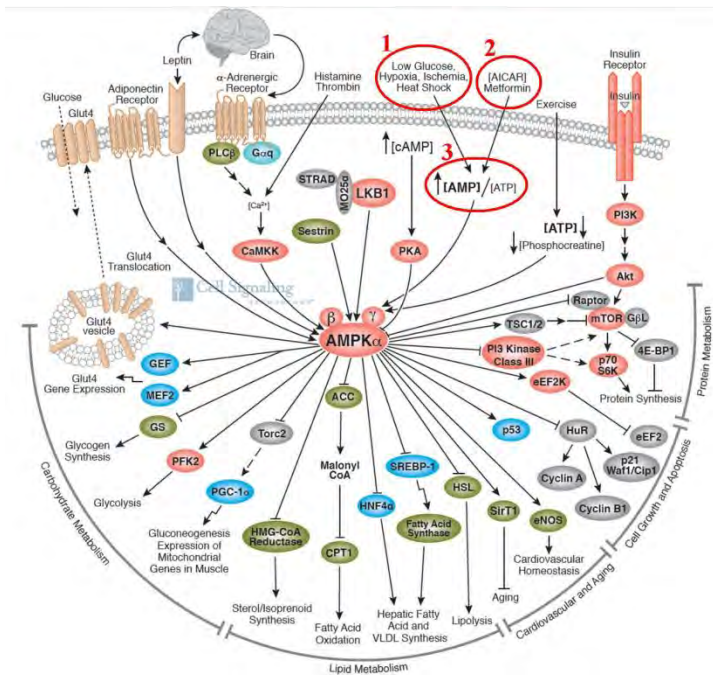
Fig. 4. The many regulatory pathways of AMPK, with the hypoxic (1), metformin (2) and ATPS suppression sites (3) labeled (http://www.cellsignal.com/).

H. AMPK

If the mechanism was indeed as we thought, then MFs would alter the production of ATP in cells. If this happened, another important intracellular enzyme, AMP-activated protein kinase (AMPK), would

9

immediately be activated, as AMPK is extremely sensitive to changes in the level of ATP. We tested this hypothesis by two supplementary assays involving metformin and resistin. As expected, MF effects were amplified by metformin, an AMPK stimulator, and attenuated by resistin, an AMPK inhibitor (Li, 2012a).

Our data therefore suggests that the karyotype contractions caused by MFs stem from interference with mitochondria's ATP synthase (ATPS), compensated by the action of AMPK. The involvement of AMPK also conveniently explains the slow restoration of karyotypes to their original level after 3 weeks, as AMPK is not only fast-acting to restore ATP levels, but slow-acting through its numerous metabolic and genetic regulation pathways (Fig. 4). It may also explain the unusual observation where increases or decreases in MF exposures can both produce KCs (Li, 2012a).

I. In the Channels

Some enzymes operate faster than predicted by classical thermodynamics, and their increased speed can be explained by tunneling of protons or electrons through activation barriers (Garcia-Viloca, 2004; Olsson, 2004). Quantum tunneling for protons over 6 nm through bridging by water molecules has been observed in tryptamine oxidation by aromatic amine dehydrogenase, for example, and tunneling in enzymatic reactions is now widely accepted in biological models (Masgrau, 2006).

It is of interest to examine how protons may flow through ATPS Fo channels. The protons trickle through a thin pipe of water molecules, propelled by an electric field of about 180 kV/cm. Adiabatic tunneling should be more efficient than non-adiabatic coupling, implying that disturbances along the channel could result in loss of channel transparency. Proton-coupled electron transfer underpins many biological reactions, and may occur as unidirectional or bidirectional, and synchronous or asynchronous, transfer of protons and electrons (Reece, 2009).
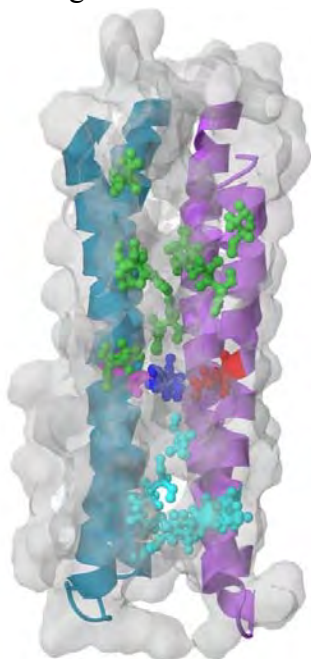
Fig. 5.   The ATPS Fo proton hydrophilic channel. Hydrophilic side chains and residues are in green and blue. (from Sasada R, Marcey D. ATP Synthase, 2010.
http://www.callutheran.edu/BioDev/omm/jmolxx/atp_synthase/atp_synthase.html#fig1).

It is probable that both electrons and protons tunnel through the channel, making theoretical analysis more complex, especially as electrons meet with different protons along a chain. Since protons are much heavier than electrons (x1836), their wavelength is 43 times shorter (inverse square root), and electrons may transfer over longer distances (Moser, 1992; Gray, 1996). Thus, electron transfer can span fractions of nano-meters, while proton transfer occurs mostly within a hydrogen bond (less than 0.197 nm). The hydrogen bond strength (23.3 kJ/mol) is just 5 times the average thermal fluctuation energy. Quantum chemical calculations show that this strength can vary as much as 90 %, depending on the level of cooperativity or anti-cooperativity within water molecule chains, which corresponds to a bond length change of 9 %, or 0.018 nm (Hus, 2012).

This limited reach of proton tunneling and its delicate dependence on water cluster structure may be major factors underlying the sensitivity of ATPS performance to MF-exposed water.

J. Water 'Remanence'

From our observations, particularly the fact that exposed cell culture medium can retain memory of past MF exposures (Li, 2012a), it does not appear that biological effects of MFs, as we detected them, are based on a direct interaction with electrons or protons, but rather, as suggested by Semikhina and Kiselev, on an interaction between MF and the structure of water, which in turn influences electron and proton tunneling. The exact structure of the water molecule arrays responsible is not known, but may be connected with long-lived hydrogen bond structures which confer particular proton transparency to ATPS Fo water channels. This structure seems vulnerable to interference by MFs over a wide range of intensities and possibly frequencies (Kiselev, 1988). Perturbations to the structure of O-H bond vibrations has even been spectroscopically detected as slow (hours) transitions in water exposed to sunlight radiation (Yokono, 2009).

This would not be the first instance of subtle changes in hydrogen bonds resulting in large influences in biology. A contemporary example relates to the selective uptake of phosphorus rather than arsenic by bacteria. The discrimination by a factor of 4,500 in phosphorus vs arsenic is based on a 4 %

11

distortion in a unique low-barrier hydrogen bond (Elias, 2012).

III.   DISCUSSION

There are similarities as well as differences between semi-conductor tunneling and ATPS tunneling. Both involve oxygen; tunneling distances, as well as the voltages applied (Fig. 6) are similar. But in semiconductor tunneling, only electrons are mobile, while protons move within ATPS. In the semiconductor, magnetic sensing is mainly through shifts in the populations of electrons with a given spin, determined by the electrodes. In ATPS, the transparency of the water channel seems determined by long-term MF exposures.

Perhaps least understood is how cells can metabolically compensate for various MF exposures over time, as shown by the restoration of their chromosome numbers after three week exposures (Li, 2012a). Anoxia leads to permanent KCs, but other KCs from MFs or other anti-oxidants are transient. Most anti-oxidant and MF KCs are larger than the atmoxic to anoxic transition KCs, possibly because some oxygen is still available to cell metabolism, even under anoxic conditions. Anoxia and MFs together are effective metabolic suppressors.
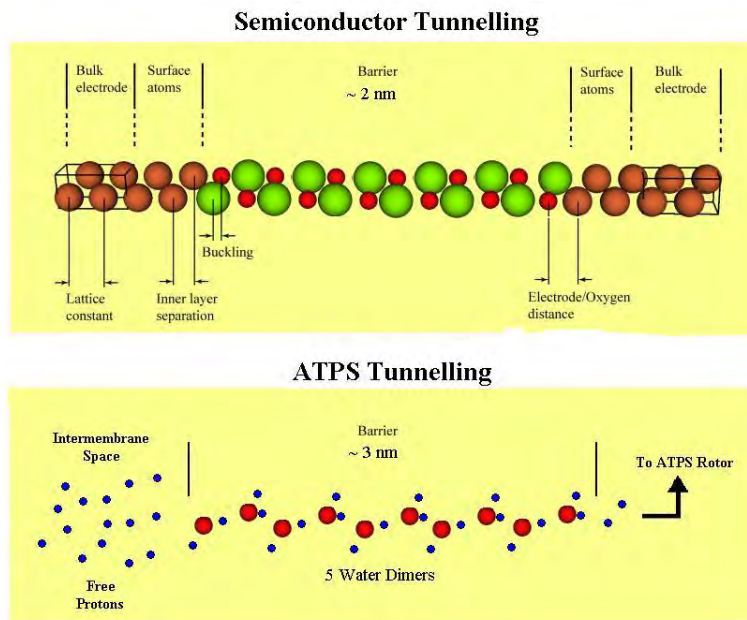

Fig. 6   Tunneling in magnetic sensors and in ATPS water channels.

12

IV. CONCLUSIONS

The particularities of hydrogen bond structures in water can justify the subtle changes detected in water structure under MF exposures. Under specific circumstances, such water changes may influence the flux of protons in ATPS channels, thus inducing some biological effects of MFs. These interactions seem to involve very small energies, and also seem to require hours to establish themselves, thus bypassing the celebrated "kT problem". These results may be environmentally important, in view of the central roles played in human physiology by ATPS and AMPK, particularly in their links to diabetes, cancer and longevity (Li, 2012a). The wide range of MF amplitudes and frequencies that can potentially disturb ATPS make this effect a global health issue. Although society seems to compile diseases with more enthusiasm that longevity (Li, 2012a), it should be remembered that MF exposures may have both undesirable and desirable effects on health.

V. REFERENCES

Binhi VN, Rubin AB. Magnetobiology: The kT paradox and possible solutions. Electromagnetic Biology and Medicine 2007; 26: 45-62.

Chernyshev A, Cukierman S. Thermodynamic view of activation energies of proton transfer in various gramicidin a channels. Biophysical Journal 2002;82;182-192.

Elias M, Wellner A, Goldin-Azulay K, Chabriere E, Vorholt JA, Erb TJ, http://www.ncbi.nlm.nih.gov/pubmed?term=Tawfik DS%5BAuthor%5D&cauthor=true&cauthor_uid=23034649 et al. The molecular basis of phosphate discrimination in arsenate-rich environments. Nature. 2012;491(7422):134-7.

Garcia-Viloca M, Gao J, Karplus M, Truhlar DG. How enzymes work: analysis by modern rate theory and computer simulations. Science 2004;303 (5655):186-95.

Gray HB, Winkler JR. Electron transfer in proteins. Annu. Rev. Biochem. 1996 65 :537-61.

Héroux P, Kyrychenko I, Bourdages M. Proliferation and apoptosis rate of living erythroleukemia cells, Microscopy and Analysis 2004;18 (3):13-15 (UK).

Huš M, Urbic T. Strength of hydrogen bonds of water depends on local environment, J. Chem. Phys. 2012;136 (2012) 14, 144305-311.

Kiselev VF, Saletskii AM, Semikhina LP. Influence of weak magnetic fields and UHF radiation on certain dielectric and optical properties of water and aqueous solutions. Translated from Teoreticheskaya i Eksperimental'naya Khimiya 1988;24(3):330-334.

Li Y, Héroux P. Extra-low-frequency magnetic fields alter cancer cells through metabolic restriction, 2012a. Available at: http://arXiv.org/abs/1209.5754

Li Y, Héroux P, Kyrychenko I. Metabolic Restriction of cancer cells in vitro causes karyotype contraction - an indicator of cancer promotion? Tumor Biology 2012;33(1):195-205.

Masgrau L, Roujeinikova A, Johannissen LO, Hothi P, Basran J, Ranaghan KE, et al. Atomic description of an enzyme reaction dominated by proton tunneling. Science 2006;312 (5771): 237–41.

Micromagnetics, 2012. Available at: http://micromagnetics.com/docs/SpinTJ_TMR_magnetic_sensors_brochure.pdf)

Moser CC, Keske JK, Warncke K, Farid RS, Dutton PL. Nature of biological electron transfer. Nature. 1992;355(6363):796-802.

Olsson MH, Siegbahn PE, Warshel A. Simulations of the large kinetic isotope effect and the temperature dependence of the hydrogen atom transfer in lipoxygenase. Journal of the American Chemical Society 2004;126 (9): 2820=2828.

Reece SY, Nocera DG. Proton-coupled electron transfer in biology: results from synergistic studies in natural and model systems. Annual Review of Biochemistry 2009;78: 673-699).

Semikhina LP, Kiselev VF. Effect of weak magnetic fields on the properties of water and ice. Russian Physics Journal 1981;31:5351-5354.

Semikhina LP, Kiselev VF, Levshin LV, Saletskii AM. Effect of weak magnetic fields on the luminescence-spectral properties of a dye in an aqueous solution. Journal of Applied Spectroscopy 1988 ;48:556-559.

Su D, Héroux P. Survey of extra-low frequency and very-low frequency magnetic fields in cell culture incubators, 2012. Available at: http://arxiv.org/abs/1211.2458

Tsymbal EY, Mryasov ON, LeClair PR. Spin-dependent tunneling in magnetic tunnel junctions. Evgeny Tsymbal Publications. Paper 19, 2003. Available at: http://digitalcommons.unl.edu/physicstsymbal/19

Yokono T, Shimokawa S, Yokono M, Hattori H. Infra-Red Spectroscopic study of structural change of liquid water induced by sunlight irradiation. 2009;Water 1(29-34).

Evidence based on EMF Medical Therapeutics

Cindy Sage, MA

Sage Associates

Santa Barbara, California

Table of Contents

I. Introduction

Electromagnetic fields are widely used in therapeutic medical applications. Proof of effectiveness has been demonstrated in numerous clinical applications of low-intensity ELF-EMF and RF-EMF, each treatment employing specific characteristics of frequency, modulation and intensity to achieve its efficacy. On the other hand, higher levels of EMFs encountered in the environment which are indiscriminately generated by technologies of the 20th and 21st centuries may result in harm. EMF levels which are allowable today under thermally-based public exposure standards do not take into account these clear indications of the sensitivities of the human body to EMFs. If we are to promulgate public exposure standards that are protective of public health, then this body of evidence on healing with EMFs is of primary importance in developing biologically-based public exposure standards.

"Although incompletely understood, tissue free radical interactions may extend to zero field levels. Emergent concepts of tissue thresholds to imposed and intrinsic magnetic fields address ensemble or domain functions of populations of cells, cooperatively whispering together in intercellular communication and organized hierarchically at atomic and molecular levels." 10

II. Therapeutic Uses for Electromagnetic Fields

Since EMFs have been shown to be effective in treating conditions of disease at energy levels far below current public exposure standards, this body of evidence forms a strong warning that indiscriminate EMF exposure is ill advised. Health concerns from indiscriminate exposure to EMF, as opposed to EMF exposures done with clinical oversight, could lead to harm as can the unsupervised use of pharmaceutical drugs.

The consequence of multiple sources of EMF exposure in daily life, with no regard to cumulative exposures or to potentially harmful combinations of EMF exposures will pose future difficulties in identifying sources of disease (because of multiple and overlapping exposures) and time-varying and geography-varying differences from person to person.

Just as ionizing radiation can be used to effectively diagnose disease and treat cancer, it is also a cause of cancer under different exposure conditions. Since EMFs are both a cause of disease,

and also used for treatment of disease, it is vitally important that public exposure standards reflect our current understanding of the biological potency of EMF exposures.

"there is an abundance of experimental and clinical data demonstrating that exogenous EMFs of surprisingly low levels can have a profound effect on a large variety of biological systems. Both electrical and electromagnetic devices have been demonstrated to positively affect the healing process in fresh fractures, delayed and nonunions, osteotomies, and spine fusion in orthopedics and for chronic and acute wound repair. These clinical results have been validated by well-designed and statistically powered double-blind clinical trials and have survived meta-analyses. The FDA has approved labeling for these biophysical devices, limited at present to these indications." "The potential clinical applications of EMF therapeutics extend far beyond those considered here and the clinical rewards are certain to be huge." "Cancer, cardiac muscle regeneration, diabetes, arthritis, and neurological disorders are just some of the pathologies that have already been shown to be responsive to EMF therapy. Successful applications of low-frequency EMFs have been reported for treatment of bronchial asthma, myocardial infarction, and venous and varicose ulcers. There is emerging research on EMF effects on angiogenesis and the manner in which this may increase stem cell survival in the treatment of Altzeimer's (sic) and Parkinson's diseases. There are also many studies that point to the possibility of the use of
EMF for peripheral nerve regeneration" and *" the treatment of cancer."* *"EMF therapy modalities are simple, safe and significantly less costly to the health care system. They offer the ability to treat the underlying pathology rather than simply the symptoms. The time is particularly opportune given the increased incidence of side effects from the use of pharmacological agents. EMF therapeutics will have a profound impact upon health and wellness and their costs worldwide."* [1]

A. Bone Repair

Clinical use of pulsed EMF has been demonstrated to achieve bone repair, particularly in fractures that do not heal on their own. Bone healing is stimulated by very weak electromagnetic fields that are far lower in strength than would produce tissue heating. The FDA approved pulsed EMF for use in bone healing in 1979. Since that time, many millions of patients have

benefited from this therapy. Since PEMF treatments are non-invasive and clinically effective, it has advantages to the patient in terms of reduced pain and suffering, reduction in health care costs, and effectiveness where other methods have failed to produce adequate clinical results.

"It is now commonplace to learn the successful use of weak, nonthermal electromagnetic fields (EMF) in the quest to heal, or relieve the symptoms of a variety of debilitating ailments. This chapter attempts to give the reader an introduction and assessment of EMF modalities that have demonstrated therapeutic benefit for bone and wound repair and chronic and acute pain." [2]

Pilla provides extensive discussion of the "clinical evidence that time-varying magnetic fields (EMF) can modulate molecular, cellular and tissue functions in a physiologically significant manner." [2] A description of the various waveforms and EMF modalities which are effective in bone and wound repair are beyond the scope of this paper, but are well documented. [2] In addition to documenting that bone repair in fractures is achieved with pulsed EMF at low intensities, Pilla also reports that pulsed EMF has been successful in promoting bone repair and healing of spine fusions for the treatment of chronic back pain from worn and/or damaged spinal discs. [3] The FDA has approved pulsed EMFs for bone healing and this is a widely recognized treatment, particularly for fractures that are slow to heal, or do not repair with conventional medical treatment. It represents one of the best documented cases in science where the body clearly responds to low-intensity EMF signals for healing purposes; these EMF signals are far below current public exposure standards and are proof of the bioactivity (in a beneficial form as applied).

Liboff describes signal shapes in electromagnetic therapies that contribute greatly to our understanding of the various forms of EMF signal delivery that are fundamental to eliciting specific bioeffects. He simply and elegantly describes electric and magnetic signal characteristics, their signature shapes and methods of delivery (time-varying, oscillatory, or modulated) which create special interactions with human tissues and organs for healing. [4]

"It is likely that the future will see combinations of such signals in therapeutic applications, especially as more information filters back from the laboratory elaborating on the nature of electromagnetic interactions with living tissue." [4]

B. Wound Repair

The clinical application of pulsed EMF has been shown to enhance wound repair and healing. [2,5] Devices that use pulsed EMF have been approved for use in the United States by the FDA. Pilla reports "*the clear clinical effectiveness of PEMF signals has resulted in significantly increased use*" in treating wounds that do not heal. [5] In Pilla's extensive summary presented on beneficial effects of EMF on wound healing, he reports pulsed EMF has been reported to reduce edema, increase blood flow, modulate upregulated growth factor receptors, enhance neutrophil and macrophage attraction and epidermal cell migration, and increase fibroblast and granulation tissue proliferation. Most wound studies were conducted on arterial or venous skin ulcers, diabetic ulcers, pressure ulcers, and surgical and burn wounds. [5] Wound repair under the influence of very low level pulsed EMFs is a second solid documentation in science that very low level EMFs are bioactive (in this case, beneficial) when applied in very specific clinical applications where the exposure variables are carefully selected.

Oschman provides an overview of the evolution of energy medicine and electromagnetic energy treatments related to bone repair, wound healing, pain relief, depression, insomnia, inflammation of tissues and other medical conditions. [6] He also underscores the counter-intuitive thesis that low-intensity EMFs can be more effective in eliciting healing responses than larger intensity exposures; and that understanding of the subtle energies and their specific interactions with human functioning is imperative.

(l)iving tissues are far more sensitive to external fields than previously realized. After a period when physicists were certain that observed sensitivities to nonionizing and nonthermal radiations wer physically impossible, we now know that biological systems defy the simple logic that larger stimuli should produce larger responses.For many living systems, extremely weak fields can be more effective than strong fields." [6]

C. Pain Management

Pulsed magnetic field (PMF) devices are also used with FDA approval for *"relief of acute and chronic pain and the reduction of edema* (swelling*), all symptoms of wounds from post-surgical procedures, musculoskeletal injuries, muscle and joint overuse, as well as for chronic wounds."* [5]

Pulsed EMF has been shown to be effective in relief of chronic pain associated with connective tissue injury (cartilage, tendon, ligaments and bone) and soft-tissue injuries associated with the joints. Both acute and chronic pain may be successfully treated with EMFs as an alternative to non-steroidal anti-inflammatory drugs (NSAIDs). Relief from chronic pain due to osteoarthritis has been reported with treatment by EMFs. [2]

Markov reports that EMF is used in treatment of pain associated with tendonitis, multiple sclerosis, carpal tunnel syndrome and some forms of arthritis. He discusses the use of pulsed EMF for headache and migraine pain relief; neck and whiplash injuries, postoperative pain, sprains, chronic pelvic pain, and nerve regeneration. Pain reduction by clinical application of pulsed EMF is achieved with non-thermal levels of exposure, and produces a nonthermal biological effect. [8]

D. Depression, Anxiety Disorders, Insomnia

"Today (2002) we are at a threshold for the acceptance of electromagnetic therapy as a clinically accepted form of therapy for such diverse diseases as unipolar depression, Parkinson's disease, and sleep disorders and the treatment of debilitating chronic and acute pain." [8]

Shealy et al (2007) detail clinical findings for treatment of depression and mood management, reduction in anxiety, and treatment of insomnia. [10] Electrical energy stimulators that deliver very low-level EMF have been reported to be clinically effective in the alteration of neurobioechemicals including serotonin and cortisol. Depression, mood disorders and insomnia have been related to disregulation of serotonin levels.

Use of EMFs to reduce symptoms of depression, anxiety and insomnia are authorized by the FDA, and have been in use since the 1970's. Shealy reports that transcranial stimulation by EMFs led to a significant relief of depression in 85% of patients who had failed pharmacological

agents, and was at least twice as effective as any known antidepressant drugs and without complications. [10]

E. Protection from Anoxia (Protection for the Heart)

The work of Albertini, Litovitz and di Carlo, Goodman and Blank, Han, Pipkin, Rasmark and Kwee, [11-17] has shown that very weak ELF-EMF and RF-EMF exposures can actually help to protect cells against tissue damage. They can induce an adaptive stress response in cells, which in turn helps the cell fight damage. The response is production of stress proteins (heat shock proteins or HSP). These stress proteins help to protect the cells against injury and death. A 20-minute exposure to electromagnetic fields at only 80 mG will start stress protein production, which helps to fight cellular damage from lack of oxygen, for example. Protection from anoxia (or lack of oxygen) is important in heart attacks. Pre-treatment with ELF-EMF (and also RF-ELF) before blocking oxygen to cells has been shown to be protective against the lack of oxygen to heart tissues. The exposure level is on the order of 80 mG ELF-EMF or far below any possible thermal heating.

This means that there are clinical applications for protection against heart attack damage that can be provided by very low-dose EMF exposures. Such protection could be vitally important in reducing damage from oxygen loss during heart attacks. It is another line of proof that low-intensity electromagnetic fields are bioactive, and when applied in specific therapeutic ways, are beneficial. It also underscores that the body can detect and decode these very weak signals, providing further evidence that thermally-based standards are incomplete because they do not take into account the sensitivity of the human body to non-thermal levels of EMF exposure.

IV. Conclusions

Since EMFs have been shown to be effective in treating conditions of disease at energy levels far below current public exposure standards, this body of evidence forms a strong warning that indiscriminate EMF exposure is ill advised.

Based on extensive clinical applications of low-intensity EMFs since at least the 1970s, it has been demonstrated beyond argument that some forms of EMFs can be medically effective in treating a wide variety of human health disorders and injuries. Since all of these treatments are conducted at energy levels that do not involve tissue heating per se, it is convincing proof that the human body both reacts to and can be affected by exposures to EMFs. Exposures can be beneficial when EMFs are applied with conscious knowledge of the exposure factors that are proven to lead to specific biological (healing) consequences. The intensity of such therapeutic exposures nearly always falls below current public exposure standards as discussed in Section 3.

V. References

1. Pilla AA. Mechanisms and Therapeutic Applications of Time-Varying and Static Magnetic Fields. Handbook of Biological Effects of Electromagnetic Fields Third Edition: Biological and Medical Aspects of Electromagnetic Fields, 2007. Barnes FS and Greenebaum B. editors, pages 392-393.

2. Pilla AA. Mechanisms and Therapeutic Applications of Time-Varying and Static Magnetic Fields. Handbook of Biological Effects of Electromagnetic Fields Third Edition: Biological and Medical Aspects of Electromagnetic Fields, 2007. Barnes FS and Greenebaum B. editors, page 352.

3. Ibid, page 356.

4. Liboff AR. Signal Shapes in Electromagnetic Therapies: A Primer. Bioelectromagnetic Medicine. 2004, Rosch PJ and Markov MS, editors, page 32.

5. Pilla AA. Mechanisms and Therapeutic Applications of Time-Varying and Static Magnetic Fields. Handbook of Biological Effects of Electromagnetic Fields Third Edition: Biological and Medical Aspects of Electromagnetic Fields, 2007. Barnes FS and Greenebaum B. editors, page 357.

6. Oschman JL. Recent Developments in Bioelectromagnetic Medicine. Bioelectromagnetic Medicine. 2004, Rosch PJ and Markov MS, editors, page 79. 77-92 entire chapter?

7. Markov MS. Magnetic and Electromagnetic Field Therapy: Basic Principles of Application for Pain Relief. Bioelectromagnetic Medicine. 2004, Rosch PJ and Markov MS, editors, page 258.

8. Prato FS. Image-Guided Electromagnetic Therapy. Bioelectromagnetic Medicine. 2004, Rosch PJ and Markov MS, editors, page 51.

9. Shealy CN Liss S Liss BS. Evolution of Electrotherapy: From TENS to Cyberpharmacology. Bioelectromagnetic Medicine. 2004, Rosch PJ and Markov MS, editors, page 93-114.

10. Adey, WR. Potential Therapeutic Applications of Nonthermal Electromagnetic Fields: Ensemble Organization of Cells in Tissue as a Factor in Biological Field Sensing. Bioelectromagnetic Medicine. 2004, Rosch PJ and Markov MS, editors, page 1.

11. Albertini A, Zucchini P, Noera G, Cadossi R, Napoleone CP, Pierangeli A. 1999. Protective effect of low frequency low energy pulsing electromagnetic fields on acute experimental myocardial infarcts in rats. Bioelectromagnetics 20:372-377.

12. Di Carlo AL, Farrell JM, Litovitz TA. 1999a. Myocardial protection conferred by electromagnetic fields. Circulation 99:813-816.

13. Goodman R, Blank M. 1998. Magnetic field stress induces expression of hsp70. Cell Stress Chaperones 3:79±88.

14. Goodman R, Blank M, Lin H, Dai R, Khorkova O, Soo L,Weisbrot D, Henderson A. 1994. Increased levels of HSP70 transcripts induced when cells are exposed to low frequency electromagnetic fields. Bioelectrochem Bioenerg 33:115-120.

15. Han L, Lin H, Head M, Jin M, Blank M, Goodman R. 1998. Application of magnetic field-induced heat shock protein 70 for presurgical cytoprotection. J Cell Biochem 71:577-583

16. Pipkin JL, Hinson WG, Young JF, Rowland KL, Shaddock JG, Tolleson WH, Duffy PH, Casciano DA. 1999. Induction of stress proteins by electromagnetic fields in cultured HL-60 cells. Bioelectromagnetics 20:347-357.

17. Raskmark P, Kwee S. 1998. The minimizing effect of electromagnetic noise on the changes in cell proliferation cause by ELF magnetic fields. Bioelectrochem Bioenerg 40:193-196.

Electromagnetic Medicine

Non-Inductive Non-Thermal Modalities

(Supplement 2012)

Abraham R. Liboff, PhD, Professor Emeritus

Department of Physics

Oakland University

Rochester Hills, Michigan   USA

I. INTRODUCTION

The area of electromagnetic medicine (EM) encompasses the applications of electricity and magnetism to medical practice. Although this includes both diagnostic and therapeutic applications, the medical community is far more familiar with the former, notably with techniques such as magnetic resonance imaging (MRI), electromyography (EMG), electroencephalography (EEG), electrocardiography (EKG), and magnetocardiography (MKG). There are historical reasons for the medical unfamiliarity (even antipathy) with electromagnetically-based therapies. One has only to look at the beginnings of modern medicine in the United States, specifically the 1910 Flexner report [1,2] that provided the basis for medical education today. Prior to this report there was widespread use of electromagnetic techniques in medicine, often little more than late 19th century versions of snake-oil cures. In great measure the present aversion to electromagnetic therapies built into modern medicine is a direct result of Victorian age quackery.

Another reason for this antipathy, apart from the constraint on the teaching curriculum, has been the extraordinary success of, first, the germ theories of Pasteur and Koch, and, second, the development of molecular biology following the work of Watson and Crick. These have engendered a sense of completeness, a feeling that there is no place for alternate, radically new approaches to the way that illness is treated. Even when electromagnetically-based therapies have proven beneficial, they have been usually ignored. There is little impetus to replace the existing approach, since it is firmly believed that nothing is more fundamental than the existing paradigm, that questions of wellness and illness are ultimately biochemical in nature.

The divisions in electromagnetic medicine are outlined in Fig. 1. Beyond the separation into diagnostic and therapeutic applications another distinction is made for applications of weak-field ELF magnetic in the treatment of illness. The description ***non-inductive non-thermal*** helps emphasize that the effects obtained by applying low intensity low-frequency electromagnetic fields to biological systems are not the result of either inductive emf generation or the delivery of thermal energies through Joule heating. By contrast, a number of clinical devices that make use of Faraday induction or Joule heating are recognized by the medical community not only because

they are effective, but also because the applied voltages, currents or heat are fully consistent with what is expected biochemically. In sharp contrast, the non-inductive non-thermal category includes clinical applications where this is not true, that is, where the electromagnetic variables that are part of the therapy fall outside those permitted by the current medical paradigm.
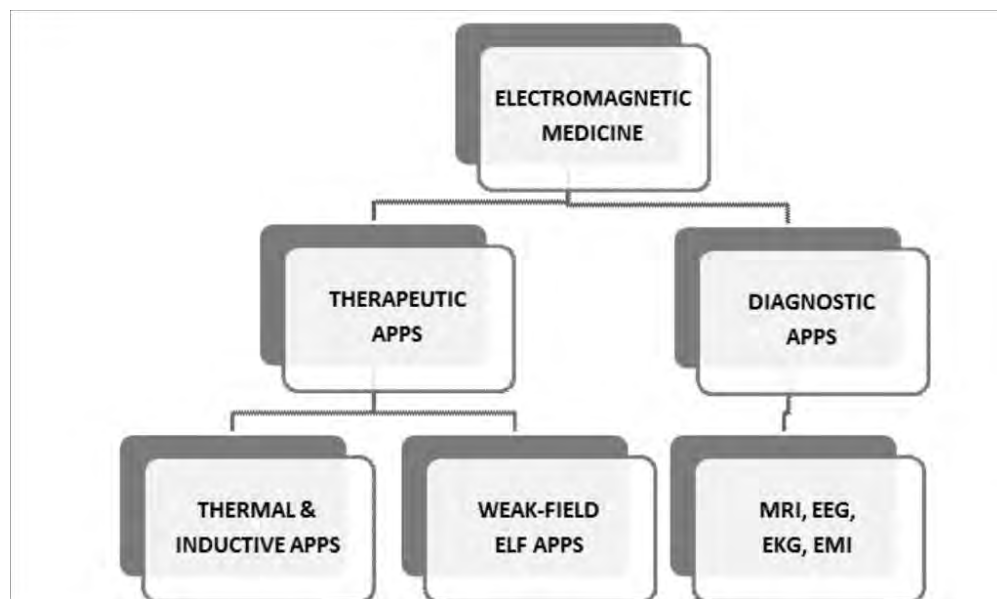

Fig. 1. Divisions comprising Electromagnetic Medicine

II. WEAK-FIELD ELF APPLICATIONS: SCIENTIFIC BASIS

There is a wealth of evidence showing that weakly intense ELF fields affect the metabolic responses in cells. It was found in the 1980s that ELF magnetic fields too weak to be considered as inductive sources of potential differences are nevertheless capable of affecting DNA synthesis in mammalian cell culture[3,4]. Since that time, there have been numerous reports (Table 1) that magnetic fields on the order of several microTesla and in the 3-300 Hz ELF frequency range can affect a wide range of biological systems. A short list of such reports, given in Table 1, emphasizes both the variety of systems in which these effects have been found, and the difficulty in providing an explanation, as evidenced by the fact that these studies have a history extending back more than 25 years. The lack of a reasonable explanation is not a trivial distinction, since there is great reluctance to accept observational evidence, regardless of replications and the number of supportive reports, without a reasonable biomolecular basis

Biological Model	YEAR	Reference
Rat behavior	1986	Thomas et al[5]
Diatom motility	1987	Smith et al[6]
Protein synthesis in salivary gland cells	1988	Goodman and Henderson[7]
Mitogenesis in lymphocytes	1989	Cossarizza et al[8]
Production of glycosaminoglycans in cartilage	1991	Smith et al[9]
Neuroblastoma cell metabolism	1992	Smith et al[10]
Expression of Insulin Growth Factor II	1995	Fitzsimmons et al[11]
Regeneration of planarians	1995	Jenrow et al[12]
Analgesia in snails	1996	Prato et al[13]
Rat EEG	1998	Vorobyov et al[14]
Growth Rate in plants	2005	Galland and Pazur[15]
Stem cell differentiation	2009	Gaetini et al[16]

Table 1. List of reports indicating that non-inductive ELF magnetic fields are biologically interactive. Note that these reports are by no means isolated. A number of these have been independently replicated, for example the studies on rat behavior, lymphocytes, planarians, and plants.

In 1998 a group led by Zhadin[17] discovered that these effects are also found at much lower intensities. AC magnetic fields as low as 40 nT can shift the electrical conductivity of polar amino acids in aqueous solutions. This work, independently replicated[18,19,20], is typified by a sharp change in conductivity at one specific frequency, as shown in Fig. 2. The explanation for this remarkable effect makes use of quantum electrodynamics to provide a means of reducing the viscosity of water sufficiently to allow Lorentz forces to be observed on solvated biological ions, thereby establishing a straightforward reason for the many difficult-to-explain magnetic stimulation reports claiming a connection to ion cyclotron resonance[21].

Ion cyclotron resonance (ICR) as it applies to biological systems was first discovered[22,23] to be a critical underlying factor in connection with previously observed[24] electromagnetically-induced changes in free calcium in brain tissue (Ca-efflux experiments). In the presence of a static magnetic field the most prominent effects are always observed for parallel AC magnetic fields with frequencies very close to the cyclotron frequency of the calcium ion. The majority of subsequent ICR cellular studies have focused on the Ca^{2+} ion. As a second messenger it is involved in regulation at all stages of growth and development, including proliferation, and in the organization of cytoskeletal elements. Indeed some of the results shown in Table 1 are examples of Ca^{2+} ICR stimulation.
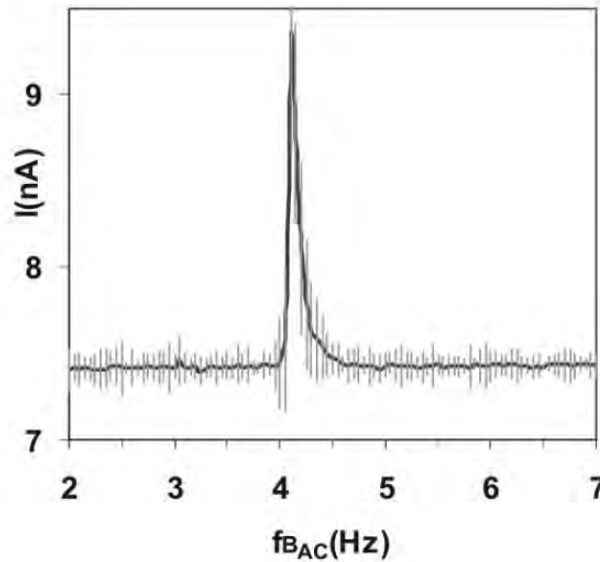
Fig. 2. Data taken by Pazur[18] illustrating the Zhadin effect[17]. A very weak AC magnetic field (40 nT) is applied to an aqueous solution of glutamic acid and the conductivity of the glu$^+$ ions is continuously monitored in terms of nA. The magnetic frequency in Hz is slowly ramped upwards. A sharp change in conductivity is observed at a frequency (4.25 Hz) close to the ion cyclotron resonance value for glu$^+$, (4.8 Hz).

The expression for the ICR resonant angular frequency is given as $w = (q/m)B_0$, where q and m are the charge and mass of the ion, and B_0 the DC magnetic field. Confirmation that the charge-to-mass ratio was explicitly involved in this effect was obtained when isotopic $_{45}$Ca was substituted for $_{40}$Ca in a study on lymphocyte proliferation[25], showing that the frequency where the maximum ICR effect on proliferation occurred was shifted down by a factor of 12%, exactly what is to be expected for a change of mass of 5 parts out of 40.

Because these ICR effects appeared to violate simplistic analysis involving magnetic induction at first they evoked much suspicion in the scientific community. Many subsequent confirmations, however, performed on different model systems in diverse experimental situations , in part listed in Table 1, proved that these weak low-frequency effects are indeed real. It is clear that magnetic field combinations when tuned to ion cyclotron resonance, can act to regulate the flow of biological information, a conclusion that has important ramifications for electromagnetic medicine. Consider the following, from a recent review[26] of this subject:

The inescapable conclusion…is that the ICR mechanism, whatever its molecular basis, is of enormous biological significance. We are able to make reproducible and consistent physiological changes of various sorts in the widest imaginable range of genera simply by applying weak magnetic fields tuned to the charge-to-mass ratio of various biological ions. It is very clear that [this] must be part of a heretofore unknown system that carries physiological information/instructions, and that better understanding will open the way to providing a radically new means of controlling wellness.

In addition to medical applications already initiated using ICR techniques there are also a number of potential advances that are likely to be further developed in the future. Consider for example the observations found in a number of ICR studies that indicate merely changing the resonance condition from one ion to another will result in the opposite result. This phenomenon was first observed by S D Smith in his studies on diatom motility[6] and later reported by others[9,27-31] (Table 2). One explanation is that this effect likely reflects the endogenous nature of bioresonance, wherein multiple ion resonances are occurring simultaneously giving rise to a balanced physiologic outcome. If this is true then it should be possible in principle to selectively reduce the undesirable in favor of the desirable. There is evidence[32] indicating that ICR applications can increase the rates of proliferation in neuroblastoma cell culture. Is It possible that there exist yet-to-be-tried ICR conditions that would have the opposite effect, namely to reduce the rates of proliferation in cancer cell lines, thereby opening the way to new cancer fighting techniques?

MODEL SYSTEM	FREQ, Hz	B_o, mT	ION	RESPONSE
Diatom motility[6]	16	20.9	Ca^{2+}	Motility✳
	16	41.0	K^+	Motility✳
Embryonic bone[9]	16	20.9	Ca^{2+}	Growth✳
	16	40.7	K^+	Growth✳
Embryonic bone[27]	16	20.9	Ca^{2+}	Growth✳
	16	40.7	K^+	Growth✳
Plant growth[28,29]	60	78.3	Ca^{2+}	Growth✳
	60	153.3	K^+	Growth✳
Rat behavior[30]	63	50	Mg^{2+}	More Active
	38	50	Ca^{2+}	More Passive
Gravitropic response[31]	35.8	46.5	Ca^{2+}	Up
	54.7	46.5	K^+	Down

Table 2. Ionic tuning can drastically alter physiological outcome. Note that specific outcomes are observed for different magnetostatic fields at the same resonant frequency, or equivalently, for different frequencies at the same static magnetic intensity.

II. PRESENT CLINICAL ELECTROMAGNETIC PRACTICE

A number of diagnostic techniques based on electromagnetic principles, such as **Magnetic Resonance Imaging** (MRI), are universally accepted by physicians, to the point where objections are heard concerning the costs to the health care system because of overuse[33]. Neurologists universally use **Electromyography** (EMG) in their practice no less than **Electrocardiography** (EKG) is used by cardiologists and internists. It also should be understood that there are efficacious electromagnetic diagnostic tools that are used outside of the United States but not permitted in the US. The US Food and Drug Administration (FDA) oversee the introduction and use of medical devices with as much zeal as it supervises pharmaceuticals. The prospect of very expensive and time-consuming procedures for new devices tends to discourage the introduction of foreign devices, regardless of their efficacy and safety. This applies to both diagnostic and therapeutic devices.

One example of a foreign diagnostic device that is presently in clinical trials in the US is the Tissue Resonance Interferometer (**TrimProbe**)[34], invented by Clarbruno Vedruccio. Following its original use as an electromagnetic device for the remote detection of land mines and for airport screening, he discovered that microwave signals in the range 400 to 1350 MHz reflect differently from cancers as compared with healthy tissue. A hand-held non-invasive probe measures the degree of interference between the incoming and reflected signals, providing instant determinative results. It has been highly successful in prostate diagnosis, proving effective in distinguishing malignancies from prostate hyperplasia and prostatitis. This technique has also been used to detect bladder cancer. Because of its non-invasiveness, its speedy application and rapid diagnosis, all within a matter of minutes, this device has great potential as a tool for screening populations at risk.

It is clearly the case that the highly specific electrical nature of the nervous system should predispose it to exogenous electrical influence. This is shown in the great variety of electric medical procedures[35] presently in use as neurotherapies. Devices such as heart pacemakers and defibrillators are so widely known that they need no description. **Vagal nerve stimulation** (VNS) is widely used as an anti-convulsant therapy. **Deep brain stimulation** (DBS) uses

electrodes in the brain to treat Parkinson's disease and other movement disorders. Chronic pain is treated using the non-invasive **Transcutaneous electrical nerve stimulator** (TENS) directly on the back or the **Cranial electrothermal stimulator** (CES) on the head. Insomnia is treated with **Low-energy emission therapy** (LEET) using an electrode positioned in the mouth. In general these devices are employed as surrogates for already existing physiological endogenous mechanisms that require a boost or improvement, with the cardiac pacemaker serving to regulate the timing of heart contractions as an illustrative example. Presently there is an extension of this concept, with widespread ongoing research aimed at mimicking the electric signals needed to restore eyesight and muscle function that may have been lost because of disease or accident.

Less well known are a number of medical accepted EM therapies that are sufficiently energetic to be acknowledged as based either on Faraday induction or Joule heating. **Transcranial Magnetic Stimulation** (rTMS)[36,37] is used to treat depression. In this procedure, approved by the FDA as efficacious and safe, a large pulsed current is sent through a coil placed strategically over the head, thereby inducing a current through the brain. In part, this serves as a modern alternative to the much older (1938) use of applied currents to treat depression, namely **ElectroConvulsive Therapy** (ECT), wherein pulses or sinusoidal voltages are applied to the scalp through electrodes, producing power levels of several hundreds of watts directly into the brain.

Another purely inductive device, **Pulsed Magnetic Field** therapy (PMF), has found great success in treating bony nonunions, a rather common problem in which fractures do not knit properly. This device was introduced by Bassett and Pilla[38] following a long history showing that living bone enjoys remarkable electric properties[39] that can be used to advantage in growth and repair processes[40]. In a very real sense, the PMF work on bone in the 1970s was the springboard for the development 25 years later of rTMS.

Electromagnetically-induced hyperthermia (**Oncotherm**)[41] and **Electrochemical Treatment** (EChT)[42] have both been found useful in treating late-stage cancers, the former mostly in Europe and Asia, and the latter in China. The Oncotherm device applies carefully directed radiofrequency devices to tumor sites, slightly elevating the local temperature, which has the

interesting effect of killing off cancer cells without affecting healthy tissues. Neither procedure has as yet been approved by the FDA.

A much older device, dating back to the 1930s, **Diapulse**, applies radiant Joule heat deep into tissues. Because this device was introduced prior to the establishment of the FDA, its acceptance was "grandfathered", that is, allowed to be advertised and marketed on the basis of earlier widespread use. Electromagnetic energy is directed to specific areas of the body in the form of 600 pulses/s with each pulse lasting 65 ms. Although it was originally used to provide pain relief the extent of the therapeutic claims now includes "neurologically associated problems". Along with a number of other devices making therapeutic claims related to radiofrequency use, the prominent frequency employed was 27.15 MHz, which has no special biological qualities, but is merely a frequency of choice permitted by the Federal Communications Commission (FCC).

This 27.15 MHz frequency has also appeared as the carrier wave in a similar arrangement to that used in the LEET insomnia device mentioned above, where one electrode is again placed in the mouth, in this case to treat cancer[43]. A much lower frequency, in the tens of Hz, modulates the 27.25 MHZ carrier. Presumably this ELF component represents the active anti-oncogenic component in this device.

Even higher frequencies, at 50 GHz and larger have also been reported as therapeutic aides. These devices, generally described as **Microwave Relaxation Therapy** (MRT)[44] machines are widely used in Russia and the Ukraine for mood behavior, and (anecdotally) to strengthen the immune system.

The author has previously attempted[45] to characterize neuroelectromagnetic therapies as falling into three categories: **subtle, gross, and disruptive**. The procedures of rTMS and ECT can be regarded as **disruptive**, considering that seizures have been associated with both, either deliberately or by accident. Similarly **gross** neurotherapies properly describe the great number of neural stimulators in use today. The term **subtle** is meant to convey the great difficulty in understanding how vanishingly small electric and magnetic signals are able to affect biological

systems. It is abundantly clear that such signals cannot be the result of either Faraday induction of voltage or thermal changes due to Joule heating.

III. NON-INDUCTIVE NON-THERMAL MEDICAL APPLICATIONS

The question of subtle electromagnetic effects in biology is not new. Observations indicating that minutely small electric currents, at levels far weaker than allowed by simple energetic estimates, are capable of profound biological effects. These were first reported in connection with living bone. Electret applications[46], likely supplying no more than a few hundred nanoAmperes, were found to significantly affect growth rates in bone. This fact was subsequently used in a number of orthopedic devices operating at 1-2 mA to repair bony non-unions[47]. The great advantage of the PMF techniques mentioned above was that currents at this level could be introduced at the repair site in a completely non-invasive way.

More recently, the FDA-approved application of ion cyclotron resonance magnetic fields to the problem of bone repair[48] has all but replaced the use of both weak electric currents and PMF pulses. Magnetic fields from a portable coil tuned jointly to Ca^{2+} and Mg^{2+} are applied for 30 minutes a day over a period of weeks. It should be emphasized that the efficacy of this application, achieving repair rates of 70% or more, remains unexplained, except insofar as one considers ion cyclotron resonance phenomena as empirically factual.

Adey also recognized the fact that such signals caused effects that were not readily explained. In attempting to understand results obtained in his laboratory showing a distinctly nonlinear response in connection with the calcium-efflux experiments, he suggested that low-energy transmission occurs at cell membranes by means of solitonic waves[49].

The results listed in Table 1 for effects related to ELF magnetic fields have their counterparts in experiments conducted with AC electric fields. In some ways these are unexpected. Unlike the transparency of biological matter to low-frequency magnetic fields polarization effects in the extracellular medium and the large electric field at the cell membrane make it difficult to apply AC electric fields to cells. Some of the weak AC electric-field clinical approaches involve the

use of invasive electrodes. Nonetheless these are noteworthy, considering the poor prognoses attached to illnesses such as glioblastoma.

Thus, one recent very promising therapy entails the use of electric fields at frequencies equal to or less than hundreds of kHz (**Tumor-Treating Fields**, or TTF) to treat aggressive glioblastoma and lung cancer[50,51]. Low-intensity electric fields, on the order of 1-2 V/cm, are found to slow the proliferation of all cells, cancer cells included. This is particularly advantageous in the treatment of brain cancer, because healthy brain cells tend not to proliferate in any case. Therefore the application of such fields is effective in slowing the increases in cancer cell production while leaving healthy cells unaffected. A somewhat similar effect has been discovered, but for applications at 50 Hz instead of hundreds of kHz. In this approach[52], a weak applied AC electric field is also used to fight cancer, not by reducing the proliferation of cancer cells, but by reducing their resistance to multidrug chemotherapy.

It is important to point out that these findings on the effectiveness of AC electric fields on cancer cell proliferation help illuminate why possible similar results that might be obtained using magnetic fields are so interesting. For one thing, there are problems related to AC electric field polarization effects that add constraints on how the cells are stimulated. By contrast because of tissue transparency to ELF magnetic fields, their clinical use will not only always be non-invasive, but also capable of being applied in more general ways.

Comparable effects of the sort observed using AC electric fields have already been observed using weak ELF magnetic fields. A number of reports have found changes in cell proliferation[8], particularly in lymphocytes, as a result of weak magnetic field stimulation. Further, in direct contrast to the electric-field <u>reduction</u> in chemotherapeutic resistance Liburdy discovered[53] that the resistance of breast cancer cells to tamoxifen was <u>increased</u> using 60 Hz magnetic fields.

Two interesting reports by Novikov highlight the clinical potential of weak magnetic fields. In the first case[54] he found that Ehrlich ascites cancer in rats can be dramatically reduced through the use of combined, ostensibly cyclotron-resonance tuned magnetic fields. In the second case[55] he demonstrated that these fields can also be used to hydrolyze, that is, break down, polypeptides by merely tuning to the charge-to-mass ratios of the constituent amino acids. One obvious

11

clinical direction suggested by this work is to use this approach to break down the b-amyloid plaque protein associated with Alzheimer's disease. Experiments have indicated that this is indeed possible in animal models, but it is not yet clear if this plaque is a cause of this disease or simply one of its symptoms.

The last entry in Table 1 indicating that weak ELF magnetic fields can play an important role in stem cell applications[16] is particularly exciting. The most difficult aspect to treating heart failure is the inability of damaged heart muscle to regenerate, leading when possible to heart transplants. Stem cell regeneration of heart tissue is an obvious remedy to this problem but the results to date have in general been slow. This stalemate has been dramatically changed through the use of weak ICR magnetic fields. It was demonstrated that cardiac stem cells from humans when exposed for five days to ELF resonance fields tuned to Ca^{2+} enjoyed significantly greater proliferation and differentiation, perhaps paving the way for a minimally manipulative means of regenerating diseased hearts. Because of this result there is now heightened interest in the use of ELF magnetic fields to enhance the implementation of regenerative medicine and tissue engineering.

A very different approach to ICR medical therapy is found in the **Seqex** device[56] which applies an oscillating magnetic field to the patient's entire body while simultaneously taking advantage of the local parallel vertical component of the earth's magnetic field to achieve resonance. Its most celebrated use has been to treat the debilitating depression that often accompanies chemotherapy following cancer remediation[57], but there have also been numerous anecdotal reports claiming success in treating other diseases, for example multiple sclerosis. There is reason to believe that the efficacy of this device may be related to its dramatic effect on antioxidants. In addition to the fact that this device employs holistic application of the combined fields, it is unique in that the applied ICR frequency is not calculated from ionic charge-to-mass ratios, but is determined by first finding in a prior separate evaluation the specific frequency conditions that sharply alters the whole-body bioimpedance. Once determined this frequency information is stored on a "smart card" for future treatments on that patient. It is worth noting that the change in whole-body bioimpedance at resonance is consistent with the sharp changes in ionic conductivity that were observed by Zhadin and others. This device has not as yet been introduced into the United States for clinical evaluation.

IV. WELLNESS AND ILLNESS: THE ELECTROMAGNETIC PERSPECTIVE

The medical community continues to regard therapeutic regimens based on weak magnetic fields with great suspicion. This fact is best illustrated by contrasting the interest shown in the use of AC electric fields to treat cancer while similar results using magnetic fields have all but been ignored. We do not seek to diminish the potential importance of these electric field effects, but it is apparent that ELF magnetic field research is still thought of as too far outside the mainstream. One useful rationalization in trying to explain the AC electric field effects has been to implicate voltage-dependent ion channels as the key interaction site. This allows one to avoid the thorny question surrounding the intrinsic difficulty in the lack of penetration of AC electric fields into the cell. By contrast, even though there appears to be no such thing as magnetically responsive ion channels, ELF magnetic fields are not impeded by the large electric field of the cell membrane, reaching all compartments inside the cell equally.

One alternate view, when looking at electromagnetic effects, may be to regard a common parameter found in both the electric and magnetic cases, perhaps involving frequency or some function of frequency, as the key distinction. This has already been hinted at in connection with ICR biological interactions.

Recently the author and colleagues[26] advanced a radical new view of electromagnetic effects in biology, suggesting that these strange new electromagnetic interactions can be explained in terms of an endogenously available substrate resonantly coupled to biological ions that enables information transfer for purposes of regulation. In this approach the tweaking of biological systems with weakly energetic electromagnetic signals reveals an underlying order to organisms, one in which the electromagnetic is elevated above the biochemical.

However, even if this generalized concept of systemic electromagnetic wellness is correct, there still remains unexplained the molecular basis that might tell us why nanoAmpere currents can help initiate bone formation or why nanoTesla magnetic fields can hydrolyze proteins. These fully replicated observations are well outside the simplistic electrical engineering that is so often used to discuss such effects. For example, it is inappropriate to express this work in terms of

Specific Absorption Ratio (SAR), because a different yardstick is required. The low levels of power absorbed by the biological system are literally many orders of magnitude below the 1 Watt/kg prescribed as safe. We know that very low levels of electromagnetic can affect biological systems, but do not know how this happens. One clearly obvious truth yet to be generally accepted, yet of vital importance to everyone, is that these effects are profoundly quantum mechanical in nature[17-21], and have little connection to the traditional safety limitations imposed by electrical engineers.

V. CONCLUSIONS

There can be little doubt that weakly energetic electromagnetic fields are biologically interactive to the point where they can be usefully applied in medically relevant therapeutic procedures. Not only does this fact suggest a bright future for the role of electromagnetism in medicine, but it also underscores the need to be very cautious when examining the effects of low-level electromagnetic fields on people. This conclusion, slightly rephrased, was expressed by the author when he wrote[58]:

> *In the long run, [weak-field exposures for medical purposes] may be the only way to prove the case for biological plausibility among those who presently choose to deny that weak field low frequency magnetic fields do indeed interact with biological systems.*

VI. REFERENCES

1. Flexner A, 1910. Medical education in the United States and Canada. A report to the Carnegie Foundation for the Advancement of Teaching, New York. Carnegie Foundation for the Advancement of Teaching .
2. Cooke M, Irby D M, Sullivan W, and Ludmerer K M, 2006. American medical education 100 years after the Flexner Report. New Eng J Med 355: 1339-1344.
3. Liboff A R, Williams T Jr, Strong D M, and Wistar R Jr, 1984. Time-varying magnetic fields: effect on DNA synthesis. Science 223: 818-820.
4. Takahashi K, Kaneko I, Date M, and Fukada E, 1986. Effect of pulsing electromagnetic fields on DNA synthesis in mammalian cells in culture. Cell Mol Life Sci 42: 185-186.
5. Thomas J R, Schrot J, and Liboff A R, 1986. Low-intensity magnetic fields alter operant behavior in rats. Bioelectromagnetics 7: 349-357.
6. Smith S D, McLeod B R, Liboff A R, and Cooksey K E, 1987. Calcium cyclotron resonance and diatom motility. Bioelectromagnetics 8: 215-227.
7. Goodman R, and Henderson A S, 1988. Exposure of salivary gland cellsto low-frequency electromagnetic fields alters polypeptide synthesis. Proc Natl Acad Sci USA 85: 3928-3932.
8. Cossarizza A, Monti D, Bersani F, Cantini M, Cadossi R, Sacchi A, and Franceschi C, 1989. Extremely low frequency pulsed electromagnetic fields increase cell proliferation in lymphocytes from young and aged subjects. Biochem Biophy Res Comm 160: 692-698.
9. Smith S D, Liboff A R, and McLeod B R, 1991. Effects of resonant magnetic fields on chick femoral development in vitro. J Bioelect 10: 81-89.
10. Smith S D, Liboff A R, McLeod B R, and Barr E J. 1992. Effects of ion resonance tuned magnetic fields on N-18 neuroblastoma cells. In M.J. Allen M J, Cleary S F, Sowers A E, and Shillady D D, Eds, Charge and Field Effects in Biosystems-3, Birkhauser, Boston.
11. Fitzsimmons R J, Ryaby J T, Mohan S, Magee F P, and Baylink D G, 1995. Combined magnetic fields increase insulin-like growth factor II in TE-85 human osteosarcoma bone cell cultures. Endocrinology 136: 3100-3106.
12. Jenrow K A, Smith C, and Liboff A R, 1995. Weak ELF fields and regeneration in the planarian Dugesia tigris. Bioelectromagnetics 16: 106-112.
13. Prato F S, Kavaliers M, Carson J J, 1996. Behavioral evidence that magnetic field effects in the land snail, Cepaea nemoralis, might not depend on magnetite or induced electric currents. Bioelectromagnetics. 17:123-30.
14. Vorobyov V V, Sosunov E A, Kukushkin N I, and Lednev V V, 1998. Weak combined magnetic field affects basic and morphine-induced rat's EEG. Brain Res 781: 182-187.
15. Galland P, and Pazur A, 2005. Magnetoreception in plants. J Plant Res 118: 371-389.
16. Gaetani R, Ledda, M, Barile L, Cimenti, I., De Carlo F, Forte E., Ionta V, Giuliani L, D'Emilia, E, Frati G, Mirali F, Pozzi D, Messina E, Grimaldi S, Giacomello A, and

Lisi A, 2009. Differentiation of human adult cardiac stem cells exposed to extremely low-frequency electromagnetic field. Cardiovasc Res 82: 411-420.

17. Zhadin M N, Novikov V V, Barnes F S, and Pergola N F, 1998. Combined action of static and alternating magnetic fields on ionic current in aqueous glutamic acid solutions. Bioelectromagnetics 19: 41-45.

18. Pazur A, 2004. Characterization of weak magnetic field effects in an aqueous glutamic acid solution by nonlinear dielectric spectroscopy and voltammetry. Biomag Res and Tech 2: 8. doi: 10.1186/1477-044x-2-8.

19. Comisso N, Del Giudice E, De Ninno A, Fleischmann M, Giuliani L, Mengoli G, Merlo F, and Talpo G, 2006. Dynamics of the ion cyclotron resonance effect on amino acids adsorbed at the interfaces. Bioelectromagnetics 27:16-25.

20. Alberto D, Busso I, Crotti G, Gandini M, Garfagnini R, Giudice P, Gnesi I, Manta F, and Piragino G, 2008. Effects of static and low-frequency alternating magnetic fields on the ionic electrolytic currents of glutamic acid aqueous solution. Electromag Biol Med 27: 25-39.

21. Del Giudice E, Fleischmann M, Preparata G, and Talpo G, 2002. On the "unreasonable" effects of ELF magnetic fields upon a system of ions. Bioelectromagnetics 23: 522-530.

22. Liboff A R, 1985. Geomagnetic cyclotron resonance in living things. J Biol Physics 13: 99-102.

23. Blackman C F, Benane S G, Rabinowitz J R, House D E, and Joines W T, 1985. A role for the magnetic field in the radiation-induced efflux of calcium ions from brain tissue *in vitro*. Bioelectromagnetics 6: 327-337.

24. Bawin S M, Adey W R, and Sabbot I M, 1978. Ionic factors in release of 45Ca2+ from chicken cerebral tissue by electromagnetic fields. Proc Natl Acad Sci USA 75: 6314-6318.

25. Rozek R J, Sherman M L, Liboff A R, McLeod B R, and Smith S D, 1987. Nifedipine is an antagonist to cyclotron resonance enhancement of 45Ca incorporation in human lymphocytes. Cell Calcium 8: 413-427.

26. Foletti A, Grimaldi S, and Liboff A R, 2012. Electromagnetic medicine: The role of resonance signaling. Electromag Biol Med (in press).

27. Regling C, Brueckner C, Liboff A R, and Kimura J H, 2002. Evidence for ICR magnetic field effects on cartilage and bone development in embryonic chick bone explants (abstract), 48th Ann mtg, Orthopedic Res Soc, Dallas.

28. Smith S D, McLeod B R, and Liboff A R, 1993. Effects of CR-tuned 60 Hz magnetic fields on sprouting and early growth of *raphanus sativus*. Bioelectrochem and Bioenergetics 32: 67-76.

29. Smith S D, McLeod B R, and Liboff A R, 1995. Testing the ion cyclotron resonance theory of electromagnetic field interaction with odd and even harmonic tuning for cations. Bioelectrochem and Bioenergetics 38: 161-167.

30. Zhadin M N, Deryugina O N, and Pisachenko T M, 1999. Influence of combined DC and AC magnetic fields on rat behavior. Bioelectromagnetics 20: 378-386.

31. Belova N A, and Lednev V V, 2000.Activation and inhibition of gravitropic response in plants by weak combined magnetic fields. Biophysics 45: 1069-1074.

32. Smith S D, Liboff A R, McLeod B R, and Barr E J. 1992. Effects of ion resonance tuned magnetic fields on N-18 neuroblastoma cells. In M.J. Allen, S.F. Cleary, A.E.

Sowers, and D.D. Shillady, Eds, Charge and Field Effects in Biosystems-3, Birkhauser, Boston.

33. Saslow L, Aug. 7, 1994. The boom in M.R.I.s: concerns grow on costs and overuse. N Y Times.

34. Gervino G, Autino E, Kolomoets E, Leucci G, and Balma M, 2007. Diagnosis of bladder cancer at 465 MHz. Electrom Biol Med 26: 119-134.

35. Liboff A R, and Jenrow, K A, 2002. Physical mechanisms in neuroelectromagnetic therapies. Neurorehabilitation 17: 9-22.

36. Barker AT, Jalinous R, and Freeston IL, 1985. Non-invasive magnetic stimulation of human motor cortex. The Lancet 325: 1106–1107.

37. George M S, Wasserman E M, Williams W A, Callahan A, Ketter T A, Basser P, Hallett M, and Post R M, 1995. Daily repetitive transcranial magnetic stimulation (rTMS) improves mood in depression. Neuroreport 2: 1853-1856.

38. Bassett C A L, Pawluk R J, and Pilla A A, 1974. Augmentation of bone repair by inductively coupled electromagnetic field. Science 184: 575-579.

39. Fukada E, and Yasuda I, 1957. On the piezoelectric effect of bone. J Phys Soc Japan 12: 1158-1162.

40. Bassett C A L, 1993. Beneficial effects of electro-magnetic fields. J Cell Biochem 51: 387-393.

41. Andocs G, Szasz O, and Szasz A, 2009. Oncothermia treatment of cancer: from the laboratory to the clinic. Electromag Biol Med 28: 148-165.

42. Chou C-K, McDougall J A, Ahn C, and Vora N, 1997. Electrochemical treatment of mouse and rat fibrosarcomas with direct current. Bioelecromagnetics 18: 14-24.

43. Costa F P, de Oliveira A C, Meirelles R, Machado M C C, Zanesco T, Surjan R, Chamms M C, de Souza Rocha M, Morgan D, Cantor A, Zimmerman J, Brezovich I, Kuster N, Barbault A, and Pasche B, 2011. Treatment of advanced hepatocellular carcinoma with very low levels of amplitude-modulated electromagnetic fields. Brit J of Cancer 105: 640–648.

44. Devyatkov N D, 1973. Influence of the millimeter wavelength range of electromagnetic radiation upon biological objects. Soviet Physics Uspekhi 110: 452-454 (in Russian).

45. Jenrow K A, and Liboff A R, 2004. Electromagnetic techniques in neural therapy. Chap. 14, Rosch P, and Markov M. (Eds) Bioelectromagnetic Medicine, Dekker, NY.

46. Fukada E, Takamatsu, T and Yasuda I, 1975. Callus formation by electret. Japan J Appl Physiol 14: 12.

47. Lavine L, Lustrin I, Shamos M H, Rinaldi R A, and Liboff A R, 1972. Electric enhancement of bone healing. Science 175: 1118-1121.

48. Diebert M C, McLeod B R, Smith S D, and Liboff A R, 1994. Ion resonance electromagnetic field stimulation of fracture healing in rabbits with a fibular ostectomy. J Orthop Res 12: 878-885.

49. Adey W R, 1993. Biologic Effects of Electromagnetic Fields. J Cell Biochem 4: 410-416.

50. Kirson E D, Dbaly V, Tovarys F, Vymazal J, Soustiel J F, Itzhaki A, Mordechovich D, Steinberg-Shapira S, Gurvich Z, Schneiderman R, Wasserman Y, Salzberg M,

Ryffel B, Goldsher D, Dekel E, Palti Y, 2007. Alternating electric fields arrest cell proliferation in animal tumor models and human brain tumors. Proc Natl Acad Sci USA 104: 10152-10157.

51. Kirson E D, Giladi M, Gurvich Z, Itzhaki A, Mordechovich D, Schneiderman R S, Wasserman Y, Ryffel B, Goldsher D, Palti Y, 2009. Alternating electric fields (TT fields) inhibit metastatic spread of solid tumors to the lungs. Clin Exp Metastasis 26: 633-640.

52. Janigro D, Perju C, Fazio V, Halkene K, Dini G, Agarwal M K, and Cucullo L, 2006. Alternating electrical stimulation enhanced chemotherapy: a novel strategy to bypass multidrug resistance in tumor cells. BMC Cancer doi: 10.1186/1471-2407-6-72.

53. Harland J D, and Liburdy,R P, 1997. Environmental magnetic fields inhibit the antiproliferative action of tamoxifen and melatonin in a human breast cancer cell line. Bioelectromagnetics 18: 565-562.

54. Novikov V V, Novikov G V, and Fesenko E E, 2009. Effect of weak combined static and extremely-low-frequency alternating magnetic fields on tumor growth in mice inoculated with the Ehrlich ascites carcinoma. Bioelectromagnetics 30: 343-351.

55. Bobkova N V, Novikov V V, Mevinskaya N I, and Fesenko E E, 2005. Reduction in the b-amyloid level in the brain under the action of weak combined magnetic fields in a model of Sporadic Alzheimer's disease. Biophysics 540: 52-57.

56. Liboff A R, 2007. Local and holistic electromagnetic therapies. Electromag Biol Med 26: 315-325.

57. Rossi E W, Corsetti M T, Sukkar S, and Poggi C, 2007. Extremely low frequency electromagnetic fields prevent chemotherapy induced myeloyoxicity. Electromag Biol Med 26: 277-281.

58. Liboff A R, 2010. Weak low-frequency electromagnetic fields are biologically interactive. European J Oncology 5: 51-62.

Electromagnetic Field Exposure Effects (ELF-EMF and RFR) on Fertility and Reproduction

Prof. Jitendra Behari, PhD

Bioelectromagnetics Laboratory

School of Environmental Sciences

Jawaharlal Nehru University

New Delhi, India

Dr. Paulraj Rajamani, PhD

Bioelectromagnetics Laboratory

School of Environmental Sciences

Jawaharlal Nehru University

New Delhi, India

I. INTRODUCTION

Electromagnetic fields and radiofrequency radiation (RFR) interact with human tissues and may have adverse effects on fertility and reproduction. This review presents evidence for ELF-EMF and RFR effects on many parameters of male sperm function; leading to questions about the genotoxicity and carcinogenicity of such exposures on fertility and reproduction in men. Much of the evidence comes from human and animal studies on sperm and male fertility factors, but there are also studies showing adverse effects on fertility and miscarriage in women.

During the last four decades or so there has been a growing concern on the effects of electromagnetic radiations on biological systems in general. This is because of the global introduction of electronic devices on a massive level for communications and data transmission, personal wireless devices, air surveillance systems, industry applications, medical/diagnostic and therapeutic purposes that are now new sources of electromagnetic fields (ELF-EMF) and radiofrequency microwave radiation (RFR). This has added another layer of pollutant (electropollution) to a growing list of environmental contaminants in air, water, soil and from noise pollution which can adversely affect human health.

There are many sources of EMF in our environment and this non-ionizing radiation interacts with the human body. Use of electronic household items and cell phones are reported to decrease fertility potential in men by decreasing sperm count, motility, viability, inducing pathological changes in sperm and testes morphology, and so on (Erogul et al. 2006). In accordance with this, several authors (Agarwal et al. 2008, 2009; Kumar et al. 2010, 2011a; Pourlis 2009; Kesari et al. 2010, 2011, 2012) focused mainly on the male reproduction patterns. It involves the development from undifferentiated diploid stem cells to highly differentiated haploid stem cells. Spermatogenesis is a complex process and it is influenced by many genes and hormones. It takes place in the testis, which may be exposed to various microwave frequencies which are currently in use (Behari and Kesari 2006). Among various factors of infertility, oxidative stress has become the main focus of interest as a potential cause of male infertility (Agarwal and Said 2003; Aitken and Roman, 2008; Kumar et al, 2010, 2011a). Male infertility is commonly associated with high rates of DNA (deoxyribonucleic acid) damage in the spermatozoa and such damage is correlated with a wide range of adverse clinical outcomes. Several studies, especially at power frequency 50/60

Hz magnetic field have found an association of exposure to human health, with emphasis on a range of clinical conditions including childhood leukaemia, brain tumours, genotoxicity and neurodegenerative disease, infertility, birth defects, increased risk of miscarriage, childhood morbidity and de novo mutations (Hardell and Sage 2008; Gharagozloo and Aitken 2011; Garcia et al. 2008; Huss et al. 2008; O'Carroll and Henshaw 2008; International Agency for Research on Cancer (IARC) Monographs of the Evaluation of Carcinogenic Risks to Human 2002; California Health Department Services (CHDS) Report 2002). Sperm DNA damage is therefore regarded as a potential risk factor to the development of normal human embryos leading to impaired embryonic development.

II. THE BIOPHYSICS OF EXTREMELY LOW FREQUENCY FIELDS

Whenever a body having finite conductivity (biological body) is intercepted by EMF it induces electric fields and circulating electric currents, which in turn competes with endogenous current and voltages, thus disturbing normal physiological balance. The depth of penetration within the body depends upon its frequency and the electric properties of the exposed portion in the body. If the current density exceeds a certain threshold value, excitation of muscles and nerves due to membrane depolarization is possible. The mode of interaction of non-ionizing radiation with biological systems can be broadly divided into two parts: extremely low frequency and radiofrequency/microwaves.

Whenever an electric field interacts with a biological body the incident field will be distorted, such that the external field will be nearly perpendicular to the boundary surface. At 60 Hz

$$E_{internal} / E_{external} \approx 4(10^{-8}). \quad (1)$$

Thus a 60 Hz external field of 100 kV/m will produce an average internal E field of the order of 4mV/m.

As far as the magnetic components of the extremely low frequency fields are concerned, magnetic permeability μ of most biological materials is practically equal to that of free space $(4\pi.10^{-7})$ H/m. This signifies that ELF H field 'inside' will be practically equal to the H field 'outside'. Only exceptions could be those biological materials that have magnetic particles inside. A time varying magnetic field (also electric field) can also induce electric currents into stationary conducting objects. Thus, all modes of interaction of time varying E fields with living matter may be triggered by time-varying (not by static) magnetic field. According to Faraday's law of electromagnetic induction time varying magnetic flux will induce E fields with resulting electrical potential differences and "eddy" currents through available

conducting paths. Sources generating low frequency electric and magnetic fields are more likely to produce physiologically significant internal E fields through the mechanism of magnetic induction. If an erect person is targeted by a vertical electric field it will be considerably "enhanced" at the top of the person's head and shoulder, and one would predict therefore that the field in the tissue would also be enhanced above that of a flat slice exposed to the same field (Deon, 1982). In a 60 Hz electric field of 1kV/m in air, the current densities (Am/m^2) in neck, waist and ankle turn out to be 0.591x10$^{-3,}$ 0.427 x^{-3} and 3.35x10^{-3} respectively (Polk 1986).

III. THE BIOPHYSICS OF RADIOFREQUENCY AND MICROWAVE FIELDS

The biological bodies are inhomogeneous, having tissue-specific dielectric properties and the complexity of the shape; which make the computations of the induced field difficult. The fields induced inside the body act differently depending upon the frequency and more particularly on (L/λ), (where L is the length of the biological body and λ the wavelength of the incident field) upon, but are not limited to the following parameters:

   (i)   The location of the field with respect to the surroundings, e.g. if there are metallic objects around, the person is grounded or otherwise.
   (ii)   Polarisation of the incident wave with respect to the orientation of the human body.
   (iii)   Size of the human body (L) with respect to the wavelength (λ) of the incident radiations (L/λ).
   (iv)   The portion of the human body.
   (v)   The electrical properties of the tissue in question.

In free space propagation of electromagnetic field the power density is given by

   Power density $= E^2/1200\ \Pi$ mW/cm^2 (1)

Where, E is the electric field strength.

   The frequency in the radio frequency-microwave region are somewhat penetrated inside the biological body interacting with the tissues inside.

From simple biophysical considerations, it follows that each body has a characteristic resonant frequency depending upon the length of the long axis. Correspondingly, for the same level of incident exposure the average value of power absorbed is dependent upon the length of the body, the degree of decoupling decreasing the average value of SAR by more than an order of magnitude. It is suggestive that absorbed RF energy can be converted into other form of energy and can cause interference with the functioning of the biological systems. A significant portion of this energy is converted into heat (absorption). The biological effects are frequency dependent. Well below 100 KHz, the induced fields can even stimulate nervous tissue.

IV. FERTILITY AND REPRODUCTION EFFECTS: ELF-EMF FIELD EXPOSURE

Since the biological body is diamagnetic it is transparent to the static magnetic field. It can therefore interact with the motional activity of paramagnetic materials. Amara et al (2006) has shown that adult male rats exposed to such fields (128 mT, 1hr/day for 30 days) show a decrease in testosterone levels and induced DNA oxidation. Subchronic exposure failed to alter spermatogenesis in rat testis. In a similar study Hong et al (2005) also concluded that 50 Hz EMFs (0.2 mT or 6.4 mT, exposed for a period of 4 weeks) may have the potential to induce DNA strand breakage in testicular cells and sperm chromatin condensation in mice.

Al-Akhras et al (2006) also treated male adult rats to 50 Hz sinusoidal magnetic field ($25\mu T$ or 250 mg) for 18 consecutive weeks. They reported no significant effects on the absolute body weight and the weight of the testis of the exposed rats. However the weight of the seminal vesicles and preputial glands were significantly reduced in the exposed male rats, along with significant reduction in sperm count of the exposed rats. There was no significant effect on the serum levels of male follicle stimulating hormone (FSH) during the 18 weeks of exposure period. On the other hand there was a significant increase in the serum levels of male luteinizing hormone (LH) after 18 weeks of exposure ($p<0.005$) while testosterone levels were significantly decreased after 18 weeks of exposure period. These results suggest that long term exposure of ELF could have adverse effects on mammalian fertility and reproduction.

Different results have been presented by Chung et al (2005) where animals exposed in-utero and subsequent neonatal exposure to a 60 Hz EMF(field strength 500 μT or 5000 mG) from

day 6 of gestation to day 21 of lactation, did not produce any detectable alteration in offspring spermatogenesis and fertility.

Akdag et al (2006) examined the effects of ELF magnetic fields (1.35 mT) on sperm count, malondialdehyde concentration, the histology of organs as: testes, brain, liver, and kidney tissues, p53 immunoreactivity of bone marrow and the serum concentrations of Cu^{2+}, Zn^{2+},Mn^{2+} and Fe^{3+} in rats. These authors found no statistically significant alteration except in Mn^{2+} concentrations ($p<0.001$).

Influence of ultrasound (frequency 2,4 and 8 MHz) and constant magnetic field (7T) on gametes, zygotes and embryos of the sea urchin were studied by Drozdov et al (2008). Magnetic field exposure interrupts the process of the gamete fusion but did not influence gametes, embryos, or embryonic development. The nature of these two stimuli is of different type. Ultrasound may heat up the water if is of sufficient power, by way of increase in water temperature and cavitation temperature, which may also break the cellular structure. The effect of magnetic field is connected to the response of the cortical cytoskeleton, which consists of bundles of actin microfilaments. The rearrangement of the cortical cytoskeleton occurs during the first 20 minutes after the contact of sperm with the egg.

Kim et al (2009) examined the effect of a 16-week continuous exposure to ELF magnetic field (MF) of 14 or 200 μT (140 or 2000 mG) on testicular germ cell apoptosis in mice. They reported no significant adverse effects of MF on body weight and testosterone levels in mice. In TUNEL staining (in situ terminal deoxynucleotidyl transferase-mediated deoxy-UTP nick end labelling), germ cells show a significantly higher apoptotic rate in exposed mice than in sham controls ($P<0.001$). TUNEL-positive cells were mainly spermatogonia. In an electron microscope study, degenerating spermatogonia showed condensation of nuclear chromatin similar to apoptosis. These results indicate that apoptosis may be induced in spermatogenic cells in mice by continuous exposure to 60 Hz of 14 MF μT (140 mG).

Roychoudhury et al (2009) examined the effects of 50 Hz extremely low frequency electromagnetic field on in vitro rabbit spermatozoa motility. These authors also studied the effects after insemination. Pooled semen samples and a control were exposed to 50 Hz ELF EMF. The difference of the test groups G1 and G2 with the control group CG (75.56%) for spermatozoa motility were found to be significant ($P<0.01$). Differences were significant ($P<0.01$) for curvilinear velocity (VCL) between the test group G3 (122.38 μ/s). Hormonally simulated adult (9-12 months) females (n=140) were inseminated with semen samples from G1, G2, G3 and G4 (0.88 $x10^9$ spermatozoa /0.5 ml average insemination portion)

immediately after ELF EMF exposure and fertilization (kindling) rates were calculated. For the G2 it was 54.28% data indicate 50 Hz ELF EMF induced alterations of spermatozoa motility and kindling rate in rabbits, therefore influencing fertility.

Cao et al (2009) also reported that magnetic fields at 1000 Hz or 2000 Hz may damage the testis by inducing injury to seminiferous tubules and Leydig cells, thickening the basal membrane, derangement, exfoliation, massive apoptosis and necrosis of spermatogenic cells in the lumen, epididymis, and consequently result in the absence of sperm.

Bernabo et al (2010) assessed the effect of acute (1hr) exposure of boar spermatozoa to an extremely low frequency electromagnetic field (ELF-EMF) (50 Hz, MF 0-2 mT) on early fertility outcome. They examined morpho-functional integrity of capacitated spermatozoa in vitro and reported in vitro ELF-EMF >0.5 mT induced a progressive acrosome damage, thus compromising the ability of spermatozoa to undergo acrosomal reaction after zona-pellucida stimulation and reducing the in vitro fertilization outcome. These effects became evident at 0.75 mT and reached the plateau at 1 mT. Under in vivo conditions, ELF-EMF intensity of 1 mT was able to compromise sperm function, significantly reducing the fertilization rate. In addition, the exposure of oviducts field ≥0.75 mT in the absence of spermatozoa was able to negatively affect early embryo development. In fact it was found to cause a slowdown in the embryo cleavage. It is apparent that at mentioned intensities the fields has negative effect on early fertility outcome in a predictive animal model.

Earlier these authors (Bernabo et al 2007) reported that MF-ELF influence negatively by dramatically effecting sperm morphology and function.

The blood-testis barrier is sensitive to environmental stimulation, which can affect its permeability and then result in antisperm antibody (AsAb) generation, which is a key step in male immune fertility. Wang et al (2010) reported the results of male mice exposed to electromagnetic pulse (EMP) by measuring the expression of tight-junction of associated proteins(ZO-1 and Occludin), vimentin microfilaments, and mice were sham exposed or exposed to EMP at two different intensities (200 kV/m and 400 kV/m) for 200 pulses. The testes were collected at different points after EMP exposure. Immunofluorescence histochemistry, western blot, laser confocal microscopy and RT-PCR were used in this study. Compared with sham group, the expression of ZO-1 and TGF-beta3 were significantly decreased accompanied with unevenly stained vimentin microfilaments and increased serum AsAb levels in EMP-exposed mice. These results are indicative of a potential BTB injury and immune infertility in male mice exposed to certain intensity of EMP.

Lorio et al (2011) studied the functional relationship between the energy metabolism and the enhancement of human sperm motility induced by ELF-EMF was investigated. Sperm exposure to ELF-EMF resulted in a progressive and significant increase of mitochondrial membrane potential and levels of ATP, ADP, and NAD(+) associated with sperm kinetic parameters. However no significant effects were detected on other parameters such as ATP/ADP ratio and energy change. When carbamoyl cyanide m-chlorophenyllhydrazone (CICCP) was applied to inhibit the oxidative phosphorylation in the mitochondria, the values of energy parameters and motility in the sperm incubated in the presence of glucose and exposed ELF-EMF did not change, thus indicating that the glycolysis was not involved in mediating ELF-EMF stimulatory effect on motility. By contrast, when pyruvate and lactate were provided instead of glucose, the energy status and motility increased significantly in ELF-EMF-treated sperm. Under these culture conditions, the inhibition of glycolytic metabolism by 2-deoxy-D-glucose (DOG) again resulted in increased values of energy and kinematic parameters, indicating that gluconeogenesis was not involved in producing glucose for use in glycolysis. These authors concluded that the key role in mediating the stimulatory effects exerted by ELF-EMF on human sperm motility is played by mitochondrial oxidative phosphorylation rather than glycolysis. Earlier these authors (Lorio et al 2007) reported that ELF-EMF exposure can improve spermatozoa motility and that this effect depends on the field characteristics. ELF-EMF with 50 Hz and square wave shape (amplitude 5 mT),while that of a sine wave of the same amplitude (also of 2.5 mT) and the same frequency had no such effect. Further a three hour exposure in the first case had the effect on sperm motility persisting for 21 hours.

People connected to local area networks wirelessly (Wi-Fi) were examined for human spermatozoa. These authors (Avendano et al 2012) selected sperms from 29 healthy donors for their capability to swim. This study using a laptop as a source contributed both ELF-EMF and RFR to the exposure conditions. Each sperm suspension was divided into two aliquots. One sperm aliquot (experimental) from each patient was exposed to an internet connected lap top by Wi-Fi for 4 hours, whereas the second aliquot (unexposed) was used as control and incubated under identical conditions without being exposed to the laptop. These authors evaluated sperm motility, viability, and DNA. These authors reported that normozoospermic, exposed ex vivo during 4 hour to a wireless internet –connected laptop showed a significant decrease in progressive sperm motility and an increase in DNA fragmentation. Level of dead sperm showed no significant differences between the two groups. They concluded that the effect (which is non-thermal) decreased motility and induced DNA fragmentation. It is

therefore speculated that keeping a laptop connected wirelessly to the internet on the lap near the testes may result in decreased male fertility.

Sage et al (2007) reported that personal and occupational use of personal digital assistants (PDAs or palm-held wireless units) produce high intensity bursts of ELF-EMF exposure in persons that carry a PDA close to the body (i.e., in a pocket or in a belt); or held to the head for cell phone conversations. ELF-EMF emissions of 10μT (100 mG) were recorded on PDAs during normal office use over a 24 hr test period. Results of ELF-EMF measurements show that email transmit and receive functions produce rapid, short duration ELF-EMF spikes in the 2-10μT (20 to 100 mG) range, each lasting several seconds to over a minute, depending on the download size. Switching the PDAs produced continuously elevated ELF-EMF pulses of over 90 μT on two units. Thus the user who wears the PDA may be receiving high-intensity ELF-EMF pulses throughout the day and night.

Avendano et al (2012) investigated the effect of laptop computers connected to internet through Wi-Fi on human sperm motility. Donor sperm samples, mostly normozoospermic, exposed ex vivo during 4 hours connection showed a significant decrease in progressive sperm motility and an increase in sperm DNA fragmentation due to nonthermal effect, thus showing potential risks to male fertility.

Bellieni et al (2012) has investigated a much wider issue of reproduction relating to that of fetal growth and the effect of emissions from lap top computers (LTC). Such wireless and ELF-EMF exposures may have adverse effects on the offspring. They measured magnetic field in the range 1 Hz -400 kHz range as emitted from LTC. These field have the advantage that being quasi static can penetrate inside the body and thereby induce voltage and induce currents. The authors reported that the magnetic field at dominant frequencies ranged from 1.8-6 μT (18 to 60 mG), where from the power supply ranges from 0.7 to 29.5 μT (7 to 295 mG). They found that the power supply produces strong intracorporal electric current in the fetus and in the mother, higher than ICNIRP (1998) basic restriction recommend to prevent adverse health effects. The field emissions from video terminals are reported to be low (0.1μT or 1 mG) and the effect of higher exposures needs to be investigated (Bellieni et al 2012)

Sun et al. (2005) investigated the effects of EMR emitted by computers on human sperm quality and did not find any adverse effect.

An observation that women who use video display terminals suffers miscarriages has led to the beginning of diagnosing the possible adverse effects of electric and magnetic fields

Extremely low frequency electromagnetic fields are likely to produce greater damage to the body systems for several reasons. One that these frequencies are close to those of physiological range and hence any overlap of these can perturb on-going biological processes. When in close contact with the body the generation of eddy currents and accompanied heating are added parameters. To differentiate their respective contributions on biological system is an impossible demand.

Extremely low frequency EMF effects induced due to electric(E) blankets generate eddy currents in the body.60 Hz magnetic field exposure generate about 3-4 mG for waterbeds (W) and about 15 mG for E (Electric Blankets),as reported by (Wertheimer and Leeper 1986). They have estimated that electric fields are of the magnitude 100 V/m. E and W both have the potential for providing excessive body heating, which may have adverse effect on sperm (Van Demark and Free 1970), leading to adverse effect on the process of embryogenesis (Edwards et al 1974,Lacy et al 1981). This high temperature could also be teratogenic in humans too (Miller et al 1978, Fraser and Skelton 1978).It is obvious that either the heat or the electromagnetic fields produced by electric or bed heating might affect the fetus. These authors concluded that E or W use has a direct effect on fetal development. It is argued that heat or electromagnetic field exposure is he seasonal. Both prolonged gestation and fetal loss have been shown to be associated with high blanket settings used by the mother, but not those used by the father. Earlier workers have also pointed out that electromagnetic exposure may cause abnormal fetal development (Delgado et al 1982).Marx (1981) pointed out that current and field distribution in embryos, responsible for normal fetal development are disturbed due to the presence of externally imposed fields .

Li et al (1995) studied the effect of prenatal electromagnetic field exposure on the risk of congenital urinary tract anomalies (CUTAs) among women with a history of subfertility as well as in general population. These authors found no consistent relation between the risk of CUTAs and prenatal exposure to electromagnetic fields from E,W ,and video display terminals among all cases of controls. The risk appeared to increase with increasing duration of use and was greatest among women who used Es during the first trimester .CUTA cases

exposed to Es prenatally appeared more likely to have anomalies of the ureter, bladder than unexposed cases. However there is an absence of association with the risk of electrically heated water beds and video display terminals and demands further investigations. They further pointed out that only women with a history of subfertility were subject to said exposure ,since the positive association between potential E use and risk of CUTAs was observed in this group. They concluded that out of the three E,W and video terminals, E has the maximum capacity,keeping in view the proximity with all parts of the body and duration of exposure. Women with subfertility history are more prone to adverse pregnancy outcome.

Juutilainen et al (1993) carried out case control study, although on a small number ,on women .They measured magnetic field at the front door and reported a five-fold increase in preclinical miscarriage. Lee et al (2001) conducted a case control study nested in a miscarriage study. They defined cases as women who had a clinical miscarriage before 20 weeks of gestation and controls as women who had a live birth. They observed a gradient in miscarriage risk as the number of environmental parameters increased above the 50th percentile. Their findings are not consistent with the results of mechanistic and mammalian studies (Portiere and Wolfe 1987) ,while some laboratory results supports alterations in the development of chick embryos exposed to EMF.(Farrell et al 1997). While numerous data have been generated but are inconclusive and the possibility of more funding seems remote.

In summary the possibility of immediate abortion has not found favour with the researchers. However a weak link is possible. A temperature rise causing adverse effect on sperm is possible and certainly avoidance is recommended more so for pregnant women. Another point of interest would be to see if any adverse effects are reversible.

The area certainly demands more investigations.

A summary of these data is presented in Table 1 (Studies on Effects of ELF-EMF on Fertility and Reproduction).

Table 1: Table showing the overall Effect of Extremely Low frequency electromagnetic field effects on reproduction and fertility

Organism used	Mode of exposure	Parameters studied	Conclusion	Reference
Human sperm	internet-connected laptop by Wi-Fi for 4 hours	sperm motility and an DNA fragmentation	Decrease in motility and increase in DNA fragmentation	Avendano et al, 2012
Human sperm	ELF -EMF	Sperm kinematics	Increase in mitochondrial membrane potential	Lorio et al 2011
Mice	4h d 2 m at 3 mT EMF with Polygonum aviculare	Sperm motility and morphology	Motility affected. With *P. aviculare* is sperm quality increased	Milan et al. 2011
Boar spermatozoa	Acute (1h) 50 Hz ELF	Early embryo development	Reduction in fertilization rate, Affect embryo development	Bernabo et al. 2010.
NMRI mice (Naval Medical Research Institute)	50 Hz, 0.5 mT EMF 4 h for 2 weeks	Fertility and height of epithelial cells	Decrease in blastocyte and increase in the height of epithelial cells	Rajaei et al.2010
Rabbit spermatozoa	50 Hz ELF	Spermatozoa motility	Change in motility and kindling rate	Roychoudhury et al.2009
ICR mice	X- ray, 1000 Hz and 2000Hz	Sperm motility	Affect testis function	Cao et al. 2009
BALB/c mice	ELF 60 Hz ,0.1 or 0.5 mT 14 or 200 mT	Apoptosis	Induced apoptosis	Kim et al. 2009
Balb C mice	Electromagnetic pulse (EMP)	Tight-junction-associated proteins,transforming growth factor-beta and AsAb level in serum	Decrease in expression of protein	Wang et al 2010

Table 1 continued …

human spermatozoa	ELF-EMF 5 mT and frequency of 50 Hz.	sperm motility	Square waveform of 5 mT amplitude and frequency of 50 Hz increase sperm motility.No change in 5 mT sine wave (50 Hz) and a 2.5 mT square wave (50 Hz	Lorio et al 2007
Sprague – Dawley rat	ELF 2hour for 2 months	Sperm count, histology, p53 immunoreactivity of bone marrow	No adverse effect. Increase in Mn2+.	Akdag et al 2006
Rat	static magnetic field (SMF) and cadmium	Antioxidant enzymes activity	SMF with Cd disrupt antioxidant response	Amara et al 2006
Mice	50 Hz .02,3.2or 6.4 mT for 2 weeks or 4 weeks	Testicular histology, weight quantity and motility of sperm	Reduced testicular weight, decreased sperm motility. High rate of deformity in sperm	Hong et al 2003
Pregnant women	Case control study (Magnetic field)	Miscarriage	Miscarriage before 20 weeks of gestation	Lee et al 2001
Sperm	12.5, 25, 50 and 100 cGy X-rays	DNA damage	Increase in DNA migration	Singh and Stephens 1998
Pregnant women	Electric blanket, electric heated water bed, and video display terminal	Congenital urinary tract abnormality(CUTA)	Increased risk of CUTA	Li et al 1995
Human	Extremely low frequency EMF(60Hz)	Abortion rate, Fetal development	Excess abortion	Wertheimer and Leeper(1986)

V. FERTILITY AND REPRODUCTION EFFECTS REPORTED FOR RADIO-FREQUENCY AND MICROWAVE EXPOSURE

Nakamura et al. (2000) found that exposure to 2.45 GHz continuous wave (CW) microwave at 2mW/cm^2 power density for 90 min decreased uteroplacental blood flow, increased progesterone and PGF$_2\alpha$ in pregnant rats. Dasdag et al. (2003) reported the decrease in seminiferous tubule diameter in male rat testes after exposure. They used commercially available 890-915 MHz GSM (global signal module) with 0.141 W/kg whole body SAR. More recently, Aitken et al. (2005) found significant damage to mitochondrial and nuclear genome in epididymal spermatozoa of mice, when exposed to RF 900 MHz EMW, 12 hr a day for 7 days. Several authors (Fejes et al. 2005; Ji-Geng et al. 2007; Kesari and Behari, 2008) have also observed that carrying the mobile phones near reproductive organs for longer time may have negative effects on the sperm motility and male fertility.

Aitken et al (2005) exposed mice to 900 MHz radiofrequency electromagnetic radiation at a SAR of 90 mW/kg inside a waveguide for 7 days (12 hr/day). Following exposure DNA damage to caudal epididymal spermatozoa was assessed. These authors reported no gross evidence of single-or double strand DNA breakage in spermatozoa taken from treated animals. However an analysis of DNA integrity revealed significant damage to both the mitochondrial genome (P<0.05) and the nuclear beta-globin locus (P<0.01). This study suggests that while RF EMR does not have a dramatic impact on male germ cell development, a significant genotoxic effect on epididymal spermatozoa is seen.

Kilgalton and Simmons (2005) report decreased semen quality with prolonged use of cell phones with negative effects on sperm motility characteristics (Fejes et al, 2005). It has been shown that sperm DNA damage is not repaired, because of chromatin structure (Singh and Stephens 1998).

Yan et al (2007) studied the effects of cellular phone emissions on sperm motility in rats. Rats were exposed to two 3-hr periods of daily cellular phone emissions for 18 weeks, sperm samples were then collected for evaluation. These authors concluded that exposed group of

rats exhibited a significantly higher incidence of sperm cell death than control group rats. In addition, abnormal clumping of sperm cells was present in rats exposed to cellular phone emissions and absent from control group rats. A study carried out in Poland (Wdowiak et al 2007) on the population using mobile phone (GSM equipment), spread over a period (1-2 years) indicates sperm quality is lowered. The authors report a decrease in the percentage of sperm cells with normal motility in the semen. The decrease in motility correlates with the frequency of using mobile phones. These two finding seem to be mutually supportive.

However there are also reports indicating no effects (Panagopoulos and Margaritis 2008, 2009, 2010).

Overall, the evidence from various laboratories studying fertility and reproduction effects over the last ten years is important enough raise questions about possible public health consequences of chronic, long-term exposure to mobile phone use, and when carried on the body close to the reproductive organs. While assessing the biological implications of mobile phone radiofrequency exposures, field based experiments are not possible. Sham exposure controls cannot be obtained. Therefore it is imperative to fall back upon laboratory experiments performed in a variety of situations (e.g. animals at different distances from the mobile phone and head) while also simulated variable distances and angles for the mobile phone variation while in actual use.

Gutschi et al (2011) studied human sperm obtained from 2110 patients attending clinics from 1993 to 2007. Semen analysis was performed in all patients. Serum free testosterone (T), follicle stimulating hormone (FSH), luteinising hormone (LH) and prolactin (PRL) were collected from all patients. Information on cell phone use from each patient was collected and the subjects were divided into two groups according to their cell phone use. Group A: cell phone use (n=991), Group B: no use (n=1119). Patients with cell phone use showed a significant higher T and lower LH levels than those who did not use a cell phone. However no significant difference was observed regarding FSH and PRL values. These authors concluded that cell phone use had a negative effect on sperm quality in men.

Kesari et al (2011) assessed free radical formation due to mobile phone exposure (2 hr a day for 35 days) and examined fertility patterns in 70-days old male Wistar rats. The specific absorption rate of the mobile phone was 0.9 W/kg. An analysis of anti-oxidant enzymes glutathione peroxidise(p<0.001) and superoxide dismutase (p<o.007) showed a decline, while

an increase in catalase (p<0.005) was observed. Malondialdehyde (p< 0.003) showed an increase and histone kinase (p=0.006) showed a significant decrease in the exposed group. Correspondingly, micronuclei also showed a significant decrease (p<0.002). A change in sperm cell cycle of $G_0 - G_1$ (p=0.42) and G_2/M (p=0.022) was recorded. These authors concluded that changes occurred due to overproduction of ROS and oxidative damage, leading to infertility.

Yan et al (2007) studied the effects of cellular phone emissions on sperm motility in rats. Rats were exposed to two 3-hr periods of daily cellular phone emissions for 18 weeks. After the exposure period, sperm samples were collected for evaluation. The authors concluded that exposed group of rats exhibited a significantly higher incidence of sperm cell death than control group rats. In addition, abnormal clumping of sperm cells was present in rats exposed to cellular phone emissions and absent from control group rats.

A related issue is the corresponding effect on male infertility.

Sommer et al (2009) undertook a very exhaustive study where male and female mice were chronically exposed (life-long, 24 hr/day) to mobile phone frequency EMF at 1966 MHz (UMTS). They studied their development and fertility patterns over four generations by investigating histological, physiological, behavioural and reproductive functions. They tested SAR from the time of mating at 0 (sham), 0.08, 0.4 and 1.3 W/kg. Power densities were kept constant for each group (0, 1.35, 6.8 and 22 W/m^2), resulting in varying SARs due to different number of adults and pups. The results show no harmful effects of exposure on the fertility and development of the animals. The number and the development of the pups were not affected by the exposure. These authors concluded no harmful effects occurred with long-term exposure of mice to UMTS mobile phone frequency radiation over several generations.

DeIuliis et al (2009) used purified human spermatozoa for exposure to electromagnetic radiation at 1.8 GHz with specific absorption rates varying from 0.4 to 2.75 W/kg. These investigators reported that motility and vitality were significantly reduced after RFR exposure, while the mitochondrial generation of reactive oxygen species and DNA fragmentation was significantly elevated (P<0.001). They also found a highly significant relationship between SAR, the oxidative DNA damage biomarker 8-OH-dG, and DNA fragmentation after exposure. These results have bearing on safety of people of reproductive age, and wellbeing of their offspring. Erogul et al (2006) also support these finding by showing effect on sperm motility and that long-term exposure may lead to behavioural or

structural changes of the male germ cell. These may appear later in life and need investigation on a longer term basis.

As a follow up of the above, Otitoloju et al (2010) exposed male mice to radiofrequency radiations at mobile phone (GSM) base station-level RFR. Sperm head abnormalities occurred in 39% to 46% of exposed mice, but in only 2% of the controls ($P<0.005$). The major abnormalities observed were knobbed hook, pin head and banana-shaped sperm head. The abnormalities were also found to be dose-dependent. This may have severe consequences for the off spring.

Gul et al (2009) investigated toxicity of microwaves (as emitted by cellular phones on ovaries in rats. In this study 82 female rats of aged 21 days (43 in the study group and 39 in the control group) were used. Pregnant rats exposed to mobile phones that were kept underneath the cages during the whole period of pregnancy. A mobile phone in a standby position for 11 hr and 45 min was turned on to speech position for 15 min every 12 hr and the battery was charged continuously. On the 21st day after the delivery , the female rat pups were killed and the right ovaries were removed. The volumes of the ovaries were measured and the number of follicles in every tenth section was counted. These authors found that the number of follicles in pups exposed to mobile phone microwaves suggest that intrauterine exposure has toxic effects on ovaries.

Salama et al (2010) examined the accumulating effects of exposure to electromagnetic radiation emitted by a conventional mobile phone (800 MHz, standby position, kept opposite to the testis) on the testicular function and structure. The animals were exposed 8 hr daily for a period of 12 weeks in a specially designed cage. Semen analysis and sperm function tests were conducted weekly. Other parameters examined were histological testicular sections and serum total testosterone. When compared with other two groups (stress control and ordinary), the exposed animals showed a drop in sperm concentration at week 6, which became significant at week 8. Mobile sperm population showed similarity amongst the three study groups until week 10 when it declined significantly, and thereafter in phone and stress control groups, with more significant decline in the exposed animals (50.6% and 72.4%, respectively). Histological examination showed a significant decrease in the diameter of seminiferous tubules in the exposed group vs the stress and ordinary controls (191 μm vs. 206 and 226 μm, respectively). The authors concluded that the pulsed radiofrequency emitted by a conventional mobile phone kept in the standby position could affect the testicular function and structure in the adult rabbit.

Falzone et al (2011) evaluated the effect of RF-EMF on sperm characteristics to assess the fertilizing potential of sperm. They exposed highly motile human spermatozoa to 900 MHz for an hour (SAR =2.0 W/kg) and examined effects at various time after exposure. The acrosome reaction was evaluated using flow cytometry. They did not find any effect on sperm propensity for the acrosome reaction. They obtained significant reduction in sperm head area (21.5±4% vs 35.5±11.4%) was obtained when compared among exposed and unexposed samples. Sperm zona binding was assessed directly after exposure. The mean number of zona-bound sperm of the test hemizona and controls was 22.8±12.4 and 31.8±12.8 (p<0.05) respectively. They concluded that though the radiation exposure did not adversely affect the acrosome reaction, it had a significant effect on sperm morphometry. They also observed a significant decrease in sperm binding to the hemizona. These data point toward sperm fertilization potential. These studies are in contradiction that fertility impairment was not caused by the induction of apoptosis in spermatozoa (Falzone et al 2010).

In a study undertaken by Ribeiro et al (2007), while experimenting with male Wistar rats, they exposed testis in the frequency and in the range of intensity (1835-1856 MHz, 0.04-1.4 mW/cm^2). The authors reported that the total body weight and absolute and relative testicular and epididymal weight did not change significantly, nor did the epididymal sperm count.

Human spermatozoa are known to be known to be vulnerable to oxidative stress because of abundant availability of substrates for free radical attack, and the lack of cytoplasmic space to accommodate antioxidant enzymes. The ROS generation does DNA damage, besides reducing fertility. The former has been linked with poor fertility, incidence of miscarriage and possible morbidity in the offspring, including childhood cancer.

There are other reports showing lack of effect on testicular function in experimental animals in the non-thermal range. They concluded that the responses are identical to those produced by hyperthermia caused by mere heating(Ribeiro et al 2007, Sommer et al 2009).

Comparison between non-modulated (DTX) and Modulated (Talk Signal) GSM Radiation

In an experimentation with insects, Panagopoulos (2011) divided these into two groups: a)the exposed (E) and b) the sham exposed (control) group (SE). Each of the two groups consisted of ten female and ten male newly emerged adult flies. The sham exposed groups had identical treatment as the exposed ones, except that the mobile phone during the "exposures" was turned off. The duration of exposure was 6 min per day in one dose extending over a period of 5 days.

In the first part of the exposure (1A) the insects were exposed in non-modulated GSM 900 MHz radiation (TDX-discontinuous transmission mode –signal) while in the second part (1B) they were exposed to modulated GSM 900 MHz radiation (or GSM talk signal). In both cases, the exposures were performed with the antenna of the mobile phone in contact with the walls of the glass vials containing the insects.

The difference between the modulated and the corresponding non-modulated GSM radiation is that the intensity of the modulated radiation is about ten times higher than the intensity of the corresponding non-modulated from the same handset (mobile phone) and additionally that the modulated radiation includes more and larger variations in its intensity within the same time interval, than the corresponding non-modulated one (Panagopoulos and Margaritis 2008). The power level of exposure for the modulated signal was 0.436 ± 0.060 mW/cm^2 and the corresponding mean value for the non-modulated emission was (0.041 ± 0.006) mW/cm^2. The measured ELF mean values of electric field intensity of the GSM signals excluding the ambient fields of 50 Hz were 6.05 ± 1.02 V/m for modulated signal and 3.18 ± 1.10 V/m for the non-modulated signal.

Experiments with the non-modulated GSM 900 MHz radiation (non-speaking mode of transmission) showed that this radiation decreased insect reproduction by an average of 18.24%. Correspondingly experiments with modulated GSM at 900 MHz (GSM "talk" signal) exposure shows that the radiation decreases reproduction by an average of 53.01 %. Above results indicate that the decrease in population is linked with intensity of the radiation. These authors concluded that between 900 MHz and 1800 MHz, the former is more bioactive owing to the difference in radiation intensity. Performing experiments at various distances (0 to 100cm) from mobile phone, Panagopoulos (2011) reported that the distance dependence is not linear. At the distances at 0 and 30 cm (intensity 378 μW/cm^2 and 10 μW/cm^2 respectively) show a maximum of decrease in reproductive capacity (window of maximum bioactivity). Correspondingly for GSM 1800 MHz at 0 and 20 cm (intensity 252μW/cm^2 and 11μW/cm^2 respectively) bioactivity is maximum (decrease in reproduction, window of maximum bioactivity) i.e. in the vicinity of free space wavelength of the corresponding radiation. For distances greater than 20 cm (up to 80 cm) the effect decreases rapidly and becomes very small for distances longer than 40 cm, but it is still evident for distances up to 80 cm (intensity down to 1.1μW/^{2}). These authors have further pointed out that it is the intensity which is primarily important rather than the frequency or the distance as such.

These distances (30 and 20 cm from GSM 900 MHz and GSM 1800 MHz correspond to the same RF intensity ($10\mu W/cm^2$) and also to the same electric field intensity of about 0.6-0.7 V/m. Maximum bioactivity is attributed to a distance of 0 cm or at approximately the two nodes of the wavelength, after which the effect declines. These authors reported no temperature increase inside any of the vials. They further concluded that the ELF components of digital mobile telephony signals that play a key role in their bioactivity, alone or in combination with the RF carrier signal . This also suggest that low frequency signals are more bioactive than higher frequency ones. Accordingly, electric field of the order of 10^{-3} V/m are able to disrupt cell function, perhaps by irregular gating of electrosensitive ion channels on the cell membranes. We conclude that both the GSM signal at 900 MHz and 1800 MHz fields appear to possess sufficient intensity for this for distances up to 50 cm from the antenna of a mobile phone (or about 50 m from a corresponding base station antenna). Therefore the restrictions being imposed on emission standards are with respect to continuous wave frequencies, but not with respect to a pulsed type, the latter being important in transmitting any intelligent information. Moreover real GSM signals are not constant in frequency and intensity. This distance of 20-30cm from the mobile phone corresponds to a distance of 20 to 30 m from a base station antenna. Panagopoulos et al (2010) showed that the bioactivity of GSM radiation in regard to short-term exposure is evident for radiation intensities down to $1\mu W/cm^2$. This value of radiation intensity is encountered at about 1m distance from a cell phone or about 100 m distance from a corresponding base station antenna. This radiation intensity is 450 times and 900 times lower than the ICNIRP limits for 900 and 1800 MHz respectively (ICNIRP,1998). It has been estimated by Panagopoulos (2011) that people may be exposed to this level of radiation for long distances so, a factor of ten could be added as a safety factor, thereby bringing down the above figure to 0.1 $\mu W/cm^2$, suggesting a limit for public exposure. These results support the findings that GSM radiation caused increased permeability of the blood –brain barrier in rat nerve cells and the strongest effect was produced by the SAR values which correspond to the weakest radiation intensity (Eberhardt et al.2008). The concept of window has earlier been described by Bawin et al (1978), Blackman et al (1980,1989). They have reported that the reproductive capacity decreases as the duration of exposure (1-21 minutes) increases(almost proportionally), for either of the two radiation types. Using statistical analysis they have confirmed that this variation is not because of the randomness of the subject, but because of the radiation exposure.

Several other authors have echoed a wide range of damaging effects on the male reproductive system and sperm parameters and cause significant changes in the sperm cell cycle (Derias et al 2006; Ji-Geng. 2007; Gutschi et al, 2011).

Non-genotoxic effects of Radiofrequency Radiation

Several studies reported no effect of RF fields on cell cycle kinetics (Vijayalaxmi et al 2001, Higashikubo et al 2001; Zeni et al, 2003; Miyakoshi et al, 2005; Lantow et al, 2006c). Alteration in cell proliferation was described only in a few reports (Pacini et al, 2002, Capri et al, 2004b).

Apoptosis is an important mechanism of protection against cancer. Several studies have reported RF field effects on human peripheral blood mononuclear cells (Capri et al, 2004a), lymphoblastoid cells (Marinelli et al, 2004), epidermis cancer cells (Caraglia et al 2005), and human Mono Mac 6 cells (Lantow et al, 2006c) and in Molts4 cells (Hook et al, 2004). No difference in apoptosis induction was detected between sham exposed and RF field exposed cells by Hook et al (2004). On the other hand, Marinelli et al (2004) have reported better survival rate of T lymphoblastoid leukaemia cells exposed to 900 MHz non-modulated RF fields and Carglia et al (2005) found apoptosis induction in human epidermoid cancer cells after exposure to 1.95 GHz fields. The European REFLEX study (Nikolova et al, 2005) reported no effects of RF fields on cell cycle, cell proliferation, cell differentiation, apoptosis induction, DNA synthesis and immune cell functionality. These authors described some findings after RF exposure on the transcript level of genes related to apoptosis and cell cycle control; however these responses were not associated with detectable changes of cell physiology. Analysis on whole genome cDNA arrays show alterations in gene expression after various RF exposure conditions using different cell types, but no consistent RF-signature such as stress response could be identified (Remondini et al, 2006).

Heat shock proteins act primarily as molecular chaperones to eliminate unfolded proteins, which can also appear from cellular stress. This stress response can be induced by many different external factors, including temperature, chemicals, oxidative stress, heavy metals, ionizing and non-ionizing radiation and ultrafine carbon black particles. Hsp70 has been shown to interfere with post mitochondrial events to prevent free radical mediated apoptosis (Gotoh et al 2001). An increased expression level of Hsp70 can thus offer protection against stress. Heat shock proteins are also involved in oncogenic processes (Jolly et al, 2000; Inoue et al, 1999; French et al, 2001).Some investigators have described increased heat shock

protein level after RF exposure (Leszczynski et al, 2002; Kwee et al, 2001). However, these results are controversial, because there are negative findings also (Cotgreave 2005).

Nikolova et al (2005) described modulation in gene regulation after RF field's exposure at a SAR of 1.5 W/kg in p53-deficient embryonic stem cells. Proteomic analyses of human endothelial cell lines showed RF fields induced changes in this expression and phosphorylation state of numerous proteins including the hsp27.

Mitochondrial generation of ROS : DNA fragmentation and Effects

Free radical formation and their interaction with biological system is a matter of major concern for it has health implications. There is evidence of free radical generation after RF-microwave exposures (Phillips et al 2009; De lullis et al 2009;Kesari and Behari 2012,Kesari et al 2012).

Mitochondrial respiratory chain is the major site for the generation of superoxide radicals (O_2 and H_2O_2). It is possible that EMF may affect the mitochondrial membranes to produce large amount of radicals ROS under experimental conditions. EMF may disturb ROS metabolism by increasing the production of ROS or by decreasing the activity of antioxidant enzymes. From the data presented here it is obvious that such a change in testes that is highly dependent on oxygen to drive spermatogenesis and yet highly susceptible to the toxic effects of reactive oxygen metabolites, activity of anti-oxidant enzymes, and increases in ROS production. Reactive oxygen species (ROS) such as superoxide anions (O^-), hydroxyl radicals (OH^-) and hydrogen peroxide ($H_2 O_2$) may influence the structural integrity and function of sperm, such as motility, capacitation, and sperm-oocyte fusion (Griveau et al 1995). Spermatozoa are particularly vulnerable to oxidative stress because their plasma membrane is rich in polyunsaturated fatty acids (PUFAS) and membrane bound NADPH oxidase. Increased ROS production has been shown to correlate with reduced male fertility (Iwasaki and Gagnon 1992), to cause perioxidative damage to the sperm plasma membrane (Hughes et al 1996), and induce both DNA strand breakages and oxidative base damage in human sperm (Kodama et al 1997). A decrease in total antioxidant capacity of seminal plasma has been correlated with a reduction in sperm quality, such as concentration, motility and morphology (Smith et al 1996).

Since the most abundant molecule in biological cells is that of water (H_2O) microwave radiation can generate free radicals like OH^-, O^-_2, H, and H^-. These molecules are extremely reactive, having a tendency to react with different biomolecules including DNA, because of an unpaired electron that they comprise, which try to give up this extra charge and go into the

paired mode. Also hydrogen peroxide (H_2O_2), a product of oxidative respiration in the mitochondria, which can be converted by electromagnetic radiation(EMR)into hydroxyl free radical via the Fenton reaction catalyzed by iron within the cells:

$$H_2O_2 + (EMR)\text{-----}\rightarrow OH^- + OH^-$$
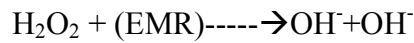
ROS generated by mobile phone exposure if not scavenged may lead to widespread lipid, protein, and DNA damage (Jajte et al 2002).

A summary of these results on Effects of Radiofrequency Microwave Radiation on Fertility and Reproduction is presented in Table 2.

The sequence of events leading toward infertility

A wide range of studies extending up to 50 GHz (Kesari and Behari 2009)) suggest that the DNA interaction with EMF is similar in nature across wide frequency ranges. DNA appears to possess the two structural characteristics of fractal antennas, electronic conduction and self- symmetry (Blank and Goodman 2011). These properties contribute to greater reactivity of DNA with EMF in the environment. The DNA damage could account for cancer promotion.

While damage to DNA has been confirmed in numerous scientific studies, it is argued that DNA repair is an on-going process and the damaged chromosomes can be reconstituted. However, this proposition is not without risk. There is no guarantee that these will replicate in the manner they were originally present. Pieces may be left out (deletions), joined in the backwards (inversions), swapped between different parts of the chromosomal (translocations)

Table 2: Overall effect of microwave radiation on reproduction and fertility

Organism used	Mode of exposure	Parameters studied	Conclusion	Reference
Fetus in the womb	laptop computers (LTCs)	induced currents in the body	power supply produces strong intracorporal electric current in the fetus and in the mother	Bellieni et al 2012
Sperm	Cell phone	Serum free testosterone (T), follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL)	Higher T and lower LH levels No change in FSH and PRL values	Gutschi et al, 2011
Male Wistar rats	2.45 GHz	Creatine and caspase	Increase in caspase and creatine kinase ; decreases in testosterone and melatonin	Kesari et al, 2011
human spermatozoa	900-MHz	Acrosomal reaction, Morphometric parameters	affect sperm morphometry decrease in sperm	Falzone et al, 2011
Male Sprague Dawley rat	1.95 GHz 5 h/d for 5 weeks	SOD, CAT, GPx, histone kinase,Apoptosis	No testicular toxicity.	Imai et al. 2011
male mice	mobile phone base stations	sperm head abnormalities	knobbed hook, pin-head and banana-shaped sperm head	Otitoloju et al, 2010
Drosophila melanogaster	GSM 900MHz and DCS 1800MHz	Reproductive capacity	cumulative effects on living organisms.	Panagopoulos and Margaritis, 2010

Table 2 continued ..

Drosophila melanogaster	900 MHz	ovarian size	Significant reduction in size of ovary	Panagopoulos and Margaritis 2010
Male Wistar rat	900 MHz 2 h d for 45 day	Sperm count, apoptosis	Reduced sperm count and increased apoptosis	Kesari et al 2010
Male Wistar rat	50GHz	SOD, CAT, GPx, histone kinase,Apoptosis	Decreased SOD,GPX and Histone kinase, increased CAT and apoptosis	Kesari and Behari 2010
Male rabbit	800 MHz 8 h /d 12 weeks	Sperm count, weights of testis, epididymis, seminal vesicles, and prostate	Drop in sperm count	Salama et al 2010
Male and female mice (C57BL)	1966 MHz (UMTS)	Semen analysis and sperm function tests	No change	Sommer et al 2009
Rat	mobile phones	volumes of the ovaries and follicles	reduction in number of follicles	Gul et al, 2009
human spermatozoa	1.8 GHz	motility and vitality	mitochondrial reactive oxygen species generation	De Iuliis et al , 2009
Wistar albino male rats	900 MHz 2 h/day (7 days/week) for 10 months	Apoptosis of testes	No effect on caspase-3 levels	Dasdag et al. 2008

Table 2 continued…

Male Wistar rat	50-GHz microwave radiation 2 h a day for 45 days at a power level of 0.86 μW/cm^2	DNA strand break, Apoptosis	Increased apoptosis and DNA strand break	Kesari & Behari, 2008
Male Sprague-Dawley rats	cellular phone emissions	sperm motility, sperm cell morphology, total sperm cell number, and mRNA levels	abnormal clumping of sperm cells	Yan et al 2007
Male Sprague-Dawley rats	cellular phone emissions for 18 weeks	sperm motility, sperm cell morphology, total sperm cell number, and mRNA levels	sperm cell death and , abnormal clumping of sperm cells	Ji-Geng et al , 2007
Mice	1800 MHz	Serum testosterone	No detectable changes	Forgács et al.2006
Human semen	cell phone	Semen analyses	negative effects on the sperm motility	Fejes, et al 2005
Male NMRI mice	1800 MHz(100μW 2 h	Steroidogenic Leydig cells	No change	Forgács et al 2005
Drosophila melanogaster	900-MHz	Reproductive capacity	decrease cellular processes during gonad development	Panagopoulos et al 2004
Pregnant rats	915MHz microwaves	uteroplacental circulation, and in placental endocrine and immune functions	No effects on blood estradiol and progesterone,	Nakamura et al, 2000
Sprague-Dawley rats	cellular phones 20 min per day (7 days a week) for 1 month	malondialdehyde ,p53 immune reactivity, sperm count, morphology,	No significant alteration	Dasdag et al, 2003

or even attached to the wrong chromosome. The effect may also be frequency dependent. In most cases, the new arrangement can work for a while if most of the genes are still present and any metabolic deficiencies can often be made good by the surrounding cells. However, things may be different if it comes to meiosis. During meiosis, the chromosomes line up in pairs (one from each original parent) along their entire length so that corresponding parts are adjacent and can be exchanged. Malformed pairs are torn apart in the later stages of meiosis so that eggs or sperms have an incomplete or unbalanced set of genes, may not function properly and so reduce fertility and other physiological functioning. There is a possibility that this may lead to permanent genetic damage, which though may not be visible in the first generation but may be thereafter. A summary of these results on Effects of Radiofrequency Microwave Radiation on Fertility and Reproduction is presented in Table 3.

Table 3: Overview of effects of Microwave radiation on reproductive patterns

Parameter studied	900 MHz	2.45GHz	10GHz	50GHz
PKC	↓	-	-	-
SOD	↓	↓	↓	↓
CAT	↑	↑	↑	↑
GPx	↓	↓	↓	↓
H1K	↓	-	↓	↓
DNA damage	↑	↑	↑	-
ROS	↑	↑	↑	-
CK	↑	↑	↑	-
Testosterone*	↓	↓	↓	-
Caspase*	↑	↑	↑	-

↑ Indicates significant increase   ↓ Indicate significant decrease

(PKC: Protein kinase C; ODC: Ornithine decarboxylase; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; H1K: Histone kinase, CK: creatine kinase, ROS: reactive oxygen species)
* Some studies have reported that there is no significant changes in reproductive system.
* Forgács et al 2005,2006 (1800 MHz)
* Dasdag et al. 2008 (900 MHz)
* Imai et al. 2011 (1.95 GHz)
* Sommer et al 2009 (1966 MHz, UMTS)

VI. PRUDENT AVOIDANCE AND GUIDANCE FOR SAFETY LIMITS

While it appears to have been convincingly established that electromagnetic fields have adverse biological effects on fertility and reproduction, the emphasis is on 'use with caution' rather than no use at all. Children in the age 12 years and younger are more prone to the

damage because of their developing nervous system. Senior citizens and persons who are ill should also exercise caution and use wireless devices only in a most demanding situation. Mobile phones should thus be carried in close proximity of the body only in an OFF position (not ON and transmitting on standby). This is so because in an "standby" mode the phone emits signal intermittently - every few minutes they emit a periodic signal lasting a few seconds long - to maintain connection with the nearest base station antenna. These periodic signals are as powerful as the usual "talk signal" during a conversation. The user must make use of mobile phone speaker mode and keep the handset at least 40 cm away from their heads and other most sensitive organ like the head, heart and reproductive organs. Another method of protection (e.g. wired ear phones) are less effective, because of the existence of intensity window. The base station antennas should not be located within or near residential areas or near heavily populated areas. If antenna placement in the vicinity of residential zones is essential, they should be made to operate at substantially lowered power. Powerful wireless antennas should be placed on the hilltops and far from populated areas . The focus thus then shifts to prudent avoidance i.e. on to reduce the frequency and length of phone calls and keep away from these devices when not in use.

Bellieni et al (2012) have quoted that levels of exposure from "laptop" computers are higher than exposures that can be found in the proximity of high-voltage power lines and transformers or the domestic video screens .It has been observed that the magnetic field strength from power supplies is higher than that recommended by ICNIRP (1998) guidelines but that from LTC are within safe limits. It is thus suggested that use of LTC in an inclined

position below the table level be avoided because it may cause increase in genital temperature ,besides causing back pain and fatigue. Moreover 'laptop' is a misnomer for its use in close proximity to the body is harmful.

Guidelines for Safety Limits

While considering the far field exposures, there are two sources: one is the microwave exposure from the base stations. While mobile phone exposure is localized, intermittent and is under voluntary control of the user, radiation from base towers is involuntary, whole-body and occurs 24 hours a day. While both the exposures may involve the same carrier frequency, the exposures are basically different in type and duration. On the whole it can be concluded that long term exposure near base stations can affect well-being of populations around them. Symptoms mostly associated with such exposures are headaches, tremor, restlessness and sleeping disorders.

The question of laying down the criteria for safe exposure is a problematic one, because the dose needs to be assessed not just as external field frequency (and spectrum), intensity, but also as cumulative exposure, as well as SAR, for whole body and specific anatomical sites. Accurate knowledge of RF exposure in a given scenario is needed for several parameters. The effect is not immediately visible but acts as silent killer. Any epidemiological studies for a long period (ten years or more) are difficult to carry under controllable situation, and few unexposed populations can serve as controls (non-exposed). Moreover the basic restrictions are expressed in quantities that are internal to the body and are not measured such as SAR. On the other hand, the reference levels are expressed (measured) in the free space situation, such as electric field. It is evident that SAR-concept alone is insufficient to define the safety guidelines for chronic exposure from mobile communications.

VI. CONCLUSIONS

Though causal evidence of one or more mechanism(s) are not yet fully refined, it is generally accepted that oxidative stress and free radical action may be responsible for the recorded genotoxic effects of EMFs which may lead to impairments in fertility and reproduction. Free radical action and/or hydrolytic enzymes like DNAase induced by exposure to EMFs may constitute the biochemical actions leading to adverse changes in hormones essential in males and female reproduction, DNA damage, which in turn causes damage to sperm motility, viability, and sperm morphology. Such exposures are now common in men who use and who wear wireless devices on their body, or use wireless-mode laptop computers. It may also account for damage to ovarian cells and female fertility, and miscarriage in women (ELF-EMF at 16 mG intermittent exposure).

VIII. REFERENCES

Agarwal A, Tamer M. Said TM. Role of sperm chromatin abnormalities and DNA damage in male infertility Human Reproduction Update 2003;9:331-345.

Agarwal A, Deepinder F, Sharma RK, Ranga G, Li J. Effect of cell phone usage on semen analysis in men attending infertility clinic: an observational study. Fertil Steril. 2008;89(1):124-8.

Agarwal A, Desai NR, Makker K, Varghese A, Mouradi R, Sabanegh E, et al. Effect of radiofrequency electromagnetic waves (RF-EMF) from cellular phones on human ejaculated semen: an in vitro study. Fertility Sterility 2009;92(4):1318-1325.

Aitken RJ, Bennetts LE, Sawyer D, Wiklendt AM, King BV. Impact of radio frequency electromagnetic radiation on DNA integrity in the male germline. Int J Androl. 2005 Jun;28(3):171-9.

Aitken RJ, Roman SD. Antioxidant systems and oxidant stress in the testes. Review. Oxidative Med. Cell Longevity. 2008;1:15-24

Akdag MZ, Dasdag S, Aksen F, Isik B, Yilmaz F. Effect of ELF magnetic fields on lipid peroxidation, sperm count, p53, and trace elements. Med Sci Monit. 2006;12 (11):BR366-71.

Al-Akhras MA, Darmani H, Elbetieha A. Influence of 50 Hz magnetic field on sex hormones and other fertility parameters of adult male rats. Bioelectromagnetics 2006; 27(2):127-131.

Amara S, Abdelmelek H, Garrel C, Guiraud P, Douki Travant JL, et al. Effects of subchronic exposure to static magnetic field on testicular function in rats. Arch Med Res. 2006;37(8):947-52.

Avendano C, Mata A, Sanchez Sarmiento CA, Doncel GF. Use of laptop computers connected to internet through Wi-Fi decreases human sperm motility and increases sperm DNA fragmentation. Fertility Sterlity 2012;97(1):39-45.

Bawin S, Adey W, Sabbot I. Ionic factors in release of 45 Ca2+ from chicken cerebral tissues by electromagnetic fields, In Proc. Natl. Acad. Sci. 1978;75(12):6314-6318.

Behari J, Kesari KK. Effects of microwave radiations on reproductive system of male rats. Embryo Talk 2006;1 (Suppl.1):81-5.

Bellieni CV, Pinto I, Bogi A, Zoppetti N, Andreuccetti D, Buonocore G. Exposure to electromagnetic fields from laptop use of "laptop" computers, Arch Environ Occup Health, 2012;67:1:31-36

Bernabo N, Tettamant E, Pistilli MG, Nardinocchi D, Beradinelli P, Mattioli M, Barboni B. Effects of 50 Hz extremely low frequency magnetic field on the morphology and function of boar spermatozoa capacitated in vitro. Theriogenology. 2007;67(4):801-815.

Bernabo N, Tettamant E, Pistilli MG, Nardinocchi D, Beradinelli P, Mattioli M, et al. Extremely low frequency electromagnetic field exposure affects fertilization outcome in swine animal model. Theriogenology. 2010;73(9):1293-1305.

Blackman CF, Benane SG, Elder JA, House DE, Lampe JA, Faulk JM.Induction of calcium-ion influx from tissue by radiofrequency radiation : Effect of sample number and modulation frequency on the power-density window. Bioelectromagnetics 1980;1:35-43.

Blackman CF, Kinney LS, House DE, Joines WT. Multiple power density windows and their origin. Bioelectromagnetics 1989;10(2):115-128.

Blank M, Goodman R. DNA is a fractal antenna in electromagnetic fields. Int J Radiation Biol 2011;87:409-415.

Cao XW, Zhao TD, Wang CH, Zhou Q, Li LQ, Yao HG, Zhang SQ, Tang, JT, Wei W. Alternating magnetic field damages the reproductive function of murine testes. Zhonghua Nan Ke Xue. 2009;15(6):530-533.

Capri M, Scarcella E, Fumelli C. Bianchi E, Salvioli S, Mesirca P. et al. In vitro exposure of human lymphocytes to 900 MHz CW and GSM modulated radiofrequency: studies of proliferation, apoptosis and mitochondrial membrane potential. Radiat Res. 2004a;162, 211-218.

Capri M, Scarcella E, Bianchi E, Fumelli C, Mesirca P, Agostini C, et al. 1800 MHz radiofrequency (mobile phones, different Global System for Mobile communication modulations) does not affect apoptosis and heat shock protein 70 level in peripheral blood mononuclear cells from young and old donors. Int J Radiat Biol. 2004b;80:389-397.

Caraglia M, Marra M, Mancinelli F, D'Ambrosio G, Massa R, Giordano A. et al. Electromagnetic fields at mobile phone frequency induce apoptosis and inactivation of the multi-chaperone complex in human epidermoid cancer cells. J Cell Physiol. 2005; 204:539-548.

Roychoudhury S, Massanyi P, Slamecka J, Chlebec I, Trandzik J, et al. In vitro gossypol induced spermatozoa motility alterations in rabbits. J Environ Sci Health B. 2009 Sep;44(7):730-41.

Chung MK, Lee SJ, Kim YB, Park SC, Shin DH, Kim SH, Kim JC. Evaluation of spermatogenesis and fertility in F1 male rats after in utero and neonatal exposure to extremely low frequency electromagnetic fields. Asian J Androl. 2005, 7(2):189-94.

Cotgreave IA. Biological stress responses to radio frequency electromagnetic radiation: are mobile phones really so (heat) shocking?, Arch Biochem Biophys. 2005;435:227–240.

Dasdag S, Akdag MZ, Aksen F, Yilmaz F, Bashan M, Dasdag M, Salih Celik M. Whole body exposure of rats to microwaves emitted from a cell phone does not affect the testes, Bioelectromagnetics 2003;24(3):182-188.

Dasdag S, Akdag MZ, Ulukaya E, Uzunlar AK, Yegin D. Mobile phone exposure does not induce apoptosis on spermatogenesis in rats. Arch Med Res. 2008 Jan;39(1):40-4.

Delgado JMR, Leal J, Monteagudo JL, Gracia MG. Embryological changes induced by weak, extremely low frequency electromagnetic fields. J Anat (Lond) 1982;134:533–552.

DeIullis GN, Newey RJ, King BV, Aitken RJ. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. PLos One 2009;4(7):e6446.

Deno DW, Zaffanella LE. Field effects of overhead transmission lines and stations, In Transmission Line Reference Book. 345 kV and above, 2nd edition , J J Ed. Project UHV, Technical Resource Operations. Large Transformer Division. General Electric Company, Pinsfield Mass. 1982;329/625.

Derias EM, Stefanis P, Drakeley, A, Gazvani R, Lewis_Jones DI. Growing concern over the safety of using mobile phones and male fertility. Arch. Androl. 2006;521:9-14.

Drozdov KA, Khlistun OA, Drozdov AL. The influence of ultrasound and constant magnetic field on gametes, zygotes, and embryos of the sea urchin. Biofizika. 2008; 53(3):513-518.

Eberhardt JL, Persson BR, Brun AE, Salford LG, Malmgren LO. Blood-brain barrier permeability and nerve cell damage in rat brain 14 and 28 days after exposure to microwaves from GSM mobile phones. Electromagn Biol Med. 2008;27(3):215-29.

Edwards MJ , Mulley R, Ring S, Warmer RA. Mitotic cell death and delay of mitotic activity in guinea pig embryos following brief material hyperthermia. J Embryol Exp Morphol 1974;32:593-602.

Erogul O, Oztas E, Yildirim I, Kir T, Aydur E, Komesli G, Irkilata HC, IrmakMK, Peker AF. Effects of electromagnetic radiation from a cellular phone on human sperm motility:an vitro study. Arch Med Res 2006;37(7):840-3.

Falzone N, Huyser C, Franken DR, Leszezynski D. Mobile phone radiation does not induce pro-apoptosis effects in human spermatozoa. Radiation Res 2010;174(2):169-76.

Falzone N, Huyser C, Becker P, Leszezynski DR, Franken DR. The effect of pulsed 900 MHz GSM mobile phone radiation on the acrosome reaction, head morphometry and zona binding of human spermatozoa. Int J Androl 2011;34(1):20-6.

Farrell JM Litovitz TL, Penafiel M, Montrose CJ, Doinov P, Barber M, et al. The effect of pulsed and sinusoidal magnetic fields on the morphology. Bioelectromagnetics. 1997;18:431-438.

Fraser FC, Skelton J (1978) Possible tetragenicity of maternal fever. Lancet 2:634.

Fejes I, Zavacki Z, Szollosi J, Koloszar Daru J, Kovacs L, Pal A. Is there a relationship between cell phone use and semen quality ? Arch Androl. 2005;51, 385-393.

Forgács Z, Kubinyi G, Sinay G, Bakos J, Hudák A, Surján A, Révész C, Thuróczy G. Effects of 1800 MHz GSM-like exposure on the gonadal function and hematological parameters of male mice. Magy Onkol. 2005;49(2):149-51. [Article in Hungarian]

Forgács Z, Somosy Z, Kubinyi G, Bakos J, Hudák A, Surján A, Thuróczy G. Effect of whole-body 1800 MHz GSM-like microwave exposure on testicular steroidogenesis and histology in mice. Reprod Toxicol. 2006; Jul;22(1):111-7.

French PW, PennyR, Laurence JA, McKenzie DR. Mobile phones, heat shock proteins and cancer. Differentiation 2001;67, 93-97.

García AM, Sisternas A, Hoyos SP. Occupational exposure to extremely low frequency electric and magnetic fields and Alzheimer disease: a meta-analysis. Int J Epidemiol. 2008;37(2):329-40

Gharagozloo P, Aitken RJ. The role of sperm oxidative stress in male infertility and the significance of oral antioxidant therapy. Hum Reprod 2011 Jul;26(7):1628-40. Epub 2011 May 5.

Gotoh T, Terada K, Mori M. hsp70-DnaJ chaperone pairs prevent nitric oxide-mediated apoptosis in RAW 264. 7 macrophages. Cell Death Differ. 2001; 8, 357-366.

Gul A, Celebi H, Ugras S. The effects of microwaves emitted by cellular phones on ovarian follicles in rats. Archives of Gynecology and Obstetrics 2009;280(5): 729-33.

Gutschi T, AI-Ali BM, Shamloul R, Pummer K, Trummer H. Impact of cell phone use on men's semen parameters. Andrologia. 2011;43, 5, 312–316.

Heredia-Rojas JA, Caballero-Hernandez DE, Rodriguez-de la Fuente AO, Ramos-Alfano G, Rodriguez-Flores LE. Lack of alterations on meiotic chromosomes and morphological characteristics of male germ cells in mice exposed to a 60 Hz and 2. 0 mT magnetic field. Bioelectromagnetics. 2004;25(1):63-8.

Hardell L, Sage C. Biological effects from electromagnetic field exposure and public exposure standards. Biomed Pharmacother. 2008;62(2):104-9.

Higashikubo R, Ragouzis M, Moros EG, Straube WL, Roti Roti JL. Radiofrequency electromagnetic fields do not alter the cell cycle progression of C3H 10T and U87MG cells. Radiat Res. 2001; 786–795.

Hong R, Liu Y, Yu YM, Hu K, Weng EQ. Effects of extremely low frequency electro magnetic fields on male reproduction in mice. Zhonghua Lao dong Wei Sheng, Zhi Ye Bing Za Zhi. 2003;21(5):342-345.

Hong R, Zhang V, Liu Y, Weng EQ. Effects of extremely low frequency electromagnetic fields on DNA of testicular cells and sperm chromatin structure in mice. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 2005;23(6):414-417.

Hook GJ, Zhang P, Lagroye I, Li L, Higashikubo R, Moros EG, et al. Measurement of DNA damage and apoptosis in Molt-4 cells after in vitro exposure to radiofrequency radiation. Radiat Res. 2004; 161:193-200.

Hughes CM, Lewis SE, Mckelvey-Martin VJ, Thompson W. A comparison of baseline and induced DNA damage in human spermatozoa from fertile and infertile men, using a modified comet assay. Mol Hum Reprod. 1996; 13, 1240-1247.

Huss A, Spoerri A, Egger M, Röösli M and for the Swiss National Cohort Study. Residence near power lines and mortality from neurodegenerative diseases: longitudinal study of the Swiss Population. Am J Epidemiol. 2008;15, 169, 167-175.

ICNIRP. Guidelines for limiting exposure to time varying electric, magnetic, and electromagnetic fields (upto 300 GHZ) 1998. Health Phys. 1998;74:494-522.

Imai N, Kawabe M, Hikage T, Nojima T, Takahashi S, Shirai T. Effects on rat testis of 1. 95-GHz W-CDMA for IMT-2000 cellular phones. Syst Biol Reprod Med. 2011; Aug;57(4):204-9.

Inoue Y, Sato Y, Nishimura M, Seguchi M, Zaitsu Y, Yamada K. et al. Heat-induced drug resistance is associated with increased expression of Bcl-2 in HL60. Anticancer Res. 1999;19:3989-3992.

Iwasaki A, Gagon C. Formation of reactive oxygen species in spermatozoa of infertile patients. Fertil Steril. 1992; 57:409-416.

Jajte J, Grzegorczyk J, Zmyslony M, Rajkowska E. Effect of 7 mT static magnetic field and iron ions on rat lymphocytes: apoptosis, necrosis and free radical processes. Bioelectrochemistry. 2002;57:107-111.

Yan JG, Agresti M, Bruce T, Yan YH, Granlund A, Matloub HS. Effects of cellular phone emissions on sperm motility in rats. Fertility Sterility, 2007;88(4):957-964.

Jolly C, Morimoto RI. Role of the heat shock response and molecular chaperones in oncogenesis and cell death. J Natl Cancer Inst. 2000;92:1564 -1572.

Juutilainen J, Matilainen P, Saarikoski S, Läärä E, Suonio S. et al. Early pregnancy loss and exposure to 50 Hz magnetic fields. Bioelectromagnetics 1993;14:220-236.

Kesari KK, Behari J. Comparative study of 900MHz and 2. 45 GHz radiation effect on reproductive system of male rats. In: Recent Advances and Challenges in Reproductive Health Research. (RS Sharma, A Rajanna, M Rajalakshmi. Proceedings of the conference on "Recent Advances and Challenges in Reproductive Health Research (Feb 19-21, 2007 New Delhi) ICMR Publication, 2008.

Kesari KK, Behari J. Fifty gigahertz microwave exposure effect of radiation on rat brain. Appl Biochem Biotechnol 2009;158:126-139.

Kesari KK, Behari J. Microwave exposure affecting reproductive system in male rats. Appl Biochem Biotechnol. 2010;31(6):495-498.

Kesari KK, Behari J. Evidence for mobile phone radiation exposure effects on reproductive pattern of male rats: Role of ROS. Electromagnetics Biology Medicine. 2012;31(3):213-222.

Kesari KK, Kumar S, Nirala J, Siddiqui MH, Behari J. Biophysical evaluation of radiofrequency electromagnetic field effects on male reproductive pattern. Cell Biochem Biophys 2012;Aug 29;DOI 10. 1007/s12013-012-9414-6

Kesari KK, Kumar S, Behari J. Effects of radiofrequency electromagnetic wave exposure from cellular phones on the reproductive pattern in male Wistar rats. Appl Biochem Biotechnol 2011;164(4):546-59.

Kim YW, Kim HS, Lee JS, Kim YJ, Lee SK, Seo JN, Jung KC, Kim N, Gimm YM. Effects of 60 Hz 14 µT magnetic field on the apopotosis of testicular cell in mice. Bioelectromagnetics 2009;30(1):66-72.

Kilgalton SJ, Simmons LW. Image content influences men's semen quality. Biol Lett. 2005; 1, 385-393.

Kodama H, Yamaguchi R, Fukada J, Kasai H, Tanaka T. Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. Fertil Steril. 1997;68, 519-524.

Kumar S, Kesari KK, Behari J. Evaluation of genotoxic effect in male wistar rats following microwave exposure. Ind J. Exp Biology 2010;48, 586-592.

Kumar S, Kesari KK, Behari J. The therapeutic effect of a pulsed electromagnetic field on the reproductive pattern of male wistar rats exposed to a 2. 45 GHz microwave field. Clinics 2011;66(7)1237-1245.

Kumar S, Kesari KK, Behari J. The influence of microwave exposure on male fertility. fertility and sterility. 2011a;95 (4); 1500-1502.

This foregoing document was electronically filed with the Public Utilities

Commission of Ohio Docketing Information System on

12/14/2015 2:39:03 PM

in

Case No(s). 14-1160-EL-UNC

Summary: Public Comment Part 2 filed on behalf of various consumers electronically filed by Ms. Donielle M Hunter on behalf of PUCO Docketing