

and 2.2 W/kg exposure, while in the second experiment the incidence at 1.4 W/kg was significantly reduced.

The experiment by Anane et al. [25] is inconclusive not only because of the divergent results of the two experiments at the same exposure condition (1.4 W/kg SAR) but mainly because of the insufficient size of experimental groups. With a 70% background tumor incidence as observed in this investigation even for an increase to 100% in the exposed group the power to detect this difference at a significance level of 5% is less than 60%. Furthermore, considering experimental and biological variation substantial differences may occur by chance simply due to different distribution of background risk between experimental groups. Therefore, in contrast to the statement of authors that relevant differences would be detected with 16 animals per group, the study was severely underpowered and prone to spurious effects from uneven distribution of background risk. Also stress from confinement of animals could have contributed to the ambiguous results.

Yurekli et al. [26] report an experiment in male Wistar albino rats with the aim to analyze oxidative stress from whole-body exposure to a GSM 945 MHz signal at a SAR level of 11.3 mW/kg. In a gigahertz transverse (GTEM) cell a base station exposure in the far field was simulated. Two groups of rats, 9 animals in each group, were either exposed 7 h a day for 8 days or sham exposed. At the end of the exposure blood was withdrawn and malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD) were measured. MDA as well as SOD was significantly increased after exposure compared to sham, while GSH was significantly reduced. These results indicate that exposure may enhance lipid peroxidation and reduce the concentration of GSH which would increase oxidative stress. A disadvantage in this experiment was that the experiments were carried out sequentially and therefore animals differed in weight and no blinding could be applied.

In a series of experiments conducted in the Kashima Laboratory, Kamisu, Japan, different *in vitro* assays were applied to test whether irradiation with 2.1425 GHz, which corresponds to the middle frequency allocated to the down-link signal of IMT-2000 (International Mobile Telecommunication 2000, a 3G wide-band CDMA system), leads to cellular responses relevant for human health [27–29]. In the first experiment phosphorylation and gene expression of p53 was assessed [27]. In the second experiment heat-shock protein expression was evaluated in the human glioblastoma cell line A172 and human IMR-90 fibroblasts [28]. The effect of exposure of BALB/T3T cells on malignant transformation, on promotion in MCA (3-methylcholanthrene) treated cells, and on co-promotion in cells pretreated with MCA and co-exposed to TPA (12-O-tetradecanoylphorbol-13-acetate) was investigated by Hirose et al. [29]. In none of these experiments applying the same exposure regimen but different intensities and exposure durations (80 mW/kg SAR up to 800 mW/kg SAR, 2 h to several weeks) an effect of exposure was observed. Exposure facility comprised of two anechoic chambers allowing blinded simultaneous exposure of an array

of 7 × 7 dishes in each chamber. Dishes were placed in a culture cabinet located in the anechoic chamber and exposed to radiation from a horn antenna whose signals were focused by a dielectric lens to obtain homogenous irradiation of the dishes. Details of the exposure protocol were not disclosed. It is stated that an IMT-2000 signal at a chiprate (a chip is a byte of information) of 3.84 Mcps was used for exposure. Assuming that it did not contain any low-frequency components as typically present in actual exposures the implications of the findings are unclear. It is rarely supposed that the high-frequency components of RF-EMFs itself are able to elicit any relevant effects in the 'low-dose' range. Rather low-frequency modulation may contribute to biological responses. Therefore, results of these Japanese investigations are of limited value for risk assessment, conditional on them having no such biologically relevant exposure attributes.

4. Discussion

Although there is considerable public concern about adverse health effects from long-term exposure to microwaves from mobile phone base stations there are only few studies addressing this issue. Several reasons can be identified for the scarcity of scientific investigations. First of all, WHO has discouraged studies of base stations, at least concerning cancer as endpoint, because retrospective assessment of exposure was considered difficult. Also COST 281 did not recommend studies of base stations and stated in 2002: "If there is a health risk from mobile telecommunication systems it should first be seen in epidemiological studies of handset use."

It is not appreciated that there are substantial and important differences between exposure to handsets and base stations. The typically very low exposure to microwaves from base stations, rarely exceeding 1 mW/m², was deemed very unlikely to produce any adverse effect. Assuming energy equivalence of effects a 24 h exposure at 1 mW/m² from a base station would be roughly equivalent to 30 min exposure to a mobile phone operating at a power of 20 mW (average output power in areas of good coverage). Because we do not know whether time-dose reciprocity holds for RF-EMF and whether there is a threshold for biological effects, there is no *a priori* argument why such low exposures as measured in homes near base stations could not be of significance for wellbeing and health. As an example from a different field of environmental health consider noise exposure: it is well known that at noise levels exceeding 85 dB(A) a temporary shift of hearing threshold occurs and that, besides this short-term effect, after years of exposure noise induced hearing loss may occur. On the other hand, at a sound pressure of more than a factor of 1000 below, when exposure occurs during the night, exposed individuals will experience sleep disturbances that could affect health in the long run. From this example it follows that exposure may have qualitatively different effects at different exposure levels.

The most important difference between mobile phone use and exposure from base station signals is duration of exposure. While mobile phones are used intermittently with exposure duration seldom exceeding 1 h per day, exposure to base stations is continuous and for up to 24 h a day. It has also to be mentioned that the exposure of mobile phone users is in the near field and localized at the head region, while base stations expose the whole body to the far field. Strictly speaking exposure from mobile phones and their base stations have almost nothing in common except for the almost equal carrier frequency that is likely of no importance for biological effects.

Concerning reconstruction of exposure to base station signals there is no greater difficulty than for retrospective assessment of exposure to mobile phones. It is not always necessary to determine exposure precisely. For epidemiological investigations it often suffices to have a certain gradient of exposures. As long as any two persons can be differentiated along such a gradient epidemiological investigations can and should be carried out.

There are seven field studies of wellbeing and exposure to base station signals available to date. Two were in occupational groups working in a building below [11] or below as well as opposite a building with a roof-mounted base station antenna [10]. The other five were in neighbors of base stations: Santini et al. [5,6], Navarro et al. [8], Hutter et al. [9], Blettner et al. [7], and Thomas et al. [12]. Studies had different methodologies with the least potential for bias in the studies of Hutter et al. [9] and Blettner et al. [7]. All other studies could be biased due to self-selection of study participants. One study explored personal dosimetry during 24 h [12] but results were inconclusive due to insufficient power and omission of nighttime measurements. The study of Blettner et al. [7] had an interesting design with a first phase in a large population based representative sample and a second phase with individual measurements in the bedrooms of participants that were a subgroup of the larger sample. Unfortunately this second sample did not contain a sufficiently large fraction of individuals with relevant exposure (99% had bedside measurements below 0.3 mW/m^2).

Despite some methodological limitations of the different studies there are still strong indications that long-term exposure near base stations affects wellbeing. Symptoms most often associated with exposure were headaches, concentration difficulties, restlessness, and tremor. Sleeping problems were also related to distance from base station or power density, but it is possible that these results are confounded by concerns about adverse effects of the base station, or more generally, by specific personality traits. While the data are insufficient to delineate a threshold for adverse effects the lack of observed effects at fractions of a mW/m^2 power density suggests that, at least with respect to wellbeing, around $0.5\text{--}1 \text{ mW/m}^2$ must be exceeded in order to observe an effect. This figure is also compatible with experimental studies of wellbeing that found effects at 2.7 and 10 mW/m^2 .

There are regular media reports of an unusually high incidence of cancer in the vicinity of mobile phone base stations. Because there are several hundred thousand base stations operating all over the world some must coincide by chance with a high local cancer incidence. Regionally cancer incidence has a distribution with an overdispersion compared to the Poisson distribution. Overdispersion is predominantly due to variations in the distribution of age and gender. Therefore, a much higher number of cases than expected from average incidences can occur by chance. Unfortunately there are no multi-regional systematic investigations of cancer incidence related to mobile phone base stations available to date. Only studies in a single community, one in Bavaria [14] and one in Israel [15], have been published that reported a significantly increased incidence in an area of 400 and 350 m around a base station, respectively. Although incidence in proximity to the base station strongly exceeded the expected values and was significant even considering overdispersion in the case of the Neila study in Bavaria, still no far reaching conclusions can be drawn due to the ecological nature of the studies. However, both studies underline the urgent need to investigate this problem with an appropriate design. Neubauer et al. [30] have recommended focusing initially on short-term effects and 'soft' outcomes given the problems of exposure assessment. However, as has been mentioned previously, the problems of exposure assessment are less profound as often assumed. A similar approach as chosen in the study of leukemia around nuclear power plants [31] could be applied also for studying cancer in relation to base station exposure. Such a case-control design within areas around a sufficiently large sample of base stations would provide answers to the questions raised by the studies of Eger et al. [14] and Wolf and Wolf [15].

In 2003 the so-called TNO study [19] had received wide publicity because it was the first experimental investigation of short-term base station exposure in individuals that rated themselves sensitive to such signals. A lot of unfounded criticism was immediately raised such as complaints about the limited sample size and the not completely balanced design. But also valid arguments have been put forward. The consecutive tests with all experimental conditions presented one after the other could result in sequential effects that may not be completely removed by balancing the sequence of exposures. In several countries follow-up studies were initiated two of which have already been published [21,23]. One of these experiments partly supported the TNO study the other found no effect. While the study of Regel et al. [21] closely followed the conditions of the previous experiment only avoiding the shortcomings of a sequential within-day design and improvements by including two intensities of UMTS exposure, the study of Eltiti et al. [23] had a different procedure and included physiological measurements. Regel et al. [21] applied the same questionnaire as has been used in the TNO study. Because non-sensitive participants and sensitive participants during sham exposure (despite their almost 10 years younger age) reported considerably lower wellbeing,

it is possible that the experimental setup was more adverse and imposed too much stress such that these conditions confounded the effect of the base station exposure. Results of the other replication experiment of Eltiti et al. [23] may be compromised by an imbalance in the sequence of experiments with more sensitive participants receiving UMTS exposure in the first session. Hence, based on available evidence, it cannot be firmly decided whether such weak signals as applied in these experiments to simulate short-term base station exposure affects wellbeing.

Concerning animal experiments and in vitro investigations the data base is insufficient to date. While in vivo exposure of Wistar albino rats [26] imply an induction of oxidative stress or an interaction with antioxidant cellular activity, in vitro experiments [27] found no indication of cellular stress in human glioblastoma cells and fibroblasts. While some may be inclined to attribute effects in the low-dose range to experimental errors there is the possibility that the characteristics of the exposure that are relevant for an effect to occur simply vary in the experiments and lead to ambiguous results. As long as these decisive features of the exposure (if they actually exist) are unknown and in particular the type and components of low-frequency modulation vary across experiments, it is impossible to coherently evaluate the evidence and to come to a science based conclusion.

Overall results of investigations into the effects of exposure to base station signals are mirroring the broader spectrum of studies on handsets and on RF-EMF in general. There are indications from epidemiology that such exposures affect wellbeing and health weakly supported by human provocation studies and an inconclusive body of evidence from animal and in vitro studies.

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Critique interphone 11/11 critique

Review

Estimating the risk of brain tumors from cellphone use: Published case–control studies

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Abstract

This paper reviews the results of early cellphone studies, where exposure duration was too short to expect tumorigenesis, as well as two sets of more recent studies with longer exposure duration: the Interphone studies and the Swedish studies led by Dr. Lennart Hardell. The recent studies reach very different conclusions. With four exceptions the industry-funded Interphone studies found no increased risk of brain tumors from cellphone use, while the Swedish studies, independent of industry funding, reported numerous findings of significant increased brain tumor risk from cellphone and cordless phone use. An analysis of the data from the Interphone studies suggests that either the use of a cellphone protects the user from a brain tumor, or the studies had serious design flaws. Eleven flaws are identified: (1) selection bias, (2) insufficient latency time, (3) definition of 'regular' cellphone user, (4) exclusion of young adults and children, (5) brain tumor risk from cellphones radiating higher power levels in rural areas were not investigated, (6) exposure to other transmitting sources are excluded, (7) exclusion of brain tumor types, (8) tumors outside the cellphone radiation plume are treated as exposed, (9) exclusion of brain tumor cases because of death or illness, (10) recall accuracy of cellphone use, and (11) funding bias. The Interphone studies have all 11 flaws, and the Swedish studies have 3 flaws (8, 9 and 10). The data from the Swedish studies are consistent with what would be expected if cellphone use were a risk for brain tumors, while the Interphone studies data are incredulous. If a risk does exist, the public health cost will be large. These are the circumstances where application of the Precautionary Principle is indicated, especially if low-cost options could reduce the absorbed cellphone radiation by several orders of magnitude.

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Keywords: Electromagnetic field; Cellphone; Brain tumor; Mobile phone; Cellular phone; Cordless phone; Glioma; Acoustic neuroma; Meningioma; Funding; Interphone

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1. Introduction

This review covers all case-control studies on the risk of brain tumors from cellphone use published up to March 2009 and does not include epidemiological studies on the risk of brain tumors from exposure to other sources of electromagnetic fields (EMFs). It examines the strengths and weaknesses of these studies and what can be learned from differences in the findings. Because certainty is not possible in science, much less in epidemiology, the indication of a possible risk of brain tumors from cellphone use suggests that the Precautionary Principle be applied.

In almost all epidemiological investigations of rare diseases, such as brain tumors, researchers use what is known as a case-control design. Cases are subjects who have the disease and controls are randomly chosen subjects without the disease. Typically controls are matched to the cases by age, gender, geographical area, and income. Subjects are asked a set of questions, which for a cellphone study would include questions about their cellphone use.

The Odds Ratio (OR), the increased risk ($OR > 1.0$), or decreased risk ($OR < 1.0$) of brain tumors as a result of exposure to cellphone radiation is reported. A two-by-two table is used to calculate the Odds Ratio. In Table 1, Case and Control subjects are in the rows and Exposed and Unexposed subjects are in the columns. The Odds Ratio = (Exposed Cases) \times (Unexposed Controls)/(Exposed Controls) \times (Unexposed Cases).

Actual studies use sophisticated statistical regression analysis to adjust for confounding effects (age, gender, smoking, etc.), but the basic concept is the same. Additionally, along with the Odds Ratio, a 95% confidence interval (CI) is reported.

In this discussion cellphone studies are grouped into early studies and later studies. The later studies are presented as

two sets of studies. Since each set uses a common protocol, each can be considered a single study: The two sets are the industry-funded Interphone studies and the independently funded Swedish studies led by Dr. Lennart Hardell.

2. Early case-control cellphone studies

The salient fact of these early studies is the short duration of cellphone use. It would have been surprising to find any risk of a brain tumor, because an increased risk would have required a short latency time between exposure and diagnosis. Indeed, none of these studies reported finding a significant risk ($p \leq 0.05$) of a brain tumor from cellphone use. Yet, as can be seen in Table 2, each study did find a non-significant ($p > 0.05$) increased risk including two near-significant findings of increased risk ($p < 0.10$). And, Auvinen et al. found that for each year of cellphone use a significant 20% increased risk of a brain cancer (glioma). Table 2 summarizes these studies [1–5].

Perhaps these early studies that found no significant risk had actually found an early warning of trouble ahead.

3. The industry-funded Interphone study

The Interphone study is a 13-country case-control study on the risk of brain and salivary gland tumors from cellphone use. The Interphone study uses a standard protocol such that all individual country results can be pooled together to increase the power of the study. This discussion is limited to the brain tumors studies.

As of December 2008 there have been 11 single-country and 3 multi-country Interphone brain tumor studies published [6–19]. The multi-country studies will not be discussed

Table 1
Simple example of increased risk.

	Exposed	Unexposed	Totals
Cases	60	40	100
Controls	49	51	100
Totals	109	91	200
Odds Ratio	1.56		

Table 2
Early cellphone case-control studies.

Study	Cases	Eligibility		Av. use time years	Major findings			Comments	
		Start	End		OR	95% CI	p value		
Hardell et al., May 2000 [1]	209	1994–1996 (Uppsala-Orebro) 1995–1996 (Stockholm)		Not reported	2.42	0.97	6.05	0.053 [†]	Temporal, parietal, occipital lobes—ipsilateral use (number of cases not reported)
Muscat et al., December 2000 ^a [2]	469	1994	1998	2.8	2.1	0.9	4.7	0.073 [†]	Neuroepithelial cancer (35 cases)
Inskip et al., January 2001 [3]	782	June-94	August-98	Not reported	1.9	0.6	5.9	0.26	Acoustic neuroma, ≥ 5 years of use (5 cases)
Muscat et al., May 2002 ^a [4]	90	1997	1999	4.1	1.7	0.5	5.1	0.36	Acoustic neuroma, 3–6 years of use (11 cases)
Auvinen et al., May 2002 ^a [5]	398		1996	Av. 2–3 (analog) <1 (digital)	1.7	0.9	3.5	0.12	Glioma, >2 years of use (number of cases not reported)
					1.2	1.0	1.4	0.050	Glioma, increase risk per year (number of cases not reported)

Bold indicates statistically significant ($p \leq 0.05$).

^a Industry funded study.

[†] Near-significant ($p \leq 0.10$).

Table 3
Summary data of the 11 Interphone studies investigated.

Study, Country	Tumor	Dx eligibility range years	% Cases ≥ 10 years	% of eligible controls refusing participation	"Regular" use from abstract	
					OR	CI
Lönn et al. 2004, Sweden	AN	3.0	9.5%	27.9%	1.0	0.6 to 1.5
Christensen et al. 2004, Denmark	AN	2.0	1.4%	36.0%	0.90	0.51 to 1.57
Lönn et al. 2005, Sweden	G	2.0	6.7%	29.5%	0.8	0.6 to 1.0
	M		4.4%		0.7	0.5 to 0.9
Christensen et al. 2005, Denmark	L-g G	2.0	5.6%	36.0%	1.08	0.58 to 2.00
	H-g G		3.4%		0.58	0.34 to 0.90
	M		3.4%		1.00	0.54 to 1.28
Schüz et al. 2006, Germany	G	3.0	3.3%	39.0%	0.98	0.74 to 1.29
	M		1.3%		0.84	0.62 to 1.13
Takebayashi et al. 2006, Japan	AN	3.9	8.2%	15.8%	0.73	0.43 to 1.23
Klaeboe et al. 2007, Norway	AN	2.0	0.0%	31.0%	0.5	0.2 to 1.0
	G		0.0%		0.6	0.4 to 0.9
	M		0.0%		0.8	0.5 to 1.1
Hours et al. 2007, France	AN	2.5	0.0%	28.8%	0.92	0.53 to 1.59
	G		0.0%		1.15	0.65 to 2.05
	M		0.0%		0.74	0.43 to 1.28
Hepworth et al., 2007, United Kingdom	G	3.3	6.8%	65.5%	0.94	0.78 to 1.13
Schlehofer et al., 2007, Germany	AN	3.1	0.0%	45.1%	0.67	0.38 to 1.19
Takebayashi et al. 2008, Japan	G	3.9	2.3%	56.3%	1.22	0.63 to 1.27
	M		4.5%	12.9%	0.70	0.42 to 1.16
Weighted average (by cases)		2.7	6.2%	40.7%		
Wt. Av. (by cases) excluding Lönn (2004) and Hepworth		2.7	6.2%	33.4%		

AN = acoustic neuroma; G = glioma; M = meningioma; L-g G = low-grade glioma; H-g G = high-grade glioma. Bold OR indicates statistically significant protection.

further because they overlap the single-country studies [17–19]. Table 3 summarizes the 11 Interphone studies included in this analysis. Three studies had no cases who had used a cellphone for ≥ 10 years [12,14,16]. For Odds Ratios (ORs) on the risk of brain tumors from “regular” cellphone use (reported in the abstracts) there were 15 ORs < 1.0 —a protective result (4 with significant protection), and 2 ORs were > 1.0 —a result indicating increased risk. The cumulative binomial probability of having 15 ORs < 1.0 and 2 ORs > 1.0 is highly unlikely ($p = 0.0012$) and indicates a significant protective effect.

Table 3 summarizes the 11 Interphone studies analyzed in this paper. It shows the years available for case diagnosis (Dx) to be eligible for participation in the study, the percentage of cases that used a cellphone for 10 or more years, the percentage of selected controls that refused to participate in the study, and the Odds Ratios of brain tumors for “regular” cellphone use reported in the abstract of each paper. Finally, weighted (by cases) averages are presented for the Dx years for eligibility in the studies, the % of cases that used a cellphone for ≥ 10 years, and the % of eligible controls that refused participation.

All 14 of these Interphone brain tumor studies have found that use of a cellphone *protects* the user from a brain tumor. The 11 studies reported a total of 284 statistically independent ORs; 217 ORs < 1.0 and 67 ORs > 1.0 ($p = 6.2 \times 10^{-20}$). There are two possibilities to explain such an incredulous result: (1) Either use of a cellphone provides protection from a brain tumor, or (2) the Interphone Protocol [20] has serious design flaws.

Eleven design flaws have been identified. The consistent findings of protection can be explained because 8 of these 11 flaws underestimate the risk of brain tumors.

3.1. Flaw 1: selection bias

In a case–control cellphone study both cases and controls are asked if they would like to participate in the study. It is reasonable to assume controls who use a cellphone are more likely to participate than controls who do not use a cellphone. This would result in selection bias. And, such selection bias would result in an underestimation of risk.

The impact of selection bias increases as the percentage of controls that refuse to participate increases. The Interphone control weighted-average refusal rate was a remarkably high 41%. Dr. Sam Milham, an occupational epidemiologist with over 100 published papers, states that in the past, science journals would not accept a study with such a high refusal rate [21].

One Interphone study investigated the possibility of selection bias by asking controls that refused participation if they used a cellphone; 34% said they used a cellphone and 59% said they did not use a cellphone, confirming selection bias in that study [6].

How could selection bias have been mitigated? First, do not tell subjects the study is a cellphone study. Second, pay

Table 4
Odds Ratios, with and without selection bias.

	Exposed	Unexposed	Totals
With selection bias			
Cases	60	40	100
Controls	60	40	100
Totals	120	80	200
Odds Ratio	1.00		
Without selection bias			
Cases	60	40	100
Controls	49	51	100
Totals	109	91	200
Odds Ratio	1.54		

Exposed Controls = (60 user “participating” controls) \times (59% participation) + (34 cellphone users among non-participating controls) \times (41% non-participants) = 49.

the subjects for participation in the study. The result would be a higher participation rate, and more importantly, control participation would not be biased for use, or non-use of a cellphone. However, given the funding provided, paying subjects was not considered.

Table 4, using semi-hypothetical data (i.e., data that approximates actual Interphone data), illustrates how the Odds Ratios will change when selection bias exists and when the selection bias has been eliminated. As can be seen the Odds Ratio increases from 1.00 (no risk) to 1.54. Inversely stated, with selection bias a finding of no risk would mask an actual risk.

3.2. Flaw 2: insufficient latency time

The known latency time (the time between exposure and diagnosis) for brain tumors is 30+ years [22], similar to lung cancer from smoking [23] and mesothelioma from asbestos exposure [24]. Ten or more years was the longest cellphone use time reported. The weighted-average of brain tumors cases with ≥ 10 years of cellphone use was 6.2% of all cases, or 16 cases per study. Not including sufficient numbers of longer-term cellphone users results in an underestimation of risk.

To resolve this problem would require about a 3-fold increase in subjects. Because the weighted-average diagnosis eligibility time was only 2.7 years (the date range for cases to be diagnosed with a brain tumor to be eligible for the study), only a small number of subjects were available. There was insufficient funding to increase the eligibility time.

It is worth noting, two independently funded cellphone case–control studies, used a 6-year eligibility time. These two studies showed a consistent risk of brain tumors for ≥ 10 years of cellphone use [25,26].

3.3. Flaw 3: definition of “regular” cellphone user

The Interphone Protocol defines “regular” cellphone use as use for at least once a week for 6 months or more with any cellphone use 1 year prior to diagnosis excluded. Based on UK cellphone subscriber data [27] and the UK study’s Dx eligibility dates [13], the rapid rise of cellphone use finds 85% of “regular” UK users had used a cellphone for less than 5 years; 98% of “regular” UK users had used a cellphone for less than 10 years (all Interphone countries have similar rapid increases in cellphone users). Given known latency times how could any risk of brain tumors be expected for “regular” users? Inclusion of such a large proportion of short-term users underestimates the risk of brain tumors.

Dr. Elizabeth Cardis, the head of the Interphone study stated, “Reporting ‘regular’ user [data] was not intended to be a risk factor.” [28]. Yet, the abstract of every Interphone brain tumor study highlights that there is no risk of brain tumors from “regular” cellphone use.

3.4. Flaw 4: exclusion of young adults and children

The Interphone Protocol requires subjects to be between 30 and 59 years of age (some studies have included ages as low as 20). There is strong evidence that the young adults and children are at greater risk from exposure to carcinogens than mature adults suggesting that the young, with greater cell growth, are more vulnerable to genetic mutations.

Two cellphone studies report higher brain tumor risks in young adults (20–29 years of age) compared to mature adults. The first study found a 7-fold increased risk of brain tumor compared to a 1.40-fold risk for all adults [29], and the second study found a 3.2-fold risk of brain tumor [30] compared to 2-fold risk in older adults. An ionizing radiation brain tumor study found the younger a child’s age, the greater the risk of brain tumors (4.6-fold/Gy risk of brain tumors for children less than 5 years of age; 3.2-fold/Gy risk for children 5 to 9 years of age, and; 1.47-fold/Gy risk for children 10 or more years) [22].

Inclusion of additional cases below 30 years would have provided greater insight into risk, but the additional cases would have increased the cost of the study.

3.5. Flaw 5: brain tumor risk from cellphones radiating higher power levels in rural areas were not investigated

Because rural users are farther away from the cell towers compared to urban users, the cellphone’s radiated power is higher [31]. Unfortunately the Interphone studies selected mostly metropolitan areas to locate brain tumor cases. When higher radiated power is not included there is an underestimation of risk.

In order to have sufficient cases to achieve statistical power, the total number of cases and controls who live in rural areas would have to be increased. This would require additional funding compared to what was provided.

Table 5
Change in Odds Ratios cordless phone use is not included and when it is included.

	Exposed	Unexposed	Totals
Cordless phone exposure treated as unexposed			
Cases	43	57	100
Controls	27	73	100
Totals	70	130	200
Odds Ratio	2.0		
	Truly exposed	Unexposed	Totals
Cordless phone exposure treated as exposed			
Cases	64	36	100
Controls	40	60	100
Totals	104	96	200
Odds Ratio	2.6		

Truly Exposed Controls = (27 “Exposed” Controls) × (64 truly exposed cases/43 “Exposed” Cases) = 40.

3.6. Flaw 6: exposure to other transmitting sources are not considered

Subjects who use cordless phones, walkie-talkies, Ham radio transmitters, etc. are treated as unexposed in the Interphone study when in fact they are exposed. Again, it is important to note that two independently funded cellphone case-control studies treated cordless phone use as exposed, and found that cordless phone use results in an increased risk of brain tumors. [25,26]. Treating exposed subjects as unexposed, once again, underestimates the risk of brain tumors.

Table 5 illustrates how the Odds Ratio would change if cordless phone users had been treated as exposed subjects. The first Odds Ratio table assumes a 2.0-fold risk. Additionally it assumes that 57% of cases did not use a cellphone (without considering cordless phone use). The second table assumes when cordless phone use is considered that the number of unexposed cases decreases to 36%. An additional assumption is cordless phones have the same risk of brain tumors as do cellphones. Given these assumptions we see that the inclusion of cordless phone use as an exposure increased the 2.0-fold risk to a 2.6-fold risk.

3.7. Flaw 7: exclusion of brain tumor types

The Interphone study includes three brain tumor types: acoustic neuroma, glioma and meningioma. Other types are excluded (e.g. brain lymphoma, neuroepithelial, etc.). Exclusion of these other tumors underestimates the risk of brain tumors.

Interestingly, as shown in Table 2 above, another industry-funded study reported a 2.1-fold risk of a neuroepithelial brain tumor [2] and an industry-funded cellphone study showed an excess risk of lymphoma in mice [32]. Given this knowledge it is surprising that all brain tumor types were not included.

3.8. Flaw 8: tumors outside the cellphone's radiation plume are treated as exposed

The radiation plume's volume is a small proportion of the brain's volume. Treating tumors outside the radiation plume as exposed tumors results in an overestimation of risk (the only flaw that overestimates risk).

The adult brain absorbs the cellphone's radiation almost entirely on the side of the head where the cellphone is held (ipsilateral); almost no radiation is deposited on the opposite side of the head (contralateral). In adults the ipsilateral temporal lobe absorbs 50–60% of the total radiation and is ~15% of the brain's volume. The ipsilateral cerebellum absorbs 12–25% of the total radiation and is ~5% of the brain's volume. Thus, 62–85% of the cellphone's radiation is absorbed by ~20% of an adult's brain's volume [33]. Because a child's brain absorbs far more radiation than an adult's brain, this data are not applicable for a child's brain.

Table 6, using semi-hypothetical data, shows how the Odds Ratio will change when all tumors are treated as exposed and when only tumors within the cellphone's radiation plume are treated as exposed. This hypothetical example assumes there is a 2.0-fold risk when all tumors are treated as exposed, and assumes that only 20% of the tumors are actually exposed. Per these assumptions, the apparent 2.0-fold risk is reduced to a 1.6-fold risk.

Because the proportion of all brain tumors to the tumors within the radiation plume is small, a larger (roughly 5-fold) number of subjects would be required. However, the funding provided, did not allow for such a large increase in subjects.

A recent paper showing changes in the brain's blood brain barrier (BBB) permeability reported, counter-intuitively, that the effect of the highest permeability of the BBB (highest leakage) occurs at lower exposures [34]. The effect of this phenomenon is that almost all the leakage from a GSM cellphone occurs deep in the brain and on the contralateral side.

Table 6
Odds Ratios with all tumors exposed and without all tumors exposed.

	Exposed	Unexposed	Totals
With flaw 8 design error			
Cases	75	25	100
Controls	60	40	100
Totals	135	65	200
Odds Ratio	2.0		
	Truly exposed	Unexposed	Totals
Without flaw 8 design error			
Cases	15	70	85
Controls	12	88	100
Totals	27	158	185
Odds Ratio	1.6		

Truly Exposed Cases = (75 "exposed" cases) × (20% brain exposed) = 15.
Truly Exposed Controls = (60 "exposed" controls) × (20% brain exposed) = 12.

Whether this is similar for the induction of brain tumors is unknown. However, whether or not it is similar does not negate the fact that the cellphone's radiation plume is in a small proportion of the total brain's volume.

3.9. Flaw 9: exclusion of brain tumor cases because of death or too ill to respond

A large number of brain cancer (glioma) cases died before they could be interviewed or were too ill to be interviewed. Common practice would be to interview a proxy (e.g., a spouse). The Interphone Protocol requires use of proxies in case of death [20], yet 3 of the 7 glioma studies excluded deceased, or too ill to be interviewed cases from their studies [9,12,13] and a 4th did not use proxies for all of the cases who were too ill to be interviewed or who had died [10]. The weighted average of these exclusions was 23% of all glioma cases. This flaw limits determining the risks, if any, from the most deadly and debilitating brain tumors from cellphone use.

Another study found significant risks for high-grade glioma (the most deadly), but not for low-grade glioma (the least deadly) [35].

3.10. Flaw 10: recall accuracy of cellphone use

Memory accuracy, particular in the distant past, is limited at best. The Interphone project investigated this problem by asking cellphone users to recall their cellphone use, and then compared the recall to billing records.

The study reported that light cellphone users tend to underestimate their use and heavy users tend to overestimate their use. This results in an underestimation of risk [36].

Accurate data for the Interphone study could have been obtained by accessing subjects' cellphone-billing records as was done in the study of recall bias [36]. It is reasonable to assume that the available funding did not support the gathering of billing records.

3.11. Flaw 11: funding bias

If studies are funded by an entity with a financial interest in the findings, it has been shown that, more often than not, the findings of such a study are favorable to the financial interest compared to studies where the funding has no financial interest.

Dr. Henry Lai at Washington University in Seattle maintains a database of cellphone biological studies. The results (Table 7) from his database (July 2007) report the magnitude of funding bias. The industry-funded studies found an effect in 28% of the studies and the independently funded studies found an effect 67% of the time. The probability that this is a chance finding is extraordinarily minute ($p = 2.3 \times 10^{-9}$).

A study on the source of funding of cellphone studies and the reported results reported, "We found that the studies funded exclusively by industry were indeed substantially less likely to report statistically significant effects on a range of

Table 7
Industry-funded and independently funded cellphone biological studies.

		Cellphone biological studies					
		Effect found		No effect found		Studies	% all studies
		Studies	% all studies	Studies	% all studies		
Industry funded	No.	27	8.3%	69	21.2%	96	29.4%
	%	28.1%		71.9%			
Independently funded	No.	154	47.5%	76	23.5%	230	70.6%
	%	67.0%		33.0%			
Totals		181	55.5%	145	44.5%	326	100.0%

$\chi^2 = 39.8$ ($p = 2.3 \times 10^{-9}$) 11 July 2006.

end points that may be relevant to health" (probability of industry-funded study reporting at least one significant result is 0.11, CI: 0.02–0.78) [37].

Financial bias is pervasive across all fields of science. It is sufficiently pervasive that books have been written on the subject and science journals have brought it to the attention of their readers. A search for books about "Funding Bias in Science" at Amazon.com found 86 titles [38].

In a review of the book "Science in the Private Interest: Has the Lure of Its Profits Corrupted Biomedical Research?" by Sheldon Krinsky, Dr. Roger Porter wrote, "The major theme of this superb book, therefore, is the degradation of the academic scientist, who is lured to the pecuniary gains offered by industry and now asks the scientific questions posed by industry instead of independently pursuing scientific investigation of public needs." [39].

A news report in the British Medical Journal reported, "Four German public health scientists have been publicly criticised in Der Spiegel magazine for accepting funding from the tobacco industry in return for supporting tobacco friendly research projects and policies in the 1980s." [40].

A substantial portion of the Interphone study funding comes from the cellphone industry. For European studies, industry has provided more than €3.2 million (\$5.1M) [27], another \$1 million came from the Canadian Wireless Telecommunications Association [41] and it is unknown if industry funding has been provided for studies in Japan, Australia and New Zealand.

In addition to the €3.2 million the Interphone Exposure Assessment Committee received funding from the UK Network Operators (O2, Orange, T-Mobile, Vodafone, '3') and French Network Operators (Orange, SFR, Bouygues) [36]. At least one member of this Committee is employed by a cellphone company: Dr. Joe Wiart from France Telecom [20].

Beyond the €3.2 million available to the European Interphone studies, the French study [12] received funding from "Orange, SFR, Bouygues Télécom." [42]; the UK study received funding from O2, Orange, T-Mobile, and Vodafone, and [13]; the Danish study received funds from the for-profit International Epidemiology Institute (IEI). The source of the IEI funds is not stated [9].

Funding for the 5-country Interphone study of acoustic neuroma also came from O2, Orange, T-Mobile, Vodafone, '3' [18].

The Muscat et al. studies [2,4] received around \$600,000 from the Cellular Telecommunication Industry Association (CTIA) via the organization CTIA created and funded, Wireless Technology Research (WTR) [43]. For the Auvinen et al. study "Finnish mobile phone manufacturers contributed to the funding for the TEKES research program." [5].

4. Increased risk Interphone laterality findings

So far the discussion had pertained to the aggregate results of the 11 Interphone brain tumor studies. It is important to note that when significant findings of risk were examined for ≥ 10 years of cellphone use it was found that 2 studies had 3 significantly increased risk results (all 3 were for ipsilateral use). The Swedish Lönn et al. acoustic neuroma study had two significant results showing an increased risk: OR = 3.9 (CI: 1.6 to 9.5) for ≥ 10 years since first ipsilateral use (based on 12 cases), and OR = 3.1 (CI: 1.2 to 8.4) for ≥ 10 years of ipsilateral use (based on 9 cases) [6]. The UK Hepworth et al. glioma study reported OR = 1.24 (CI: 1.02 to 1.52) for ≥ 10 years of ipsilateral use (based on 278 cases) [13].

If we examine Table 3 there is little difference between the parameters of these studies relative to the weighted average of all 11 studies. The Lönn et al. acoustic neuroma study had the third smallest control refusal rate (27.9%), yet the Hepworth et al. glioma study had the highest control refusal rate (65.5%).

When the weighted averages are calculated without the Lönn (2005) and Hepworth studies (see Table 3) the Dx years and the % of cases who used a cellphone for >10 years remained unchanged. However, the % of controls who refused to participate is 33.4% (from 40.7%). This is because the Hepworth study has the highest number of controls of any of the 11 studies (1716 controls, 24% of all controls) and the highest control refusal rate (65.5%) of the 11 studies.

Possibly the increased risk of brain tumors could be the result of laterality recall bias. Yet, as would be expected the ipsilateral ORs are greater than the contralateral ORs in all

3 findings suggesting there was little or no laterality recall bias.

Of the remaining 9 Interphone brain tumor studies, 3 studies had no cases that had used a cellphone for ≥ 10 years, and 3 of the studies with cases that had used a cellphone for ≥ 10 years did not report laterality results [7,9,10]. The Lönn et al. study did find non-significant glioma increased risks for ≥ 10 years since first ipsilateral use and for ≥ 10 years duration of "regular" ipsilateral use (OR = 1.6, CI: 0.8 to 3.4, $p = 0.19$, based on 15 cases, and OR = 1.8, CI: 0.8 to 3.9, $p = 0.14$, based on 14 cases, respectively), and similar increased meningioma risks were found for ≥ 10 years of ipsilateral use and ≥ 10 years duration of "regular" ipsilateral use (OR = 1.3, CI: 0.5 to 3.9, based on 5 cases, and OR = 1.4, CI: 0.4 to 4.4, based on 4 cases, respectively) [8]. Every Interphone Odds Ratio for ≥ 10 years of ipsilateral cellphone use reported an increased risk (OR > 1.0).

This suggests that when the two highest exposures reported in the Interphone study are combined (≥ 10 years of use and ipsilateral use), the resultant increased risk offsets the overall systemic protective skew resulting from the Interphone Protocol's flaws, and an increased risk is found in spite of the systemic protective skew. If true, whatever the reported risk, the actual risk (flaws removed) is larger.

5. The independently funded Swedish studies led by Dr. Lennart Hardell

These studies had virtually no industry funding and were entirely within a single-country: Sweden. Table 8 compares both sets of studies. Clearly the Interphone studies have more cases than the Hardell studies. However, the Hardell studies have more cases that used a cellphone for 10 or more years. Almost certainly, the larger number of long-term users is because of the considerably longer diagnosis eligibility range (range of brain tumor diagnosis dates when cases are eligible to participate in a study).

Because selection bias increases as the control refusal rate increases, the substantially smaller control refusal rate in the Swedish studies mitigates against any significant selection bias while the 3.6-fold larger Interphone studies control refusal rates enhances the problem of selection bias.

The Swedish studies, with some exceptions: did not examine risks in regions exposed to the cellphone's radiation plume (flaw 8), excluded cases who had died or were too ill to be

interviewed (flaw 9), and did not use subjects' billing records (flaw 10). The Interphone studies had all 11 flaws but did include a portion of the cases that had died or were too ill to be interviewed (flaw 9).

In contrast to the Interphone studies results, which appear to be incredulous (i.e., use of a cellphone protects the user from a brain tumor), the Hardell team results are internally consistent if wireless phones (cellphones and/or cordless phones) use is a risk of brain tumors.

- The higher the cumulative hours of use, the higher the risk [35];
- The higher the radiated power, the higher the risk [44];
- The higher the number of years since first use, the higher the risk [35];
- The higher the exposure (tumor on the same side of the head where the cellphone or cordless phone was held), the higher the risk [25,26], and;
- The younger the user, the higher the risk [29].

6. Role of industry

There has been a long history of industry using "science" to counter findings of risk by industry independent scientists [45]. Over many decades multiple industries have perfected a series of techniques used to diminish or delay effective action that is perceived as harmful to their interests [45].

If we examine, the history of tobacco, ionizing radiation, asbestos, and more recently cellphones, we see there has been an extraordinarily long time between first warnings (followed by many more warnings) and the eventual Public Health acknowledgement that there is a problem (Tobacco: 1856–1964; Ionizing Radiation: 1896–1998; Asbestos: 1911–1996; Cellphones: 1993–?) [46].

6.1. Insufficient funding

Arguably, inadequate funding of research projects is the most common reason why previous studies had been unable to detect what was later seen as an obvious risk. Given insufficient funding, many naïve researchers accept the grant and proceed with the best possible study given the financing provided. Here are two examples.

At the 2005 meeting of the Bioelectromagnetics Society a study was presented of rats exposed to cellphone radiation.

Table 8
Comparison of Interphone studies and Swedish Hardell studies.

Study	Total cases	≥ 10 years of use cases	Controls	Participation refusal rate		Dx eligibility range years	Industry funding	Identified flaws
				Cases	Controls			
Interphone ^a	4378	172	7229	14.1% ^b	40.7% ^b	2.69 ^b	\$6.1M+	1 to 11
Hardell	2159	289	2162	11.2% ^c	11.3%	6.00	\$0	8, 9, 10

^a Based on 11 single-country Interphone studies published to date (March 2009).

^b Weighted-average of 11 single country Interphone studies published to date (March 2009).

^c Weighted-average of two pooled studies, "benign" and malignant brain tumors.

The study used 13 rats in two groups: 5 for cellphone radiation effects on rat brains, and 8 for cellphone radiation effects on rat skin [47]. As would be expected, with such a small group of animals, no effects were found. When the presenter was asked why she had used so few animals, she said France Telecom had not given her sufficient funding to use more animals.

A second example, is the Interphone study, with more than €3.2 million (\$5.1M) of industry money for European research teams [27], and another \$1 million from the Canadian Wireless Telecommunications Association (CWTA) [40]. The overwhelming majority of significant Interphone study findings found cellphone use *protects* the user from brain tumors. As discussed above, adequate funding could have eliminated or substantially mitigated the numerous flaws that can account for this incredulous result.

7. Potential public health impact

What is the potential public health impact if cellphone use induces brain tumors? The answer is we do not know, but it is possible to make a rough estimate based on information we have. We can use the CTIA cellphone subscriber data by year [48], and assume that there is a 30-year time delay between first cellphone use and the diagnosis of a brain tumor (latency time). We can also assume that 10% of long-term cellphone users will be diagnosed with a brain tumor, similar to 10% of long-term smokers diagnosed with lung cancer. The result is Fig. 1, which estimates the potential number of cellphone-induced brain tumors by year in the United States. Since Fig. 1 is based on a mathematic model, it can be legitimately challenged (even by this author), and the numerical assumptions adjusted. However, this author finds the shape of the graph, a long time delay followed by a rapid increase in brain tumors, to be highly credible.

As can be seen in Fig. 1, for many years, only a minute number of cellphone-induced brain tumors would be predicted each year (invisible on the scale of the graph). By 2004, the most recent year US brain tumor diagnosis data is available there remains an imperceptible ~1900 cellphone-induced brain tumors. In 2004 the model calculates there would be about 1900 cellphone-induced brain tumors out of ~50,000 brain tumors diagnosed that year [49]. By 2009 an increase can be seen in the graph (but the incidence of brain tumors would not be reported by the government until 2013). After 2009 there is a very rapid increase. The model predicts there will be ~380,000 cellphone-induced brain tumors in 2019.

This would overwhelm the United States public health system. The cost of treating brain tumor patients is on the order of \$250,000 per patient [50]. This translates to a \$9.5B cost in 2019. Since this would also require roughly a 7-fold increase in neurosurgeons within the next 11 years, surgery for the vast majority of cases would not be an option, so the estimated \$9.5B cost would be far less due to lack of treatment resources.

8. Precautionary Principle

Simply put, the Precautionary Principle (PP) is a policy that says if there is some evidence that a problem may exist and low, or no-cost remedial actions are possible, then these actions should be undertaken. Colloquially, we say, "Better safe than sorry." If cellphones induce brain tumors the potential public health costs are large. There is also a simple action that can reduce the absorbed cellphone radiation by several orders of magnitude.

Cellphone radiation decreases as the square of the distance from the phone. As a result even small changes in distance

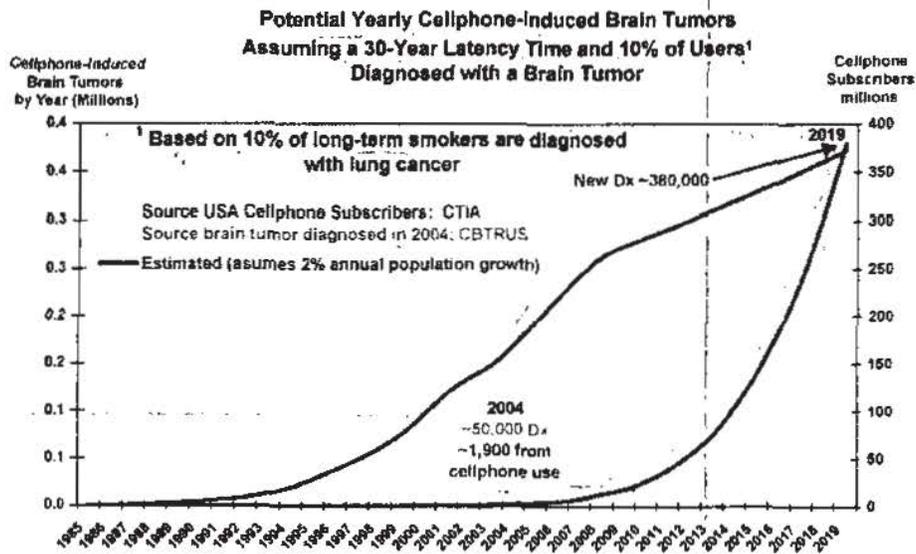


Fig. 1. Long-delay followed by sudden onset of brain tumor epidemic.

have a dramatic effect. For example, when the speaker on the cellphone is placed to the ear, the cellphone is 2 mm from the head and if the cellphone is held 200 mm (100 times) from the head, this change in distance would result in a 10,000-fold reduction in the radiation absorbed by the head.

With use of a headset (not a wireless headset) connected to a cellphone the cellphone is not held directly against the ear and thus the absorbed cellphone radiation could be reduced by several orders of magnitude.

An appropriate PP action would be to mandate cellphone manufacturers to remove the existing cellphone speaker that is placed to the ear and replace it with a headset directly connected to the cellphone. The cost would be near zero (potentially a net cost savings): remove one cellphone speaker—add another speaker (AKA headset).

9. Conclusions

The industry-funded Interphone study has assured the public there is no risk of brain tumors from cellphone use. Yet, a closer analysis of the data leads to the incredulous conclusion that cellphone use protects the user from brain tumors ($p = 6.2 \times 10^{-20}$). A more likely explanation of the data is that the studies were flawed and that there is a link between cellphone use and brain tumors. The Swedish team studies, independent of industry funding, have reported increased brain tumor risk from cellphone use and cordless phone use.

The long history of corporate funded “science” delaying effective action against toxic agents, in some cases up to 100 years, argues convincingly for application of the Precautionary Principle. This is especially true in light of the potentially enormous public health impact should cellphones be shown to cause brain tumors.

The Precautionary Principle clearly applies in this case, since the problem is possible but not certain, and low cost ameliorating actions are easily implemented by industry. With over 3 billion people using cellphones, and with children among the heaviest users, it is time for governments to mandate precautionary measures to protect their citizens.

Conflicts of interest

None.

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Review

Long-term exposure to magnetic fields and the risks of Alzheimer's disease and breast cancer: Further biological research

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↑ Aβ
↓ melatonin (cat)

Abstract

Objective: Extremely low frequency (ELF) and radio frequency (RF) magnetic fields (MFs) pervade our environment. Whether or not these magnetic fields are associated with increased risk of serious diseases, e.g., cancers and Alzheimer's disease, is thus important when developing a rational public policy. The Bioinitiative Report was an effort by internationally recognized scientists who have spent significant time investigating the biological consequences of exposures to these magnetic fields to address this question. Our objective was to provide an unbiased review of the current knowledge and to provide our general and specific conclusions. **Results:** The evidence indicates that long-term significant occupational exposure to ELF MF may certainly increase the risk of both Alzheimer's disease and breast cancer. There is now evidence that two relevant biological processes (increased production of amyloid beta and decreased production of melatonin) are influenced by high long-term ELF MF exposure that may lead to Alzheimer's disease. There is further evidence that one of these biological processes (decreased melatonin production) may also lead to breast cancer. Finally, there is evidence that exposures to RF MF and ELF MF have similar biological consequences. **Conclusion:** It is important to mitigate ELF and RF MF exposures through equipment design changes and environmental placement of electrical equipment, e.g., AC/DC transformers. Further research related to these proposed and other biological processes is required.

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Keywords: Extremely low frequency (ELF); Magnetic fields (MFs); Amyloid beta (Aβ); Melatonin; Alzheimer's disease (AD)

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1. Introduction

In this review, we emphasize (a) two proposed biological models “explaining” the apparent relationship between high, long-term exposure to extremely low frequency (ELF) magnetic fields (MFs) and Alzheimer’s disease (AD), one of which also relates to breast cancer and (b) areas of biological research needed to confirm or refute these models. Prior to this discussion, we provide the conclusions from our detailed review chapter (Section 12: Davanipour and Sobel [1]) in the Bioinitiative Report [2] related to epidemiologic research, which initially identified these relationships. We refer the reader to Section 12 and supporting, peer-reviewed papers for details of the epidemiologic studies discussed in that section. Other papers in this issue of Pathophysiology (e.g., on the stress response and DNA strand breaks) demonstrate that exposures to ELF MF and radio frequency (RF) MF often have the same biological consequences.

2. Epidemiologic studies presented in the Bioinitiative Report related to Alzheimer’s disease and breast cancer

The conclusions reached from our detailed review of the literature in Section 12 in the Bioinitiative Report (see references for URL) on long-term significant ELF MF exposure and Alzheimer’s disease and breast cancer are provided below [1]. The section references below refer to sub-sections of Section 12 of the Bioinitiative Report.

Melatonin production (Section II). Eleven of the 13 published epidemiologic residential and occupational studies are considered to provide (positive) evidence that high long-term ELF MF exposure can result in decreased melatonin production. The two negative studies had important deficiencies which may certainly have biased the results. Thus, 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.

2.1. Alzheimer’s disease

Section 12 of the Bioinitiative Report provides the details of the following conclusions.

- There is initial evidence that (i) a high level of peripheral amyloid beta, generally considered the primary neurotoxic agent when aggregated, is a risk factor for AD and (ii) medium to high MF exposure can increase peripheral amyloid beta. High brain levels of amyloid beta are also a risk factor for AD and medium to high MF exposure to brain cells likely also increases these cells’ production of amyloid beta (Section IIIA).
- 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 (Section IIIB).
- There is strong epidemiologic evidence that long-term exposure to ELF MF is a risk factor for AD. There are seven studies of ELF MF exposure and AD that met our inclusion criteria. Six of these studies are more or less positive and only one is negative. The negative study has a serious deficiency in ELF MF exposure classification which results in subjects with rather low exposure being considered as having significant exposure. Several published studies were excluded from further consideration due to serious deficiencies, primarily diagnostic inaccuracy (e.g., use of death certificates for diagnosis of AD) and/or serious exposure assessment problems. These latter studies likely had risk estimated seriously biased towards the null hypothesis of no risk. It should be noted, however, that even some of these studies were positive (Sections IIIC and IIID).

2.2. Breast cancer

There is sufficient evidence from *in vitro* and animal studies, from human biomarker studies, from occupational and light at night case-control studies, and the only two longitudinal studies with appropriate collection of urine samples to conclude that high ELF MF exposure may certainly be a risk factor for breast cancer (Section IV). Note that at the time the Bioinitiative Report was made public, there was only one longitudinal study with appropriate collection of urine samples. There are now two such studies [3,4].

Seamstresses. Seamstress is, in fact, one of the most highly ELF MF exposed occupations, with exposure levels generally well above 10 mG over a significant proportion of the workday. Seamstresses have been consistently found to be at higher risk of Alzheimer’s disease and breast cancer. This occupation deserves specific attention in future studies. We are unaware of any measurements of RF MF among seamstresses (Section V and throughout Section 12).

3. Biological hypotheses relating ELF MF exposure to Alzheimer’s disease and breast cancer

Two biological hypotheses are discussed. The first one relates ELF MF exposure to increased amyloid beta (A β) production and subsequent development of AD. The second one relates ELF MF exposure to decreased melatonin production. Decreased melatonin production appears to have differing deleterious consequences related to AD and breast cancer development.

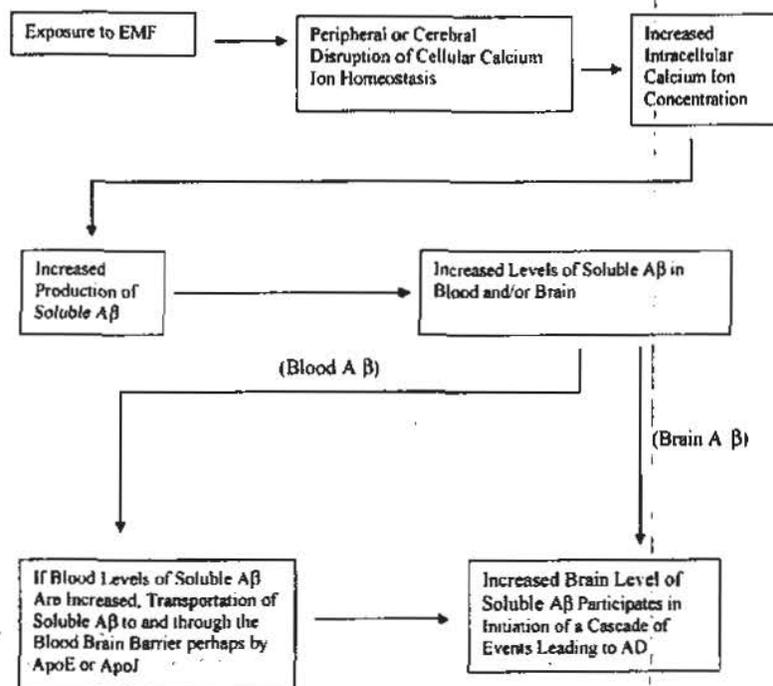


Fig. 1. Hypothesized biological pathway from MF exposure to AD Development (from Sobel and Davanipour [5]).

3.1. ELF MF exposure and peripheral and brain production of amyloid beta (Fig. 1)

The ELF MF exposure and increased amyloid beta hypothesis was developed by Sobel and Davanipour as a result of our initial findings that long-term ELF MF occupational exposure was a risk factor for AD [5] (see Fig. 1). Seamstress was the most common occupation among subjects with AD in the five databases we investigated [6–8]. ELF MF exposure among seamstresses had not been measured prior to our 1995 study [6]. Beginning in 1994, we measured a very large number of seamstresses working in either a factory setting or individually. Their exposures were very high, particularly when using an industrial sewing machine. The highest exposures were, however, not to the brain, because the motor on industrial machines is located at the knees. The motor or AC/DC transformer in home sewing machines is in the machine arm located near the operator's chest and right arm. This peripheral exposure led us to consider how peripheral ELF MF exposure might be associated with development of amyloid plaques in the brain.

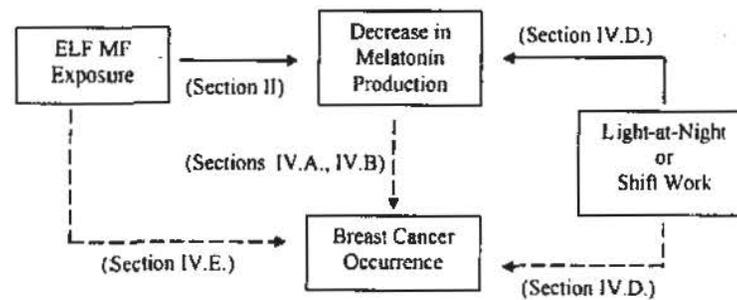
Our biologically plausible hypothesis relating MF exposure to AD is based on the independent work of many researchers in several different fields. Details and references are provided in Sobel and Davanipour [5]. Briefly, the hypothesized process involves increased peripheral or brain production of A β as a result of MF exposure causing voltage-gated calcium ion channels to be open longer than normal. This results in abnormally high intracellular levels of calcium ions which in turn results in the production of A β . The result-

ing A β is quickly secreted into the blood. If peripheral, the A β is then transported to and through the blood–brain barrier, perhaps best chaperoned by the $\epsilon 4$ isoform of apolipoprotein E (apoE). (Note that this might be one reason why the $\epsilon 4$ isoform is a risk factor for AD.) Fig. 1 provides a schematic outline of the hypothesis. Each step in the proposed pathway is supported by *in vitro* studies.

At the time of publication of this hypothesis, no human studies related to this hypothesis had been conducted. There are now two groups that have published relevant studies, without apparently any knowledge of our hypothesis—or at least no reference to the hypothesis: (1) high levels of peripheral A β_{1-42} , the more neurotoxic version of A β , has been found to be a risk factor for AD [9,10]; acute exposure to ELF MF increases peripheral A β [11]. Details may also be found in the Bioinitiative Report (Section IIIA) [1].

3.2. Melatonin—background

Melatonin is found in every cell of the body and readily crosses the blood–brain barrier. It scavenges reactive oxygen species (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 [12,13].



Note: Dashed lines indicate studies directly relating ELF MF exposure, light-at-night or shift work, or lower melatonin production to breast cancer occurrence. Section references refer to Section 12 of the Bioinitiative Report [1].

Fig. 2. Outline of the evidence that ELF MF exposure causes breast cancer through decreases in melatonin production—with section references to Section 12. Bioinitiative Report [1]. Note: Dashed lines indicate studies directly relating ELF MF exposure, light-at-night or shift work, or lower melatonin production to breast cancer occurrence.

3.3. Low melatonin production and Alzheimer's disease

Numerous *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 found that melatonin has the following effects:

- Inhibition of the neurotoxicity and cytotoxicity of A β , including in mitochondria [14–19];
- Inhibition of the formation of β -pleated sheet structures and A β fibrils [20–25];
- Reversal of the profibrillogenic activity of apolipoprotein E ϵ 4, an isoform conferring increased risk of AD [21];
- Inhibition of the oxidative stress *in vitro* and in transgenic mouse models of AD, if given early [23,26,27], but not necessarily if given to old mice [28];
- Increase in survival time in mouse models of AD [23];
- Reduction of oxidative stress and of proinflammatory cytokines induced by A β _{1–40} in rat brain *in vitro* and *in vivo* [29–31];
- Decrease of the prevalence of A β _{1–40} and A β _{1–42} in the brain in young and middle aged mice [32];
- Improvement of memory and learning in rat models of AD pathology [33,34], but not necessarily in A β -infused rat models [35].

Note that transgenic mouse models of AD mimic senile plaque accumulation, neuronal loss, and memory impairment. There have been several reviews, e.g., [36–39]. Thus, chronic low levels of melatonin production may be etiologically related to AD incidence [40].

3.4. Low melatonin production and breast cancer

See Fig. 2 for a diagram of the discussed relationships between ELF MF exposure and breast cancer risk.

In vitro studies related to prevention of oxidative damage. Well over 1000 publications have found that melatonin neu-

tralizes hydroxyl radicals and reduces oxidative damage. For reviews see Tan et al. [41] and Peyrot and Ducrocq [42]. Melatonin has also been shown to act synergistically with vitamin C, vitamin E and glutathione [43] and stimulates the antioxidant enzymes superoxide dismutase, glutathione peroxidase and glutathione reductase [44]. Furthermore,

- melatonin neutralizes hydroxyl radicals more efficiently than does reduced glutathione [45,46];
- melatonin reduces oxidative damage to macromolecules in the presence of free radicals [47,48] due at least to its free radical scavenging properties [49];
- melatonin increases the effectiveness of other antioxidants, e.g., superoxide dismutase, glutathione peroxidase, and catalase [50–54];
- melatonin has protective effects against ultraviolet and ionizing radiation [55–57];
- melatonin has been found to be a more potent protector from oxidative injury than vitamin C or vitamin E (micro-moles/kg) (for a review of the evidence, see: Tan et al. [43];
- melatonin was also found *in vitro* to scavenge peroxy radicals more effectively than vitamin E, vitamin C or reduced glutathione [58], although melatonin is not a very strong scavenger of peroxy radicals [49].

Animal studies of melatonin and mammary tumor prevention. 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., [50–63]). Tan et al. [64,65] found that melatonin at both physiological and pharmacological levels protected Sprague–Dawley rats from saffrole induced liver DNA adduct formation. Melatonin and retinoic acid appear to act synergistically in the chemoprevention of animal model tumors [66] and *in vitro* systems [67].

Melatonin prevents oxidative DNA damage by estradiol and radiation. Karbownik et al. [68] found that melatonin

protects against DNA damage in the liver and kidney of male hamsters caused by estradiol treatment. Several studies have found that laboratory animals are protected by melatonin from lethal doses of ionizing radiation (e.g., [69–71]). Vijayalaxmi et al. [70] and Karbownik et al. [71] also investigated markers of oxidative DNA damage and found significant decreases in these markers in the melatonin treated animals.

Melatonin: Scavenger of $\cdot\text{OH}$ and Other ROS. Melatonin is a powerful, endogenously produced scavenger of reactive oxygen species (ROS), particularly the hydroxyl radical ($\cdot\text{OH}$). Other ROS which melatonin scavenges include hydrogen peroxide (H_2O_2), nitric oxide (NO^\cdot), peroxytrite anion (ONOO^-), hypochlorous acid (HOCl), and singlet oxygen ($^1\text{O}_2$) [50,72–75]. $\cdot\text{OH}$ 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. $\cdot\text{OH}$ is the most reactive and cytotoxic of the ROS [76]. $\cdot\text{OH}$ appears not to be removed by antioxidative enzymes, but is only detoxified by certain direct radical scavengers such as melatonin [77].

4. Discussion and future research

Other papers in this special issue of Pathophysiology provide evidence that RF MF exposure and ELF MF exposure may have similar biological consequences.

We primarily limit our discussion of future research to studies in humans with experimental medicine components, emphasizing the latter. However, we initially discuss limiting exposures.

It should be noted that ELF MF exposure may also be associated with other cancers. This may be because of the decrease in melatonin production and melatonin's varying antioxidant, anti-inflammation, and immune response enhancement properties.

4.1. Epidemiologic studies

The incidence rates of Alzheimer's disease and breast cancer are increasing. These increases are certainly in part due to our living longer, at least for AD, if not better lives. However, environmental exposures are likely to play important roles. At the same time, ELF and RF MF exposure is becoming more and more common in our world. In our three published studies of MF and AD, approximately 7.4–12.0% of the cases and 3.4–5.3% of the controls had primary occupations associated with medium or high ELF MF exposure [6–8]. Many more subjects may have had exposures from sources generally not identified in epidemiologic studies, because individualized 'on-site' exposure assessment is usually not feasible. We give two examples coming from 'onsite' inspections we have performed: a subject who had developed amyotrophic lateral sclerosis (ALS) had spent many years with a 75 mG ELF MF exposure due to having his foot on

a deadbolt lock/unlock foot device for his office door under his desk; a subject who had developed AD who spent over 25 years sitting at his home desk for at least 4 h per day in a chair backed up to a wall with a fuse box directly on the other side of the wall which produced a very high ELF MF exposure to his back and head. (Note that there is also significant epidemiologic evidence that ELF MF exposure is a risk factor for ALS.) The frequencies of such exposures in studies are unknown.

As is often the case, more research is required. However, the designs of this future research should be informed and directed by the results of previous research. Future epidemiologic studies should use subjects for whom it is unequivocally known that the ELF MF and/or RF MF exposure is high and matched subjects for whom such exposure is known to be low. Matching criteria should include age, gender, and residential environment so as to at least partially exclude other exposures.

There should be additional studies related to the levels of production of peripheral amyloid beta, particularly $\text{A}\beta_{1-42}$, and melatonin, on the one hand, and both MF exposure and the risk of AD, on the other hand. Such studies need to be able to investigate the possible associations between peripheral amyloid beta and melatonin levels and both earlier/concurrent MF exposure and subsequent development of AD. Similar studies need to be carried out for breast cancer, excluding the amyloid beta component. This effort will likely require both retrospective and longitudinal studies. There are only two known longitudinal studies [3,4] which collected urine samples at baseline so that overnight pre-morbid melatonin production was reliably estimated. These studies found an association between low melatonin production and breast cancer. These studies may also be able to provide important additional information if it is possible to determine MF exposures with reasonable accuracy and follow-up AD status on a sufficient number of participants.

Case-control studies of melatonin as a risk factor for AD and breast cancer are hampered by the fact that biological sequelae of both AD and breast cancer result in a decline of melatonin production to an unknown extent. (In breast cancer patients, there is a melatonin production rebound when tumors are surgically removed. In AD patients, the production of serotonin, the precursor of melatonin, is decreased and noradrenergic regulation becomes dysfunctional [78].) However, melatonin production is partially under genetic control. We have conducted a study of relatively healthy members of nuclear families and melatonin production (DOD Congressionally Directed Medical Research Program Grant: DAMD17-00-1-0692). The production of melatonin of the mother was successfully modelled as a function of the melatonin of a daughter, after adjusting for both the daughter's age and the influence of the father. This work allows for the design of case-control studies of the influence of long-term MF exposure on both melatonin production and the risks of breast cancer and AD.

4.2. ELF and/or RF MF exposure mitigation

It is also vital to mitigate both the extent of MF exposure and the effects of such exposure. Mitigation means efforts to both locate and shield or move the sources of MF away from individuals and design equipment which produces lower levels of MF. Little effort has apparently been spent on design issues. There are simple things that can be done. For example, almost all AC/DC transformers emit about 75 mG ELF MF fields. The exception, in our experience, has been a few transformers for Apple laptops measured about 10 years ago. AC/DC transformers are now everywhere, specifically under and around office desks and in nearly every room in a residence, often near the heads of beds. Maximizing one's distance from a transformer is important, because the strength of the MF field drops off with the square or cube of the distance from the source.

Seamstress is a very common profession and being a seamstress is clearly a risk factor for AD and quite possibly for breast cancer also. Seamstresses experience higher ELF MF exposure than members of almost any other profession. Older industrial sewing machines are extremely common all over the world. They produce extremely strong MFs, but it is possible to design "covers" for the motor to interfere with these fields, much as "headphones" can mitigate sound waves. Newer computer driven home sewing machines produce MF because of the AC/DC transformer. These transformers are placed in the arm of the machine, which results in high MF exposure to the operator. Simply by connecting the transformer to the machine by an electrical cord about three or more feet from the operator would mitigate a significant percentage of the MF exposure.

4.3. Biological mechanisms/experimental medicine research

We argue that, to the extent possible, research should now be conducted in humans. We list the following research questions as important examples of studying the biological effects of ELF and/or RF MF exposure:

1. Generation of peripheral amyloid beta
 - a. Determination of intracellular Ca^{2+} ion concentration changes as a consequence of ELF or RF MF exposure.
 - b. Measurement of the amount of $\text{A}\beta_{1-42}$ and $\text{A}\beta_{1-40}$ produced by and secreted from cells.
 - i. This could be done at least by measuring blood levels of amyloid before and after ELF and/or RF MF exposure.
 - ii. Perhaps there are more sophisticated experimental designs.
 - c. Determination of which cell types in fact produce more amyloid beta after or during ELF and/or RF MF exposure.
 - d. Determination of the dose response relationship(s) between ELF and/or RF MF exposure and cellular amyloid beta production.
 - e. Measurement of the accumulation of amyloid beta in the brain, perhaps using PET scans [79,80].
2. Decrease in melatonin production

Note: it is known that the pineal gland, the primary source of melatonin, has a tendency to become calcified and, perhaps, this is the reason why generally there is a reduction of melatonin production during aging.

 - a. Determination of the extent of intracellular calcium within the pineal gland as a result of acute ELF and/or RF MF exposure.
 - b. Determination of the extent of calcification of the pineal gland as a result of varying levels of long-term ELF and/or RF MF exposure.

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Disturbance of the immune system by electromagnetic fields—A potentially underlying cause for cellular damage and tissue repair reduction which could lead to disease and impairment

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Abstract

A number of papers dealing with the effects of modern, man-made electromagnetic fields (EMFs) on the immune system are summarized in the present review. EMFs disturb immune function through stimulation of various allergic and inflammatory responses, as well as effects on tissue repair processes. Such disturbances increase the risks for various diseases, including cancer. These and the EMF effects on other biological processes (e.g. DNA damage, neurological effects, etc.) are now widely reported to occur at exposure levels significantly below most current national and international safety limits. Obviously, biologically based exposure standards are needed to prevent disruption of normal body processes and potential adverse health effects of chronic exposure.

Based on this review, as well as the reviews in the recent Bioinitiative Report [<http://www.bioinitiative.org/>] [C.F. Blackman, M. Blank, M. Kundi, C. Sage, D.O. Carpenter, Z. Davanipour, D. Gee, L. Hardell, O. Johansson, H. Lai, K.H. Mild, A. Sage, E.L. Sobel, Z. Xu, G. Chen, The Bioinitiative Report—A Rationale for a Biologically-based Public Exposure Standard for Electromagnetic Fields (ELF and RF), 2007)], it must be concluded that the existing public safety limits are inadequate to protect public health, and that new public safety limits, as well as limits on further deployment of untested technologies, are warranted.

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1. Introduction

Around the world, for a number of years, there has been an active debate involving the general public, scientists, journalists, politicians, and people from the electric power and telecom companies, all trying to answer the basic question: Is biology compatible with the ever-increasing levels of electromagnetic fields (EMFs)? Or, to put it in more layman's terms: Can we, as human beings, survive all the radiation? Are we built for a 24-h, whole-body irradiation life? Are we immune to these signals, or are we actually playing with our planet's future, putting life at stake? The answers appear to be: *No, we are not designed for such EMF exposure loads. We are not immune. We are gambling with our future.*

Very often the biggest threat from EMF exposure is said to be cancer. However, this is not the most horrifying scenario.

Just imagine if some basic and general molecular and/or cellular mechanism were altered. For instance, imagine if one morning the nitrogen-binding bacteria in the soil or the honey bees in the air had been destroyed beyond repair. Or, as this paper will indicate, imagine if our immune system, trying to cope with the ever-increasing electromagnetic signals, finally could not do so any longer!

Is the immune system designed to deal with “allergens” never present before, but now being invented, manufactured and used? Is it likely that our immune system, by some enormously intelligent ‘glitch’ in the evolutionary process has that capacity? Is that even remotely likely? *Of course, not.*

The recommended safe exposure levels have not taken this into account, since the existing standards are only based on the immediate heating of cells and tissues [most often evaluated in fluid-filled plastic dolls!]. They certainly do not take into consideration long-term effects or non-thermal effects that occur before heating can be detected. Furthermore, the recommendations do not take into account all available sci-

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entific reports. *The recommended exposure levels are not in any sense safe levels and are entirely inadequate.*

2. Basic concepts and components of the immune system

The human immune system is part of a general defense barrier towards our surrounding environment. We live in a biological system, the world, dominated by various microorganisms, including microbes and viruses, many of which can cause harm. The immune system serves as the primary line of defense against invasion by such microbes. As we are, practically speaking, built as a tube, the outer surface – the skin – and the innermost surface – the gastrointestinal tract – are the major borders between us and the outside world. These borders must be guarded, protected and constantly repaired since any damage to them could be fatal. In addition to these major borders there are number of other organ/tissue interfaces at which cellular conduct is monitored, evaluated and dealt with 24 h around the clock. Damage that is not detected and properly repaired in time can develop into cancer; something well known for ultraviolet light overexposure.

The skin and the mucous membranes are part of the innate or non-adaptive immune system. However, if these barriers are broken (e.g. after cutting a finger), then microbes, including potential pathogens (i.e. harmful microbes) can enter the body and begin to multiply rapidly in the warm, moist, nutrient-rich environment. The cut may not be as abrupt as a knife cut, it could also very well be an internal leakage, such as the one found after microwave exposure of the fragile blood–brain barrier [2]. Such a leakage could indeed be fatal, causing nerve cell damage and followed by cellular death [3].

One of the first cell types encountered by a foreign organism after a cut in the skin is the phagocytic white blood cell. These cells congregate within minutes and begin to attack the invading foreign microbes. The next cell type to be found in the area of such a local infection will be the so-called neutrophils. They are also phagocytic and use pattern-recognizing surface receptor molecules to detect structures commonly found on the surface of bacteria. As a result, these bacteria – as well as other forms of particulate materials – will be ingested and degraded by the neutrophils. Various other protein components of serum, including the complement components may bind to the invader organisms and facilitate their phagocytosis, thereby further limiting the source of infection/disease. Other small molecules, the interferons, mediate an early response to viral infection by the innate system.

The innate immune system is often sufficient to destroy invading microbes. If it fails to clear an infection, it will rapidly activate the adaptive or acquired immune response, which – as a consequence – takes over. The molecular messenger connection between the innate and the adaptive

systems are molecules known as cytokines. (The interferons are part of this molecular family.)

The first cells in this cellular orchestra to be activated are the T- and B-lymphocytes. These cells are normally at rest and are only recruited when needed, i.e. when encountering a foreign (=non-self) entity referred to as an antigen. The T- and B-lymphocytes, together with a wide spectrum of other cell types, have antigen receptors or antigen-recognizing molecules on their surface. Among them you find the classical antibodies (=B-cell antigen receptors), T-cell antigen receptors as well as the specific protein products of special genetic regions (=the major histocompatibility complexes). The genes of humans are referred to as human leukocyte antigen (HLA) genes and their protein products as HLA molecules. The antibodies – apart from being B-cell surface receptors – are also found as soluble antigen-recognizing molecules in the blood (immunoglobulins). The adaptive immune response is very highly effective but rather slow; it can take 7–10 days to mobilize completely. It has a very effective pathogen (non-self) recognition mechanism, a molecular memory and can improve its production of pathogen-recognition molecules during the response.

A particularly interesting set of cells are the various dendritic cells of the skin as well as elsewhere. In the outermost cutaneous portion, the epidermis, you find both dendritic melanocytes, the cells responsible for the pigment-production, as well as the Langerhans cells with their antigen-presenting capacity. In the deeper layer, the dermis, you find corresponding cells, as well as the basophilic mast cells, often showing a distinct dendritic appearance using proper markers such as chymase, tryptase or histamine. All these cells are the classical reactors to external radiation, such as radioactivity, X-rays and UV light. For that reason, our demonstration [4] of a high-to-very high number of somatostatin-immunoreactive dendritic cells in the skin of persons with the functional impairment electrohypersensitivity is of the greatest importance. Also, the alterations found in the mast cell population of normal healthy volunteers exposed in front of ordinary household TVs and computer screens [5] are intriguing, as are the significantly increased number of serotonin-positive mast cells in the skin ($p < 0.05$) and neuropeptide tyrosine (NPY)-containing nerve fibers in the thyroid ($p < 0.01$) of rats exposed to extremely low-frequency electromagnetic fields (ELF-EMF) compared to controls. This indicates a direct EMF effect on skin and thyroid vasculature [[6–8]; for further details and refs., see below]. In the gastrointestinal tract, you will find corresponding types of cells guarding our interior lining against the outside world.

The immune system can react in an excessive manner and it can cause damage to the local tissue as well as generally to the entire body. Such events are called hypersensitivity reactions and they occur in response to three different types of antigens: (a) infectious agents, (b) environmental disturbances, and (c) self-antigens. The second one is, as you will

see, of utmost importance when we discuss the impact of the new electromagnetic fields of today's world.

For environmental substances to trigger hypersensitivity reactions, they must be fairly small in order to gain access to the immune system. Dust triggers a range of responses because the particles are able to enter the lower extremities of the respiratory tract, an area that is rich in adaptive immune-response cells. These dust particles can mimic parasites and may stimulate an antibody response. If the dominant antibody is IgE, the particles may subsequently trigger immediate hypersensitivity, which is manifest as allergies, such as asthma or rhinitis. If the dust stimulates IgG antibodies, it may trigger a different kind of hypersensitivity, e.g. farmer's lung [9].

Smaller molecules sometimes diffuse into the skin and these may act as haptens, triggering a delayed hypersensitivity reaction. This is the basis of contact dermatitis caused by nickel [9].

Drugs administered orally, by injection or onto the surface of the body can elicit hypersensitivity reactions mediated by IgE or IgG antibodies or by T-cells. Immunologically mediated hypersensitivity reactions to drugs are very common and even very tiny doses of drugs can trigger life-threatening reactions. These are well classified as idiosyncratic adverse drug reactions.

In this respect, electromagnetic fields could be said to fulfil the most important demand: they penetrate the entire body.

The hypersensitivity classification system was first described by Coombs and Gell [cf. ref. 10]. The system classifies the different types of hypersensitivity reaction by the types of immune responses involved. Hypersensitivity reactions are reliant on the adaptive immune system. Prior exposure to antigen is required to prime the adaptive immune response to produce IgE (type I), IgG (type II and III) or T-cells (type IV). Because prior exposure is required, hypersensitivity reactions do not take place when an individual is first exposed to antigen. In each type of hypersensitivity reaction the damage is caused by different adaptive and innate systems, each of which has its respective role in clearing infections.

In essence, the immune system is a very complex one, built up of a large number of cell types (B- and T-lymphocytes, macrophages, natural killer cells, mast cells, Langerhans cells, etc.) with certain basic defense strategies. It has evolved during an enormously long time-span and is constructed to deal with its known enemies. *Among the known enemies one will not find modern electromagnetic fields, such as power-frequency electric and magnetic fields, radiowaves, TV signals, mobile phone or WiFi microwaves, radar signals, X-rays or artificial radioactivity.* They have been introduced during the last 100 years, in many cases during the very last decades. They are an entirely new form of exposure and could pose to be a biological "terrorist army" against which there are no working defences. They penetrate the body, and some have already proven to be fatal. Today no-one would consider having a radioactive wrist watch with glowing digits (as you

could in the 1950s), having your children's shoes fitted in a strong X-ray machine (as you could in the 1940s), keeping radium in open trays on your desk (as scientists could in the 1930s), or X-raying each other at your garden party (as physicians did in the 1920s). In retrospect, that was just plain madness. However, the persons doing so and selling these gadgets were not misinformed or less intelligent. The knowledge at the time was deficient, as was a competent risk analysis coupled to a parallel analysis of public needs.

3. Electromagnetic fields – now and previously

The electromagnetic spectrum covers a broad range of frequencies (over 20 orders of magnitude), from low frequencies in electricity supplies, radiowaves and microwaves, infrared and visible light, to X-rays, radioactivity and cosmic rays. Electromagnetic fields are present everywhere in our environment, and except for the visible spectrum, they are invisible to the human eye.

An electromagnetic field consists of an electrical part and a magnetic part. The electrical part is produced by a voltage gradient and is measured in volts/metre. The magnetic part is generated by any flow of current and is measured in Tesla. Magnetic fields as low as around 0.2 μ T (a millionth of a Tesla) can produce biological effects. For comparison, using a mobile (cell) phone or a PDA exposes you to magnetic pulses that peak at several tens of microTesla [11,12], which is well over the minimum needed to give harmful effects. Because mobile phones and other wireless gadgets are held close to the body and are used frequently, these devices are potentially the most dangerous sources of electromagnetic radiation that the average person possesses.

Even the extremely low frequencies (ELF) that are widely used in powerlines and domestic appliances should be viewed with caution. In June 2007, the World Health Organization (WHO) pointed out that they are believed to be one of the causes for children's leukemia. Pulsed or amplitude-modulated, at a biologically active lower frequency (i.e. when the radio signal strength rises and falls in time with the lower frequency), high frequencies are the hallmark of mobile phones, WiFi systems, PDAs, etc. At radiofrequencies, electric and magnetic fields are closely interrelated and we typically measure their levels as power densities in Watts per square metre (W/m^2).

4. Electromagnetic fields and health

Life on Earth, since its beginning more than 3.5 billion years ago, has developed under the influence of the practically static geomagnetic field and the radiation from the sun. All living organisms that have not been able to directly cope with these influences are either gone or have adapted in one of several ways. Living under-ground, only being active during night, living in the deeper waters (at least from 1 m and down

below) of our oceans and lakes, under the foliage of the jungle trees, or having developed a skin (or, for plants, a cortex) containing a pigment (animals and plants have very similar ones) that will shield from some heat and some sunshine. Any fair-skinned Irish or Scandinavian person learns very early to avoid even the bleak sun up-north to avoid a nasty sunburn. That sunburn will develop into a postinflammatory hyperpigmentation, that may have cosmetic value, but will also cause a redness of the skin as well as heat and pain/itch sensations.

But, during the last 100 years we have found that the pigment in our skin does not furnish any protection against other frequencies. Cosmic rays, radioactivity, X-rays, UVC, UVB and now even UVA are considered, together with radar-type microwaves to be very dangerous to health. We are translucent to power–frequency magnetic fields as well as mobile phone and WiFi microwaves, but this does not mean that they are without possible effects, through thermal or non-thermal mechanisms.

For me, as a scientist, this poses the main relevant questions: Is it possible to adapt our biology to altered exposure conditions in less than 100 years, or do we have to have thousands of years – or longer – for such an adaptation? And, in the meantime, what kind of safety standards must we adopt? A 'prudent avoidance' strategy, ALARA, recommendation levels based only on thermal effects, or is the only actual safe safety level for such exposures 0 (zero) Watts/kg until we really know? Or is "human progress", profit and greed more important than possible damage to our health? How far can we push the Russian roulette? And who should decide about this? Who should be held responsible if something goes wrong?

Our limited understanding of the biological effects of the vast majority of frequencies gives reason for concern. Although there is still a debate in this regard, *tinnitus, brain tumours and acoustic neuroma clearly are associated with cell phones and mobile phones, as is childhood leukemia with powerlines* (for references, see Blackman et al. [1]).

Communications and radar antennae expose those who live or work near these installations to their emissions. The radiation travels through buildings, and can also be conducted along electrical wires or metal plumbing. Wireless communications create levels within buildings that are many orders of magnitude higher than natural background levels. The same is true for appliances using power frequencies.

There are four phenomena that emerge from the use of electricity: ground currents; "electromagnetic smog" from communications equipment; electric and magnetic fields from power supplies and specialized equipment; and high frequencies on powerlines or so-called "dirty electricity". They may all be potential environmental toxins and this is an area of research that must be further pursued.

It is worth noting that off-gassing of electrical equipment may also contribute to sensitivities. Different sorts of technology (e.g. various medical equipment, analogue or digital telephones; flat screen monitors and laptop com-

puters or larger older monitors) may vary significantly in strength, frequency and pattern of electromagnetic fields. One challenging question for science is to find out if, for instance, 50- or 60-Hz ELF pure sine wave, square waves or sawtooth waveform, ELF-dirty (e.g. radiofrequencies on powerlines), ELF-modulated radiofrequency fields, continuous wave radiofrequency radiation and particularly pulsed radiofrequency signals are more or less bioactive, e.g. as neurotoxic, immune-disrupting and/or carcinogenic environmental exposure parameters. As will be discussed below, hazardous effects on the immune system of this potential environmental toxin must be seriously considered.

5. Effects of electromagnetic fields on the immune system

An ever-increasing number of studies has clearly shown various biological and medical effects at the cellular level due to electromagnetic fields, including power–frequency, radiofrequency and microwaves. Such fields are present in everyday life, at the workplace, in homes and places of leisure.

5.1. *The functional impairment electrohypersensitivity (EHS)*

One of the first observations of a direct effect on the immune system was the finding in the 1980s of persons with the functional impairment electrohypersensitivity (EHS), namely those who claim to suffer from subjective and objective skin- and mucosa-related symptoms, such as itch, smarting, pain, heat sensation, redness, papules, pustles, etc., after exposure to visual display terminals (VDTs), mobile phones, DECT telephones, WiFi equipments, as well as other electromagnetic devices. Frequently, symptoms from internal organ systems, such as the heart and the central nervous system, are also encountered [13].

Persons with EHS experience facial skin symptoms (sensory sensations of the facial skin including stinging, itching, burning, erythema, rosacea), eye irritation, runny or stuffy nose, impaired sense of smell, hoarse dry throat, coughing, sense of pressure in ear(s), tinnitus, fatigue, headache, "heaviness" in the head, sleeplessness, nausea/dizziness, cardiac symptoms and difficulties in concentrating. In the Cox [14] report on electrical hypersensitivity in the United Kingdom, mobile phone users' symptoms included headaches (85%), dizziness (27%), fatigue (24%), nausea (15%), itching (15%), redness (9%), burning (61%), and cognitive problems (42%). For those individuals reporting EHS symptoms in the UK population, the percentage of persons with symptoms from cell phone masts was 18%, DECT cordless phones (36%), landline phones (6%), VDTs (27%), television (12%) and fluorescent lights (18%). In addition, Fox [15] reported that a questionnaire survey of EHS individuals revealed symptoms of nausea, and of dizziness/disorientation.

Levallois et al. [16] in 2002 reported on their study of prevalence of self-perceived hypersensitivity to EMF in California. They found that about 3% of the population reports to be electrohypersensitive. About 0.5% of the population reported the necessity to change jobs or remain unemployed due to the severity of their symptoms. Underestimation of these percentages is discussed, since the population surveyed was found through contact with either an occupational clinic or a support group, and electrohypersensitive people very frequently cannot engage in normal outings (go out, travel, meet in buildings with EMF exposures, etc.). The study concludes that while there was no clinical confirmation of the reported symptoms of electrohypersensitivity, the perception is of public health importance in California, and North America. The results were based on a telephone survey among a sample of 2072 Californians. Being "allergic or very sensitive" to getting near electrical devices was reported by 68 subjects resulting in an adjusted prevalence of 3.2% (95% confidence interval: 2.8–3.7). Twenty-seven subjects (1.3%) reported sensitivity to electrical devices but no sensitivity to chemicals. Alleging that a doctor had diagnosed "environmental illness or multiple chemical sensitivity" was the strongest predictor of reporting being hypersensitive to EMF in this population (adjusted prevalence odds ratio = 5.8, 95% confidence interval: 2.6–12.8). This study confirms the presence of this self-reported disability in North America.

A recent German survey [17] suggests that the prevalence of subjects who attribute health complaints to EMF exposures is not negligible. In a sample of 2500 interviewees, 8% specifically attributed health complaints to exposures from mobile phone base station antennas or the use of mobile or cordless phones. In Sweden, 3.1% of the population claimed to be hypersensitive to EMF. Considerable variation across countries, regions within countries, and surveys in the same regions has been noted before. In 1997, the European Group of Experts reported that electrical hypersensitivity had a higher prevalence in Sweden, Germany, and Denmark than in the United Kingdom, Austria, and France. All these data suggest that the true number is still uncertain and requires further research (cf. Schüz et al. [18]).

Roosli et al. [19,20] estimate that the proportion of individuals in Switzerland with EHS symptoms is about 5%, where the exposures of concern are cited to be mobile phone base stations (74%), followed by mobile phones (36%), cordless phones (29%), and powerlines (27%). They reported that about half the Swiss population is concerned about health effects from EMF exposures in general.

The WHO has acknowledged the condition of electrohypersensitivity, and published in 2006 a research agenda for radiofrequency fields. The WHO recommends that people reporting sensitivities receive a comprehensive health evaluation. It states: "Some studies suggest that certain physiological responses of EHS individuals tend to be outside the normal range. In particular, hyperactivity in the central nervous system and imbalance in the autonomic nervous sys-

tem need to be followed up in clinical investigations, and the results for the individuals taken as input for possible treatment". Studies of individuals with sensitivities ought to consider sufficient acclimatization of subjects as recommended for chemical sensitivities, as well as recognition of individuals' wavelength-specific sensitivities. Reduction of electromagnetic radiation may ameliorate symptoms in people with chronic fatigue.

Lyskov et al. [21] in 2004 reported that EHS individuals exhibited sensitivity to VDTs, fluorescent lights and television, all of which produce flickering light. EHS individuals who were given provocation tests with flickering light exhibited a higher critical flicker frequency (CFF) than normal, and their visual evoked potential (VEP) was significantly higher than in controls. In follow-up studies, individuals with EHS demonstrated increased CFF, increased VEP, increased heart rate, decreased heart rate variability (HRV) and increased electrodermal (EDA) reaction to sound stimuli. These results indicate an imbalance in the autonomic nervous system and a lack of normal circadian rhythms in these EHS individuals. [N.B. It may just show that they feel ill. It is very hard for me to understand how sensitivity to flickering light could account for EHS in conjunction with e.g. mobile phones and base stations.]

Mueller and Schierz [22], also in 2004, reported that soundness of sleep and well-being in the morning, but not sleep quality, were affected by overnight EMF exposure in EHS individuals. An effect was reported where EHS individuals shifted their position in the bed during sleep to the non-exposed (or probably less exposed) side of the bed, something which may have strong implications for disease development (cf. Hallberg and Johansson, submitted).

Marková et al. [23] reported that non-thermal microwave exposure from global system for mobile communication (GSM) mobile telephones at lower levels than the International Commission for Non-Ionizing Radiation Protection (ICNIRP) safety standards affect 53BP1 and γ -H2AX foci and chromatin conformation in human lymphocytes. They investigated effects of microwave radiation of GSM at different carrier frequencies on human lymphocytes from healthy persons and from persons reporting hypersensitivity to EMFs. They measured the changes in chromatin conformation, which are indicative of stress response and genotoxic effects, by the method of anomalous viscosity time dependence, and analyzed tumour suppressor p53-binding protein 1 (53BP1) and phosphorylated histone H2AX (γ -H2AX), which have been shown to co-localize in distinct foci with DNA double-strand breaks (DSBs), using immunofluorescence confocal laser microscopy. The authors reported that microwave exposure from GSM mobile telephones affect chromatin conformation and 53BP1/ γ -H2AX foci similar to heat shock. For the first time, they reported that effects of microwave radiation from mobile telephones on human lymphocytes are dependent on carrier frequency. On average, the same response was observed in lymphocytes from hypersensitive and healthy subjects. N.B. These effects occurred at non-

thermal microwave exposure levels from mobile telephones that are permissible under safety standards of ICNIRP!

The same group after having described frequency-dependent effects of mobile phone microwaves (MWs) of GSM on human lymphocytes from EHS persons and healthy persons (see above), went ahead asking themselves this: Contrary to GSM, universal global telecommunications system (UMTS) mobile phones emit wide-band MW signals. Hypothetically, UMTS MWs may result in higher biological effects compared to GSM signals because of eventual "effective" frequencies within the wideband. Based on this hypothesis they have very recently reported for the first time that UMTS MWs affect chromatin and inhibit formation of DNA double-strand breaks co-localizing 53BP1/ γ -H2AX DNA repair foci in human lymphocytes from hypersensitive and healthy persons and confirm that effects of GSM MWs depend on carrier frequency [24]. Remarkably, the effects of MWs on 53BP1/ γ -H2AX foci persisted up to 72 h following exposure of cells, even longer than the stress response following heat shock. The data are in line with the hypothesis that the type of signal, UMTS MWs, may have higher biological efficiency and possibly larger health risk effects compared to GSM emissions. No significant differences in effects between groups of healthy and hypersensitive subjects were observed, except for the effects of UMTS MWs and GSM – 915 MHz MWs on the formation of the DNA repair foci, which were different for hypersensitive ($p < 0.02$ [53BP1]/0.01[γ -H2AX]) but not for control subjects ($p > 0.05$). The non-parametric statistics used here did not indicate specificity of the differences revealed between the effects of GSM and UMTS MWs on cells from hypersensitive subjects and more data are therefore needed to study the nature of these differences.

5.2. EHS as radiation damage/the mast cell hypothesis

Persons claiming adverse skin reactions after having been exposed to computer screens or mobile phones could be reacting in a highly specific way and with a completely correct avoidance reaction, especially if the provocative agent was radiation and/or chemical emissions – just as you would do if you had been exposed to e.g. sun rays, X-rays, radioactivity or chemicals. My working hypothesis, thus, became that they react in a cellularly correct way to the electromagnetic radiation, maybe in concert with chemical emissions such as plastic components, flame retardants, etc., something later focussed upon by professor Denis L. Henshaw and his collaborators at the Bristol University [25,26]. This is also covered in great depth by the author Gunni Nordström in her latest book [27].

Very early, immune cell alterations were observed when exposing two EHS individuals to a TV monitor [4]. In this article, we used an open-field provocation, in front of an ordinary TV set, of persons regarding themselves as suffering from skin problems due to work at video display terminals. Employing immunohistochemistry, in combination with a wide range of antisera directed towards cellular and neu-

rochemical markers, we were able to show a high-to-very high number of somatostatin-immunoreactive dendritic cells as well as histamine-positive mast cells in skin biopsies from the anterior neck taken before the start of the provocation. At the end of the provocation the high number of mast cells was unchanged, however, all the somatostatin-positive cells had seemingly disappeared. This latter finding may be due to loss of immunoreactivity, increase of breakdown, etc. The high number of mast cells present may explain the clinical symptoms of itch, pain, edema and erythema.

In facial skin samples of electrohypersensitive persons, the most common finding is a profound increase of mast cells as monitored by various mast cell markers, such as histamine, chymase and tryptase [28]. From these studies, it is clear that the number of mast cells in the upper dermis is increased in the electrohypersensitivity group. A different pattern of mast cell distribution also occurred in the electrohypersensitivity group, namely, the normally empty zone between the dermo-epidermal junction and mid-to-upper dermis had disappeared in the electrohypersensitivity group and, instead, this zone had a high density of mast cell infiltration. These cells also seemed to have a tendency to migrate towards the epidermis (=epidermotrophism) and many of them emptied their granular content (=degranulation) in the dermal papillary layer. Furthermore, more degranulated mast cells could be seen in the dermal reticular layer in the electrohypersensitivity group, especially in those cases showing mast cell epidermotrophism. Finally, in the electrohypersensitivity group, the cytoplasmic granules were more densely distributed and more strongly stained than in the control group; and, generally, the size of the infiltrating mast cells was found to be larger in the electrohypersensitivity group as well. It should be noted, that increases of similar nature were demonstrated later on in an experimental situation employing normal healthy volunteers in front of visual display units, including ordinary television sets [5].

Mast cells, when activated, release a wide range of mediators, among them histamine, which is involved in a variety of biological effects with clinical relevance, e.g., allergic hypersensitivity, itch, edema, local erythema, and many types of dermatoses. From the results of the cited studies, it is clear that electromagnetic fields affect the mast cell and the dendritic cell population, and may degranulate these cells.

The release of inflammatory substances, such as histamine, from mast cells in the skin results in a local erythema, edema, and sensation of itch and pain, and the release of somatostatin from the dendritic cells may give rise to subjective sensations of ongoing inflammation and sensitivity to ordinary light. These are common symptoms reported from persons suffering from EHS/screen dermatitis. Mast cells occur in the brain [29] and their presence may, under the influence of EMF and/or radiofrequency radiation exposure lead to a chronic inflammatory response by the mast cell degranulation.

Mast cells are also present in the heart tissue and their localization is of particular relevance to their function. Data

from studies made on interactions of EMF with cardiac function have demonstrated that changes are present in the heart after exposure. Some electrically sensitive people have symptoms similar to heart attacks or strong heart palpitations after exposure to EMF.

We have also, in more detail, compared facial skin from EHS persons with corresponding material from normal healthy volunteers [30]. The aim of the study was to evaluate possible markers to be used for future double-blind or blind provocation investigations. Differences were found for the biological markers calcitonin gene-related peptide (CGRP), somatostatin (SOM), vasoactive intestinal polypeptide (VIP), peptide histidine isoleucine amide (PHI), NPY, protein S-100 (S-100), neuron-specific enolase (NSE), protein gene product (PGP) 9.5 and phenylethanolamine *N*-methyltransferase (PNMT). The overall impression in the blind-coded material was such that it turned out easy to blindly separate the two groups from each other. However, no single marker was 100% able to pin-point the difference, although some were quite powerful in doing so (CGRP, SOM, S-100). In our ongoing investigations, we have also found alterations of the Merkel cell number in the facial skin of electrohypersensitive persons (Yoshimura et al., in preparation). However, it has to be pointed out that we cannot draw any definitive conclusions about the cause of the changes observed, based upon those results. Blind or double-blind provocations in a controlled environment [5] are necessary to elucidate the underlying causes for the changes reported in this particular investigation. So far, unfortunately, I and my co-workers have not been able to attract funding for such studies.

Gangi and Johansson [31,32] have proposed models for how mast cells and substances secreted from them (e.g., histamine, heparin, and serotonin) could explain sensitivity to EMF similar to those used to explain UV- and ionizing radiation-related damages. We discuss the increasing number of persons who report cutaneous problems as well as symptoms from certain internal organs, such as the central nervous system and the heart, when being close to electric equipment. Many of these respondents are users of video display terminals, and have both subjective and objective skin- and mucosa-related symptoms, such as pain, itch, heat sensation, erythema, papules, and pustules. The nervous system-derived symptoms are, e.g., dizziness, tiredness, and headache, erythema, itch, heat sensation, edema, and pain which are also common symptoms of sunburn (UV dermatitis). Alterations have been observed in cell populations of the skin of EHS persons similar to those observed in the skin damaged due to UV light or ionizing radiation.

Dr. Shabnam Gangi and I, in two theoretical papers [31,32], have put forward a model for how mast cells and substances secreted from them could explain sensitivity to EMF. The model starts from known facts in the fields of UV- and ionizing radiation-related damages, and uses all the new studies dealing with alterations seen after e.g. power frequency or microwave EMF to propose a simple summarizing model for the phenomenon of electrohypersensitivity.

Mast cells are able to secrete an array of potent mediators which may orchestrate neuroinflammation and affect the integrity of the blood–brain barrier. The “cross-talk” between mast cells, lymphocytes, neurons and glia constitutes a neuroimmune axis which is implicated in a range of neurodegenerative diseases with an inflammatory and/or autoimmune component.

Mast cells are involved in numerous activities ranging from control of the vasculature, to tissue injury and repair, allergic inflammation and host defences. They synthesize and secrete a variety of mediators, activating and modulating the functions of nearby cells and initiating complex physiological changes. Interestingly, nitric oxide (NO) produced by mast cells and/or other cells in the microenvironment appears to regulate these diverse roles. Some of the pathways central to the production of NO by mast cells and many of the tightly controlled regulatory mechanisms involved have been identified. Several cofactors and regulatory elements are involved in NO production, and these act at transcriptional and post-translational sites. Their involvement in NO production and the possibility that these pathways are critically important in mast cell functions in EHS persons should be investigated. The effects of NO on mast cell functions such as adhesion, activation and mediator secretion ought to be examined with a focus on molecular mechanisms by which NO modifies intracellular signalling pathways dependent or independent of cGMP and soluble guanylate cyclase. Metabolic products of NO including peroxynitrite and other reactive species may be the critical elements that affect the actions of NO on mast cell functions. Further understanding of the actions of NO on mast cell activities may uncover novel strategies to modulate inflammatory conditions.

It is important to remember that mastocytosis – an abnormal accumulation of mast cells in one or more organ system – can occur secondarily to other causes, such as inflammation and some kinds of leukemia. The increase in EHS being described here is more accurately thought of as “primary” mastocytosis, meaning that the increased number of mast cells occurs independently of any other cause. However, because of the increased number of mast cells in primary mastocytosis, conditions such as osteoporosis and inflammation may arise as a result of the activity of those mast cells. The manner in which primary mastocytosis can be distinguished from secondary mastocytosis and other conditions should also be addressed in controlled studies.

Patients with mastocytosis may or may not have constitutional symptoms, including weight loss, pain, nausea, headache, malaise, or fatigue. These symptoms may be due to uncontrolled proliferation of mast cells or involvement of distinct organs, such as the stomach and intestines, or bone or bone marrow. Constitutional symptoms also can result from high levels of mast cell mediators in the blood stream. The severity of symptoms varies from mild to life threatening.

Holmboe and Johansson [33] reported on testing EHS persons for increased levels of IgE or signs of a positive Phadiatop Combi (which is a screening test for allergies towards

certain foods, pollens, insects, and other animals) both of which would be indicators of an immune system alert. Five men and 17 women participated in the study. Skin and nervous system effects were the primary symptoms reported. The most frequently reported symptoms were skin redness, eczema and sweating, loss of memory, concentration difficulties, sleep disturbances, dizziness, muscular and joint-related pain, and muscular and joint-related weakness. Headache, faintness, nasal stuffiness, and fatigue were also common. In addition, 19 of the people had disturbances of the gastrointestinal tract. All the EHS persons had tinnitus. However, no connection between IgE blood levels and symptoms was found. All EHS people had normal values (<122 kU/l). Only three people had a positive Phadiatop Combi.

In summary, it is evident from our preliminary experimental data that various biological alterations are present in EHS persons claiming to suffer from exposure to EMF. The alterations are themselves enough to fully explain the EHS symptoms, and the involvement of the immune system is evident.

Thus, it is of paramount importance to continue investigating persons with EHS. We would favour studies of EMF interaction with mast cell release of histamine and other biologically active substances, studies of lymphocyte viability, as well as studies of the newly described serotonin-containing melanocytes. Also, continued analysis of the intraepidermal nerve fibers and their relations to these mast cells and serotonin-containing melanocytes is very important. Finally, not to be forgotten, a general investigation of persons with EHS versus normal healthy volunteers regarding the above markers as well as other markers for cell traffic, proliferation and inflammation is very much needed. Such research may lay a firm foundation for necessary adjustment of accessibility, thus helping all persons with EHS.

5.3. Rat skin and thyroid: animal model studies

In addition to the studies in humans, we have also done a series of animal experiments [6–8]. These have been a collaborative effort between the Department of Biology, Faculty of Sciences, Novi Sad, Serbia, and my own research group at the Karolinska Institute, Stockholm, Sweden.

These papers go back to early observations in persons with EHS where large increases in the cutaneous mast cell count could be demonstrated as compared to normal healthy volunteers. A corresponding effect on cutaneous mast cells from normal healthy volunteers placed in front of ordinary TVs/PCs could also be shown. My working hypothesis since then is that EHS is a kind of radiation damage, since the observed cellular changes are very much the same as the ones you would find in tissue subjected to UV-light or ionizing radiation (for refs., see above).

One very fierce criticism has been that such mast cell alterations in persons with electrohypersensitivity (or in normal healthy volunteers!) can not be due to EMFs and/or airborne chemicals, but must be due to psychological or psy-

chiatric personality disturbances, cognitive malfunction, or likewise.

The aim of these studies has therefore been to investigate the influence of extremely low-frequency electromagnetic fields (ELF-EMFs) on mast cells, parafollicular cells, and nerve fibers in rat skin and thyroid gland, as seen using light and transmission electron microscopy. The experiments were performed on 2-month-old Wistar male rats exposed for 4 h a day, 5 or 7 days a week for 1 month to power-frequency (50 Hz) EMFs (100–300 μ T, 54–160 V/m). After sacrifice, samples of skin and thyroid were processed for indirect immunohistochemistry or toluidine blue staining and were then analyzed using the methods of stereology. Antibody markers to serotonin, substance P, CGRP, and PGP 9.5 were applied to skin sections and PGP 9.5, CGRP, and neuropeptide Y (NPY) markers to the thyroid. A significantly increased number of serotonin-positive mast cells in the skin ($p < 0.05$) and NPY-containing nerve fibers in the thyroid ($p < 0.01$) of rats exposed to ELF-EMF was found compared to controls, indicating a direct EMF effect on skin and thyroid vasculature.

After ultrastructural examination, a predominance of microfollicles with less colloid content and dilated blood capillaries was found in the EMF group. Stereological counting showed a statistically significant increase of the volume density of follicular epithelium, interfollicular tissue and blood capillaries as well as the thyroid activation index, as compared to the controls. The volume density of colloid significantly decreased. Ultrastructural analysis of thyroid follicular cells in the EMF group revealed the frequent finding of several colloid droplets within the same thyrocyte with the occasional presence of large-diameter droplets. Alterations in lysosomes, granular endoplasmic reticulum and cell nuclei compared to the control group were also observed. Taken together, both the light microscope and the ultrastructural results show the stimulating effect of power-frequency EMFs.

The results obtained in animal studies cannot be understood by psychological or psychiatric theories, but are clearly related to the EMF exposure. In view of recent epidemiological studies, pointing to a correlation between long-term exposure from power-frequency magnetic fields or radio-/microwaves and cancer, our data have to be taken seriously.

5.4. Cutaneous heat shock protein/stress response pathway

Recent evidence by Leszczynski et al. has indicated activation of stress-induced pathways in cultivated cells in response to microwaves [34]. Their article indicated that mobile telephone microwaves activate a variety of cellular signal transduction pathways, among them the hsp27/p38MAPK stress response pathway [34]. Whether activation of stress response pathways relates to apoptosis, blood-brain barrier permeability, or increased cancer in humans remains to be investigated. Further work reported gene and protein expres-

sion changes in human endothelial cell lines with microwave 900 MHz mobile phone exposure [35].

5.5. Childhood leukemia and power-frequency magnetic fields; CNS tumours and cell phone use

Childhood leukemia was connected to power-frequency magnetic fields already in the pioneering work by Wertheimer and Leeper [36]. More recently, Scandinavian scientists have identified an increased risk for acoustic neuroma (i.e., a benign tumour of the eighth cranial nerve) in cell phone users, as well as a slightly increased risk of malignant brain tumours such as astrocytoma and meningioma on the same side of the brain as the cell phone was held [37–40]. In addition, a clear association between adult cancers and FM radio broadcasting radiation has been noticed, both in time and location [41–43]. Initial studies on facial nevi indicate that nowadays young children can have a substantial number of these (Hallberg and Johansson, unpublished data). If, in addition to the low-frequency EMF, there is a radiofrequency and/or microwave correlation, then this must be considered in future research and safety programs.

5.6. Effects by microwaves on acute experimental allergic encephalomyelitis

Turning back to the immune system, Anane et al. [44] have studied the effects of acute exposure to GSM-900 microwaves (900 MHz, 217 Hz pulse modulation) on the clinical parameters of the acute experimental allergic encephalomyelitis (EAE) model in rats in two independent experiments: rats were either habituated or non-habituated to the exposure restrainers. EAE was induced with a mixture of myelin basic protein and *Mycobacterium tuberculosis*. Female Lewis rats were divided into cage control, sham-exposed, and two groups exposed either at 1.5 or 6.0 W/kg local specific absorption rate (SAR averaged over the brain) using a loop antenna placed over their heads. No effect of a 21-day exposure (2 h/day) on the onset, duration, and termination of the EAE crisis was seen.

5.7. Effects of electromagnetic fields on immune system parameters, including cellular markers

5.7.1. Residential exposure effects/occupational studies

The object of the study by Boscol et al. [45] in 2001 was to investigate the immune system of 19 women with a mean age of 35 years, for at least 2 years (mean = 13 years) exposed to electromagnetic fields induced by radio-television broadcasting stations in their residential area. In September 1999, the EMFs (with range 500 kHz–3 GHz) in the balconies of the homes of the women were (mean \pm S.D.) 4.3 ± 1.4 V/m. Forty-seven women of similar age, smoking habits and atopy composed the control group, with a nearby resident EMF exposure of <1.8 V/m. Blood lead and urinary trans-trans muconic acid (a metabolite of benzene), markers of exposure

to urban traffic, were higher in the control women. The EMF exposed group showed a statistically significant reduction of blood NK CD16+–CD56+, cytotoxic CD3(–)–CD8+, B and NK activated CD3(–)–HLA-DR+ and CD3(–)–CD25+ lymphocytes. In vitro production of IL-2 and interferon-gamma (INF-gamma) by peripheral blood mononuclear cells (PBMC) of the EMF exposed group, incubated either with or without phytohaemagglutinin (PHA), was significantly lower; the in vitro production of IL-2 was significantly correlated with blood CD16+–CD56+ lymphocytes. The stimulation index (S.I.) of blastogenesis (ratio between cell proliferation with and without PHA) of PBMC of EMF exposed women was lower than that of the control subjects. The S.I. of blastogenesis of the EMF exposed group (but not blood NK lymphocytes and the in vitro production of IL-2 and INF-gamma by PBMC) was significantly correlated with the EMF levels. Blood lead and urinary trans-trans muconic acid were barely correlated with immune parameters: the urinary metabolite of benzene of the control group was only correlated with CD16+–CD56+ cells indicating a slight effect of traffic on the immune system. In conclusion, this study demonstrates that high-frequency EMFs reduce cytotoxic activity in the peripheral blood of women without a dose-response effect. [Such an effect could only be considered as very serious; since this could hamper the immune system in its daily struggle against various organisms/agents.]

A more general reaction pattern was found by Dmoch and Moszczynski [46] who assessed immunoglobulin concentrations and T-lymphocyte subsets in workers of TV re-transmission and satellite communication centres. An increase in IgG and IgA concentrations, an increased count of lymphocytes and T8 lymphocytes, a decreased count of NK cells and a lower value of T-helper/T-suppressor ratio were found.

The immunoglobulins' concentrations and T-lymphocyte subsets during occupational exposures to microwave radiation were also assessed in 1999 by Moszczynski et al. [47]. In the workers of re-transmission TV center and center of satellite communications on increased IgG and IgA concentration and decreased count of lymphocytes and T8 cells was found. However, in the radar operators IgM concentration was elevated and a decrease in the total T8 cell count was observed. The different behaviors of examined immunological parameters indicate that the effect of microwave radiation on the immune system depends on the exposure. However, disorders in the immunoglobulins' concentrations and in the T8 cell count had not, so far, caused any reported clinical consequences in their workers.

Tuschl et al. [48] recorded a considerable excess of recommended exposure limits in the vicinity of shortwave diathermy devices used for medical treatment of patients. Different kinds of field probes were used to measure electric and magnetic field strength and the whole body exposure of medical personnel operating shortwave, decimetre wave and microwave units was calculated. To investigate the influence of chronic exposure on the immune system of operators,

blood was sampled from physiotherapists working with the above mentioned devices. Eighteen exposed and 13 control persons, matched by sex and age, were examined. Total leucocyte and lymphocyte counts were performed and leucocytic subpopulations determined by flow cytometry and monoclonal antibodies against surface antigens. In addition, to quantifying subpopulations of immunocompetent cells, the activity of lymphocytes was measured. Lymphocytes were stimulated by mitogen phytohemagglutinin and their proliferation measured by flow cytometry. No statistically significant differences between the control and exposed persons were found. In both study groups all immune parameters were within normal ranges.

5.7.2. Electromagnetic fields and atopy

In an attempt to understand how non-atopic and atopic fertile women with uniform exposure to toxic compounds produced by traffic immunologically react to high or low frequency electromagnetic fields (ELMF), Del Signore et al. [49] performed a preliminary study. Women were divided in group A (non-atopic, non-exposed to ELMF); B (atopic, non-exposed to ELMF); C (non-atopic, exposed to ELMF); D (atopic, exposed to ELMF). In vitro cell proliferation of PBMC of atopic women (groups B and D) stimulated by PHA was reduced. The ELMF exposed women (groups C and D) showed lower levels of blood NK CD16(+)-CD56+ lymphocyte subpopulations and of in vitro production of interferon-gamma (both spontaneously and in presence of PHA) by PBMC, suggesting that ELMF reduces blood cytotoxic activity. Serum IgE of the atopic women exposed to ELMF (group D) was higher than that of the other groups. Linear discriminant analysis including serum zinc and copper (essential enzymes for immune functions), blood lead and urinary trans-trans muconic acid, a metabolite of benzene (markers of exposure to traffic) and key parameters of immune functions (CD16(+)-CD56+ lymphocyte subset, serum IgE, interferon-gamma produced by PBMC in presence of PHA, stimulation index of blastogenesis) showed absence of significant difference between groups A and C and a marked separation of groups B and D. This datum suggests that ELMF have a greater influence on atopic women exposed to traffic than on non-atopic ones, again pointing out differing reaction capacities in the human population – possibly dependent on varying immune functions based on variations in genetic make-up. [This is of particular interest since EHS persons have certain atopic features (Liden, personal communication) that may make them more susceptible to EMFs.]

5.7.3. Animal and human cellular in vivo and in vitro studies

One very interesting set of experiments was performed by Cleary et al. [50] in 1990. Whole human blood was exposed or sham-exposed in vitro for 2 h to 27 or 2450 MHz radiofrequency (RF) radiation under isothermal conditions (i.e., $37 \pm 0.2^\circ\text{C}$). Immediately after exposure, mononuclear

cells were separated from blood by Ficoll density-gradient centrifugation and cultured for 3 days at 37°C with or without mitogenic stimulation by PHA. Lymphocyte proliferation was assayed at the end of the culture period by 6 h of pulse-labeling with 3H-thymidine (3H-TdR). Exposure to radiation at either frequency at specific absorption rates (SARs) below 50 W/kg resulted in a dose-dependent, statistically significant increase of 3H-TdR uptake in PHA-activated or unstimulated lymphocytes. Exposure at 50 W/kg or higher suppressed 3H-TdR uptake relative to that of sham-exposed cells. There were no detectable effects of RF radiation on lymphocyte morphology or viability. Notwithstanding the characteristic temperature dependence of lymphocyte activation in vitro, the isothermal exposure conditions of this study indicate that the biphasic, dose-dependent effects of the radiation on lymphocyte proliferation are not dependent on heating.

Half a decade later (1996), Cleary et al. [51] published yet another paper with the title "Effect of isothermal radiofrequency radiation on cytolytic T lymphocytes". Previous in vitro studies had provided evidence that RF radiation modulates proliferation of human glioma, lymphocytes, and other cell types. The mechanism of such cell proliferation modulation, as well as mechanisms for effects on other cell physiologic endpoints, however, was not well understood. To obtain insight regarding interaction mechanisms, they investigated effects of RF radiation exposure on interleukin 2 (IL-2)-dependent proliferation of cytolytic T-lymphocytes (CTL-2). After exposure to RF radiation – in the presence or absence of IL-2 – cells were cultured at various physiological concentrations of IL-2. Treatment effects on CTL-2 proliferation were determined by tritiated thymidine incorporation immediately or 24 h after exposure. Exposure to 2450 MHz RF radiation at specific absorption rates (SARs) of greater than 25 W/kg (induced E-field strength 98.4 V/m) induced a consistent, statistically significant reduction in CTL-2 proliferation, especially at low IL-2 concentrations. At lower SARs, 2450 MHz exposure increased CTL-2 proliferation immediately after exposure but reduced 24 h postexposure proliferation. RF radiation effects depended on the mitotic state of the cells at the time of exposure.

In 1992, Czerska et al. [52] studied the effects of continuous and pulsed, 2450-MHz radiation on spontaneous lymphoblastoid transformation of human lymphocytes in vitro. Normal human lymphocytes were isolated from the peripheral blood of healthy donors. One-milliliter samples containing one million cells in chromosome medium 1A were exposed for 5 days to conventional heating or to continuous wave (CW) or pulsed wave (PW) 2450-MHz radiation at non-heating (37°C) and various heating levels (temperature increases of 0.5, 1.0, 1.5, and 2°C). The pulsed exposures involved 1- μs pulses at pulse repetition frequencies from 100 to 1000 pulses/s at the same average SAR levels as the CW exposures. Actual average SARs ranged to 12.3 W/kg. Following termination of the incubation period, spontaneous lymphoblastoid transformation was determined with an image analysis system. The results were compared

among each of the experimental conditions and with sham-exposed cultures. At non-heating levels, CW exposure did not affect 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 transformation at non-heating levels. This finding is significant ($p < 0.002$). At heating levels PW exposure enhanced transformation to a greater extent than did conventional or CW heating. This finding is significant at the 0.02 level. It was 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.

In 2003, Dabrowski et al. [53] exposed samples of mononuclear cells isolated from peripheral blood of healthy donors ($n = 16$) to 1300 MHz pulse-modulated microwaves at 330 pps with 5 μ s pulse width. The samples were exposed in an anechoic chamber at the average value of power density of $S = 10 \text{ W/m}^2$ (1 mW/cm^2). The average specific absorption rate (SAR) was measured in rectangular waveguide and the value of SAR = 0.18 W/kg was recorded. Subsequently, the exposed and control cells were assessed in the microculture system for several parameters characterizing their proliferative and immunoregulatory properties. Although the irradiation decreased the spontaneous incorporation of 3H-thymidine, the proliferative response of lymphocytes to PHA and to Con A as well as the T-cell suppressive activity (SAT index) and the saturation of IL-2 receptors did not change. Nevertheless, the lymphocyte production of interleukin (IL)-10 increased ($p < 0.001$) and the concentration of IFN γ remained unchanged or slightly decreased in the culture supernatants. Concomitantly, the microwave irradiation modulated the monokine production by monocytes. The production of IL-1b increased significantly ($p < 0.01$), the concentration of its antagonist (IL-1ra) dropped by half ($p < 0.01$) and the tumour necrosis factor (TNF- α) concentration remained unchanged. These changes of monokine proportion (IL-1b versus IL-1ra) resulted in significant increase of the value of the LM (=the monokine influence on lymphocyte mitogenic response; cf. Dabrowski et al. [54]) index ($p < 0.01$), which reflects the activation of monocyte immunogenic function. The results indicate that pulse-modulated microwaves represent the potential of immunotropic influence, stimulating preferentially the immunogenic and proinflammatory activity of monocytes at relatively low levels of exposure!

Following these findings of G_0 phase PBMC exposed to low-level (SAR = 0.18 W/kg) pulse-modulated 1300 MHz microwaves and subsequently cultured, demonstrating changed immune activity (as of above), in 2006 Stankiewicz et al. [55] investigated whether cultured immune cells induced into the active phases of cell cycle (G_1 , S) and then exposed to microwaves will also be sensitive to EMF. An anechoic chamber containing a microplate with cultured cells and an antenna emitting microwaves (900 MHz simulated GSM signal, 27 V/m, SAR 0.024 W/kg) was placed

inside an ASSAB incubator. The microcultures of PBMC exposed to microwaves demonstrated significantly higher response to mitogens and higher immunogenic activity of monocytes (LM index) than control cultures. The results suggest that immune activity of responding lymphocytes and monocytes can be additionally intensified by 900 MHz microwaves. [The above described effects of an immune system activity-intensifying effect of 900 MHz microwaves are a very important warning signal as well as a very important piece of the explanatory jigsaw puzzle regarding EHS. In the latter, affected persons very often describe "influenza-like" sensations in their body. Maybe the mobile phones, as well as other high-frequency devices, have aroused the immune system to too high an activation level?]

Two papers of paramount importance are Donnellan et al. [56] and Harvey and French [57]. In the first, a mast cell line, RBL-2H3, was exposed to 835 MHz for 20 min, three times per day for 7 days at a power density of $8.1 \pm 3 \text{ mW/cm}^2$. From day 4 onwards, it was observed that the rate of DNA synthesis and cell replication increased, that actin distribution and cell morphology became altered, and that the amount of beta-hexosaminidase (a marker of granule secretion) released in response to a calcium ionophore was significantly enhanced, in comparison to unexposed cultures. No effects were seen on levels of cytoskeletal protein synthesis or of beta-actin mRNA. Morphological changes persisted following subculture for at least 7 days in the absence of further exposure. It is hypothesized that effects of exposure to an EMF at 835 MHz may be mediated via a signal transduction pathway. In the second publication, Harvey and French used a resonant cavity which delivered a continuous wave exposure at 864.3 MHz at an average SAR of 7 W/kg to determine non-thermal biological effects of microwave exposure. A human mast cell line, HMC-1, was used as the biological target. Cells were exposed three times for 20-min duration daily, for 7 days. The temperature of the cell culture medium during the exposure fell to 26.5 °C. Effects were seen on localization of protein kinase C, and expression of three of the 588 genes screened. The affected genes included the proto-oncogene c-kit, the transcription factor nucleoside diphosphate kinase B and the apoptosis-associated gene DAD-1. Stress response genes were variably upregulated. No significant effect on morphology or on F-actin distribution was detected. They concluded that low-power microwave exposure may act on HMC-1 cells by altering gene expression via a mechanism involving activation of protein kinase C, and at temperatures well below those known to induce a heat shock response.

Elekes et al. [58] in 1996 found a very interesting sex-difference. The effect of continuous (CW; 2.45 GHz carrier frequency) or amplitude-modulated (AM; 50 Hz square wave) microwave radiation on the immune response was tested. CW exposures (6 days, 3 h/day) induced elevations of the number of antibody-producing cells in the spleen of male Balb/c mice (+37%). AM microwave exposure induced elevation of the spleen index (+15%) and antibody-producing cell number (+55%) in the spleen of male mice. No changes were

observed in female mice. It is concluded that both types of exposure conditions induced moderate elevation of antibody production only in male mice.

Irradiation with electromagnetic waves (8.15–18 GHz, 1 Hz within, $1 \mu\text{W}/\text{cm}^2$) in vivo increases the cytotoxic activity of natural killer cells of rat spleen [59]. In mice exposed for 24–72 h, the activity of natural killer cells increased by 130–150%, the increased level of activity persisting within 24 h after the cessation of treatment. Microwave irradiation of animals in vivo for 3.5 and 5 h, and a short exposure of splenic cells in vitro did not affect the activity of natural killer cells.

Whole body microwave sinusoidal irradiation of male NMRI mice with 8.15–18 GHz (1 Hz within) at a power density of $1 \mu\text{W}/\text{cm}^2$ caused a significant enhancement of TNF production in peritoneal macrophages and splenic T-lymphocytes [60]. Microwave radiation affected T-cells, facilitating their capacity to proliferate in response to mitogenic stimulation. The exposure duration necessary for the stimulation of cellular immunity ranged from 5 h to 3 days. Chronic irradiation of mice for 7 days produced the decreasing of TNF production in peritoneal macrophages. The exposure of mice for 24 h increased the TNF production and immune proliferative response, and these stimulatory effects persisted over 3 days after the termination of exposure. Microwave treatment increased the endogenously produced TNF more effectively than did lipopolysaccharide, one of the most potential stimuli of synthesis of this cytokine. Microwaves, thus, indeed can be a factor interfering with the process of cellular immunity!

A very intriguing investigation was carried out by Gapeev et al. [61], who compared horn, dielectric and channel antennae and their matching with various types of loads, including a biological object. The channel antenna in contrast to dielectric and horn provides the uniform spatial distribution of specific absorbed rating in the frequency range used and wide-band matching with the object both in near field and far field zones of the radiator. It is shown, that low-intensity electromagnetic radiation of extremely high frequency in near field zone of the channel radiator modifies the activity of mouse peritoneal neutrophils on a quasi-resonance manner. The interaction of electromagnetic radiation with the biological object has been revealed in the narrow-band frequencies of 41.8–42.05 GHz and consists in inhibition of luminol-dependent chemiluminescence of neutrophils activated by opsonized zymosan. No frequency dependence has been found of the electromagnetic radiation effects in the far field zone of the radiator. The results obtained suggest, that the quasi-resonance dependence of the biological effect on the frequency of the electromagnetic radiation in the near field zone is conditioned by structure and nature of the electromagnetic radiation in this zone.

In 2003, Gatta et al. [62] studied the effects of in vivo exposure to GSM-modulated 900 MHz radiation on mouse peripheral lymphocytes. The aim of this study was to evaluate whether daily whole-body exposure to 900 MHz

GSM-modulated radiation could affect spleen lymphocytes. C57BL/6 mice were exposed 2 h/day for 1, 2 or 4 weeks in a TEM cell to an SAR of 1 or 2 W/kg. Untreated and sham-exposed groups were also examined. At the end of the exposure, mice were killed and spleen cells were collected. The number of spleen cells, the percentages of B- and T-cells, and the distribution of T-cell subpopulations (CD4 and CD8) were not altered by the exposure. T- and B-cells were also stimulated *ex vivo* using specific monoclonal antibodies or LPS to induce cell proliferation, cytokine production and expression of activation markers. The results did not show relevant differences in either T- or B-lymphocytes from mice exposed to an SAR of 1 or 2 W/kg and sham-exposed mice with few exceptions. After 1 week of exposure to 1 or 2 W/kg, an increase in IFN-gamma (Ifng) production was observed that was not evident when the exposure was prolonged to 2 or 4 weeks. This suggests that the immune system might have adapted to RF radiation as it does with other stressing agents. All together, from their in vivo data, they concluded that the T- and B-cell compartments were not substantially affected by exposure to RF radiation and that a clinically relevant effect of RF radiation on the immune system is unlikely to occur. [Another explanation could be that the cells were unable to deal with the exposure and the obvious follow-up question then will be: What happened with the immune cells after months and years of exposure?]

On the other hand, Kolomytseva et al. [63], in their whole-body exposure experiment designed to study the dynamics of leukocyte number and functional activity of peripheral blood neutrophils under whole-body exposure of healthy mice to low-intensity extremely high-frequency electromagnetic radiation (EHF EMR, 42.0 GHz, $0.15 \text{ mW}/\text{cm}^2$, 20 min daily), showed that such a whole-body exposure of healthy mice to low-intensity EHF EMR has a profound effect on the indices of non-specific immunity. It was shown that the phagocytic activity of peripheral blood neutrophils was suppressed by about 50% ($p < 0.01$ as compared with the sham-exposed control) in 2–3 h after the single exposure to EHF EMR. The effect persisted for 1 day after the exposure, and then the phagocytic activity of neutrophils returned to the normal within 3 days. A significant modification of the leukocyte blood profile in mice exposed to EHF EMR for 5 days was observed after the cessation of exposures: the number of leukocytes increased by 44% ($p < 0.05$ as compared with sham-exposed animals), mostly due to an increase in the lymphocyte content. The supposition was made that EHF EMR effects can be mediated via the metabolic systems of arachidonic acid and the stimulation of adenylate cyclase activity, with subsequent increase in the intracellular cAMP level.

The modification of indices of the humoral immune response to thymus-dependent antigen (sheep erythrocytes) after a whole-body exposure of healthy mice to low-intensity extremely high-frequency electromagnetic radiation was reported by Lushnikov et al. in 2001 [64]. Male NMRI mice were exposed in the far-field zone of horn antenna at a frequency of 42.0 GHz and energy flux density of $0.15 \text{ mW}/\text{cm}^2$

under different regimes: once for 20 min, for 20 min daily during 5 and 20 successive days before immunization, and for 20 min daily during 5 successive days after immunization throughout the development of the humoral immune response. The intensity of the humoral immune response was estimated on day 5 after immunization by the number of antibody-forming cells of the spleen and antibody titers. Changes in cellularity of the spleen, thymus and red bone marrow were also assessed. The indices of humoral immunity and cellularity of lymphoid organs changed insignificantly after acute exposure and a series of five exposures before and after immunization of the animals. However, after repeated exposures for 20 days before immunization, a statistically significant reduction of thymic cellularity by 17.5% ($p < 0.05$) and a decrease in cellularity of the spleen by 14.5% ($p < 0.05$) were revealed. The results show that single low-intensity extremely high-frequency electromagnetic radiation, at the frequency and energy flux density used, does not influence the humoral immune response intensity in healthy mice but influences immunogenesis under multiple repeated exposures.

Experiments have also been conducted to elucidate the effects of chronic low power-level microwave radiation on the immunological systems of rabbits [65]. Fourteen male Belgian white rabbits were exposed to microwave radiation at 5 mW/cm², 2.1 GHz, 3 h daily, 6 days/week for 3 months in two batches of seven each in specially designed miniature anechoic chambers. Seven rabbits were subjected to sham exposure for identical duration. The microwave energy was provided through S band standard gain horns connected to a 4K3SJ2 Klystron power amplifier. The first batch of animals was assessed for T-lymphocyte-mediated cellular immune response mechanisms and the second batch of animals for B-lymphocyte-mediated humoral immune response mechanisms. The peripheral blood samples collected monthly during microwave/sham exposure and during follow-up (5/14 days after termination of exposures, in the second batch animals only) were analysed for T-lymphocyte numbers and their mitogen responsiveness to ConA and PHA. Significant suppression of T-lymphocyte numbers was noted in the microwave group at 2 months ($p < 0.01$) and during follow-up ($p < 0.01$). The first batch of animals was initially sensitised with BCG and challenged with tuberculin (0.03 ml) at the termination of microwave irradiation/sham exposure and the increase in foot pad thickness (delta mm), which is a measure of T-cell-mediated immunity (delayed type hypersensitivity response, DTH) was noted in both the groups. The microwave group revealed a "better" response than the control group (delta % +12.4 versus +7.54).

Nasta et al. [66], very recently examined the effects of in vivo exposure to a GSM-modulated 900 MHz RF field on B-cell peripheral differentiation and antibody production in mice. Their results show that exposure to a whole-body average SAR of 2 W/kg, 2 h/day for 4 consecutive weeks does not affect the frequencies of differentiating transitional 1 (T1) and T2 B-cells or those of mature follicular B and marginal zone B-cells in the spleen. IgM and IgG serum levels are also

not significantly different among exposed, sham-exposed and control mice. B-cells from these mice, challenged in vitro with LPS, produce comparable amounts of IgM and IgG. Moreover, exposure of immunized mice to RF fields does not change the antigen-specific antibody serum level. Interestingly, not only the production of antigen-specific IgM but also that of IgG (which requires T–B-cell interaction) is not affected by RF-field exposure. This indicates that the exposure does not alter an ongoing in vivo antigen-specific immune response. In conclusion, the results of Nasta et al. [66] do not indicate any effects of GSM-modulated RF radiation on the B-cell peripheral compartment and antibody production.

Whole-body microwave sinusoidal irradiation of male NMRI mice, exposure of macrophages in vitro, and preliminary irradiation of culture medium with 8.15–18 GHz (1 Hz within) at a power density of 1 μ W/cm² caused a significant enhancement of tumour necrosis factor production in peritoneal macrophages [67]. The role of microwaves as a factor interfering with the process of cell immunity must, thus, be seriously considered. Furthermore, the effect of 8.15–18 GHz (1 Hz within) microwave radiation at a power density of 1 μ W/cm² on the tumour necrosis factor (TNF) production and immune response was tested by Novoselova et al. [68]. A single 5 h whole-body exposure induced a significant increase in TNF production in peritoneal macrophages and splenic T-cells. The mitogenic response in T-lymphocytes increased after microwave exposure. The activation of cellular immunity was observed within 3 days after exposure. A diet containing lipid-soluble nutrients (beta-carotene, alpha-tocopherol and ubiquinone Q9) increased the activity of macrophages and T-cells from irradiated mice. These results demonstrate that irradiation with low-power density microwaves stimulates the immune potential of macrophages and T-cells, and the antioxidant treatment enhances the effect of microwaves, in particular when the effect of irradiation is reduced.

In the experimental study by Çetin et al. [69] in 2006, the hematological effects of pulsed EMFs chronic exposure were investigated on mice. Sixty, 6-week-old male Swiss mice, weighing 40–45 g were used, and were divided into two groups: in one group, animals ($n=30$) were exposed to pulsed EMFs (60 Hz, intensity 3 μ T, 12 h by day) for a 120-day period, whereas the second group ($n=30$) was used as control. On days 15, 30, 90 and 120, samples were taken by cardiac puncture for the hematological analysis (red blood cell and white blood cell counts, leukocyte distribution). Whereas no significant difference was noted between control and exposed animals at the 15th and the 30th days, a macrocytic anemia characterized by decreases in of hemoglobin concentration, hematocrit values and erythrocyte counts and by increases in mean corpuscular volume, occurred in the exposed animals on day 90. Furthermore, they have shown significant reductions of leukocyte, lymphocyte and neutrophil counts, while monocyte counts were increased. On day 120, these leukocyte alterations were still

observed, whereas erythrocyte parameters approached control values. These results suggest that pulsed electromagnetic fields (60 Hz and 3 μ T) affect the hematological parameters of mice, probably by reducing proliferation and differentiation of marrow stem cells.

Obukhan [70] has performed cytologic investigations designed to study bone marrow, peripheral blood, spleen, and thymus of albino rats irradiated by an EMF of 2375, 2450, and 3000 MHz. Structural and functional changes in populations of megakaryocytes, immunocompetent cells as well as of undifferentiated cells, and of other types of cells that are dependent on the intensity of irradiation were revealed. The results permitted establishing the probability-threshold levels of exposure taking account of reactions of perception and physiologic adaptation together with compensatory and regenerative processes and the injury sustained. It was shown that changes in bone marrow cells differentiation and reproduction, rather than integral shifts in the peripheral blood, acquired the utmost significance. The blast cell population increased in low-intensity exposure, along with disturbances in mitosis.

The possibility of genotoxicity of radiofrequency radiation (RFR) applied alone or in combination with X-rays was recently investigated *in vitro* using several assays on human lymphocytes by Stronati et al. [71]. The chosen SAR values are near the upper limit of energy absorbed by localized tissue during the use of some cellular telephones. The purpose of the combined exposures was to examine whether RFR might act epigenetically by reducing the fidelity of repair of DNA damage caused by a well-characterized and established mutagen. Blood specimens from 14 donors were exposed continuously for 24 h to a GSM basic 935 MHz signal. The signal was applied at two SAR; 1 and 2 W/Kg, alone or combined with a 1-min exposure to 1.0 Gy of 250 kVp X-rays given immediately before or after the RFR. The assays employed were the alkaline comet technique to detect DNA strand breakage, metaphase analyses to detect unstable chromosomal aberrations and sister chromatid exchanges, micronuclei in cytokinesis-blocked binucleate lymphocytes and the nuclear division index to detect alterations in the speed of *in vitro* cell cycling. By comparison with appropriate sham-exposed and control samples, no effect of RFR alone could be found for any of the assay endpoints. In addition, RFR did not modify any measured effects of the X-radiation. In conclusion, this study has used several standard *in vitro* tests for chromosomal and DNA damage in Go human lymphocytes exposed *in vitro* to a combination of X-rays and RFR. It has comprehensively examined whether a 24-h continuous exposure to a 935 MHz GSM basic signal delivering SAR of 1 or 2 W/Kg is genotoxic *per se* or whether, it can influence the genotoxicity of the well-established clastogenic X-radiation. Within the experimental parameters of the study in all instances no effect from the RFR signal was observed. [Of course, DNA damage is a well characterized effect of electromagnetic irradiation of other cell types, including lymphoblastoid cells [72], fibroblasts [73], and brain cells [74].]

Despite the important role of the immune system in defending the body against infections and cancer, only rather few investigations on possible effects of RF radiation on function of human immune cells have been undertaken. One of these is the investigation by Tuschl et al. [75] in 2006 where they assessed whether GSM modulated RF fields have adverse effects on the functional competence of human immune cells. Within the frame of a multidisciplinary project "Biological effects of high frequency electromagnetic fields (EMFs)" sponsored by the National Occupation Hazard Insurance Association (AUVA), *in vitro* investigations were carried out on human blood cells. Exposure was performed at GSM basic 1950 MHz, an SAR of 1 mW/g in an intermittent mode (5 min "ON", 10 min "OFF") and a maximum ΔT of 0.06 °C for the duration of 8 h. The following immune parameters were evaluated: (1) the intracellular production of interleukin-2 (IL-2) and interferon (INF) gamma in lymphocytes, and IL-1 and TNF-alpha in monocytes were evaluated with monoclonal antibodies. (2) The activity of immune-relevant genes (IL-1-alpha and beta, IL-2, IL-2-receptor, IL-4, macrophage colony stimulating factor (MCSF)-receptor, TNF-alpha, TNF-alpha-receptor) and housekeeping genes was analyzed with real time PCR. (3) The cytotoxicity of lymphokine activated killer cells (LAK cells) against a tumour cell line was determined in a flow cytometric test. For each parameter, blood samples of at least 15 donors were evaluated. No statistically significant effects of exposure were found and there was no indication that emissions from mobile phones are associated with adverse effects on the human immune system.

Chagnaud and Veyret [76] in 1999 could also not demonstrate an effect of low-level pulsed microwaves on the integrity of the immune system. They investigated the effects of GSM-modulated microwaves on lymphocyte sub-populations of Sprague-Dawley rats and their normal mitogenic responses using flow cytometry analysis and a colorimetric method. No alterations were found in the surface phenotype of splenic lymphocytes or in their mitogenic activity.

[N.B. One must always be very cautious when it comes to negative findings. For example: of the 100 or so papers on genotoxic effects of RF fields, a majority has been done with peripheral blood lymphocytes. Except for special cases, these cells are highly protected from their upregulated repair enzymes. These cells are often used to investigate chemical genotoxicity, because in these cases the toxicity often occurs due to the action of the repair enzymes. Repair-deficient cells remain intact! The mechanisms of action of EMFs may not be clearly understood, but it is unlikely they mimic such chemical enzyme-induced genotoxicity. — Yet another example is the use of mice and rats for the study increases in brain tumour incidence due to mobile telephony exposure. Since the incidence data from human studies point to a needed exposure time of at least 5 years, and mice and rats do not live longer

than 2 years, the rats will die long before they have had a chance to develop the tumours!]

Irradiation by pulsed microwaves (9.4 GHz, 1 μ s pulses at 1000/s), both with and without concurrent amplitude modulation (AM) by a sinusoid at discrete frequencies between 14 and 41 MHz, was assessed for effects on the immune system of Balb/c mice [77]. The mice were immunized either by sheep red blood cells (SRBC) or by glutaric–anhydride conjugated bovine serum albumin (GA-BSA), then exposed to the microwaves at a low rms power density (30 μ W/cm²; whole-body averaged SAR approximately 0.015 W/kg). Sham exposure or microwave irradiation took place during each of 5 contiguous days, 10 h/day. The antibody response was evaluated by the plaque-forming cell assay (SRBC experiment) or by the titration of IgM and IgG antibodies (GA-BSA experiment). In the absence of AM, the pulsed field did not greatly alter immune responsiveness. In contrast, exposure to the field under the combined-modulation condition resulted in significant, AM-frequency-dependent augmentation or weakening of immune responses.

To study the possible RF effects on human lymphocyte activation, Capri et al. [78] analyzed CD25, CD95, CD28 molecules in unstimulated and stimulated CD4+ or CD8+ T-cells in vitro. Peripheral blood mononuclear cells (PBMCs) from young and elderly donors were exposed or sham-exposed to RF (1800 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, after RF exposure they observed 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 a molecule in regulation of the immune response.

In the paper by Yurekli et al. [79], a GHz transverse electromagnetic (GTEM) cell was used as an exposure environment for plane wave conditions of far-field free space EMF propagation at the GSM base transceiver station (BTS) frequency of 945 MHz, and effects on oxidative stress in rats were investigated. When EMFs at a power density of 3.67 W/m² (SAR = 11.3 mW/kg), which is well below current exposure limits, were applied, MDA (malondialdehyde) level was found to increase and GSH (reduced glutathione) concentration was found to decrease significantly ($p < 0.0001$). Additionally, there was a less significant ($p = 0.019$) increase in SOD (superoxide dismutase) activity under EMF exposure.

Since genotoxic effects of the second generation standard GSM have been reported after exposure of human cells in vitro, Schwarz et al. [80] decided to use human cultured fibroblasts of three different donors and three different short-term human lymphocyte cultures and expose them to 1950 MHz UMTS below the SAR safety limit of 2 W/kg. The alkaline comet assay and the micronucleus assay

were used to ascertain dose and time-dependent genotoxic effects. Five hundred cells per slide were visually evaluated in the comet assay and comet tail factor (CTF) was calculated. In the micronucleus assay 1000 binucleated cells were evaluated per assay. The origin of the micronuclei was determined by fluorescence labeled anticentromere antibodies. All evaluations were performed under blinded conditions. UMTS exposure increased the CTF and induced centromere-negative micronuclei (MN) in human cultured fibroblasts in a dose and time-dependent way. Incubation for 24 h at an SAR of 0.05 W/kg generated a statistically significant rise in both CTF and MN ($p = 0.02$). At an SAR of 0.1 W/kg the CTF was significantly increased after 8 h of incubation ($p = 0.02$), the number of MN after 12 h ($p = 0.02$). However, under these conditions, no UMTS effect was obtained with lymphocytes, either unstimulated or stimulated with phytohemagglutinin. The authors conclusion was that UMTS exposure may cause genetic alterations in some but not in all human cells in vitro.

Simkó and Mattsson [81] have presented a hypothesis of a possible initial cellular event affected by exposure to ELF-EMF, an event that is compatible with the multitude of effects observed after exposure. Based on an extensive literature review, they suggested that ELF-EMF exposure is able to perform such activation by means of increasing levels of free radicals. Such a general activation is compatible with the diverse nature of observed effects. Free radicals are intermediates in natural processes, like mitochondrial metabolism, and are also a key feature of phagocytosis. Free radical release is inducible by ionizing radiation or phorbol ester treatment, both leading to genomic instability. EMFs might be a stimulus to induce an "activated state" of the cell such as phagocytosis, which then enhances the release of free radicals, in turn leading to genotoxic events. Simkó and Mattsson envisaged that EMF exposure can cause both acute and chronic effects that are mediated by increased free radical levels: (1) Direct activation of, for example macrophages (or other cells) by short-term exposure to EMF leads to phagocytosis (or other cell-specific responses) and consequently, free radical production. This pathway may be utilized to positively influence certain aspects of the immune response, and could be useful for specific therapeutic applications. (2) EMF-induced macrophage (cell) activation includes direct stimulation of free radical production. (3) An increase in the lifetime of free radicals by EMF leads to persistently elevated free radical concentrations. In general, reactions in which radicals are involved become more frequent, increasing the possibility of DNA damage. (4) Long-term EMF exposure leads to a chronically increased level of free radicals, subsequently causing an inhibition of the pineal gland hormone melatonin. Taken together, these EMF-induced reactions could lead to a higher incidence of DNA damage and therefore, to an increased risk of tumour development. While the effects on melatonin and the extension of the lifetime of radicals can explain the link between EMF

exposure and the incidence of for example leukaemia, the two additional mechanisms described by them specifically for mouse macrophages, can explain the possible correlation between immune cell system stimulation and EMF exposure.

5.7.4. Effects of EMFs on the immune system at pregnancy

Nakamura et al. [82] have investigated a very important issue, namely what happens to pregnant rats exposed to microwaves. Earlier data had indicated that these microwaves produce various detrimental changes based on actions of heat or non-specific stress, although the effects of microwaves on pregnant organisms were not uniform. This study was therefore designed to clarify the effect of exposure to microwaves during pregnancy on endocrine and immune functions. Natural killer cell activity and natural killer cell subsets in the spleen were measured, as well as some endocrine indicators in blood: corticosterone and adrenocorticotrophic hormone (ACTH) as indices of the hypothalamic–pituitary–adrenal axis; beta-endorphin, oestradiol, and progesterone in six female virgin rats and six pregnant rats (9–11 days gestation) exposed to microwaves at 10 mW/cm² incident power density at 2450 MHz for 90 min. The same measurements were performed in control rats (six virgin and six pregnant rats). Skin temperature in virgin and pregnant rats increased immediately after exposure to microwaves. Although splenic activity of natural killer cells and any of the subset populations identified by the monoclonal antibodies CD16 and CD57 did not differ in virgin rats with or without exposure to microwaves, pregnant rats exposed to microwaves showed a significant reduction of splenic activity of natural killer cells and CD16+ CD57- ones. Although corticosterone and ACTH increased, and oestradiol decreased in exposed virgin and pregnant rats, microwaves produced significant increases in beta-endorphin and progesterone only in pregnant rats. So, in summary, Nakamura et al. [82] showed that microwaves at the power of 10 mW/cm² produced activation of the hypothalamic–pituitary–adrenal axis and increased oestradiol in both virgin and pregnant rats, indicating that microwaves are a great stress in pregnancy.

In the following year, 1998, the same groups of scientists published a new study [83] in which they examined the involvement of opioid systems in reduced natural killer cell activity (NKCA) in pregnant rats exposed to microwaves at a relatively low level (2 mW/cm² incident power density at 2450 MHz for 90 min). They assayed beta-endorphin (betaEP) in blood, pituitary lobes, and placenta as well as splenic NKCA in virgin and/or pregnant rats. Although microwaves elevated colonic temperatures by 0.8 °C for virgin and 0.9 °C for pregnant rats, and betaEP in blood and anterior pituitary lobes (AP) significantly, it did not change blood corticosterone as an index of hypothalamic–pituitary adrenal axis. There were significant interactions between pregnancy and microwave exposure on splenic NKCA, betaEP in both blood and AP, and blood progesterone.

Intra-peritoneal administration of opioid receptor antagonist naloxone prior to microwave exposure increased NKCA, blood, and placental betaEP in pregnant rats. Alterations in splenic NKCA, betaEP and progesterone in pregnant rats exposed to microwaves may be due to both thermal and non-thermal actions. These results suggest that NKCA reduced by microwaves during pregnancy is mediated by the pituitary opioid system.

To further clarify the effects of microwaves on pregnancy, uterine or uteroplacental blood flow and endocrine and biochemical mediators, including corticosterone, estradiol, prostaglandin E(2) (PGE(2)), and prostaglandin F(2)alpha (PGF(2)alpha), Nakamura et al. published yet another study in 2000 [84]. In this article they measured these parameters and factors in rats exposed to continuous-wave (CW) microwave at 2 mW/cm² incident power density at 2450 MHz for 90 min. Colonic temperature in virgin and pregnant rats was not significantly altered by microwave treatment. Microwaves decreased uteroplacental blood flow and increased progesterone and PGF(2)alpha in pregnant, but not in virgin rats. Intraperitoneal (i.p.) administration of angiotensin II, a uteroplacental vasodilator, before microwave exposure prevented the reduction in uteroplacental blood flow and the increased progesterone and PGF(2)alpha in pregnant rats. Increased corticosterone and decreased estradiol during microwave exposure were observed independent of pregnancy and pretreatment with angiotensin II. These results suggest that microwaves (CW, 2 mW/cm², 2450 MHz) produce uteroplacental circulatory disturbances and ovarian and placental dysfunction during pregnancy, probably through non-thermal actions. The uteroplacental disturbances appear to be due to actions of PGF(2)alpha and may pose some risk for pregnancy! [Could the above findings be part of the explanation behind the sensational findings of Magras and Xenos [85] from 1997?]

5.7.5. Synchronization of cerebral rhythms. Important for the brain-immune system axis?

Vecchio et al. [86] have reported that EMF from mobile phones affects the synchronization of cerebral rhythms. Their findings suggest that prolonged exposure to mobile phone emissions affect cortical activity and the speed of neural synchronization by interhemispherical functional coupling of EEG rhythms. This may be evidence that such exposure can affect the way in which the brain is able to process information, by interfering with the synchronization rhythms between the halves of the brain, and by deregulating the normal alpha wave 2 (about 8–10 Hz) and alpha 3 (10–12 Hz) bands. [Could such deregulation affect the brain-immune system axis? If so, what implications would it have in the short- as well as in the long-term?]

5.7.6. Classical contact allergy reactions

Finally, in addition, classical contact allergy reactions, such as chromate allergy, have been studied by Seishima et al. [87]. The background for the study was an earlier case

report about a patient with allergic contact dermatitis caused by hexavalent chromium plating on a cellular phone. The new study described the clinical characteristics and results of patch tests (closed patch tests and photopatch tests were performed using metal standard antigens) in eight patients with contact dermatitis possibly caused by handling a cellular phone. The eight patients were four males and four females aged from 14 to 54 years. They each noticed skin eruptions after 9–25 days of using a cellular phone. All patients had erythema, and seven had papules on the hemilateral auricle or in the preauricular region. Three of eight patients had a history of metal allergy. Chromate, aluminium and acrylnitrile-butadiene-styrene copolymer were used as plating on the cellular phones used by these patients. The patch test was positive for 0.5%, 0.1% and 0.05% potassium dichromate in all eight patients. The photopatch test showed the same results. One patient was positive for 2% cobalt chloride and one for 5% nickel sulfate. Based on these data, it is important to consider the possibility of contact dermatitis due to a cellular phone, possibly caused by chromate, when the patients have erythema and papules on the hemilateral auricle or in the preauricular region.

6. Effects of electromagnetic fields on other biological systems

Some parallel investigations, pointing to severe biological effects that need to be mentioned are, for instance, the results of Roux et al. [88] in 2008. Using an especially designed facility, the Mode Stirred Reverberation Chamber, they exposed tomato plants (*Lycopersicon esculentum* Mill. VFN8) to low level (900 MHz, 5 V/m) EMF for a short period (10 min) and measured changes in abundance of three specific mRNA soon after exposure. Within minutes of stimulation, stress-related mRNA (calmodulin, calcium-dependent protein kinase and proteinase inhibitor) accumulated in a rapid, large and 3-phase manner typical of an environmental stress response. Accumulation of these transcripts into the polysomal RNA also took place (indicating that the encoded proteins were translated) but was delayed (indicating that newly-synthesized mRNA was not immediately recruited into polysomes). Transcript accumulation was maximal at normal Ca(2+) levels and was depressed at higher Ca(2+), especially for those encoding calcium-binding proteins. Removal of Ca(2+) (by addition of chelating agents or Ca(2+) channel blocker) led to total suppression of mRNA accumulation. Finally, 30 min after the electromagnetic treatment, ATP concentration and adenylate energy charge were transiently decreased, while transcript accumulation was totally prevented by application of the uncoupling reagent, CCCP. These responses occur very soon after exposure, strongly suggesting that they are the direct consequence of application of radiofrequency fields, and their similarities to wound responses strongly suggests that this radiation is perceived by plants as an injurious stimulus! [Furthermore, it is

impossible to interpret these reactions as “psychological or psychiatric personality disturbances, cognitive malfunction, or likewise”.]

Also, the data from Divan et al. [89] deserve to be mentioned. They examined the association between prenatal and postnatal exposure to cell phones and behavioral problems in young children. Mothers were recruited to the Danish National Birth Cohort early in pregnancy. When the children of those pregnancies reached 7 years of age in 2005 and 2006, mothers were asked to complete a questionnaire regarding the current health and behavioral status of children, as well as past exposure to cell phone use. Mothers evaluated the child's behavior problems using the Strength and Difficulties Questionnaire. Mothers of 13,159 children completed the follow-up questionnaire reporting their use of cell phones during pregnancy as well as current cell phone use by the child. Greater odds ratios for behavioral problems were observed for children who had possible prenatal or postnatal exposure to cell phone use. After adjustment for potential confounders, the odds ratio for a higher overall behavioral problems score was 1.80 (95% confidence interval = 1.45–2.23) in children with both prenatal and postnatal exposure to cell phones. Exposure to cell phones prenatally – and, to a lesser degree, postnatally – was associated with behavioral difficulties such as emotional and hyperactivity problems around the age of school entry. [An obvious follow-up question would be “What about immune function alterations?”.] Naturally, and hopefully, these associations may be non-causal and may be due to unmeasured confounding. But if real, they would be of public health concern given the widespread use of this technology.

The exposure to non-thermal microwave EMF generated by mobile phones affects the expression of many proteins. This effect on transcription and protein stability can be mediated by the MAPK (mitogen-activated protein kinase) cascades, which serve as central signaling pathways and govern essentially all stimulated cellular processes. Indeed, long-term exposure of cells to mobile phone irradiation results in the activation of p38 as well as the ERK (extracellular signal-regulated kinase) MAPKs. Friedman et al. [90] recently have studied the immediate effect of irradiation on the MAPK cascades, and found that ERKs, but not stress-related MAPKs, are rapidly activated in response to various frequencies and intensities. Using signaling inhibitors, they delineated the mechanism that is involved in this activation. They found that the first step is mediated in the plasma membrane by NADH oxidase, which rapidly generates ROS (reactive oxygen species). These ROS then directly stimulate MMPs (matrix metalloproteinases) and allow them to cleave and release Hb-EGF (heparin-binding EGF (epidermal growth factor)). This secreted factor activates the EGF receptor, which in turn further activates the ERK cascade. Thus, their study demonstrates for the first time a detailed molecular mechanism by which electromagnetic irradiation from mobile phones induces the activation of the ERK cascade and thereby induces transcription and other cellular processes.

The terminal deoxynucleotide transferase dUTP nick end labeling (TUNEL) assay, a well known technique widely used for detecting fragmented DNA in various types of cells, was used by Panagopoulos et al. [91] to detect cell death (DNA fragmentation) in a biological model, the early and mid stages of oogenesis of the insect *Drosophila melanogaster*. The flies were exposed *in vivo* to either GSM 900 MHz or DCS 1800 MHz radiation from a common digital mobile phone, for few minutes per day during the first 6 days of their adult life. The exposure conditions were similar to those to which a mobile phone user is exposed. Previous results from the same group [92–94] had shown a large decrease in the oviposition of the same insect caused by GSM radiation. The recent results suggest that this decrease in oviposition, is due to degeneration of large numbers of egg chambers after DNA fragmentation of their constituent cells, induced by both types of mobile telephony radiation. Induced cell death is recorded for the first time, in all types of cells constituting an egg chamber (follicle cells, nurse cells and the oocyte) and in all stages of the early and mid-oogenesis, from germarium to stage 10, during which programmed cell death does not physiologically occur. Germarium and stages 7–8 were found to be the most sensitive developmental stages also in response to electromagnetic stress induced by the GSM and DCS fields and, moreover, germarium was found to be even more sensitive than stages 7–8.

7. Conclusions

- Both human and animal studies report large immunological changes upon exposure to environmental levels of modern, human-made EMFs. Some of these exposure levels are equivalent to those of wireless technologies in daily life, and often at low or very low (i.e., non-thermal) levels.
- Measurable physiological changes (mast cells increases, for example) that are bedrock indicators of allergic response and inflammatory conditions are stimulated by EMF exposures.
- Chronic exposure to such factors that increase allergic and inflammatory responses on a continuing basis may be harmful to health. The data presented here, as well as the very rapid international increase in incidence of allergies, asthma and other oversensitivities, together form a clear warning signal.
- It is, thus, possible that chronic provocation by exposure to EMF can lead to immune dysfunction, chronic allergic responses, inflammatory responses and ill health if they occur on a continuing basis over time. This is an area that should be investigated immediately.
- Specific findings from studies on exposures to various types of modern equipment and/or EMFs report over-reaction of the immune system; morphological alterations of immune cells; profound increases in mast cells in the upper skin layers, increased degranulation of mast cells and larger size of mast cells in electrohypersensitive indi-

viduals; presence of biological markers for inflammation which are sensitive to EMF exposure at non-thermal levels; changes in lymphocyte viability; decreased count of NK cells; decreased count of T-lymphocytes; negative effects on pregnancy (uteroplacental circulatory disturbances and placental dysfunction); suppressed or impaired immune function; and inflammatory responses that can ultimately result in cellular, tissue and organ damage.

- The functional impairment electrohypersensitivity is reported by individuals in the United States, Sweden, Switzerland, Germany, Belgium, Italy, The Netherlands, Norway, Denmark and many other countries of the world. Estimates range from 3% to perhaps 10% of populations, and appear to be a growing condition of ill-health leading to lost work and productivity.
- The WHO and IEEE literature surveys do not include all of the relevant papers cited here, leading to the conclusion that evidence has been ignored in the current WHO ELF Health Criteria Monograph; and the proposed new IEEE C95.1 RF public exposure limits.
- The current international public safety limits for EMFs do not appear to be sufficiently protective of public health at all, based on the studies of immune function. New, biologically based public standards are warranted that take into account low-intensity effects on immune function and health that are reported in the scientific literature. Also the accessibility needs of persons with the functional impairment electrohypersensitivity must be fully addressed and resolved as dictated by the UN 22 “Standard rules on the equalization of opportunities for people with disabilities” (about the UN 22 Standard Rules, see website: <http://www.un.org>; since 2007 they have been upgraded into the UN “Convention on Human Rights for Persons with Functional Impairments”).

The conclusion of the above must be that there are a number of very strong indications of EMFs being capable of disturbing the immune system and thus increasing disease, including cancer, risk. It is somewhat odd that professional epidemiologists for the last 50 years have not addressed the issue of reduced repair but only looked at increased cell damages from different agents and environments when trying to understand trend changes.

Based on this review as well as on the recent Bioinitiative Report [<http://www.bioinitiative.org/>] [1], it must be concluded that the existing public safety limits are inadequate to protect public health. From a public health policy standpoint, new public safety limits, and limits on further deployment of untested technologies, are warranted.

New biologically based public and occupational exposure are recommended to address bioeffects and potential adverse health effects of chronic exposure. These effects are now widely reported to occur at exposure levels significantly below most current national and international limits. Therefore, biologically based exposure standards are needed to prevent disruption of normal body processes. Effects are

reported for DNA damage (genotoxicity that is directly linked to integrity of the human genome), cellular communication, cellular metabolism and repair, cancer surveillance within the body; and for protection against cancer and neurological diseases. Also reported are neurological effects including changes in brainwave activity during cell phone calls, impairment of memory, attention and cognitive function; sleep disorders, cardiac effects; and – as reported here – serious impact on the immune function (allergic and inflammatory responses).

The current recommendation must be a biologically based exposure limit that is completely protective against e.g. extremely low frequency and radiofrequency fields which, with chronic exposure, can reasonably be presumed to result in no adverse impacts on health and well-being. Today, such a completely protective safety limit would, for many exposure situations, be zero.

Finally, attention to the above need would also mean a great gain in future public health costs for the entire electrified world. To do the opposite could turn out to be very expensive.

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Reproductive and developmental effects of EMF in vertebrate animal models

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Abstract

This paper reviews the literature data on the effects of electromagnetic fields (EMF), in the reproductive organs as well as in prenatal and postnatal development of vertebrate animals. Review articles which have been published till 2001, regarding the reproductive and developmental effects of the entire range of frequency of electromagnetic fields, were surveyed. Experimental studies which were published from 2001 onwards were summarized. Special focus on the effects of radiofrequencies related to mobile communication in the above mentioned topics has been made. According to the majority of the investigations, no strong effects resulted regarding the exposure to EMF of mobile telephony in the animal reproduction and development. However further research should be done in order to clarify many unknown aspects of the impact of EMF in the living organisms.

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1. Introduction

During the 20th century, the exposure to electromagnetic fields (EMF) became an important source of concern about the possible effects in the living organisms. The artificial sources of electromagnetic radiation have risen tremendously because of the ongoing needs on electricity, telecommunications, and electronic devices. In this context, World Health Organisation (WHO) established in 1996 the International EMF project in order to assess health and environmental effects of exposure to EMF in the frequency range from 0 to 300 GHz. For the purpose of this paper this range will be divided into static (0 Hz), extremely low frequency (ELF > 0–300 kHz), intermediate frequencies (IF > 300–10 MHz) and radiofrequency (RF 10 MHz–300 GHz) fields [J. Juutilainen, Developmental effects of electromagnetic fields, *Bioelectromagnetics* 7 (2005) S107–S115]. The mobile phone technology is based on radiofrequency radiation with transmission of microwaves carrying frequencies between 880 and 1800 MHz [P.A. Valberg, T.E. van Deventer, M.H. Repacholi, Workgroup report:

base stations and wireless networks-radiofrequency (RF) exposures and health consequences, *Environ. Health Perspect.* 115 (2007) 416–424].

The mobile telephony revolution took place in the last decade. There is an increasing number of cell phone users all over the world. Also, new technologies which use the spectrum of high frequency emissions are incorporated in many aspects of telecommunications. As a consequence, there is a lot of interest about the possible effects of the radiation emitted from the machines which are engaged in the telephony such as hand phones, base stations and transmitters.

The biological effects of EMF have been and are being investigated on different levels of organization. On the level of human populations, epidemiological studies are used whereas, on the level of individuals human, animal and plant *in vivo* experiments are carried out. Furthermore, on the level of organs, tissues and cells *in vitro* investigations are employed. Finally, on the sub-cellular level, biochemical and molecular techniques are utilized.

From another point of view, many studies have been carried out or are in progress about the various effects of radiation emissions regarding the behaviour, cancer, central nervous system, sleep, children, cardiovascular system, immune function, reproduction and development [3].

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The present paper will focus on the existing data about the reproductive and developmental effects of EMF in vertebrates. Reproduction is a critical function of the organisms and involves two body systems the male and female genital system. The development comprises a series of events which begins with fertilization, continues with implantation, embryonic growth and terms with sexual maturity. In the context of systematic zoology, the vertebrates are close to the humans. Therefore, the animal studies could provide useful information on the comprehension of interaction of EMF with the living organism and on the possible commonality with the humans.

The biological effects of EMF of interest can be broadly grouped into thermal and non-thermal [4]. The thermal effects are associated with local heat production just like the mechanism of a microwave oven. The non-thermal mechanism is triggered by an amount of energy absorption, which is not directly associated with temperature change but rather to some other changes produced in the tissues.

The goal of this paper is to present the up to date available data about the EMF and their potential effects on reproduction and development, filling the gap of information from the most recent published reviews. All the bibliographic data which will be presented were collected exclusively from scientific journals published in English and partially in other languages. The survey includes studies which were published from 2001 onward. The studies which relate to the impact of mobile phone electromagnetic fields will be presented thoroughly and independently from the date of their publication.

2. Historical background

The first paper which I found in the medical literature, regarding the effects of EMF on the development of vertebrates, was published in 1893 in an anatomical journal from Windle [5]. The author summarized the observations of three scientists and added his own about the effects of electricity on the chicken embryos. Two years later the same author [6], published an account on the effects of electricity and magnetism on development.

In 1980 two papers were published about the biological effects of microwave radiation. Cook et al. [7] published a comprehensive survey regarding the very early research on the biological effects of electromagnetic fields. The early work on short waves from 1885 to 1940 was presented. Following, the authors summarized the available data from 1940 to 1960. Leach [8] provided an account on the genetic, growth and reproductive effects of microwave radiation including early studies in this field that were published from 1959 to 1979. The majority of revised papers dealt with animals. Later, Algers and Hennichs [9] summarized the biological effects on vertebrates, of electromagnetic fields where the frequency did not exceed 100 Hz. The authors included many studies about the impact of EMF on farm animals. The same

year, a specialized review was published on the effects of non-ionizing radiation on birds [10].

Berman et al. [11], presented the results of a large multinational experimental effort (Henhouse project) regarding the low frequency EMF effects on chick embryos. Juutilainen [12], Chernoff et al. [13], Brent et al. [14] presented detailed reviews of the literature about the effects on reproduction related to low frequency EMF.

Jensh [15] reviewed behavioral teratologic studies using microwave radiation with special interest to continuous wave (CW) 915, 2450, or 6000 MHz radiation.

Verschaeve and Maes [16] reviewed the genetic, carcinogenic and teratogenic effects of RF (300 MHz–300 GHz). Regarding the effects on reproduction and teratogenesis, studies from 1961 to 1991 were surveyed. The majority of these experimental studies dealt with the exposure of animals at 2.45 GHz. The same year, Huuskonen et al. [17] reported on the teratogenic and reproductive effects of low frequency (0–100 kHz) magnetic fields associated with the use or transmission of electric power or emitted from video display terminals. The animal studies that were surveyed, have been published from 1987 to 1997 regarding the effects of alternating magnetic fields on prenatal development of rats and mice. In the same paper, studies on the effects of prenatal exposure of alternating magnetic fields on postnatal development were included. Brent [18] provided a thorough review of *in vivo* and *in vitro* studies on the reproductive and teratologic effects of low frequency EMF. The survey of reproductive effects has involved studies with chick embryos, chickens, cows, mice, and rats from 1969 to 1996. O'Connor [19] recorded the intrauterine effects in animals exposed to radiofrequency and microwave fields with a special feature. The SAR of the surveyed studies was above the limit of 0.4 W/kg.

Experimental studies on the teratologic effects or developmental abnormalities from exposure to RF electromagnetic fields in the range 3 kHz–300 GHz were reviewed from Heynick and Merritt [20]. The review included investigations with insects, birds (chicken, quails, turkeys) and mammalian species (mice, rats) as well as non-human primates which appeared from 1974 to 2000. A brief critical review on the developmental effects of extremely low frequency (ELF) electric and magnetic fields provided by Juutilainen [21]. Löscher [22] published a survey of the effects of radiofrequency electromagnetic fields on production, health and behaviour of farm animals.

Juutilainen [1] reported on the effects of EMF on animal development. In his review, he surveyed specific topics such as the Henhouse project, the interaction of LF-IMF EMF with known teratogens, and the behavioral teratology of RF. Saunders and McCaig [23] summarized the possible effects on prenatal development of physiologically weak electric fields induced in the body by exposure to extremely low frequency electromagnetic fields and of elevated temperature levels that might result from exposure to radiofrequency (RF) radiation.

Table 1

Overview of investigations on EMF effects on animal genital system.

Animal species	Exposure frequency	Exposure parameters	Exposure duration	Endpoint	Results	Comments	Reference
Mouse Swiss	50 Hz	25 mT	Continuous 90 days	Effects on reproductive ability	No effect on the fertility of male and female mice. The ovarian weight was significantly increased		[27]
Mouse CD1 (BALB/c X DBA/2)	60 Hz	2 mT	Continuous for 72 h or 8 h/day for 10 days	Sperm morphology	No statistically differences were observed	Two groups were treated with mitomycin C. Sperm abnormalities were found in the group exposed versus the group treated with mitomycin C alone	[28]
Mouse BALB/c	60 Hz	0.1 or 0.5 mT	24 h/day for 8 weeks	Germ cell apoptosis in the testes	No significant changes in testicular weights. Decrease of normal seminiferous tubules. Increase of the germ cell death		[29]
Rat Sprague-Dawley	60 Hz	5, 83.3, 500 mT	Continuous 21 h/day from day 6 of gestation to day 21 of lactation	Spermatotoxicity and reproductive dysfunction in the F1 offspring	No detectable alterations in offspring spermatogenesis and fertility		[30]
Rat Sprague-Dawley	50 Hz	25 ± 1 μT	Continuous for 18 weeks	Effects on sperm count, weights of testes, seminal vesicles, preputial glands	No effect on the weight of testes. Significant reduction of the weight of seminal vesicles and preputial glands. Significant reduction in sperm count		[31]
Rat Sprague-Dawley	50 Hz	1.35 ± 0.018 mT	2 h/day, 7 days/week for 2 months	Sperm count, morphological changes of testes	No significant alterations were observed	Funding not mentioned	[32]
Rat Wistar albino ♂♂	50 Hz	1 mT (mean value)	3 h/day for 50 or 100 days	Morphological evaluation of uterus and ovaries	Ultrastructural alterations in germinal epithelium of ovaries in the experimental group (50 days) as well as in tunica albuginea (100 days)	Ambiguous observations in the uterus	[33]
Rat Sprague-Dawley ♂♂	20 kHz	6.25 mT	8 h/day, 5 days/week for 90 days	Histopathological examination of various organs	No differences were seen in testis and ovary		[34]

Table 1 (Continued)

Animal species	Exposure frequency	Exposure parameters	Exposure duration	Endpoint	Results	Comments	Reference
Rat Wistar ♂♀	50 Hz		3 weeks in utero and 5 weeks postnatal	Testes	Morphological changes in the boundary tissue of the seminiferous tubules		[35]
Rat Sprague–Dawley ♂♂	20 kHz sine waves	6.25 mT	8 h/day for 12 or 18 months	Histopathological examination of various organs	No differences were seen in testis and ovary		[36]
Rat Wistar	30–300 GHz	>0.3 mW/cm ²	30 min for 63 days	Spermatogenesis	Morphological changes in spermatozoa	Scanty data presentation	[37]
Rat Wistar	50 Hz		8 h/day for 8 months	Histological evaluation of testes	Mean seminiferous tubule diameter and testicular weight were significantly lower in exposed group. Histologic damage score was threefold in experimental group versus control		[38]

A special topic, the effects of EMF from power lines on avian reproductive biology, was reviewed by Fernie and Reynolds [24]. Krewski et al. [25], reviewed studies referring to various disciplines regarding the effects of RF. The included literature was published between 2001 and 2003. A novelty of this paper, was a discussion of the reports of various authorities and committees about the potential health risks associated with exposure to RF fields. A gap in the literature regarding the biological effects of EMF in the intermediate frequency range was covered by the review of Shigemitsu et al. [26].

During the last decade, many reports from authorities (local, national and international) and expert panels have been uploaded on the web [2].

It is suggested that the reader refer to the above-mentioned review articles and electronic addresses, in order to assemble a more complete and detailed view of the biological effects of EMF.

3. Male genital system

The testes are very important organs situated externally to the body and enclosed by the scrotum. The testicular parenchyma is the site of an intense proliferation and differentiation of the germinal cells that will become the sperm cells. The testes are very sensitive to temperature variations and for this reason the scrotum, which contains the testicular parenchyma, has a specialized contractile structure.

Studies that have evaluated EMF effects (mainly LF) on the genital systems of the vertebrates are summarized in Table 1.

Regarding mobile telephony, the first study conducted by Dasdag et al. [39] investigated whether there are adverse effects due to microwave exposure emitted by cellular phones in male Wistar albino rats. The animals ($n = 18$) were divided in three groups (control, standby exposed group, speech exposed group). Specific energy absorption rate (SAR) was 0.141 W/kg. Rats in the experimental groups were exposed for 2 h/day for 1 month in standby position, whereas phones were turned to the speech position three times for 1 min. The decrease of epididymal sperm counts in the speech groups was not found to be significant. Differences in terms of normal and abnormal sperm forms were not observed. Histological changes were especially observed in the testes of rats in the speech group. Seminiferous tubular diameter of rat testes in the standby and speech groups was found to be lower than the sham group. Rectal temperatures of rats in the speech group were found to be higher than the sham and standby groups. The rectal temperatures of rats before and after exposure were also found to be significantly higher in the speech group.

The same group of authors [40], failed to reproduce the results of their previous work. Sixteen Sprague–Dawley rats were separated into two groups (control, experimental). They were exposed to 890–915 MHz pulsed wave (PW) daily for

20 min/day for 1 month. For 250 mW average radiated power, SAR was 0.52 W/kg. No differences were observed in the percentages of epididymal normal and abnormal sperms, the epididymal sperm count, as well as in the seminiferous tubule diameter between control and experimental groups. Also, the testicular biopsy score as evaluated by Johnson's scale did not differ significantly.

Aitken et al. [41] assessed the testis of mice irradiated with 900 MHz in a waveguide, with an exposure condition SAR 90 mW/kg for 7 days at 12 h/day. The authors did not observe abnormalities regarding the sperm number, morphology and vitality. However, they reported significant damage to the mitochondrial genome as well as to the nuclear-globin locus.

Results similar to a previous study [39] regarding the diameter of the seminiferous tubules of rat testes were obtained by Ozguner et al. [42]. During the experiment, 20 male Sprague–Dawley rats (5 months of age) were either exposed to 900 MHz CW (average power density 1 ± 0.4 mW/cm²) or not (control group). Rats exposed 30 min/day, for 5 days/week for 4 weeks. The authors also did not observe significantly different values of weight of testes, testicular biopsy score count and the percentage of interstitial tissue. However, the mean height of the germinal epithelium was found decreased in the group of rats that had been irradiated.

Forgács et al. [43] repeatedly exposed male NMRI mice to 1800 MHz GSM like microwave radiation at 0.018–0.023 W/kg whole body SAR. 11–12 sham exposed and 11–12 exposed mice were used. The animals were exposed ten times (over 2 weeks) and the duration of exposure was 2 h/day. No microwave exposure-related morphological alterations were found in testis, epididymis and prostate.

Adult male rats were examined after exposure at sub-chronic exposure to RF emitted from a conventional cell phone on their testicular function. Sixteen Wistar rats were used at age 30 days. The animals were exposed for 1 h daily during 11 weeks. The experimental group ($n=8$) was exposed to 1835–1850 MHz at 0.04–1.4 mW/cm². Total body weight and absolute and relative testicular and epididymal weights did not change significantly. Epididymal sperm count was not significantly different between the groups. Regarding the histomorphological endpoints of the study, no difference was found between the experimental and control arm [44].

The effect of cellular phone emissions on sperm characteristics in 16 Sprague–Dawley rats were studied [45]. The laboratory animals were divided in two groups (experimental, control) and exposed to four cell phones which had a personal communications service code division multiple access frequency band of 1.9 GHz (800 MHz digital and 800 MHz analog). The rats received daily (3 h–30 min rest–3 h) cell phone exposure for 18 weeks. The SAR ranged from 0.9 to 1.80 W/kg whereas the power from 0.00001 to 0.607 W, according to the specific mode of function. The authors analyzed the morphology of the sperm cells from

epididymis of rats. The percentage of deformities for the experimental group was 34.3% and the percentage of deformities for the control group was 32.1%. This difference in the occurrence of deformities between the two groups was not statistically significant ($p>.05$) through a paired *t* test. The total sperm counts from the testes were not significantly different between the two groups. None of the temperature differences between the two groups were statistically significant.

Sixteen Sprague–Dawley rats were used to evaluate the bcl-2 protein (an anti-apoptotic protein) in rat testes. The experimental group ($n=8$) was exposed to commercial (GSM) cellular phones irradiation for 20 min/day for 1 month. Average power density was measured at 0.047 mW/cm² and SAR levels changed between 0.29 and 0.87 W/kg. The testes were investigated by means of immunohistochemistry. No difference was observed between testes sections of the sham and experimental groups in terms of bcl-2 staining. These results indicate that the radiation emitted from 900 MHz cellular phones did not alter the anti-apoptotic protein in the testes of rats [46].

In order to investigate the apoptosis-inducing effect of mobile phone exposure on spermatogonia in seminiferous tubules, 31 Wistar albino male rats were divided in three groups such as cage control ($n=10$), sham exposed ($n=7$), and experimental ($n=14$). The 2 h/day (7 days/week) exposure of 900 MHz radiation (power density 0.012–0.149 mW/cm² and SAR 0.07–0.57 W/kg) over a period of 10 months was evaluated by means of immunohistochemistry. The long-term radiation did not affect the active caspase-3 levels in testes of rats. Caspase-3 is a typical feature of apoptosis [47].

4. Female genital system

Studies on the impact of RF in the female genital system are scarce. Two studies were conducted in order to evaluate the effects on endometrial apoptosis and the ameliorating effects of a combination of vitamin E and C against EMF damage.

Oral et al. [48], exposed sexually mature female rats (16 weeks old) to 900 MHz radiation, 30 min/day for 30 days. Twenty-four Wistar albino rats were divided in three groups (sham exposed, EMF exposed, EMF exposed treated with vitamin C and E). The animals were exposed at 1.04 mW/cm² (SAR 0.016–4 W/kg). The effect of microwaves was examined in rat endometrium by means of immunohistochemistry. Endometrial apoptosis was observed. Guney et al. [49], repeated the experiment with the addition of another group (control). Histological changes in endometrium, diffuse and severe apoptosis in the endometrial surface, epithelial and glandular cells were reported regarding the group exposed to EMF. Also, eosinophilic leucocyte and lymphocyte infiltration were seen in the endometrial stroma.

Table 2
Overview of investigations on EMF effects on animal development.

Animal species	Exposure frequency	Exposure parameters	Exposure duration	Endpoint	Results	Comments	Reference
Rat Sprague–Dawley	50 Hz	7, 70, 350 mT	22 h/day during 0–7 or 8–15 day of gestation	Effects on teratogenicity and embryonic development	No differences regarding embryonic deaths, fetal weight and teratogenicity		[50]
Mouse ICR	50 Hz	Sham (0.1–1 μ T), 0.5, 5 mT	9 weeks σ 2 weeks ϕ prior to mating	Effects on teratogenicity and embryonic development	No differences regarding embryonic deaths, fetal weight and teratogenicity		[51]
Mouse Swiss Webster	0 Hz–25 MHz		1 week beginning from the 18th day of pregnancy	Morphological changes in brain, thymus, adrenal gland during embryonic development	Pathological changes were observed in the neonates		[52]
Rat Sprague–Dawley	60 Hz	0 (sham group), 5, 83.3, 500 mT.	22 h/day during 6–20 day of gestation	Developmental toxicity	No differences regarding embryonic deaths, fetal weight and teratogenicity		[53]
Chicken	50 Hz	1.33–7.32 mT	24 h	Effects on teratogenicity and embryonic development	Significant difference in the percentage of abnormal embryos versus control was found in 4.19, 5.32, 5.86, and 6.65 densities. Some embryos with extra ribs, defects in ribs and vertebrae, anuria and abnormal beaks were observed	Funding not mentioned	[54]
Mouse ICR	20 kHz	6.5 mT	8 h/day from 2.5 to 15.5 days post-coitum	Effects on teratogenicity and embryonic development	No statistically significant differences in the number of implantation, embryonic death, resorption, growth retarded fetuses, external and skeletal abnormalities		[55]
Chicken Leghorn HR7	50 Hz	1 μ T, 500 μ T, 1 mT	Continuous for 15 or 21 days	Effects on embryo/fetus	At 15 days of incubation body weight was significantly lower versus controls. At 21 days of incubation the body weight and cranial diameters were smaller versus control. No differences in brain weight were observed in all groups	Funding not mentioned	[56]
Mouse ϕ	Static magnetic field	400 mT	6 min/day from 7.5 to 14.5 day of pregnancy	Teratogenic effects	Polydactylism, abdominal fissure, fused ribs, vestigial 13th rib, brain hernia, curled tail		[57]
Mouse ϕ	50 Hz	1.2 mT	8 h/day during pregnancy	Body weight of dams, development of offspring	Fetal loss, malformed fetuses, retardation of growth of the offspring in the first 2 weeks after birth	Article in chinese	[58]
Chicken White Leghorn eggs	50 Hz	1.33–7.32 mT	4 days	Morphological evaluation of embryos/fetuses	Abnormal brain cavities, spina bifida, monophthalmia, ocular defects and growth retardation		[59]