Biological Effects of Radiofrequency Fields

Experimental Animal Studies

Experimenting with animals is a classical and logical solution to investigating, in a controlled manner, the possible interactions of RF fields with whole biological organisms. This was extensively done before the 1990s, when safety thresholds for RF fields were being established. Therefore, high power densities were first utilized and lowered until the lowest levels were found to cause a disruption of behavior in certain controlled tasks, such as operant conditioning, or in observational arenas, such as open fields. We are not reviewing these studies here, since we intend to concentrate on those carried out at or below safety levels standardized by bodies such as ICNIRP and IEEE. The reader is directed to IEEE (2005, Annex B) for a thorough review on this research before the 1990s.

Animal experiments registered in the published literature can be divided roughly into three groups:

- cancer induction and promotion,
- behavioral effects
- other physiological and pathological alterations.

By the end of 2008, according to an extensive review by Swicord and Balzano (2009), there were 781 papers in the WHO EMF Health Project database reporting research on the effects of RF fields from 0.1 to 100 GHz on animals. The majority of the investigations employed laboratory rodents (mice and rats) and investigated effects of RF in the range of 900 MHz to 2.5 GHz, which is presently the most used for mobile and wireless voice and data communication. As we have mentioned before, results obtained with these animals do not necessarily translate to humans and other animals, since the absorption characteristics of RF in their internal organs are quite different, as well as various aspects of their biology. Interestingly enough, despite the importance of knowing the distribution of RF fields in the body of these animals, only one paper was concerned with dosimetry.

Due to this, mass media reporting of possible detrimental effects on humans based on animal studies can be, and were, prematurely misrepresented to the general public. This is because they omitted the methodological difficulties and caveats regarding the interpretation of results and its translation to humans, and reported mostly on single studies, many times chosen for its sensationalistic potential and not on the basis of a scientific consensus.

Animal studies cover a very large variety of organisms, structural and functional effects, and are given in the following table (adapted from Swicord & Balzano (2009), by permission):

Type and Number of Published In Vivo RF studies

In Vivo Study Type	Number Published	% Total
Animal Behavior, Brain Biochemistry, Neuropathology, Drug Interaction	140	17,9%
Teratogenicity, Reproduction, & Development	117	15,0%
Thermal Analysis	85	10,9%
Immune Function & Hematology	83	10,6%
Blood Brain Barrier, Brain (PET scan) and Other Tissue Blood Flow	56	7,2%
Eye Pathology	37	4,7%
Auditory Pathology & MW Hearing	36	4,6%
Gene & Protein Expression & Activity	29	3,7%
Micronuclei & Chromosome Aberrations	28	3,6%
Chemical-Radiation-Genetically Initiated Tumor Bioassay	27	3,5%
Oxidative Stress	24	3,1%
Blood Press., Heart Rate, Circulation, and Resp. Rate	23	2,9%
DNA Breaks, Damage & Mutation	19	2,4%
EEG, Event Related Potentials, Sleep Disturbances	19	2,4%
Long Term Rodent Bioassay	19	2,4%
Hormone Changes	12	1,5%
Calcium (and other ion) Studies	10	1,3%
Cell Line Injection Tumor Bioassay	5	0,6%
Other Animal Studies	5	0,6%
Proliferation, Growth Rate, & Cell Cycle Analysis	5	0,6%
Animal Study with Multiple Parameters Examined	1	0,1%
Experimental Dosimetry in Animals	1	0,1%
Total	**781**	

The majority of studies (about 71%) fell under the following categories:

- Thermal effects
- Animal behavior
- Brain biochemistry
- Neuropathology
- Teratogenicity
- Reproduction and development
- Immune function
- Hematopoietic system
- Blood-brain barrier

- Brain blood flow
- Visual and auditory systems

Another 14 % were related to the effects on genetic material and cell function and biochemistry under *in vivo* conditions.

Due to the large number of studies, we will focus our review on what we feel are the three most important areas: effects on the blood-brain barriers, teratogenesis (cancer induction and promotion) and long term survival under chronic exposure.

The Blood-Brain Barrier

The blood-brain barrier (BBB) has a very important function in mammals, providing a selective barrier between the blood supply to the brain and its internal milieu (extracellular fluid). This unique and complex system involves vascular membranes and supporting cells of the brain (glia), and provides a kind of selective filter that avoids undesired substances that circulate in the blood (and which could have toxic effects on neurons, for example), entering this milieu. Therefore, anything that weakens or opens the controls of the BBB might be detrimental to the health of the brain.

Reports were published in 1977 suggesting that irradiation of rats with RF at levels below current safety standards affected negatively the BBB was documented with standard techniques, using dyes or nuclide-labeled compounds which normally do not cross the barrier. Research published more recently by the group of Salford in Sweden (1993), with a series of more than 1,600 rats, showed that the BBB changed its permeability to the animal's own albumin, but not to fibrinogen, immediately as well as 7 and 14 days after being irradiated with 900 MHz GSM signals for 2 hours. His research received much press coverage and provoked alarm in the general public. Later, Salford tried to demonstrate indirectly that the albumin which passed the BBB and accumulated around neurons in the extracellular fluid of the spinal cord and the brain, could lead to lesions and neuron death in several areas of the brain (Salford *et al*, 2003), and that these lesions might be responsible for a decrease in memory observed in a small groups of irradiated rats (Nittby *et al*, 2008). Furthermore, the same group suggested a dose-response relationship between SAR level of GSM irradiation from 0.1 to 1.2 W/m^2 and that the uptake of albumin could be responsible for neuronal death (Eberhardt *et al*., 2008).

According to Swicord & Balzano, since 1990, 52 papers investigated the possible effects of RF on the disruption of BBB permeability. After clustering the multiple results of the same laboratory into 29 single studies, the score came to 11 studies showing no effect, 10 reporting thermal effects and 8 reporting other, possibly non-thermal effects (27,5%). Irradiation levels varied widely among the studies or were not documented at tissue level, making comparisons difficult.

In addition, most of the investigations were not controlled enough to rule out other possible factors present during the study, such as manipulation stress or head trauma, which are known to affect the BBB. The most possible explanation for the 8 remaining studies is that they were also due to thermal effects. For example, it was shown by Sutton & Carroll (1979) that a gradual elevation of the brain temperature to 40 ºC occurred during typical exposure of rats to RF, due to the small size and thin cranial bones of these animals,

causing increased permeability of the BBB. This effect was reversed by a perfusion of the brain with cooled blood. Merritt et al. (1978) compared the effects on the BBB of a temperature increase by either blowing hot air or RF exposure, and obtained similar effects.

More recently, Fritz *et al* (1997) and Ohmoto et *al* (1996) demonstrated experimentally that the temperature increase caused by RF heating of brain tissues might be the most likely explanation for BBB disruption in rats.

With one exception, BBB effects were not researched on larger animals, such as dogs, cats or monkeys, which have cranial configurations closer to humans. Since temperature doesn't change appreciably while using a cell phone handset for several minutes by humans, as ascertained with PET scan imaging, BBB disruptions are not to be expected (Huber *et al.* 2005).

Cancer Induction and Promotion

In vivo experimental studies on teratogenicity (induction and promotion of tumors and/or blood neoplasms) is an important line of inquiry, since this rates among the highest fears of possible long-term effects of RF exposures below safety levels, i.e., possibly due to the break-up of DNA, formation of micronuclei, etc. These studies, which were done *in vivo*, usually in small rodents, employ several techniques such as cell line injection tumor bioassays, effects on genetic material by joint chemical and RF irradiation, etc. Animals with no previous tumors are investigated (induction), as well as animals with tumors previously induced by known carcinogenics (promotion). The appearance of cellular molecular predecessors of tumorigenesis can also be investigated.

Initially, it seemed that non-thermal effects of RF could indeed be associated with experimental teratogenesis in experimental animals, because hyperthermia normally doesn't increase in tumorigenesis (Dewhirst et al, 2003). One of the first animal experimental studies along these lines was widely publicized (Chou *et al*, 1992) and reported a small increase in overall tumor incidence in rats irradiated for two years with RF. The authors considered that these results might not be biologically significant, since the survival of animals was not affected. Another study of great public impact at the time was carried out by Repacholi *et al* (1997) in Australia, found a higher incidence of follicular lymphomas in transgenic mice exposed to RF for 18 months. At this point, a review of the literature on cancer induction and promotion by Repacholi (1997) concluded that the situation was still very contradictory and inconsistent, and that more research was needed. Methodological issues regarding the exposure parameters arose, however, and replication studies by Utteridge et al. (2002) and Oberto et al (2007) failed to confirm these findings. Another investigation was carried out by Anghileri et al. (2005), who reported that RF exposure induced tumors in rats and increased their mortality, probably by causing cellular calcium alterations via non-thermal effects, as a possible triggering factor. Their results, however, could not be confirmed or replicated by other researchers, since they did not report on exposure levels, and used a small number of animals in the experimental group.

Following Repacholi's suggestion, a number of investigations followed and another review in 2003, by Elder, concluded that "*the weight-of-evidence of 18 studies shows that long term, low level exposure to RF energy does not adversely affect survival and cancer in laboratory mammals.*"

Despite this inequivocal statement, the initial positive results on cancer induction in animals continued to provoke a flurry of other studies in succeeding years. According to the review by Swicord & Balzano (2009), 40 such studies have been published since 1990. The duration of exposure ranged from a few weeks to more than two years, and most of the studies investigated continuous exposure (20 to 22 hours per day, 7 days per week) to RF frequencies, such as those commonly used for mobile communications, with various frequency and amplitude modulation schemes. The power densities and SARs employed in most studies were comparable to that generated by cell phone handsets close to the head (1 to 4 W/m^2).

Despite using SARs far above what normal users are exposed to, in terms of accumulated duration along a lifetime, and taking into account the quite different RF distributions in the crania of experimental animals compared to humans, 92.5% of the studies showed no significant effect on tumor formation.

Long Term Survival

Since no significant short term effects of RF on animals were confirmed, with the exception of intense brain and body heating by RF, other studies tried to investigate the effects of lower levels of RF exposure on the long term survival of laboratory animals. Instead of looking for specific changes in organ systems, they investigated detrimental effects in terms of reduced longevity by comparing them to non-exposed animals (control group). Chronic continuous low-level RF irradiation was employed, *i.e.*, simulating conditions similar to those of organisms living near base stations. The average survival of irradiated groups of animals was not affected in 95,8% (23 out of 24 studies), therefore no non-thermal effects could be demonstrated at this level.

Latin American Research

As expected, we could find only a very few published RF animals studies published in peer reviewed journals, all from researchers from the same Brazilian state (Rio Grande do Sul).

Ribeiro et al (2007) investigated the effects of subchronic exposure to 0,8 GHz RF emitted from a conventional GSM cellular telephone on the testicular function in adult rats 1 hour daily for 11 weeks. No statistically significant differences were found for rectal temperature measured before and after the exposure period, testicular and epididymal weight, lipid peroxidation levels in these organs, serum total testosterone and the epididymal sperm count, maturation phase spermatid retention at stage IX-X, interstitial infiltration, cellular vacuolation and multinucleate giant cells. The authors concluded that exposure didn't impair testicular function in adult rats.

Ferreira et al (2006a) investigated the occurrence of chromosomal damage in red blood cells in rat offspring exposed in utero to low level RF used in GSM communication, by using the micronucleus assay. The activity of antioxidant enzymes, total sulphydryl , protein carbonyl groups and thiobarbituric acid-reactive species were evaluated in the peripheral blood and in the liver. The authors noted a significant increase in micronuclei occurrence, but no alteration in oxidative metabolism, so they concluded that RF had genotoxic potentials in rat embryos exposed during embryogenesis, but with no explainable mechanism.

The same group (Ferreira et al, 2006b) investigated the effect of acute RF exposure on

non-enzymatic antioxidant defense and lipid and protein oxidative damage in the rat frontal cortex and hippocampus, by performing malondialdehyde (MDA) and carbonyl assays to assess lipid and protein oxidative damages, respectively. No changes in lipid and protein damage, and also in non-enzymatic defense were found in frontal cortex or hippocampus.

Conclusions

The effects of RF irradiation seems significant only when heating of internal tissues is achieved, i.e., when SARs and power densities are much above safety thresholds. Since below these levels no significant heating occur, especially in the well-protected human head. It was to be expected that any observed and consistent effects on animals could be explained on the basis of putative non-thermal effects.

The general conclusion after 20 years of animal experimentation studies is that no effects could be demonstrated. There is a remarkable consistent absence of effects of RF on intact animals, at least at RF levels below international standards. The few studies reporting effect on the BBB, cancer induction and promotion and overall survival of chronic exposure to RF were lacking, or were due to uncontrolled thermal effects.

With regard to possible mechanisms of interaction of RF fields, both in *in vitro* and *in vivo* experimental studies, ICNIRP (2009) has concluded that the examination of a very large literature database led to the conclusion that finding any low-level non-thermal effect between 150 MHz and 150 GHz is very unlikely and that finding such effects between 10 MHz and 300 GHz may not be possible.