9

Biological research in the Asia-Pacific area

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9.1 INTRODUCTION

This chapter provides an overview of past and current research conducted in laboratories and on exposed populations in the Asia-Pacific region. Countries in this region actively conducting some research include Australia, China and Japan. Other countries in the region may be conducting research but have either published locally in journals not available outside the country and are not abstracted by journals such as Current Contents, or are unknown to the author. In many cases there are only abstracts of conferences conducted in English, but no paper is subsequently published in an English journal. Only a small amount of research is being conducted in any country in the Asia-Pacific region. Thus this review concentrates on a few key results.

The World Health Organization noted in its most recent review (WHO, 1993), that, while current studies do not indicate that exposure to radio frequency fields (RF) at environment levels cause any known detriment to health, there should be more studies to make better health risk assessment from exposure to low-level RF fields. Because of the rapid technological advances made in telecommunications, particularly in the use of devices such as mobile telephones, and the potential for exposure of large segments of the population to RF fields, increasing pressure has been placed on governments and standards setting organizations to ensure that exposure to low-level RF fields has no associated adverse health consequences.

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9.2 PAST RESEARCH

9.2.1 Australia

The only research into biological effects of RF fields conducted in Australia has been by Dr. Andrew Wood and co-workers at the Swinburn University of Technology in Melbourne, Victoria, and by Dr. Michael Repacholi and co-workers at Royal Adelaide Hospital in South Australia. The latter research is being completed and will be described under current research.

Wood *et al.* (1993) have recently published a replication study on calcium efflux from toad heart. This was a replication of the Schwartz *et al.* (1990) study that reported an increase in the movement of calcium ions in frog hearts exposed to RF (240 MHz) fields amplitude modulated at 16 Hz. Other studies have reported enhanced movement of calcium ions from avian brain hemispheres (Adey, 1981; Blackman *et al.*, 1982) and Graham *et al.* (1991) had produced evidence to suggest that ELF fields modify the heart rate in human volunteers. It was important that any effect of exposure to RF fields modulated at ELF frequencies on calcium be confirmed since these ions play a critical role in the normal physiology of the heart and are responsible for excitation-contraction coupling.

Groups of 80 toad hearts were sham exposed or exposed in a TEM cell to 240 MHz fields modulated at 16 Hz, and SARs of 0.15, 0.18, 0.24, 0.30 or 0.36 mW/kg for 30 minutes. Determinations were made on the movement of calcium ions using ^{45}Ca as a tracer. Wood and co-workers concluded that there was no effect at the SARs and frequency chosen.

9.2.2 China

A large number of abstracts have been published for meetings of the Bioelectromagnetics Society. It is apparent that there are a few research groups investigating biological effects of RF fields, but rarely are the papers published in an English language journal. This makes assessment and follow-up of the results virtually impossible. Some of the investigators and institutions involved in RF research include: Liu and co-workers at the Shanxi Medical College and Beijing Air Force Hospital; Jixi and colleagues at the Northwest Telecommunications Engineering Institute, and Zhong-gi and co-workers at the Military Medical College in Xi'an; Chiang and co-workers at the Zhejiang Medical University in Hangzhou; and Zhao and co-workers at the Beijing Medical University.

In a paper providing the highlights of mm-wave bioeffects research conducted in China since 1984, Li (1988a) reported that the combined effect of haematoporphyrin derivative (HPD) and 39 GHz field-exposure (3 mW/cm^2, 30 min/day for 3 days) on MGC-80-3 gastric carcinoma cells significantly reduced cell

viability compared to HPD alone. Exposure of mice to 36 GHz fields (1 mW/cm^2, 15 or 30 min/day for 5 days) was reported to increase white-blood-cell count in peripheral blood, increase glycogen deposits in the liver, and increase pyknosis of the nuclei of megakaryocytes in the bone marrow. When the left middle fingers of 90 healthy human volunteers was exposed to 38 GHz fields at 6 mW/cm^2 for 15 minutes, extension and dilation of capillary loops, blood velocity changes and white blood cell rolling were observed.

A few researchers have been involved in determining if microwaves can cause any changes in cells in culture. Li *et al.* (1988a) report that in human hepatoma (SMMC-7721) cell cultures in monolayer, when exposed to 36 GHz fields at 1 or 2 mW/cm^2 for 15 or 30 min, morphological changes were observed using scanning electron microscopy. When the cells were exposed *in vitro* to 1, 2 or 3 mW/cm^2 for 30 min/day for 1, 2 or 3 days there was a significant reduction in cloning ability without any increase in cell mortality, and there was a loss of DNA content. The same research team reports that pre-exposure of female mice to 304 rads of ^{60}Co γ-rays and 24 hours later, exposure to 36 GHz fields at 3 mW/cm^2 for 30 min/day for 5 days, increased the formation of the colony forming unit – culture (CFU-C) in the bone marrow compared to the sham exposed group (Li *et al.*, 1988b). This result had previously been found *in vitro*. Given the shallow depth that 36 GHz fields penetrate tissue, it seems likely that the results given above would have a bearing on human exposure only if heating was the predominant mechanism.

In a pilot study to determine if 2450 MHz fields could promote colon cancer in mice initiated with dimethylhydrazine, Shao *et al.* (1988) reported that exposure to 10 mW/cm^2 (but not when tested at lower power densities of 1 or 5 mW/cm^2) significantly accelerated its development in infant mice, suggesting a thermal mechanism. In this study Balb/c mice were exposed to CW fields in an anechoic chamber in the E-orientation at 0, 1, 5 or 10 mW/cm^2, 3 h daily, 6 sessions per week for over 5 months. A tumour promoter (TPA) was administered once per week for 10 weeks, commencing on the third week. In their peer reviewed publication on this study, the authors reported that they could not find any promoting effect of microwave exposure (Wu *et al.*, 1994). The SARs in the study were estimated to be 10–12 W/kg.

Liu and co-workers report various effects from *in vivo* studies on rodents exposed to 2450 MHz fields (Liu *et al.*, 1988; Liu *et al.*, 1991a; 1991b). Groups of 20 mice were exposed to CW fields at 20, 30 or 40 mW/cm^2 (no exposure duration given) and compared to a sham exposed group and a positive control group exposed to 150 R of ionizing radiation. The authors report that the microwave exposure increased the micronucleus rate in polychromatic erythrocytes of femur bone marrow. Microwave fields also caused deformation of sperm shape, affected germs cells more than somatic cells. There was a higher sperm teratogeny rate with a tendency of increasing effect with exposure to higher power density. In a later report, Liu and Liu (1991a) conducted the same experiment using pulsed 250 MHz fields (pulsing regime not given) and

again reported changes in sperm morphology in the groups exposed to 30 and 40 mW/cm^2 but not at 20 mW/cm^2. It seems apparent that microwave heating played a major role in producing these results since the exposure levels were relatively high.

In order to determine causes of lessened libido and menstruation disorders in workers exposed to microwaves, Liu and Liu (1991b) studied the effects of 2450 MHz fields on American SD rats. Groups of 10 (half males and half females) were exposed to 20, 40, 60, or 80 mW/cm^2 for 30 min/day for 3 days. Statistically significant differences in blood testosterone and progesterone levels were found in the highest exposed group. Increases in menstruation disorders appeared. In a similar type of study, Liu *et al.* (1992a) reported increases in the blood enzyme, lipid peroxidase (LPO), with increasing dose up to 60 mW/cm^2. Exposures to 80 mW/cm^2 caused the LPO to decrease with increasing dose. The authors note that LPO is an important factor for gene mutation, chromosomal aberration and carcinogenesis and that if the LPO is high this could increase the risk of carcinogenesis. Nie and Yuan (1992) reported an increase in the activity of choline acetyltransferase (ChAT) in the brains of mice exposed to 2450 MHz microwaves at 10 mW/cm^2 (SAR 11.4 W/kg). Follow-up studies were underway; however, it should be noted that the relatively high levels of exposure would result in elevated temperatures in the mice.

Investigations of the effects of microwave exposure on humans have been reported from Chinese laboratories. In a study of 121 workers exposed to RF fields (<30 MHz), the high exposure group ($\geq$100 V/m), Liu *et al.* (1992b) reported changes in their electrocardiograms (ECG) compared to the low exposure group (<100 V/m). The investigators suggested that 100 V/m should be the limit in an RF standard. When the level of the -SH groups was observed in the serum of these high frequency workers, no difference was observed (Liu *et al.*, 1992).

9.2.3 Japan

Biological effects research commenced in Japan in the seventies. The primary objective was to determine if there were health effects resulting from RF exposure that required limits to be established in standards (Amemiya, 1994). Some of the earlier research included a study indicating reduced growth in the thymus following long-term, low-level irradiation of mice to 400 MHz and 900 MHz fields (Saito *et al.*, 1988).

In studies on anaesthetized frog, Miura and Okada (1991) provided evidence for a mechanism of vasodilation that may depend on an increase in Ca^{2+} outflow through the plasma membrane of the smooth muscle and/or an increase in Ca^{2+} influx through the sarcoplasmic reticulum. The exposure parameters leading to the effect on vasodilation were: 10 MHz fields, 1 V (peak to peak), 7.3 mG, 2.19 V/cm field applied 50% of the time at 10 kHz burst rates. Since Ca^{2+} ATPase is activated by cyclic GMP which is produced by the enzymic action

of guanylate cyclase, the authors suggested the electromagnetic field exposure may activate guanylate cyclase to facilitate cyclic GMP production.

Exposure of fertile eggs to 428 MHz fields at 5.5 mW/cm^2 for more than 20 days caused increased embryo death and teratogenic effects as well as delayed hatching (Saito *et al.*, 1991). The authors claimed that the effects were not due to any temperature increase. In a follow-up study, Saito *et al.* (1993) produced further evidence that chronic exposure to 428 MHz fields at 4 mW/cm^2 prolonged the incubation period. In a companion study on mice, Saito *et al.* (1993) reported that 1 mW/cm^2 exposure altered the immune system by suppressing cell-mediated immune competence by local delayed hypersensitivity.

In Japan there is increasing concern among the population about possible adverse health effects from exposure to chronic levels of RF fields from wireless communications. Those involved with the technology recognise the necessity for evaluation of safety through further research. Thus the level of funding for fundamental research is now increasing to answer questions related to the health problems expressed by the population. A major animal study to provide further information on the safety of cellular telephones commenced towards the end of 1995.

9.3 CURRENT RESEARCH

9.3.1 Australia

The rapid rise in the use of mobile telephones in Australia, and the associated concerns voiced by the general public about possible health effects from exposure to the RF fields from the telephone antennas and base stations, has resulted in an increased amount of research to address these concerns. This has included both *in vitro* and *in vivo* studies.

RF fields have been reported in a few animal studies to increase incidence of various cancers. However, these studies are insufficient to establish that RF field exposure can lead to an increased incidence of cancer. As a result, Repacholi and co-workers have been conducting a study to determine whether long-term exposure of female, Eμ-pim1 transgenic mice (van Lohuizen *et al.*, 1989) to 900 MHz fields can increase the normal incidence of cancer in these cancer-prone mice, especially lymphoma. 100 mice were sham exposed and 100 mice were exposed to far-field RF fields (900 MHz, pulse width 0.6 ms, pps 217 Hz) for 1 h/day for 18 months. The SARs for individual mice ranged from 0.01–4.2 W/kg. However since the mice were seen to remain for large periods in closely packed groups of 5, it was determined that mice under these conditions would be exposed to SARs in the range 0.13–1.4 W/kg. Specific pathogen free transgenic mice aged 4–6 weeks were purchased from GenPharm, California and allowed to rest for 10 days prior to entry into the study. The facility was maintained as

specific pathogen free (SPF) throughout the study. An SPF facility is free of pathogens likely to affect the test animals. The statistical analyses and review of results are now in progress and the results will be published within the next six months. It is anticipated that follow-up studies may be necessary.

Adey (1988) has suggested that, modulated RF fields may affect membranes by altering the binding characteristics of calcium on surface glycoprotein strands. This RF signal is then transmitted to the cell interior by an unidentified mechanism, presumably via transmembrane proteins, where some change in specific enzyme kinetics could affect processes connected with cell maturation and division. Whilst not acting as a primary carcinogen, it has been argued that electromagnetic fields may act as promoters or co-promoters of carcinogenesis. Thymic lymphocytes (T-cells) are important components of the immune system and respond by directly recognizing antigens. The presence of an antigen triggers enlargement of the cell, mitosis and increased DNA and RNA synthesis. Concanavalin A is thought to mimic this process. Further, cells undergoing mutations or malignant transformation may alter their antigenic properties and evoke a T-cell response. If electromagnetic fields are affecting this process then normal immune response would be impaired. With this rationale Wood and co-workers are currently investigating the effects of pulsed 915 MHz fields on thymic lymphocyte function. In particular they are studying the effect of GSM mobile telephone radiation waveforms on critical components of the immune system, as measured by cellular and membrane calcium levels in thymic lymphocytes. They are also investigating whether RF pulse modulated fields affect the normal responses of these cells to mitogens such as Concanavalin A, co-carcinogens such as phorbol myristate acetate, or antibodies such as anti-CD3 monoclonal antibodies. In addition studies will be conducted to determine whether these RF fields can affect the ability of certain substances to alter cell membrane architecture (e.g. Concanavalin A).

9.3.2 China

Several papers published in China over the past few years have reported biological effects from exposure to chronic low intensity millimetre (mm) wave electromagnetic fields. Effects such as pyknosis of nuclei of megakaryocytes, increased glycogen deposition in the liver and decreases in spermatocytes and spermatogonia in testes, have been reported. Since the mm wave (36 GHz) have a very low depth of penetration, it is possible that the effects observed may be due to an indirect mechanism. Studies by Li and co-workers are continuing to confirm these results and to determine the mechanism involved. In a study on mice exposed to 36 GHz fields, 4 mW/cm^2, 30 min/day for 5 days, histological examination of a number of tissues continued to show the above effects, even with the use of a local skin block (Novocaine) applied prior to exposure. Measurement of the rectal temperature indicated no significant elevation. The

authors suggest that these results indicate that the mechanism of action to produce the above effects must be non-thermal. Further studies are continuing.

Studies by another group of investigators in China also suggest the existence of non-thermal effects from RF exposure. Jingsui and Huai (1993) studied 450 microwave operators (no frequency of exposure given) who had worked for more than one year in this occupation and for periods of at least 3 months at any one time. These workers were compared with 249 controls. Following observations of a large number of health-related parameters from groups of workers exposed to different ranges of exposure, the authors concluded that adverse health effects appeared when exposures exceeded 1 mW/cm^2. The same authors also studied mice exposed to 2.45–3.0 GHz fields (CW 0.5–20 mW/cm^2, or pulsed 0.25–10 mW/cm^2) for 7 days and found a number of differences in health-related parameters. These results also suggested that non-thermal mechanisms were evident. Further studies are continuing to identify these mechanisms.

9.3.3 Japan

One of the most important lines of research currently being undertaken in Japan is a replication of the studies of Kues, Monahan and co-workers (Kues *et al.*, 1985; 1988; Monahan *et al.*, 1988). Kues and co-workers have reported that corneal abnormalities and vascular leakage from the iris were observed in adult cynomolgus monkeys after a 4-hour exposure to 2.45 GHz fields (CW, 20–30 mW/cm^2, local SAR 5.3–7.8 W/kg, or pulsed, 10 s pulses, 100 pps, 1 or 10 mW/cm^2, local SAR 0.26, 2.6 W/kg) for 4 h/day on 3 consecutive days. RF exposures were conducted alone and in combination with ophthalmic drugs (pilocarpine and timolol maleate) applied topically prior to exposure. Corneal damage and iris vascular leakage were observed following exposure to fields as low as 1 mW/cm^2. It was suggested that serum proteins from the iris could escape into the anterior chamber and contribute to the production of endothelial lesions.

Kamimura *et al.* (1994) have been attempting to replicate these studies using the same experimental procedure except that the monkeys were not anaesthetized as they were in the Kues *et al.* studies. CW power densities exceeding the 30 mW/cm^2 value reported by Kues *et al.* were used but the same corneal endothelial abnormalities could not be observed. Further there were no abnormalities observed in the vitreous humour, crystalline lens or retina. Further studies are anticipated to extend these results to pulsed fields.

Having previously reported that low dose RF exposure to pregnant mice causes an extension of the gestation period and reduction in the thymus weight of their progeny, Saito and colleagues are continuing their research on mouse embryos (Saito *et al.*, 1994). In their most recent study they report the production of malformations in the foetuses of pregnant rats exposed to 2.45 GHz fields from a 30 W microwave therapy apparatus. The SAR, calculated to be

34.7 W/kg, caused increases in foetal death and a variety of foetal malformations. Further studies are being conducted to determine the mechanisms for the teratogenesis caused by high levels of RF exposure. Obviously exposures at a level of 34.7 W/kg will cause a significant temperature rise.

9.4 CONCLUDING REMARKS

While the amount of research activity in the Asia Pacific Area is low, there is useeful research being conducted. Research in China however is very difficult to evaluate because of the lack of full details on which to make any assessment. It is particularly important that there is replication of results by independent laboratories. This is being carried out and will continue. The results challenging the calcium efflux studies and the reports of enhanced damage to eyes when RF exposure is conducted in combination with ophthalmic drugs is of great interest. New challenges are being presented by the Chinese research that should be investigated further.

REFERENCES

Adey, W.R. (1981) Tissue interactions in nonionizing electromagnetic fields. *Physiol Rev*, **61**, 435–514.

Adey, W.R. (1988) Cell membranes: the electromagnetic environment and cancer promotion. *Neurochem Res*, **13**, 671.

Amemiya, Y. (1994) Research on biological and electromagnetic environments in RF and microwave regions in Japan. *IEICE Trans Communications*, **E77-B**(6), 693–698.

Blackman, C.F., Benane, S.G., Kinney, L.S. *et al.* (1982) Effects of ELF fields on calcium-ion efflux from brain tissue *in vitro*. *Radiation Res.*, **92**, 510–520.

Chiang, H. (1988) *Study of microwave conception and its genetic effects.* Abstract: Bioelectromagnetic Society Meeting, Stamford, Connecticut, June 19–23.

Graham, C., Cohen, H.D., Cook, M.R. *et al.* (1991) *Human cardiac activity in 60 Hz magnetic fields.* Abstract: Bioelectromagnetic Society Meeting, Salt Lake City, Utah, June 23–27.

Jingsui, P. and Huai, C. (1993) *The non-thermal effects caused by microwave radiation.* Abstract: Bioelectromagnetic Society Meeting, Los Angeles, California, June 13–17.

Kamimura, Y., Sato, K., Saiga, T. *et al.* (1994) Effect of 2.45 GHz microwave irradiation on monkey eyes. *IEICE Trans Communications*, **E77-B**(6), 762–765.

Kues, H.A., Hist, L.W., Lusty, G.A. *et al.* (1985) Effects of 2.45 GHz microwaves on primate corneal endothelium. *Bioelectromagnetics*, **6**, 177–188.

Kues, H.A. and Macleod, D.S. (1988) *Histological evaluation of microwave-induced vascular leakage in the iris.* Abstract: Bioelectromagnetic Society Meeting, Stamford, Connecticut, June 19–23.

Li, J.X., Nui, Z-Q., Zhu, P-J. *et al.* (1988a) *Bioeffects of 36.11 GHz mm-wave field on human hepatoma monolayer cultures.* Abstract: Bioelectromagnetic Society Meeting, Stamford, Connecticut, June 19–23.

Li, J.X., Nui, Z-Q., Zhu, P-J. *et al.* 1988b) *Effects of mm-wave irradiation on murine bone marrow cells damaged by gamma-rays.* Abstract: Bioelectromagnetic Society Meeting, Stamford, Connecticut, June 19–23.

Li, J. (1988) *MM-wave bioeffect investigation in China.* Abstract: Bioelectromagnetic Society Meeting, Stamford, Connecticut, June 19–23.

Liu, W-K. and Liu, F. (1991a) *Effects of microwave radiation on sperm morphology in mice.* Abstract: Bioelectromagnetic Society Meeting, Salt Lake City, Utah, June 23–27.

Liu, W-K. and Liu, F. (1991b) *Effects of microwave on sex hormone in rats.* Abstract: Bioelectromagnetic Society Meeting, Salt Lake City, Utah, June 23–27.

Liu, W-K., Tan, X-T., and Sun, T-Y. (1992a) *Effects of microwave radiation on LPO in rats.* Abstract: 1st World Congress for Electricity and Magnetism in Biology and Medicine, Lake Buena Vista, Florida, June 14–19.

Liu, W-K., Tan, X-T., and Sun, T-Y. (1992b) *Influence of non-ionizing radiation on lymphocyte micronucleus rate in the human blood.* Abstract: 1st World Congress for Electricity and Magnetism in Biology and Medicine, Lake Buena Vista, Florida, June 14–19.

Liu, W-K. and Syu, Z-L. (1992) *The observation of -SH in the serum of high frequency workers.* Abstract: 1st World Congress for Electricity and Magnetism in Biology and Medicine, Lake Buena Vista, Florida, June 14–19.

Liu, W-K., Qi, Y. *et al.* (1988) *Effects of microwave radiation on incidence of micronucleus of polychromatic erythrocytes (PCE) in the bone marrow and the shape of sperm in mice.* Abstract: Bioelectromagnetic Society Meeting, Stamford, Connecticut, June 19–23.

van Lohuizen, M., Verbeek, S., Krimpenfort, P. *et al.* (1989) Predisposition to lymphomagenesis in pim-1 transgenic mice: Cooperation with c-myc and N-myc in murine leukaemia virus-induced tumors. *Cell*, **56**, 673–682.

Miura, M. and Okada, J. (1991) Non-thermal vasodilation by radiofrequency burst-type electromagnetic field radiation in the frog. *J Physiol.*, **435**, 257–273.

Monahan, J.C., Kues, H.A., Macleod, D.S. *et al.* (1988) *Lowering of microwave exposure threshold for induction of primate ocular effects by timolol maleate.* Abstract: Bioelectromagnetic Society Meeting, Stamford, Connecticut, June 19–23.

Nie, S. and Yuan, G. (1992) *Effects of microwave irradiation on ACH level and CHAT activity in the mouse brain.* Abstract: The First World Congress for Electricity and Magnetism in Biology and Medicine, Lake Buena Vista, Florida, June 14–19.

Saito, K., Goto, N., Ogasawara, T. *et al.* (1988) Growth patterns in several organs in mice under low-level exposure. *J Growth*, **27**(1), 1–6.

Saito, K., Suzuki, K. and Motoyoshi, S. (1991) Lethal and teratogenic effects of long-term low-intensity radio frequency radiation at 428 MHz on developing chick embryo. *Teratology*, **43**, 609–614.

Saito, K. and Suzuki, K. (1994) Teratogenic effect of microwave irradiation on mouse embryos. *J Robotics Mechatronics*, **6**, 51–54.

Saito, K., Tsuchida, Y., Yamada, K. *et al.* (1993) Effect of exposure to RF of fertilized chicken eggs and pregnant mice on hatchability, organ-weight, and locally delayed