# EFFECTS OF 2450 MHZ MICROWAVE RADIATION ON MEIOSIS AND REPRODUCTION IN MALE MICE.

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We reported previously that repetitive exposures of male mice over 12 days, i.e. the duration of the first meiotic prophase (P I) of the spermatocyte I stage of spermatogenesis, to pulsed 9.4 GHz (Manikowska et al.,1979) or continous 2450 MHz (Manikowska-Czerska et al., 1985) microwaves induces chromosomal aberrations, primarily multivalent configurations, in germ cells. The experiments described below were designed to examine effects of exposure over consecutive stages of P I, and to ascertain whether the induced aberrations may be transmitted to the offspring, and affect their development. The results indicate that aberrations may be induced by exposures over the duration of initial prophase and early pachytene, and only to a limited extent after exposure over later stages of P I. An increase in fetal postimplantation loss and live balanced translocation carriers were observed among the litters sired by exposed males.

## MATERIAL AND METHODS.

Adult male ICR mice were exposed 30 min. daily to continous 2450 MHz microwaves in an environmentally controlled waveguide system (Ho et al.,1973). Whole body average specific absorption rate (WBA-SAR) was determined from measurements of forward, reflected, and transmitted power. The animals were immobilized in polyethylene containers, and exposed tail to head to 0 (sham), 1 and 10 W/kg WBA-SAR, resulting in a local SAR to the testes about 2 - 2.5 times higher than the WBA. Increases in colonic temperature after exposure did not exceed 0.5$^{o}$C. In the 1$^{st}$ series of experiments the spermatogenic cell population was synchronized by prior administration of hydroxyurea and Trenimon (Oud et al., 1979). The animals were exposed for 3 consecutive days timed so, as to expose spermatocytes I over initial prophase, early pachytene, mid- and late pachytene, or diplotene of P I. The animals were sacrificed when the cells reached the metaphase of the first meiotic division (M I), i.e. 10, 7, 4 or 1 day after termination of exposures. Chromosome preparations from testes were made as described previously (Manikowska-Czerska et al., 1985), and M I plates were examined for aberrations.

In the 2$^{nd}$ and 3 $^{rd}$ series mice were exposed for 2 weeks, six days a week, 10 animals per exposure level, a total of 60. A heritable translocations test (HTT) was performed on animals of the 2$^{nd}$ series (Manikowska-Czerska et al., 1984). The males were mated with unexposed females during the 4$^{th}$ and 5$^{th}$ weeks after termination of exposure, i.e. at the time cells exposed during PI reached the ejaculate (Oakberg, 1956). Their male offspring was kept until sexual maturation and caged for a week with 2 unexposed females. Males which produced normal size litters were

rejected, the remaining ones were again caged with another pair of unexposed females, the selection procedure was repeated, and the males were mated once more. Males selected after the second mating, were mated again. Males which proved sterile or produced litters of reduced size over 3 consecutive matings were sacrificed, and their testes were examined for chromosomal aberrations. A dominant lethal test (DLT) was performed on animals of the 3rd series (Czerski et al., 1985). After termination of exposure the males were caged with successive pairs of unexposed females for consecutive 3-day periods over 9 weeks. Pregnant females were dissected the 14±1 day of gestation and the numbers of total, live, and dead implants, and of resorptions were determined. The significance of data was examined using multivariate analysis of variance techniques and Duncan's multiple range test by Dr P.M.Silverman of CDRH/FDA.

RESULTS AND DISCUSSION.

In the first series of experiments at least 20 M I plates were analysed per animal. No chromosomal aberrations were seen in 16 sham exposed males,i.e. in a total of 328 M I plates. After exposure at 1 W/kg aberrations (multivalent configurations) were seen in 7 out of 8 males, 4 exposed during initial prophase (leptotene/zygotene) and 4 exposed during early pachytene. 8 M I plates out of 168 were abnormal. In 8 animals exposed during later stages (4 at mid- and late pachytene, and 4 at diplotene) no aberrations were seen in a total of 185 M I plates. Following exposures at 10 W/kg during initial prophase and early pachytene aberrations were seen in 7 out of 8 males, i.e. in 11 out of 178 M I plates. After exposure during mid- and late pachytene multivalent configurations were seen in 1 male out of 4 ( 1 M I plate out of 94). A similar result was obtained following exposure during diplotene: multivalent configurations were seen in 1 male out of 4, i.e. in 1 M I plate out of 96. At this level of exposure aberrations were seen in 9 out of 16 animals, i.e. in 13 plates out of 368. The small number of animals precludes a meaningful statistical analysis, and the test should be evaluated as a qualitative one. In these terms, it seems that early prophase, and mid- and late pachytene are more susceptible to induction of chromosome abnormalities by microwave exposure, than are later P I stages.

In the second series (HTT) 10 sham exposed males sired 174 $F_1$ males, 4 of these were selected for cytological examination of testes based on the outcome of 3 matings. No chromosomal abnormalities were found. 10 males exposed at 1 W/kg sired 148 $F_1$ males, 7 of these were selected for cytological examination, and no aberrations were detected. 10 animals exposed at 10 W/kg sired 119 males, 5 translocation carriers were observed among 11 $F_1$ males selected because of sterility or semisterility. This result indicates that translocations induced by exposure can be recovered from offspring of irradiated males. Translocations carriers occurr among laboratory mice naturally, Leonard (1972) reports their incidence as 2.3 to 9.1 per 10 000 animals.

In the third series the overall pregnancy rate was about 60%. No significant differences between the sham exposed and microwave exposed groups were seen in DLT results following matings during the 1st, 3rd, 6th, and 8th weeks after termination of exposures. The test was not carried out during the 7th week. The results obtained following matings during the remaining weeks are shown in the table below:

| Males exposed at (W/kg) | Week | N° litters | Total implants* | Fractional postimplantation loss* |
|---|---|---|---|---|
| 0 | 2 | 29 | 14.8±0.40 | 0.09±0.017 |
| 1 | 2 | 30 | 13.0±0.67x | 0.14±0.038 |
| 10 | 2 | 41 | 12.5±0.57x | 0.12±0.024 |
| 0 | 4 | 38 | 13.7±0.52 | 0.06±0.013 |
| 1 | 4 | 41 | 13.3±0.48 | 0.17±0.022x |
| 10 | 4 | 39 | 12.2±0.83 | 0.19±0.032x |
| 0 | 5 | 37 | 15.1±0.51 | 0.05±0.009 |
| 1 | 5 | 34 | 13.4±0.42x | 0.15±0.026x |
| 10 | 5 | 39 | 14.9±0.40 | 0.11±0.018x |
| 0 | 9 | 36 | 15.1±0.47 | 0.07±0.012 |
| 1 | 9 | 38 | 15.1±0.43 | 0.11±0.015 |
| 10 | 9 | 34 | 14.1±0.52 | 0.14±0.037x |

* mean ± standard error, x difference significant at 0.05 level when compared with the sham (0 W/kg).

The preimplantation loss (decrease in total implants) noted the 2nd week indicates that late spermatid stages were affected. The increase in postimplantation loss observed the 4th and 5th week indicates effects on P I, and thus is in agreement with the results obtained in series 1 and 2, and in previous experiments (Manikowska et al., 1979, Manikowska-Czerska et al., 1985). There is no obvious dose-dependence of the effects. The 9th week, however, an increase in postimplantation loss was seen only after exposure to the higher SAR.

Only a few reports on effects of microwaves on male meiosis are available. Goud et al. (1982) exposed Swiss albino to 170 $mW/cm^2$ for 70 seconds and found effects on sperm morphology, and an increased postimplantation loss during 3 weeks after exposure. These results are in agreement with our observations. However, Beechey et al.(1986) attempted to verify the results reported by us earlier (Manikowska-Czerska et al., 1985) exposing male hybrid C3H/HeH mice to 2450 MHz radiation amplitude modulated at 100 Hz in an anechoic chamber using the same exposure regime WBA-SARs as in our experiments. The mice were restrained, and could not change their position. The authors did not indicate, however, whether the animals were exposed head to tail or tail to head which significantly affects the dose to the testes, the difference between those two positions being about a factor of 4

to 5 in local SAR. Chromosomal aberrations consisting in exchanges and presence of fragments were more frequent in the group exposed at the highest level ( 20 W/kg) when in other groups and shams combined, but did not exceed the incidence in shams alone. Thus Beechey et al.(1986) did not confirm our findings. Apart from a difference in exposure conditions (100 Hz amplitude modulation), a different strain of mice was used. It is well known (Lovell et al., 1987) that various mouse strains respond differently to chemical germ cells mutagens, and this may also be the case with effects of microwaves. Kowalczuk et al. (1983) and Saunders et al.(1983) exposed rear halves of male C3H mice for 30 minutes to 42-44 W/kg 2450 MHz and found later sperm morphology alterations. The DLT revealed a significant increase in preimplantation loss during weeks 2, 3, 5, 6, and 8 post exposure, the pregnancy rate was reduced to 10-13% of control values during weeks 4 and 5. Postimplantation loss did not increase. However it was reduced during weeks 4 and 5, possibly indicating that aberrations induced were so severe as to lead to early embryonal death.

In conclusion, it seems that single high level or repeated 2450 MHz exposure may induce chromosomal aberrations in male germ cells, spermatocyte I (early prophase I) and late postmeiotic cells being the most sensitive stages. More dosimetric studies and exploration strain and species specific differences in reponses are needed, before any extrapolations are made, and conclusions about the relevance of these findings to the human situatiuon are drawn (see Czerski and Davis, 1987).

REFERENCES:

**1.** Beechey C.V., Brooker D., Kowalczuk C.I., Saunders R.D., Searle A.G. (1986): Int.J.Rad.Biol. 50, 909-918. **2.** Czerski P., Davis C.C.(1987): In: Blank M., Findl E., eds (1987): **Mechanistic Approaches to Interactions of Electric and Electromagnetic Fields with Living Systems.** Plenum, New York and London, pp. 231-247. **3.** Czerski P., Glaser Z.R., Krop B.A., Silverman P.M., Manikowska-Czerska E. (1985): 7th Ann. Mtg. BEMS, San Francisco, abstr. p.49. **4.** Goud S.N., Usha Rani M.V., Reddy P.P., Rao M.S., Saxena V.K. Mutation Res. 103, 39-24. **5.** Ho H.S., Ginns E.L., Christman C.L.(1973): IEEE Trans MTT 21, 832-840. **6.** Kowalczuk C.I., Saunders R.D., Stapleton H.R. (1983): Mutation Res. 122, 155-161. **7.** Leonard E.(1972): In: Hollaender A., de Serres F.J.: **Chemical Mutagens. Vol.3**, Plenum, New York, pp. 21-56. **8.** Lovell D.P., Anderson D., Jenkinson P.C.(1987): Mutation Res. 187, 37-44. **9.** Manikowska E., Luciani J-M., Servantie B., Czerski P., Obrenovith J., Stahl A. (1979): Experientia 35, 388-390. **10.** Manikowska-Czerska E., Czerski P., Krop B.A., Leach W.M. (1984): 6th Ann. Mtg. BEMS, Atlanta, abstr. p. 34. **11.** Manikowska-Czerska E., Czerski P., Leach W.M. (1985): J. Heredity 76, 71-73. **12.** Oakberg E.E. (1956): Am. J. Anat. 99, 507-516. **13.** Oud J.l., de Jong J.H., de Rooij D.G. (1979): Chromosoma 71, 237-248. **14.** Saunders R.D., Darby S.C., Kowalczuk C.I. (1983): Mutation Res. 117, 345-356.