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Project Summary

Behavioral Effects of Microwaves: Relationship of Total Dose and Dose Rate

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The goal of this research was to compare the relationship of whole body averaged specific absorption rate (SAR) and specific absorption (SA) to determine if dose rate or dose was a better predictor of biological effects. Sperm positive Long-Evans female rats were exposed to 2450 MHz CW microwave radiation for 1-3 hours at approximately 10 W/kg. The maternal subjects were then observed for natural delivery of their litters. Sensitivity to thermally induced seizures and huddling were studied in the offspring. Analyses revealed that there were no statistically significant differences between exposed and control offspring on the behavioral indices. The behavior did not appear to be effected by prenatal exposure to microwave radiation at these levels. The huddle sizes became smaller as the pups aged both in exposed and control offspring.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The purpose of the investigation was to define the relationship of exposure duration and average whole body specific absorption rate (SAR) of continuous wave (CW) radiofrequency radiation (RFR) using biological endpoints The research was conducted in two phases. In the first phase, female Long-Evans rats were exposed at SARs of 2, 4, 6, or 8 W/kg for durations of 1, 2, 3, 4, 5, or 6 hours. Subsequently, CF-1 mice were exposed at 2, 4, and 8 W/kg for durations of 1, 2, 3, 4, 5, or 6 hours. Colonic temperatures were taken immediately before and immediately following each exposure session.

In the second phase of the research sperm-positive Long-Evans female rats were exposed at SARs of 10 W/kg for durations of 1 and 3 hours. The exposures occurred on gestational days 12 through 18. The maternal subjects were irradiated and then observed for natural delivery of their litters. The pups constituted the subject pool for subsequent behavioral studies. The behavioral tests included sensitivity to thermally induced seizures and huddle size.

Experimental Procedures

All maternal exposures occurred in a Lindgren 4-Shield RFR anechoic chamber The sham chamber was located adjacent to the microwave exposure facility and shared the same air flow system with the anechoic chamber. The temperature at the location of the subject differed by no more than 1°C between the anechoic and sham chambers 2450-MHz CW microwave radiation was emitted from a horn antenna mounted on the ceiling of the anechoic chamber. During exposure, the animals were placed in Plexiglas cylindrical containers that were positioned on top of a bilayer styrofoam platform. The pup seizures in the second

phase were performed using a 2450-MHz circularly polarized waveguide placed inside the sham chamber to provide ambient temperature and humidity control.

The SAR was determined using twin well calorimetry. The power settings for the estimated range of masses were determined from measurements of the temperature change of equal masses of Ringer's solution in plastic bags Animal carcasses were then used to determine if the selected power levels did in fact produce equivalent SAR measurements. In the second phase, the exposure levels were determined using a pre-established table for the desired SAR of 10 W/kg based upon the mass of the maternal subject. The power level was changed each day according to the body mass in order to provide a constant SAR throughout the exposure period

The subjects were female Long-Evans rats and female CF-1 mice from the animal colony at The University of Tulsa. The rats used in the first phase of the study ranged from 64 to 100 days of age while the mice ranged from 56 to 133 days of age The rats were chosen from the available animals based on a body mass between 180 and 190 g while the mice had body masses between 28 and 30 g. The rats for the second phase were primaparous females between 90 and 180 days of age at the time of breeding. The day on which a sperm plug was detected was considered Day 1 of gestation.

The schedule for obtaining measurements at durations of 1, 2, 3, 4, 5, or 6 hours for each of the SAR levels was determined randomly. Animals were exposed singly and all of the exposures were scheduled such that the end of the exposure occurred at the same hour of the day (15:00). The body mass of the animals available for exposure was measured between 8:00 and 9.00 h. Colonic temperatures were taken immediately prior to placing the animal in the Plexiglas holder for exposure and again at the end of the exposure period. In the first phase, the rat exposure schedule was repeated three times resulting in three rats in each of the 24 conditions. The mouse exposure schedule was repeated twice resulting in two mice in each of the 18 exposure conditions.

In the second phase, the same procedures were followed for the 1 and 3 hour exposure periods. The exposures began on Day 12 of gestation and continued through Day 18. Each day, a 1 hour and a 3 hour exposure session was conducted. Morning and afternoon exposures were counterbalanced for exposure groups. After the exposure session on Day 18, the maternal subject was placed in a plastic maternity cage and monitored until the day of birth of the litter At this time the pups were counted, weighed, and assigned to either the seizure or huddle study.

On day two of age the pups were counted and all pups in a litter over seven were chosen randomly and used in the seizure study. The seizures were observed by placing the pup in a beaker inside the circularly polarized waveguide. The pre-exposure skin temperature, the post-exposure skin temperature, the condition of the pup, and the ambient temperature were all recorded.

The huddle sizes were measured at 5, 10, and 15 days of age On each of the three days, six pups were removed from the home cage and placed in a plastic test cage. After a 15 minute acclimation period, huddles were photographed from directly overhead using a tripod-mounted 35 mm camera. One photograph was taken every 15 minutes until four photographs had been taken of each litter. The developed slides were projected onto a chalkboard and the outer circumference of the huddles was traced onto a sheet of paper taped to the board. Two methods for measuring the size of the huddle were used The method termed perimeter measures traced the perimeter of the huddle as if the pups were enclosed in a rubberband resulting in a convex polygon entirely enclosing all pups in the litter. The other measure is referred to as the individual pup measure and was obtained by tracing the circumference of the huddle including the outline of the pup without extended tails and limbs.

Results and Discussion

In general the average temperature change (post-minus pre-exposure colonic temperature) for the exposed Long-Evans rats reflected increased colonic temperatures. With two exceptions, (SAR 2W/kg for 4 or 5 hours) the colonic temperature increased during the exposure. For a given SAR, the temperature change did not vary considerably as an effect of duration of exposure. However, the sham exposed rats showed a decrease in colonic temperature that is more evident for longer exposure durations. Any exposure longer than one hour resulted in a colonic temperature decrease in the sham exposed rats.

The correlation between pos exposure colonic temperature and SA i significant (r = 0 46, p < 0.0001) as i the correlation between post exposur colonic temperature and SAR (r = 0.7 p < 0.0001). It is of interest to note tha SAR is a much better predictor of pos exposure temperature in the ra accounting for 62% of the variance While SA is significant, it only account for 21% of the variance. Of th correlations between duration o exposure and post exposure colon temperature at each of the four SA levels, only the 2 W/kg condition wa significant (r = -0 55, p < 0 01), an this correlation was negative.

These same comparisons wer made for the CF-1 mice. For duration of exposure longer than one or two hours all of the mice had lower post exposur colonic temperatures The averag temperature change for the shar exposed mice also reflected a decrease

The post exposure colon temperature was not related significantl to SA and was only mildly related to SA (r = 0.43, p < 0 005). The correlation between duration of exposure and pos colonic temperature were all negative an only the 2 W/kg condition was significar (r = -0 57, p < 0.03) The pos exposure temperature was lower than th pre-exposure temperature for all but fiv mice and the relationship between pos exposure temperature and duration o exposure (r = -0 57, p < 0 0001) a well as the relationship betwee temperature change and duration o exposure (r = -0.35, p < 0.02) wer significant.

The average for the whole bod average SAR was 10.45 W/kg (± 1.0 SD) The highest SAR value was 12. W/kg and the lowest was 8.5 W/kg. Dat from 59 gravid dams were used in th analyses; 18 cage control, 21 shar exposed and 20 microwave exposed

All treatment variables associate with the characteristics of the materna subjects and their exposure as well a the variables associated with the litte characteristics and behavioral measure were analyzed using a three facto analysis of variance. Post hoc test (Tukey HSD) were performed only whe a significant main effect was followed b a significant interaction

Although the average mass of th dams in the different treatment group did not differ, there was a statistical significant difference in the mass gaine from Day 1 through Day 18 (F = 5 87, < 0 01). This difference was due to th sham exposed groups gaining more tha

the microwave exposed groups. The cage control group was not different in weight gain from either the sham exposed or the microwave exposed group. Time of day of exposure and duration of exposure were not significant.

As expected, the microwave exposed groups experienced an increase in colonic temperature during exposure. There was a significant difference in temperature change associated with all three of the major variables: exposure condition ($F = 289.98$, $p < 0.001$), duration of exposure ($F = 11.8$, $p < 0.002$), and time of day of exposure ($F = 4.21$, $p < 0.05$). The average temperature change for the microwave exposed groups was 2.04°C. The sham exposed groups had a decrease in temperature with an average of -0 51°C. The one hour groups increased more than the 3 hour groups while the morning exposure groups increased less than the afternoon Groups. There were no significant interactions.

Litter sizes for the pups at birth were not significantly different with averages of 11 22, 11.76, and 10 60 pups for the cage control, sham exposed and microwave exposed groups. Pups from each litter were selected for the seizure study when they were 2 days of age. There were no differences for the mass of the pups, the pre- and post-treatment skin temperature, the difference between the post- and pre-treatment skin temperature or the average latency to seizure.

Huddle sizes were analyzed on days 5, 10, and 15 of age. Both the perimeter measurement ($F = 3.51$, $p < 0\ 05$) and the individual pup measurement ($F = 40.42$, $p < 0\ 001$) produced significant differences based upon the age of the pups. Older pups form smaller and smaller huddles. The proportion of variance accounted for by the two measurement techniques was calculatedusing the eta^2 method suggested by other investigators The perimeter measure accounted for 8% of the variance in huddle size across the three age groups while the individual measure accounted for 57%.

Conclusions and Recommendations

Under identical exposure conditions and identical rates of energy deposition (SAR) the thermal response as measured by colonic temperature is dramatically different for the mouse and the rat. Mice actually decrease their body temperature while being dosed with energy at 2, 4, and 8 W/kg even when the duration of exposure is as long as 6 hours. The thermal response of the microwave exposed mice is not distinguishable from that of sham exposed mice when measured by colonic temperature.

The rats did not display this efficient thermal regulation of body temperature under these conditions of exposure. Levels of radiation at 2, 4, 6, and 8 W/kg raised body temperature to a given degree and the rats maintained this increased temperature for durations of exposure as long as 6 hours Sham exposed rats showed a reduction in body temperature at all durations of exposure greater than one hour.

The use of colonic temperature as an indication of thermal responsivity results in opposite results in sham exposed and microwave exposed rats but does not indicate differences in sham and microwave exposed mice. Such a dramatic species difference under identical laboratory exposure conditions argues for extreme caution in attempting to generalize to human exposure conditions. In particular, biological effects observed in only one non-human species should be used in establishing human exposure conditions only when the procedure allows for the inclusion of considerable caveats regarding the lack of essential data. The procedure should also ensure that these caveats will be included in the tables or figures used by practitioners in attempting to abide by a recommendation.

In phase 2 there were significant differences in weight gain between the microwave and sham exposed maternal subjects. However, this difference was not accompanied by differences in either litter size or pup mass.

The results of the second phase indicated that post-natal measures of thermally induced seizure sensitivity or huddle size in pre-natally exposed rat pups were not significantly effected by the microwave exposure at 10 W/kg. Two measures were used to determine huddle size. The age of the pups was a significant variable and the proportion of variance accounted for was much greater for one of the two measures. Behavioral research often demonstrates the importance of operational definitions in which variables and procedures are defined by the methods used to measure them. Like species differences, the importance of operational definitions in laboratory research and its interpretation is a basic experimental principle learned by most investigators in the most elementary research methods courses. However, bioelectromagnetics research is replete with investigations relying on one species and one experimental measurement technique. Some of the ambiguities in the data base might be explained by these two basic experimental principles.

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The complete report, entitled "Behavioral Effects of Microwaves: Relationship of Total Dose and Dose Rate," (Order No. PB 89-118 640/AS; Cost: $15.95, subject to change) will be available only from:
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