Reproduction, Growth, and Development of Rats during Chronic Exposure to Multiple Field Strengths of 60-Hz Electric Fields

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Reproduction, Growth, and Development of Rats during Chronic Exposure to Multiple Field Strengths of 60-Hz Electric Fields. ROMMEREIM, D. N., ROMMEREIM, R. L., SIKOV, M. R., BUSCHBOM, R. L., AND ANDERSON, L. E. (1990). Fundam. and Appl. Toxicol. 14, 608-621. A study with multiple exposure groups and large group sizes was performed to establish whether exposure to 60-Hz electric fields would result in reproductive and developmental toxicity. A response model was developed from previous results and tested in groups of rats exposed to electric fields at various field strengths. Female rats were mated, and sperm-positive animals randomly distributed among four groups: sham-exposed or exposed to 10, 65, or 130 kV/m, 60-Hz vertical electric fields. Animals were exposed for 19 hr/day throughout the experiment. During gestation, exposure to the higher field strengths resulted in slightly depressed weight gains of dams. Offspring were born in the field and remained with their dams through the suckling period. Numbers of pups per litter and pup mortality did not differ among the exposure groups. Dams exposed at 65 kV/m lost slightly more weight through the lactation period than the control group. Male pups exposed to higher field strengths gained slightly less weight from 4 to 21 days of age than did sham-exposed animals. At weaning, two F₁ females per litter (randomly selected) continued on the same exposure regimen were mated at 11 weeks of age to unexposed males, and euthanized at 20 days of gestation. Uterine contents were evaluated, and all live fetuses were weighed and examined for external, visceral, and skeletal malformations. Fertility and gestational weight gain of F₁ females were not affected by exposure, nor was prenatal viability or fetal body weight. No significant increase in the incidence of litters with malformations was observed. Although no developmental toxicity was detected, exposures produced physical changes in the dams, evidenced as a rust-colored deposit on the muzzle and ears (chromodacryorrhea) that increased in incidence and severity at 65 and 130 kV/m. Incidence of chromodacryorrhea was not significantly different between sham-exposed rats and those exposed at 10 kV/m.

Effects on survival, growth, and development of laboratory animals (mice and rats) exposed to electric fields during gestation or neonatal life have previously been reported (Knickerbocker et al., 1967; Marino et al., 1967, 1980; Sikov et al., 1984). However, the magnitudes of observed changes were small, and effects have been inconsistent across studies.

More recently, a multigeneration screening study utilizing Hanford Miniature swine was used to detect effects of electric field exposure on reproduction and development in a larger species (Sikov et al., 1987). Swine were chronically exposed to a 30 kV/m, 60-Hz electric field over two generations. Maternal weight gain during pregnancy, number of embryonic implants, and number of offspring per litter were not affected. Survival rates of the offspring and their growth curves were also indistinguishable between groups.
However, comparison of offspring from dams exposed or sham exposed for 18 months showed mean body weights of male fetuses and mean body weights of female piglets to be less in the exposed group. In addition, an increase in frequency of malformations was observed in fetuses of exposed females. A clear association of the changes with exposure, per se, could not be demonstrated because of a number of inconsistencies in response patterns and the possibility of interacting factors (Sikov et al., 1987).

Subsequently, rats were used to investigate the possible effects of electric field exposure on development and reproduction. Two, essentially identical, multiple-generation rat experiments were conducted (Rommereim et al., 1987). Copulatory behavior, gestational weight gains, pup weights at birth, and their subsequent growth curves were not affected by exposure. In one of the replicate experiments, there was a statistically significant decrease in fertility and a significant increase in the fraction of litters with malformed fetuses among exposed animals. However, in the second replicate, no significant developmental effects were detected in fetuses of dams exposed throughout gestation. A clear association of reproductive or developmental changes with exposure could not be demonstrated. Effects detected in the first experiment were not seen in the second and were attributed to either normal biological variation or a response threshold near 65 kV/m for induction of malformations.

Accordingly, in the study reported here we tested for a treatment-related response, utilizing multiple exposure groups, including 65 kV/m (effective field strength) as used in our previous experiments; 130 kV/m which was twice as strong as that used in the previous work and had been shown to be tolerated by rats (Rommereim et al., 1989); the lowest level of exposure (10 kV/m) was chosen to delineate the response curve at lower field strengths or possibly to determine a no-observed-effect level; and the fourth group was sham-exposed as a control. Additionally, the chance of obtaining equivocal results due to biological variation was minimized by using large group sizes with the initial group populations of 68 female rats per treatment group. To obtain a statistical power of 0.8 or greater in comparisons of malformation incidence between groups, the study was designed to obtain a minimum of 80 litters for teratological evaluation per exposure group.

METHODS

Exposure system. Rats were exposed to 60-Hz electric fields on systems consisting of five parallel-plate electrodes. The metal plates were rectangular and electrically insulated from one another. When energized, they provided uniform (±5%), vertical, 60-Hz electric fields at the strengths utilized in this experiment. Four such exposure systems (racks), electrically isolated from each other, were located within one room.

Four tiers of rat cages were provided on each system; each tier contained eight polycarbonate modules, each with six individual rat cages (12.5 cm wide, 25.0 cm long, 10 cm high). During parturition and litter-rearing, rats were housed in modules divided into three compartments, so that the cages were then twice as large as the standard cages. Floors of the cages were made of wire mesh and were an integral part of the lower electrode so that rats were in electrical contact with the reference ground. A small amount of Antron III (a conductive carpet material from E. I. Dupont Co.) was placed in the cages during the period in which litters were delivered and reared.

The exposure system design minimizes sources of phenomena associated with electric fields of intensities used in this experiment (Dietrich and Patterson, 1988). Current conduction, transient charge, and transient energy through the water system to a rat while drinking were tested, and values were below known levels of perception. Electrode voltages and spacing were such that flashover from rats within cages did not occur. Corona, as detected by radio noise, was not present. Harmonic distortions of input voltages were less than 0.5%. Cage floor vibration velocity was less than 0.5 mm/sec measured at the voltages used in this study. Rats were exposed to one of four field strengths (0, 10, 65, 130 kV/m) for 19 hr daily, 7 days per week. Animals and their respective exposure regimens were rotated weekly from one system to another to limit the potential effect of rack location within the room on rat growth and development. Personnel performing animal care, manipula-
FIG. 1. Multiple field strength experiment: Schematic representation of experimental protocol. Exposure began as females are bred (first arrow) and continued for 130 consecutive days through the subsequent experimental events.

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ute days for females in which sperm was not detected. Females that were determined to have mated were not returned to males. After the breeding phase of the experiment was completed, females in which sperm was not detected were euthanized (13 days after the last day of possible mating), and their pregnancy status was determined (Salezowski, 1964). Body weight was recorded at 0, 7, 15, and 20 days of gestation (dg). Animals were killed at 20 dg for teratological evaluations.

Teratologic evaluation: maternal and fetal examination. Females that mated were killed on 20 dg with carbon dioxide. Their body cavities were opened, and the uterus, ovaries, and abdominal and thoracic viscera were examined visually. Corpora lutea were counted, and maternal liver and uterus were weighed. Uteri were externally examined for hemorrhage, removed from the peritoneal cavity, and incised longitudinally to expose their contents. All live and dead fetuses and resorption sites were detected were euthanized (13 days after the last day of possible mating). Body weight was recorded at 0, 7, 15, and 20 days of gestation (dg). Animals were killed at 20 dg for teratological evaluations.

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TABLE 1
EFFECTS OF 60-HZ ELECTRIC FIELD EXPOSURE ON PREGNANCY AND POSTNATAL SURVIVAL OF RATS

| Maternal (F₀) data | Sham-exposed | 10 | 65 | 130 |
|-------------------|--------------|----|----|-----|
| No. mated         | 68           | 68 | 68 | 68  |
| No. pregnant (%)  | 65 (96)      | 60 (88) | 65 (96) | 61 (90) |
| Body mass, at 0 days of gestation (dg) | 272 ± 2.0 | 274 ± 2.1 | 274 ± 2.1 | 271 ± 2.1 |
| Gestational weight gain a | 173 ± 2.7 | 174 ± 2.9 | 168 ± 3.1 | 164 ± 2.8 |
| Maternal (F₀) data | 0            | 1  | 2  | 1   |
| Pup (F₁) data     | No. offspring per litter, Day 1 b | 14.2 ± 0.4 | 14.5 ± 0.3 | 14.5 ± 0.3 | 13.7 ± 0.4 |
| Neonatal mortality c | 3.0 ± 0.7 | 1.7 ± 0.5 | 2.1 ± 0.5 | 1.6 ± 0.4 |
| Juvenile mortality d | 1.8 ± 1.2 | 0.2 ± 0.2 | 1.0 ± 0.5 | 0.2 ± 0.2 |
| Percent male, Day 1 e | 47.2 ± 1.7 | 48.9 ± 1.7 | 48.5 ± 1.5 | 44.6 ± 1.6 |

a 21 dg weight minus 0 dg weight (g ± SE).
b Mean ± SE.
c Mean percentage per litter that died between 1 day and 4 days of age (±SE).
d Mean percentage per litter that died between reduction of litter size to eight individuals at 4 days of age and weaning at 21 days of age (±SE).
e Mean percentage male pups per litter (±SE).

age and between 4 and 21 days of age were similar in all groups. The sex ratio (mean percentage of male pups) in each litter was not different among the treatment groups.

Pup mean body masses were similar among the groups for both sexes at 1 and 4 days of age (Table 2). Growth curves were similar for all groups within sex, although male pups exposed at the two higher field strengths tended to gain slightly less weight (p < 0.05) in the period between 4 and 21 days of age than did those in the other groups. Mean weight gains of female pups (females selected to continue on experiment after 21 days of age) did not differ among the groups. Mean body weights of dams at Day 1 after parturition were similar in the four groups. Rats exposed at 65 kV/m lost significantly more weight during lactation than did those of the other groups (Table 2).

Throughout the postweaning period measures of toxicity or animal well-being were equivalent among groups for the selected female rats that continued on study. Only two rats died during this period, of causes unrelated to exposure (broken tooth, bladder stones), and mean body weights among the groups were the same.

Breeding procedures began when the F₁ female rats reached 11 weeks of age. Approximately equal numbers of females were mated daily from each group, and curves, calculated from the cumulative numbers of rats mated daily, did not differ among groups. Over a 12-day period, mating was detected to have occurred in 81 to 91% of the rats in the four groups, the pregnancy rate of which varied between 87 to 94%. These values were not significantly different among groups (Table 3). Mean numbers of consecutive daily mating periods were approximately equal among groups.

Mean body weights at 0 day of gestation were not significantly different among groups.
TABLE 2

EFFECT OF 60-HZ ELECTRIC FIELD EXPOSURE OF RATS ON OFFSPRING GROWTH AND DAM LACTATIONAL WEIGHT LOSS

| No. litters | Sham-exposed | Field strength (kV/m) | 10 | 65 | 130 |
|-------------|--------------|-----------------------|----|----|-----|
| Male (F₁) offspring body weights (g)* | | | | | |
| 1 day | 6.8 ± 0.1 | 7.0 ± 0.1 | 6.8 ± 0.1 | 6.9 ± 0.1 |
| Gain, Days 1–4 | 3.0 ± 0.1 | 3.0 ± 0.1 | 2.9 ± 0.1 | 3.0 ± 0.1 |
| Gain, Days 4–21 | 49.6 ± 0.6 | 50.0 ± 0.5 | 48.0 ± 0.6* | 48.0 ± 0.5* |
| Female (F₁) offspring body weights (g) | | | | | |
| 1 day | 6.4 ± 0.1 | 6.6 ± 0.1 | 6.4 ± 0.1 | 6.5 ± 0.1 |
| Gain, Days 1–4 | 2.9 ± 0.1 | 2.9 ± 0.1 | 2.8 ± 0.1 | 2.8 ± 0.1 |
| Gain, Days 4–21 | 47.1 ± 0.6 | 47.9 ± 1.0 | 45.8 ± 0.5 | 46.0 ± 0.5 |
| Gain, 3–11 weeks | 218 ± 2 | 220 ± 2 | 216 ± 2 | 216 ± 2 |
| Maternal (F₀) body weights (g) | | | | | |
| Day 1 following parturition | 344 ± 3 | 343 ± 3 | 343 ± 3 | 337 ± 3 |
| Loss, Days 1–21 | −39.4 ± 2.2 | −39.2 ± 2.1 | −46.2 ± 2.4* | −40.4 ± 2.5 |
| Percentage weight loss, Days 1–21 | −11.4 ± 0.6 | −11.3 ± 0.6 | −13.4 ± 0.7 | −11.9 ± 0.7 |

* Mean ± SE.

a Significantly different from controls (p < 0.05).

TABLE 3

MATERNAL (F₁) REPRODUCTIVE PERFORMANCE AND TOXICITY IN RATS CHRONICALLY EXPOSED OR SHAM-EXPOSED TO 60-HZ ELECTRIC FIELDS*

| No. paired with males | | | | 10 | 65 | 130 |
|---|---|---|---|----|----|-----|
| No. (%) mated | | | | | | |
| No. (%) pregnant | | | | | | |
| No. days mated* | 4.4 ± 0.3 | 4.7 ± 0.3 | 4.8 ± 0.3 | 4.3 ± 0.3 |
| Body weight, 0 dg (g)* | 289 ± 2.8 | 282 ± 2.9 | 274 ± 3.1 | 273 ± 2.6 |
| Gestational weight gain, 20 dg-0 dg (g) | 137 ± 2.2 | 139 ± 1.9 | 136 ± 2.1 | 134 ± 2.1 |
| Gravid uterus weight (g) | 66.9 ± 1.7 | 66.9 ± 1.8 | 67.2 ± 1.8 | 64.0 ± 1.8 |
| Extrargestational weight gain (g)* | 70 ± 1.6 | 73 ± 1.4 | 69 ± 1.5 | 70 ± 1.4 |
| Maternal liver weight Absolute (g) | 15.3 ± 0.2 | 15.2 ± 0.1 | 15.2 ± 0.2 | 14.7 ± 0.2* |
| Relative (%)* | 3.7 ± 0.03 | 3.6 ± 0.03 | 3.7 ± 0.04 | 3.6 ± 0.03 |

* Data expressed as means ± SE except as noted.

+ One rat removed due to technician error.

* Mean number of daily periods paired with males to achieve copulation.

+ Days of gestation; data shown only for rats that were pregnant.

* Gestational weight gain minus uterine weight.

Liver weight divided by 20 dg body weight × 100.

* p < 0.05 versus sham-exposed, Tukey's test.
TABLE 4

DEVELOPMENTAL TOXICITY IN RATS CHRONICALLY EXPOSED OR SHAM-EXPOSED TO 60-HZ ELECTRIC FIELDS*

| Litters examined at 20 days of gestation | 0 | 10 | 65 | 130 |
|-----------------------------------------|---|----|----|-----|
| No. Corpora lutea/dam                   | 15.8 ± 0.28 | 16.2 ± 0.23 | 15.7 ± 0.27 | 15.3 ± 0.31 |
| No. implantation sites/dam              | 14.9 ± 0.4  | 15.0 ± 0.4  | 15.1 ± 0.3  | 14.2 ± 0.4  |
| Percentage implantation sites/Corpus luteum | 93.6 ± 1.5 | 92.1 ± 1.7  | 95.6 ± 1.4  | 92.6 ± 1.7  |
| No. resorptions/litter                  | 63.6         | 70.2         | 69.6         | 64.4         |
| Percentage implantations resorbed       | 7.3 ± 0.8    | 8.1 ± 0.9    | 9.9 ± 1.2    | 8.4 ± 0.9    |
| No. live fetuses/litter                 | 13.8 ± 0.4   | 13.8 ± 0.4   | 13.6 ± 0.4   | 13.0 ± 0.4   |
| Fetal body weight (g)*                  | 2.98 ± 0.02  | 2.96 ± 0.03  | 3.01 ± 0.04  | 3.01 ± 0.02  |
| Placental weight (g)*                   | 3.12 ± 0.02  | 3.16 ± 0.03  | 3.20 ± 0.04  | 3.19 ± 0.03  |
| Percentage males/litter                 | 48.5 ± 1.5   | 49.7 ± 1.4   | 51.4 ± 1.6   | 53.8 ± 1.8   |

* Data expressed as means ± SE except as noted.  
* One dam aborted before litter evaluation.  
* One dam was excluded from evaluation due to dental malocclusion.  
* Data expressed as mean of litter means.

(p = 0.075). Mean weight gain through pregnancy was not affected by exposure, and extragestational weight gain (an indicator of dam well-being through pregnancy) was also not affected by exposure.

In teratological examinations, mean gravid uterine weights were not affected by treatment (p = 0.52). Absolute mean liver weight was significantly less (p = 0.05) in rats exposed at the highest field strength than in the sham-exposed group. However, ratios of mean liver weight to body weight did not differ.

No significant differences were observed among treatment groups with respect to the total number of corpora lutea per dam, implantation sites per dam, or preimplantation loss as indexed by implantation sites per corpus luteum (Table 4). Postimplantation embryonic death was not affected by exposure, as indicated by the fact that none of the measures of implant resorption differed among groups. Mean number of live fetuses per litter was less in rats exposed to the 130 kV/m field, but it was not statistically different from the control group (p = 0.33). Mean fetal body weight and placental mass were the same among the groups. The sex ratio (number of males/litter) appeared to increase with field strength but did not differ significantly among groups (p = 0.08) and was within the range of expected variation.

A total of 5076 fetuses in 375 litters were examined. In all cases, the number of litters examined per treatment group exceeded the 80 litters required for a statistical power of 0.8 or greater for comparison of malformation incidence. A wide variety of malformations were recorded, as would be consistent with the large number of litters examined (Table
### TABLE 5

**FETAL MALFORMATIONS OBSERVED IN LITTERS OF SECOND-GENERATION RATS CHRONICALLY EXPOSED OR SHAM-EXPOSED TO 60-HZ ELECTRIC FIELDS**

| Malformations—external* | Field strength (kV/m) | 0  | 10  | 65  | 130 |
|-------------------------|-----------------------|----|-----|-----|-----|
| Anotia                  | 0                     | 1/1|     |     |     |
| Acaudal or vestigial tail | 0                   | 1/1|     |     |     |
| Edema                   | 0                     | 1/1|     |     |     |
| Imperforate anus        | 0                     | 1/1|     |     |     |
| Umbilical hernia        | 0                     | 0  |     |     |     |
| Retrognathia            | 0                     | 1/1|     |     |     |
| Malformations—visceral* |                       |    |     |     |     |
| Anophthalmia or microphthalmia |       | 1/1|     |     | 3/3 |
| Cleft palate            | 0                     | 0  |     |     |     |
| Hydrocephaly            | 2/2                   | 0  | 3/3 |     | 4/3 |
| Major vessel malformation | 1/1                | 1/1|     |     |     |
| Malformations—skeletal* |                       |    |     |     |     |
| Hemivertebra with rib agenesis |         | 1/1|     |     |     |
| Branched rib            | 1/1                   | 0  |     |     |     |
| Vertebral agenesis      | 0                     | 1/1|     |     |     |
| Cleft sternum           | 0                     | 0  |     |     | 2/1 |
| Fused sternebrae        | 1/1                   | 0  | 8*/1|     | 7*/1|
| Wavy, bent, or knobby rib | 1/1               | 1/1|     | 2/2 |     |
| Fused ribs              | 0                     | 0  |     |     | 1/1 |
| Kinked tail             | 0                     | 0  |     | 3/1 |     |
| Total affected, absolute| 6/5                   | 3/3| 16*/9| 11/5|     |
| Total affected, mean percentage per litter ± SE | 0.43 ± 0.20 | 0.27 ± 0.16 | 1.44 ± 0.76 | 0.80 ± 0.52 |

* Data presented as No. of fetuses affected/No. of litters affected.

* A single fetus may be represented more than once if multiple defects were present.

* $p < 0.05$ versus sham-exposed, $\chi^2$ test.

5). Several malformations were recorded in litters from exposed groups which did not occur in the sham-exposed animals: anotia, acaudal or vestigial tail, edema, imperforate anus, umbilical hernia, retrognathia, cleft palate, rib agenesis, cleft sternum, fused ribs, and kinked tail. However, the incidence of each was low and did not differ significantly between sham-exposed and exposed groups. The predominant major vessel malformation observed was a retroesophageal aortic arch with abnormal origin of the right subclavian artery. Vertebral agenesis occurred in the sacral region. Malformation incidence was low overall, with the exception of fused sternebrae, which occurred in one fetus in the sham-exposed group versus eight in one litter of the 65 kV/m group and seven in one litter in the 130 kV/m group. Incidence of fused sternebrae was significantly higher ($p < 0.05$) on a per fetus but not on a per litter basis. Evaluation of total malformation incidence on a per fetus basis showed a significantly higher number of malformed fetuses in the
group exposed at 65 kV/m than was observed in the other groups. However, on a per litter basis the total incidence of malformations did not differ significantly among groups. The mean percentage of malformed fetuses per litter was the same among the treatment groups ($p = 0.34$).

A few incidences of fetal variations were significantly higher for specific site/exposure group combinations, but no clear correlation of increasing incidence with increasing field strength was evident. For example, incidence of dilated ureter was significantly higher in fetuses of dams exposed to 65 kV/m electric fields on both a per fetus and a per litter basis (Table 6). The associated variations of renal pelvic cavitation were also higher on a per fetus basis. Incidence of an additional ossification site at the first lumbar vertebra was significantly higher per fetus (but not per litter) in animals exposed to 65 kV/m; the incidence was not higher in rats exposed to 130 kV/m.

Likewise, significantly increased incidences of reduced ossifications in skull, sternum, and vertebrae were recorded on a fetus and litter basis in rats exposed at 10 kV/m. Incidence of this condition in rats exposed at higher field strengths did not differ from that of the sham-exposed group.

During routine observation of the F₀ dams, a rust-colored deposit (chromodacryorrhea) was observed on the muzzles and ear hair. Subsequently, the deposits were also observed on F₁ females during the 3 months of exposure following birth and the incidence and severity of chromodacryorrhea were recorded at termination. A statistically significant trend ($p < 0.0001$) toward an increased incidence of deposits was recorded with increasing field strength in both lactating and pregnant animals. Seventy-eight percent of nursing dams exposed at the highest field strength had detectable amounts of such deposits (Table 7). Further details on these observations are reported elsewhere (Leung et al., 1989).
TABLE 7
FREQUENCY OF CHROMODACRYORRHEA SEVERITY SCORES IN PREGNANT FEMALE RATS
CHRONICALLY EXPOSED OR SHAM-EXPOSED TO 60-HZ ELECTRIC FIELDS

| Field strength (kV/m) | 0 | 10 | 65 | 130 |
|-----------------------|---|----|----|-----|
| Lactating dams (F₀)   |  |    |    |     |
| Number examined       | 65 | 59 | 63 | 61  |
| Number cases (%)      | 4 (6) | 8 (14) | 28 (44) | 47 (78) |
| Pregnant rats (F₁)    |  |    |    |     |
| Number examined       | 99 | 83 | 92 | 101 |
| Score                 |    |    |    |     |
| 0 Without deposits    | 86 (86.9) | 69 (83.1) | 48 (52.2) | 39 (38.6) |
| 1 Slight              | 13 (13.1) | 14 (16.9) | 33 (35.9) | 29 (28.7) |
| 2 Moderate nose or ears | 0 (0.0) | 0 (0.0) | 11 (12.0) | 28 (27.7) |
| 3 Moderate nose and ears | 0 (0.0) | 0 (0.0) | 0 (0.0) | 4 (4.0) |
| 4 Significant         | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (1.0) |
| 5 Extreme             | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |

Note. Number (%) scored within each category.

* Amount of deposits defined by subjective scores as: 0, without deposits; 1, slight amounts, light dots; 2, moderate areas on nose or ears, approximately 1 to 3 mm in diameter; 3, moderate areas on nose and ears, each approximately 1 to 3 mm in diameter; 4, significant areas on nose and ears, approximately 3 mm in diameter or larger, with interrupted areas extending from nose to eyes; 5, large areas on entire head and ears.

a Significantly different from controls (p < 0.0001).

DISCUSSION

Throughout the study there were no indications of disease or confounding factors unrelated to exposure other than an occasional transient reduction in body mass of individual animals as a result of water-dispenser malfunction. Effects of this transient deficiency are judged to be minimal. In general, mean body weights were consistent among the groups, and body weight gains were comparable to those in previous experiments.

Growth

Our observation of slightly reduced gestation body weight gain of F₀ dams exposed at 130 kV/m was unexpected. In our previous work, gestational body weight gain in females exposed to 150 kV/m did not differ from that of sham-exposed animals (Rommereim et al., 1989). Differences between the two experiments may be explained in that dams in this study received no acclimation to the electric field prior to breeding in contrast to a 1-month exposure in the previous experiment. Reproductive outcome was largely unaffected, as indicated by the fact that the mean number of live pups per litter did not differ significantly among the groups. The reduced gestational weight gain of the dams in the high exposure group is consistent with a numerically lower, albeit nonsignificant, number of live pups per litter in the high exposure group; however, a chance association is an equally plausible explanation.

Overall, no evidence of detrimental effects on survival or growth of the offspring was observed in animals exposed to electric fields. No maternal mortality was associated with electric field exposure; only two deaths occurred and those were attributable to factors...
other than the field. Most of the rats that did not deliver at the expected time were not pregnant, and three of the F₀ dams that did not litter had only a single large pup in utero. The low incidence of this situation and its distribution among groups indicate that it is not attributable to electric-field exposure.

The weight loss of lactating dams exposed to the 65 kV/m field was slightly greater than that in other groups. However, the weight loss was not proportional to increased field strength and is therefore not considered of consequence.

**Teratology**

In the F₁ generation females, measures of reproductive capability were unaffected by exposure: the percentage mating and rate at which rats mated (cumulative proportion on consecutive days) did not differ among the treatment groups.

Although not statistically significant, the number of live fetuses per litter was numerically less (0.8 pup per litter) in rats exposed to 130 kV/m than in other groups (Table 4). This deficit is not accounted for by prenatal death; rather, it reflects fewer than normal ovulations (indicated by the number of corpora lutea per dam) and thus fewer implantation sites per dam. The electric field may have decreased the number of ovulations, but chance is an equally tenable explanation, as the values did not differ significantly.

Low fetal body weights generally provide an excellent indicator of deleterious effects. Equivalence of this parameter among groups gives no indication of fetal toxicity. The lack of malformation differences between groups, with the litter as the basis for comparison (Fig. 2), indicates that exposure was not teratogenic. It should be noted that the number of affected fetuses was significantly elevated in the 65 kV/m group and a specific malformation (fused sternebrae) occurred at a significantly higher incidence in fetuses exposed to 65 and 130 kV/m (Table 5). However, these findings do not provide credible evidence of electric-field exposure effects because the incidence of fused sternebrae in the 65 and 130 kV/m groups was limited to one litter in each group. Nor was this finding unique to litters exposed to electric fields, as fused sternebrae also occurred in an unexposed fetus. Historical control incidence of fused sternebrae approximates incidences recorded in the sham-exposed group (1 in 2600 fetuses, 1 in 170 litters).

Examination of mating records among litters with malformations did not reveal significant relationships of sire or grandam to litters with malformations or to type of malformation. Two litters with malformations in the 65 kV/m group were sired by the same male rat; both litters had a single malformed fetus with affected ribs. This male sired two additional litters in this group that had no malformed fetuses and two litters in other exposure groups that were likewise without malformations. Litters with incidences of fused sternebrae were sired by different males. Two litters in the 65 kV/m group had the same grandam, and each had one affected fetus but with different malformations. No other litters with malformations had the same grandam. Because the F₀ generation was purchased from an animal supplier our genetic
information is limited, but from the available data, no significant relationships are evident.

Although a few types of malformations differ between groups, these findings are not considered to be treatment-related effects because of the absence of increased incidence in (1) total number of litters with one or more malformed fetuses (litters rather than fetuses are the unit of analysis (Manson et al., 1984)), (2) specific malformation or category of malformations when compared by litters, or (3) any response by increasing field strength.

The teratological assay yields several parallels with results of our previous replicate experiment. In the earlier experiments (Rommereim et al., 1987) incidences of litters with malformed fetuses (combined data from replicate experiments) from 3-month-old F1 dams were 5.0% for controls and 9.5% in litters from animals exposed at 65 kV/m. In the results reported here, malformation incidence was doubled in rats exposed at 65 kV/m as predicted by the statistical model (Fig. 2). However, lack of a comparably increased percentage of affected litters in animals exposed at 130 kV/m invalidates the hypothesis that increased field strength would result in increased numbers of terata. Results of this experiment clearly demonstrate that developmental changes did not increase with field strength exposures above 65 kV/m, mediating against the existence of a threshold for altered development. One could argue that there is a window of effect around the 65 kV/m exposure level. At this time, however, a biological explanation for such an event does not exist.

Chromodacryorrhea

The only overt sign of an effect of electric fields in adult rats was a significant increase at 65 and 130 kV/m in the incidence and severity of a rust-colored deposit on the face and ears of mature rats exposed to 65 or 130 kV/m (Fig. 3). These deposits occurred in the absence of any detectable effects on body weight. The material is thought to originate in the Harderian gland, located within the eye-socket, behind the eye. Phenomena of a similar nature have been documented in rats as a response to various stressors and were called chromodacryorrhea by Harkness and Ridgway (1980). This effect appears to indicate a physiological response to exposure, perhaps to chronic irritation or stress, but the mechanism for stimulating production of the exudate by electric fields is unknown at this time.

Because a significant increase in the incidence of chromodacryorrhea was not reported in earlier experiments, it is probable that exposure at 130 kV/m (twice that used in the previous work) intensified a subtle effect, prompting closer investigation and, thus, detection at 65 kV/m. Occasionally, the severity of chromodacryorrhea in individual sham-exposed females was equal to that of exposed rats (Table 7). Furthermore, not all exposed rats displayed the colored deposits, making detection of a subtle effect among groups difficult at the lower field strengths.

A similar chromodacryorrhea response has been reportedly produced by various stressors (Scialli, 1988), and stress in rodents has been shown to produce adverse effects on implantation and embryo viability, resulting in terata when sufficiently intense (Cook et al., 1982; Rozenzweig and Blaustein, 1970; Barlow et al., 1975). Additionally, the incidence of chromodacryorrhea in rats exposed at 65 and 130 kV/m but not at 10 kV/m clearly indicates a response to electric field exposure; however, the fields were not stressful enough to produce developmental toxicity. The incidence of chromodacryorrhea was not significantly elevated in litters with malformations ($p = 0.54$).

If the chromodacryorrhea produced by electric field exposure is an indicator of stress, then stress is a potential factor which may have contributed to the developmental
changes reported in the earlier studies. For example, when combined with other factors (disease, diet, housing, etc.), electric field induced stress may have acted synergistically to produce the developmental changes observed in the swine experiment. The effects of electric fields on developmental integrity when combined with stressors have not been specifically examined.

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