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Effects of Low-Frequency Magnetic Fields on Fetal Development in CBA/Ca Mice

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Effects of alternating magnetic fields (MFs) on the embryonic and fetal development in CBA/Ca mice were studied. Mated females were exposed continuously to a sinusoidal 50 Hz (13 μT or 0.13 mT root mean square) or a sawtooth 20 kHz (15 μT peak-to-peak) MF from day 0 to day 18 of pregnancy for 24 h/day until necropsied on day 18. Control animals were kept under the same conditions without the MF. MFs did not cause maternal toxicity. No adverse effects were seen in maternal hematology and the frequency of micronuclei in maternal bone marrow erythrocytes did not change. The MFs did not increase the number of resorptions or fetuses with major or minor malformations in any exposure group. The mean number of implantations and living fetuses per litter were similar in all groups. The corrected weight gain (weight gain without uterine content) of dams, pregnancy rates, incidences of resorptions and late fetal deaths, and fetal body weights were similar in all groups. There was, however, a statistically significant increase in the incidence of fetuses with at least three skeletal variations in all groups exposed to MFs. In conclusion, the 50 Hz or 20 kHz MFs did not increase incidences of malformations or resorptions in CBA/Ca mice, but increased skeletal variations consistently in all exposure groups. Bioelectromagnetics 19:477–485, 1998.

Key words: electromagnetic fields; embryogenesis; teratology; low frequency magnetic fields

INTRODUCTION

The possible adverse effects of low-frequency magnetic fields (MFs) on reproductive outcome have been studied both epidemiologically and experimentally [for reviews see Juutilainen, 1991; Chernoff et al., 1992]. Experimental results suggest that low-frequency MFs may disturb the development of chick embryos, but the results are inconclusive [Berman, 1990; Berman et al., 1990; Juutilainen, 1991].

Results in mammalian teratological studies are inconsistent. In several experiments, pregnant rats or mice have been exposed to sinusoidal 50–60 Hz MFs at intensities from 2 μT to 30 mT or to 15–20 kHz fields with sawtooth waveform (similar to fields emitted by video displays) at 5.7–66 μT [Tribukait et al., 1987; Frölen et al., 1993; Wiley et al., 1992; Stuchly et al., 1988; Ryan et al., 1996; Huuskonen et al., 1993; Mevissen et al., 1994; Kowalczuk et al., 1994]. The results show that the MF exposures used do not induce gross malformations, and many studies have found no evidence of teratogenic or embryotoxic effects. The only finding that shows some consistency is slightly increased minor skeletal anomalies seen in several studies in rats [Stuchly et al., 1988; Huuskonen et al., 1993; Mevissen et al., 1994] and mice [Kowalczuk et al., 1994], at 50–60 Hz and/or 15–20 kHz.

Another interesting finding is the increase of early resorptions seen in CBA/S mice exposed to 20 kHz MFs with sawtooth waveform [Frölen et al., 1993]. The increase was statistically highly significant and was replicated in several large experiments in the same laboratory.

Most data on genotoxicity of MFs are negative [Murphy et al., 1993]. However, 50 Hz, 30 μT, both

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TABLE 1. Study Setup in Terms of Groups and Exposure to Electromagnetic Field

Phase	Exposure groups	Group size	Geomagnetic field (μT)
1–3	Control	41/45/64[a,b]	34–40
	50 Hz, 13 μT (sinusoidal)	55/56/66	30–34
	20 kHz, 15 μT (sawtooth)	53/56/65	37–40
4	Control	34/34/35	45–50
	50 Hz, 0.13 mT (sinusoidal)	31/33/35	50–55

[a]Number of litters with at least 1 live fetus/number of litters/number of copulated females.
[b]Phases 1–3 are combined.

continuous and intermittent, MFs induced chromosomal aberrations in human amniotic cells [Nordenson et al., 1994]. In human lymphocytes in vitro, chromosomal aberrations were found after exposure to pulsing electromagnetic fields [Khalil and Qassem, 1991]. Strong 50 Hz electric fields induced micronuclei in somatic cells in mice [El Nahas and Oraby, 1989].

To further investigate the possible embryotoxic effects of MFs, we performed a series of studies with CBA/Ca mice exposed to sinusoidal 50 Hz or sawtooth 20 kHz MFs. The CBA/Ca strain was selected because the results of Frölen et al. [1993] suggest that MF exposure might increase resorptions in the closely related strain CBA/S. Because the data on mutagenicity of MFs are elusive, we also analyzed micronuclei in the bone marrow erythrocytes of the dams as a measure of genotoxicity.

MATERIALS AND METHODS

Study Design

The study was conducted in 4 phases. Phases 1–3 were identical and their results were combined (Table 1). One group of animals (in phases 1–3) was exposed to an alternating MF with a triangular (sawtooth) waveform similar to that used in the previous experiments with mice [Tribukait et al., 1987; Frölen et al., 1993] and rats [Huuskonen et al., 1993]. The field characteristics were frequency 20 kHz; peak-to-peak flux density 15 μT; rise time 5 μs; and fall time 45 μs. Another group of mice was exposed to a 50 Hz sinusoidally oscillating field similar to that reported to affect the development of chick embryos [Juutilainen et al., 1987] and to induce skeletal anomalies [Huuskonen et al., 1993]. The time-average (root mean square) magnetic flux density was 13 μT (MF strength 10 A/m). The third group of animals was sham-exposed and served as controls.

In phase 4, a group of animals was exposed to a 50 Hz sinusoidal MF at 0.13 mT. Sham-exposed animals were used as controls.

Exposure

In all cases the exposure was continuous (24 h/day) throughout pregnancy (from day 0 to day 18) up to cesarean section at the end. The MFs were produced by sets of rectangular coils as described earlier [Huuskonen et al., 1993]. The 2 active exposure systems were separated by a distance of 2 m, and the sham-exposed control animals were at least 3.5 m from the nearest active exposure system. The background alternating MF strength was below 0.1 μT. The static (geomagnetic) flux density in the exposure systems is shown in Table 1. The inclination of the geomagnetic field was between 60° and 80°.

Animals, Housing, and Mating

SPF-quality mice of CBA/Ca-strain (B&K Universal Limited, North Humberside, England) were used. The nulliparous females were 10–13 weeks old with average group mean body weights of 19–21 g at the time of matings. Males were at least 2 weeks older and of proven fertility. The experiments were conducted in an animal room containing 3 separate cubicles for animal cages. Each exposure group was located in its own cubicle. Females and males were housed before mating in groups of 5 or 6 animals of the same sex in transparent Macrolon cages (type III). The cages contained granular aspen chip bedding (Tapvei Ltd., Kaavi, Finland).

The temperature and the relative humidity of the animal rooms were 21 ± 1 °C and 50 ± 10%, respectively. The room was illuminated with artificial light for 12 h daily (7 a.m. to 7 p.m.) and, during the dark period, a dim ''pilot'' light was used. Commercial standard rat pellet diet [R36, Astra-Ewos (Lactamin), Södertälje, Sweden] and tap water were available at all times throughout the experiment.

Females were mated for 2–3 h before light period with males polygamously (3 females with 1 male). Vaginal plugs were observed on the following morning, day 0 of gestation. The mated females were randomized to experimental and control groups by the stratified body weight procedure. The animals were identified by ear puncture. The animals were housed maximally 6 animals per cage (Macrolon III) during exposure. Exposure was started on the morning after mating. During the exposure period the animals were observed daily for clinical signs. Body weights were recorded on days 0, 6, 13, and 18 of pregnancy.

Termination

Dams were killed on the morning of the 18th day of pregnancy by CO_2 asphyxiation. From a portion of

the animals heparinized blood samples were drawn from the heart for hematology. The uterine contents and ovaries were examined immediately for the number of corpora lutea, weight of the uterus with its contents, number of implantation sites, sex and number of viable fetuses, number of dead fetuses, number of resorptions, individual fetal weights, external abnormalities, and placental weights. If 2 fetuses seemed to share the same implantation scar in the uterus, the number of implantation sites was calculated as a sum of numbers of live and dead fetuses and resorptions.

Resorptions

Resorptions were classified as follows:

R0 = resorption that is detected only after ammonium sulfide staining
R1 = a small placental remnant
R2 = a placental remnant with fetal membranes $\leq$ 0.3 cm
R3 = a placental remnant with fetal membranes $>$ 0.3 cm $<$ 0.5 cm
R4 = a placental remnant with fetus $\leq$ 0.5 cm
R5 = a placental remnant with fetus $>$ 0.5 cm $\leq$ 1.0 cm
R6 = a placental remnant with fetus $>$ 1.0 cm, the fetus partly macerated

Resorptions were described by size and color.

Examination of Fetuses

Fetuses were examined for external (all phases) and skeletal (phases 1, 2, and 4) abnormalities. Abnormalities were classified to malformations, minor malformations, variations, and other findings. A fetus was classified as a variant if it had at least 3 skeletal variations, e.g., wavy ribs, irregular sternebrae, and reduced cranial ossification [Huuskonen et al., 1993]. Malformations were defined as very rarely occurring or obviously lethal changes, and minor malformations as slight, relatively rarely occurring structural changes that are obviously not detrimental. Variations were defined as common structural changes (usually occurring spontaneously in more than 5% of the population). Other findings included, for instance, slight edema and hemorrhages. Malformations in dead fetuses (and the fetuses born too early) were not included in the number of fetuses with malformations.

Classification of abnormal fetuses was based on the most serious abnormality of that fetus (also in litters) so that each fetus is counted only once. Fetuses with multiple major malformations are fetuses that have several malformations or at least one malformation and one minor malformation. Fetuses with multiple minor malformations are fetuses that have several minor malformations or at least one minor malformation and one of the other findings.

Every live fetus was preserved first in ethanol. Sex was confirmed a few hours later by checking the sex organs by opening the abdominal cavity. The skeletons were prepared by clearing them in potassium hydroxide, stained with Alcian blue and Alizarin red S [Kimmel and Trammel, 1981; Dawson, 1926], and stored to be analyzed later. Dead fetuses were preserved in Bouin fixative for possible later necropsy under the microscope. The empty uteri were placed into a solution of ammonium sulfide to visualize hemorrhagic alterations in implantation sites of very early resorptions [Salewski, 1964].

Ten females were excluded from the litter data. In phase 2, 8 females (from the control group) were excluded because one night at early pregnancy water bottles had leaked, wetting the bedding material. These females had total resorptions or were not pregnant. One female (from the control group) was necropsied on a wrong necropsy date. In phase 1, 1 female (50 Hz, 10 A/m group) had begun to deliver during the night before necropsy (4 fetuses were born and they were partly cannibalized, 1 resorption was found in the same uterine horn, 1 normal live fetus was found in the other horn). This dam is included in the data of all pregnant females as a litter with stillbirths, but not included in the calculations where data are given from females with at least 1 live fetus.

Necropsy of Dams, Hematology, and Micronucleus Analysis

A limited necropsy focusing on the thoracic and abdominal cavities was performed on each dam. Blood samples were collected by cardiac puncture from a portion of the dams in phase 4 (control and 50 Hz, 0.13 mT) for hematological analysis and differential leukocyte counts. The hematological parameters analyzed from whole blood were leukocytes, erythrocytes, hemoglobin, hematocrit, erythrocyte mean cell volume, erythrocyte mean cell hemoglobin, erythrocyte mean cell hemoglobin concentration, and thrombocytes. Differential leukocyte count included unsegmented neutrophil leukocytes, segmented neutrophil leukocytes, eosinophils, basophils, lymphocytes, and monocytes.

The method introduced by Schmid [1975] was modified to make the slides for micronucleus analysis in the bone marrow of the dams in phase 1. Briefly, the method was as follows. Both femora were removed from each animal and cleaned of muscle. A small opening was cut in the proximal end of the bones and the bone marrow was flushed through the opening with 1 ml of inactivated fetal calf serum into a tube con-

taining 2 ml of the serum. The sample was centrifuged (5 min at 1000 rpm) and at least 2 smear slides were prepared from each pellet. The slides were air-dried, fixed for 5 min in methanol, and air-dried. Fixed slides were coded, stained with acridine orange [Hayashi et al., 1983], and analyzed at ×1000 magnification by fluorescent microscopy. One thousand polychromatic erythrocytes were analyzed for micronuclei in 13–16 dams per group. The recorded micronuclei were sharply contoured, from round to oval in shape, and exhibited strong green-yellow fluorescence. The proportion of polychromatic erythrocytes to normochromatic cells in the total erythrocyte population was analyzed by counting the number of cells until the score for one cell type reached 1000.

Statistical Analysis

For each litter, preimplantation loss and postimplantation loss were calculated as a percentage according to the formulas:

$$\text{Preimplantation loss: } (a - b)/a \times 100$$

$$\text{Postimplantation loss: } (b - c)/b \times 100$$

where a = number of corpora lutea, b = total number of implantations, and c = number of live fetuses.

The statistical analyses were performed using the SPSS/PC+ test package for the IBM PC/XT/AT (SPSS Inc., Chicago, IL). The mean maternal, fetal, and placental weights per litter and the mean values of implantations and living fetuses per litter were analyzed by one-way analysis of variance followed by Scheffé's test or by Student's t-test. Because of the non-normal distributions, pre- and postimplantation losses and group mean values of litter percentages of anomalies were analyzed by Mann-Whitney U-non-parametric test. Hematological data were analyzed by Student's t-test. Other data were analyzed by chi-squared tests.

RESULTS

Dams

No notable maternal effects were observed during the study. There were no treatment-related clinical signs in dams and the terminal body weights, weight gain, and the corrected body weight gain (weight gain without uterine content) of the exposed groups did not differ from those of the control animals (data not shown). The mean weight of uteri with and without fetuses and placental weights were also similar in all groups (data not shown).

In necropsy, opacity in the right eye in 1 control dam (phase 1), hair loss in 1 dam (phase 4, 50 Hz, 0.13 mT), swollen mammary glands in 1 control dam (phase 3), and enlarged Payer patches in 3 dams (phase 3, 50 Hz, 13 μT) were observed. One dam had begun the delivery before the necropsy (phase 1, 50 Hz). These necropsy findings are sporadic and do not have clear connection to MF treatment. It therefore appears unlikely that maternal toxicity has contributed to any differences between litters.

Fertility

MFs did not affect significantly the fertility of the animals. No statistically significant differences were noted in the fertility ratio (percentage of pregnant females of mated females), number of corpora lutea, sex ratio, and preimplantation loss between groups (Table 2). The mean number of implantation sites per litter and the mean number of live fetuses per litter in all pregnant animals were slightly but not statistically significantly increased in the 50 Hz, 13 μT group (Table 2). This difference in the number of live fetuses per litter results mainly from the higher rate of total resorptions in the control group (4 females) than in the 50 Hz, 13 μT group (1 female) (Table 2). This is substantiated by the observation that if the dams with total resorptions are excluded from the data, the mean number of implantation sites and live fetuses per litter were similar in all groups (Table 2). The number of animals with total resorptions was 3 in the 20 kHz group and 0 and 2 in the control and the 50 Hz (0.13 mT) groups in the phase 4 study, respectively (Table 2).

As in the case of implantation sites, there was no difference in the number of litters with resorptions or in resorptions between groups when total resorptions are omitted. The resorption frequency as percentage of resorptions of the implantation sites was 11.2, 10.0, and 10.5% in the control, 50 Hz (13 μT), and 20 kHz groups, respectively, in females with at least 1 living fetus (Table 2). In phase 4, the percentage of resorptions was 9.0% in the control group and 7.7% in the 50 Hz (0.13 mT) group in females with at least 1 live fetus (Table 2). MF exposures did not affect the timing of the resorptions. The numbers of early and late resorptions and late fetal deaths were similar in all groups (Table 2). Early resorptions (a small placental remnant without visible fetal membranes) were the most common resorption type. Altogether, the data indicate that these MFs did not cause resorptions in CBA/Ca mice.

Offspring

MFs did not cause external major malformations in fetuses (Table 3). Only a few live fetuses had external major malformations and their frequency did not differ significantly between the MF-treated groups and the controls. The major malformations were micro-

TABLE 2. Reproduction and Litter Data: Phases 1–3 and 4

Parameter	Exposure group				
	Control	50 Hz (13 μT)	20 kHz	Control	50 Hz (0.13 mT)
Pregnant females[a]	45 (70)	55 (83)	56 (86)	34 (97)	33 (94)
Litters with live fetuses[b]	41 (91.1)	55 (100)	53 (94.6)	34 (100)	31 (93.9)
Litters with resorptions[b]	28 (62.2)	32 (57.1)	35 (66.0)	20 (58.8)	16 (48.5)
Litters with total resorptions[b]	4 (8.9)	1 (1.8)	3 (5.7)	0	2 (6.1)
Litters with stillbirths	0	1	0	0	0
Implantations	368	485	466	321	306
Live fetuses[c]	305 (82.9)	422 (87.0)	403 (86.5)	291 (90.7)	263 (85.9)
Resorptions[c]	60 (16.3)	60 (12.4)	59 (12.7)	29 (9.0)	41 (13.4)
Late fetal deaths[c]	3 (0.8)	3 (0.6)	4 (0.9)	1 (0.3)	2 (0.7)
Corpora lutea/litter[d]	8.6 ± 0.3	9.0 ± 0.2	9.1 ± 0.3	9.8 ± 0.3	9.7 ± 0.3
Implantations/litter[d]	8.2 ± 0.3	8.8 ± 0.2	8.3 ± 0.3	9.4 ± 0.3	9.3 ± 0.3
Live fetuses/litter[d]	6.8 ± 0.4	7.7 ± 0.2	7.2 ± 0.3	8.6 ± 0.3	8.0 ± 0.5
Dead fetuses/litter[d]	0.07 ± 0.04	0.05 ± 0.03	0.07 ± 0.04	0.03 ± 0.03	0.06 ± 0.04
Non-live implants/litter[d,e]	1.4 ± 0.3	1.1 ± 0.3	1.1 ± 0.2	0.9 ± 0.2	1.3 ± 0.4
Preimplantation loss (%)[d]	−0.9 ± 3.7	0.9 ± 2.0	4.5 ± 3.2	1.9 ± 3.2	2.3 ± 3.0
Postimplantation loss (%)[d]	20.3 ± 4.3	12.1 ± 2.2	16.1 ± 3.1	9.2 ± 1.6	13.8 ± 4.2
Resorptions/pregnant dam[d]	1.3 ± 0.3	1.1 ± 0.3	1.1 ± 0.2	0.9 ± 0.2	1.2 ± 0.4
R0	0.36 ± 0.24	0.24 ± 0.24	0.21 ± 0.15	0	0
R1	0.78 ± 0.17	0.75 ± 0.13	0.73 ± 0.11	0.68 ± 0.13	1.09 ± 0.40
R2	0.07 ± 0.04	0.07 ± 0.04	0.05 ± 0.04	0.06 ± 0.04	0
R3	0.07 ± 0.04	0.04 ± 0.03	0.02 ± 0.02	0.03 ± 0.03	0
R4	0	0	0	0.06 ± 0.04	0.12 ± 0.07
R5	0.04 ± 0.03	0	0.04 ± 0.03	0.03 ± 0.03	0
R6	0.02 ± 0.02	0	0	0	0.03 ± 0.03
Females with at least 1 live fetus					
Litters with malformed fetuses[g]	17 (37.8)	20 (35.7)	22 (39.3)	4 (11.8)	7 (2.3)
Live fetuses[c]	305 (87.9)	422 (89.4)	403 (88.6)	291 (90.7)	263 (91.6)
Malformed fetuses[h]	20 (6.6)	25 (5.9)	24 (6.0)	4 (1.4)	7 (2.3)
Resorptions[c]	39 (11.2)	47 (10.0)	48 (10.5)	29 (9.0)	22 (7.7)
Affected implants[c,i]	62 (17.9)	75 (15.9)	76 (16.7)	34 (10.6)	31 (10.8)
Implantations/litter[d]	8.5 ± 0.2	8.7 ± 0.2	8.6 ± 0.2	9.4 ± 0.3	9.3 ± 0.3
Live fetuses/litter[d]	7.4 ± 0.3	7.8 ± 0.2	7.6 ± 0.2	8.6 ± 0.3	8.5 ± 0.3
Non-live implants/litter[d]	1.0 ± 0.2	0.9 ± 0.1	1.0 ± 0.1	0.88 ± 0.16	0.77 ± 0.17
Sex ratio	0.50	0.51	0.53	0.55	0.48
Preimplantation loss (%)[d]	−2.9 ± 3.7	1.2 ± 2.0	2.9 ± 2.8	1.9 ± 3.2	3.7 ± 3.0
Postimplantation loss (%)[d]	12.5 ± 2.2	10.5 ± 1.4	11.4 ± 1.6	9.2 ± 1.6	8.2 ± 1.7

[a]Percentage of mated females in parentheses.
[b]Percentage of pregnant females in parentheses.
[c]Percentage of implantations in parentheses.
[d]Mean ± SEM.
[e]Resorptions and late fetal deaths.
[f]R0–R6 = classification of resorptions by size.
[g]Percentage of litters in parentheses.
[h]Percentage of live fetuses in parentheses.
[i]Resorptions, late fetal deaths, and malformations.

phthalmia, club foot, exencephalia, cleft palate, gastroschisis, scoliosis, and umbilical hernia. The number of fetuses with minor external malformations, such as wavy tail, irregular eyes, and irregular palate, did not differ between groups (Table 3). Other observed abnormalities included slackness of fetuses and hemorrhages.

The proportion of skeletal variants (fetuses with at least 3 skeletal variations) was statistically significantly higher ($P \leq .05$) in the MF-treated groups (both 50 Hz and 20 kHz groups) than in the respective control groups (Tables 4, 5). The skeletal variations at increased 20 kHz were irregular ribs (at least 1 rib folded, shorter, or otherwise abnormal), decreased ossification of caudal vertebral centra, irregular sternebrae (less than 4 ossified sternebrae or at least 4 irregularly ossified sternebrae or bipartite sternebrae), decreased cranial ossification, and decreased ossification in phalangeals in forelegs or in rearlegs.

At 50 Hz, 13 μT, the variations increased were wavy ribs (at least 1), ossification of caudal vertebral centra, irregular sternebrae, decreased cranial ossification, poorly ossified bone, decreased ossification in metatarsals or metacarpals, proximal fingers, and proximal toes. At 50 Hz, 0.13 mT, wavy ribs, decreased cranial ossification, poorly ossified bone, decrease in

TABLE 3. External Malformations in Fetuses

Observations	Phases 1–3			Phase 4	
	Control	50 Hz (13 μT)	20 kHz	Control	50 Hz (0.13 mT)
No. of examined live fetuses	305	422	403	291	263
No. of litters	41	55	53	34	31
Malformations	5 (5)[a] (1.6%)	3 (3) (0.7%)	6 (5) (1.5%)	1 (1) (0.3%)	1 (1) (0.4%)
Minor malformations	15 (12) (4.9%)	22 (17) (5.2%)	18 (17) (4.5%)	4 (4) (1.4%)	6 (6) (2.3%)
Others	6	6	5	5	4
Fetuses with multiple major malformations	2 (2)	2 (2)	2 (2)	0	0
Fetuses with multiple minor malformations	3 (3)	1 (1)	4 (4)	0	0

[a]Number of litters affected.

TABLE 4. Skeletal Anomalies: Phase 1–2[†]

Observation	Exposure group		
	Control	50 Hz (13 μT)	20 kHz
No. of fetuses	182	291	287
No. of litters	24	38	36
Minor malformations			
Camptodactyia	0	0	1.0 ± 0.8
Irregular jaw	3.7 ± 1.6	4.0 ± 1.1	4.0 ± 1.1
Bended or wavy tail	0.6 ± 0.6	0.7 ± 0.5	0.6 ± 0.4
Skeleton anomaly	0	0.3 ± 0.3	0
Variations			
Tibia, abnormal	0	1.0 ± 0.6	0.3 ± 0.3
Irregular clavicle	9.1 ± 2.2	9.1 ± 2.3	8.9 ± 1.9
Wavy ribs	6.1 ± 2.1	9.4 ± 1.7	8.8 ± 1.9
Irregular ribs	24.6 ± 4.6	25.6 ± 3.5	34.3 ± 4.1
Highly cartilaginous cervical vertebrae	0	0	0.3 ± 0.3
Non-ossified cervical centra	23.3 ± 3.6	27.0 ± 3.1	26.8 ± 3.4
Vertebral centra, at least 1 unossified	3.0 ± 1.3	2.1 ± 0.8	2.3 ± 0.9
Caudal vertebrae, less than 4 ossified	15.1 ± 3.8	22.8 ± 3.1	22.0 ± 3.0
Irregular, non-ossified or bipartite sternebrae	9.8 ± 3.1	17.4 ± 2.8	16.5 ± 2.5
Poorly ossified cranium	12.1 ± 3.2	19.8 ± 3.2	21.8 ± 3.5
Poorly ossified bone	15.1 ± 4.6	19.2 ± 4.4	11.4 ± 2.6
Irregular hyoid	2.0 ± 1.6	2.5 ± 0.9	1.7 ± 0.8
Poorly ossified metatarsals or metacarpals	5.2 ± 1.9	8.3 ± 2.1	7.0 ± 2.0
Proximal fingers, less than 3 ossified	12.7 ± 3.1	17.1 ± 2.7	20.2 ± 2.6
Proximal toes, less than 2 ossified	30.1 ± 4.6	43.8 ± 4.0*	44.1 ± 3.9*
Fetuses with at least 3 skeletal variations	8.8 ± 2.7	15.4 ± 2.0*	15.7 ± 2.4*

[†]The figures represent percentage of affected fetuses of the living fetuses per litter. Mean ± SEM.
*Mann-Whitney U-non-parametric test, $P \leq .05$, compared with the control group.

ossification in metatarsals or metacarpals were found. Most of the variations were due to decrease in ossification, more clearly at 50 Hz groups. Both field types induced quite similar skeletal variations. The frequency of the variations was not related to the field strength.

Hematology and Micronucleus Analysis

The 50 Hz (0.13 mT) MF did not affect the total number of blood cells or the differential counts of leukocytes of the dams (data not shown). Also, the number of micronuclei in polychromatic or normochromatic erythrocytes in the bone marrow did not differ significantly between the MF-exposed and the control groups (Table 6, phase 1), suggesting that MF did not have a clastogenic effect in the mice.

DISCUSSION

In the present study, the only effect clearly related to MF treatment was the increased incidence of fetuses

TABLE 5. Skeletal Anomalies: Phase 4[†]

Observation	Exposure group	
	Control	50 Hz (0.13 mT)
No. of fetuses	291	263
No. of litters	34	31
Minor		
Bended tail	0	0.5 ± 0.5
Irregular jaw	0	1.4 ± 0.8
Variations		
Tibia anomaly	0	0.4 ± 0.4
Irregular clavicle	14.6 ± 2.5	16.3 ± 2.8
Wavy ribs	8.0 ± 1.7	12.2 ± 2.0
Irregular ribs	48.6 ± 3.1	51.4 ± 4.0
Non-ossified cervical centra	15.0 ± 1.9	16.7 ± 3.2
Vertebral centra, at least 1 unossified	0.3 ± 0.3	1.5 ± 0.7
Caudal centra, less than 3 ossified	11.6 ± 1.8	14.0 ± 3.2
Irregular, non-ossified or bipartite sternebrae	13.7 ± 2.2	13.8 ± 2.7
Poorly ossified cranium	10.5 ± 1.8	18.4 ± 2.7*
Poorly ossified other bone	0.3 ± 0.3	3.2 ± 1.1*
Irregular hyoid	0.3 ± 0.3	1.1 ± 0.6
Poorly ossified metatarsals or metacarpals	2.9 ± 1.1	4.9 ± 1.6
Proximal fingers, less than 3 ossified	8.9 ± 1.7	10.2 ± 2.2
Proximal toes, less than 2 ossified	29.6 ± 3.6	34.0 ± 4.1
Fetuses with at least 3 skeletal variations	12.1 ± 2.1	20.1 ± 2.8*

[†]The figures represent percentage of affected fetuses of the living fetuses per litter. Mean ± SEM.
*Mann-Whitney U-non-parametric test, $P \leq .05$, compared with the control group.

TABLE 6. Micronucleus in Femur Bone Marrow Erythrocytes of Mice*

Exposure group	Animals	MNPCE (‰)	MNNCE (‰)	PCE/PCE + NCE
Control	14	2.00 ± 1.41	1.62 ± 1.63	0.71
50 Hz, 13 μT	13	1.23 ± 1.54	1.10 ± 1.92	0.63
20 kHz	16	1.31 ± 1.35	1.43 ± 2.12	0.67

*One thousand polychromatic erythrocytes (PCE) were analyzed per animal. MNPCE, micronucleated polychromatic erythrocytes; MNNCE, micronucleated normochromatic erythrocytes; NCE, normochromatic erythrocytes.

with skeletal variations in all MF exposure groups, both at 50 Hz at the field strengths of 13 μT and 0.13 mT and at 20 kHz at the field strength of 15 μT. This finding is in concordance with the previous observations of increased numbers of fetuses with minor skeletal anomalies and/or variations in rats [Stuchly et al., 1988; Huuskonen et al., 1993; Mevissen et al., 1994] and mice [Kowalczuk et al., 1994; Chiang et al., 1995] exposed to MFs.

The skeletal abnormalities seem to be exposure, species, and strain dependent. In CD-1 fetuses, the incidence of fetuses with 1 or more cervical ribs was statistically significantly increased following exposure to a 20 mT, 50 Hz sinusoidal MF. The affected fetuses seemed to be clustered in a few litters [Kowalczuk et al., 1994]. In Swiss Webster mice, reduced skeletal calcification or loss of the skeleton was found in the occipital bone and in the fifth and sixth thoracic vertebrae, in the fifth rib, and in the second thoracic vertebrae after prenatal exposure to a sawtooth 15.6 kHz MF with a peak magnetic flux density of 40 μT [Chiang et al., 1995].

Mevissen et al. [1994] suggested that the skeletal changes (increased ossification) in rats indicated accelerated prenatal development. Our current results suggest mostly decreased ossification. The present study used a much weaker field strength than Mevissen et al. [1994], which may explain the different changes. In our previous rat study with similar field strengths as in the current study in phases 1–3 [Huuskonen et al., 1993] there were indications of both decreased and increased ossification.

Several studies have established that extremely low-frequency electric and MFs can cause bone-related biological changes, and these fields have been used successfully for facilitating bone healing in humans

[Bassett et al., 1982]. Effects on bone formation during development are thus feasible. Alterations of bone cell growth rates [Bassett et al., 1982] and inhibition of bone cell response to parathyroid hormone have been observed [Luben et al., 1982]. The biological mechanisms of action of MF in bone formation are not known. Effects on signal transduction at the receptor and intracellular level have been described. For example, alterations in the cellular level of cyclic AMP have been observed [Luben et al., 1982; Cain et al., 1987; Luben, 1991; Brighton and McCluskey, 1987; Brighton and Townsend, 1988; Schimmelpfeng and Dertinger, 1993; Knedlitschek et al., 1993].

Pulsed electromagnetic fields have also been shown to alter the synthesis of extracellular matrix components during chondrogenesis in the embryonic chick limb [Archer and Ratcliffe, 1983; Norton et al., 1988]. Exposure to pulsed electromagnetic fields can produce skeletal pattern abnormalities in regenerating newt limbs that are quite similar to congenital skeletal limb deformities that appear in human neonates [Landesman and Douglas, 1990]. The developing limb and the regenerating limb do share some common morphogenetic processes.

The results indicate that 50 Hz sinusoidal or 20 kHz sawtooth MFs do not cause major malformations or increase the number of resorptions or late fetal deaths in CBA/Ca mice. Fetal body weight and gross fetal development were also in the normal range. In this respect, the results are different from those of the previous studies in C3H or CBA/S mice where increased malformations, resorptions, or decreased body weight or size were found [Tribukait et al., 1987; Frölen et al., 1993; Svedenstål and Johanson, 1995]. We have previously reported suggestive evidence of increased resorptions in CBA/S but not CBA/Ca mice [Juutilainen et al., 1997]. The reasons for different responses in CBA/S and CBA/Ca strains are at present not known.

El Nahas and Oraby [1989] reported a dose-dependent increase of micronucleated bone marrow polychromatic erythrocytes in male Swiss mice exposed for 24 h to 50 Hz electric fields. The electric field intensities were 100, 170, 220, and 290 kV/m. The negative results of the present study suggest, however, that low-strength MFs do not have clastogenic and/or spindle toxic effects in pregnant CBA/Ca mice. That the number of blood cells, proportion of immature red blood cells in total erythrocyte population, and differential count of leukocytes in blood did not change indicates that MFs did not affect the development of blood cells in the bone marrow. Decreased leukocyte count and alterations of white blood cells have been described earlier [Stuchly et al., 1988; Picazo et al., 1994].

In conclusion, exposure to a sinusoidal 50 Hz, 13 μT or 0.13 mT or a sawtooth 20 kHz MF was not teratogenic or embryotoxic in CBA/Ca mice. Nevertheless, the consistent increase of skeletal variations in all exposure groups indicates that low-frequency MFs exert subtle biological effects on prenatal development. The significance of these minor changes for human health risk assessment is not known.

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