JOURNAL OF BIOELECTRICITY, 9(2), 213-231 (1990)

SHORT COMMUNICATION

*META-ANALYSIS OF MULTI-GENERATIONAL STUDIES OF MICE EXPOSED TO POWER-FREQUENCY ELECTRIC FIELDS

Andrew A. Marino
Department of Orthopaedic Surgery
LSU Medical Center
Shreveport, LA 71130

ABSTRACT

Eight multi-generational studies involving mice exposed to 60-Hz electric fields, 0.5–100 kV/m were performed during the past 15 years to help evaluate potential health risks. A statistically significant influence of field exposure on development and variance in development occurred in all 8 experiments. There was an *a posteriori* probability of 0.304 that the field would alter development: the average effect on body weight was 6.6 ± 3.6%, and the respective probabilities for observing an increase and decrease in weight (compared with the controls) were 0.57 and 0.43. The *a posteriori* probability of the effect on variance was 0.367; increases and decreases in the field-exposed animals were equally probable. A significant effect on survival was observed in half the experiments. None of the reported effects exhibited a dose-response relationship.

INTRODUCTION

The issue of health risks from powerline electromagnetic fields (EMFs) matured in the United States in 1974 during hearings in New York involving 765-kV powerlines. Disparate views emerged regarding the existence of EMF-induced bioeffects, their health implications, and the mechanism that might subserve them. The original conception of the physiological mechanism of environmental EMFs was that they were stressors (1); changes in exposed subjects — including development of disease — were seen as linked to chronic activation of the neuroendocrine system. Even before the New York hearing ended, investigators at Battelle Northwest Laboratories (BNL) proposed a dose-response theory (2). The effort to establish the

*Presented at FASEB 74th Annual Meeting, April 1-5,1990, Washington, DC.

validity of either (or both)of these theories has been a dominant theme of environmental electromagnetism for the last 15 years.

If the same dose of an independent variable is applied to independent groups, occurrence of the same change in a dependent variable is one possible result; in such cases the magnitude of the dependent variable usually changes proportionately with changes in the independent variable. When the dependent variable does consistently exhibit the same value following presentation of the independent variable (irrespective of all other somatic and internal factors that might exert an influence on the dependent variable), or, when the dependent variable exhibits values proportional to different levels of the independent variable, the link between the variables is described as a dose-response (DR) relationship.

Banal as it may sound, non-DE relationships are also real. If the environmental temperatures of a stone and a mouse are increased, the stone's temperature will increase proportionately and consistently but the temperature of the mouse will depend on its internal state. Stressors including temperature, are detected by the body's sensory systems which produce afferent signals to the thalamus. For whatever reason is embedded in the body's wisdom, efferent signals to the neuroendocrine and immune systems are then elicited, thereby affecting the serum levels of hormones, cytokines, and many other factors. Since the body's sensory and internal control systems continue to function whether or not the stressor of interest to the investigator is applied, any particular dependent variable typically will endure multiple influences from many external and internal factors. A stressor may have only a minor impact (compared with that resulting from uncontrolled factors) on a dependent variable in a particular experiment, and this impact may not be observable in every instance. It may, however, be observable in some instances. A fundamental distinction regarding bioeffects associated with environmental EMFs is whether they are DE or non-DE bioeffects.

One may infer the existence of a biological effect when a dependent variable in the exposed animals is observed to differ from that which would have been measured if the independent variable were not applied (or, if the controls are appropriately chosen, to differ from the level in the control animals). If many comparisons involving dependent variables are made, statistical significance ($P < 0.05$) may be observed by chance; consequently, the occurrence of one or more such successes is not necessarily an indication that an effect actually occurred. But if the observed number of successes is too high to be ascribed to chance, then an influential role may be attributed to the independent variable. If the frequency of the effects in independent trials exceeds a certain threshold, or if the pattern of change in the magnitude of the dependent variable is related to that of the independent variable, or both, the relation between the variables may be characterized as being of the DE type; otherwise, it is a non-DE relationship. In this manner, the relation between any variables may be studied (by replicating experiments or varying the dose) without the necessity of assuming that it be of one particular type. Since 1975, my colleagues and I have pursued this approach in the context of a multi-generation study of mice exposed to 60-Hz electric fields. Similar studies were performed at BNL. In the following analysis of the published results of these studies, I show that 60-Hz electric fields of 0.5–100 kV/m consistently affected survival, development, and variance in development in mice, and that each effect exhibited a non-DE relationship with the electric field.

METHODS

Five multi-generation (MG) studies (total of 8 experiments) involving exposure of mice to 60-Hz electric fields were performed during the last 15 years (3-10). In MG-1 (3) Ha/ICR mice were divided into vertical-exposure (VE), horizontal-exposure (HE), and control (C) groups and housed in plastic cages, 15x30x15 cm high. A metal cage top was used in the VE group, and water was provided via a water bottle (located outside the cage) with a metal tube that extended downward 5 cm from the cage top; food was provided via a trough that extended downward about 7 cm from the cage top. The field was applied by grounding the cage top and energizing an insulated metal plate underneath the cage; the undisturbed electric field (away from the water tube and food trough) was 15 kV/m. The cage top in the HE group was plastic, but the method of providing food and water to the exposed animals was similar to that used in the VE group. An electric field having an undisturbed strength of 10 kV/m was applied to the HE group by energizing pairs of parallel metal plates placed outside each cage. The mice in the C group were housed in plastic cages with metal cage tops, but were not subjected to an applied electric field. The bedding material in each cage was a 2-inch layer of sanitized wood shavings.

In MG-2 (4) and MG-3 (5) mice were divided into vertical-exposure (VE), horizontal-exposure (HE), vertical-control (VC), and horizontal-control (HC) groups, and were maintained in specially constructed housing units: One unit held the HE and HC groups, but separate units were built for the VE and VC groups. Food was placed on the wood bedding, and the water bottle was placed inside the cage with the mice. Metal was not present in the environment of any of the four groups. The respective 60-Hz electric fields in MG-2 and MG-3 were 3.5 kV/m and 0.5 kV/m.

In MG-4 and MG-5 (6-10) Swiss-Webster mice were divided into VE and VC groups and were housed in plastic modules 12.7x25.4x5.1 cm high. The exposure system consisted of two identical units, each of which consisted of 5 vertically-arranged metal plates that accommodated the modules. In one unit the plates were energized with phase-controlled voltages to produce 100 kV/m in the interplate regions. The bottom of each module was stainless-steel mesh (about 0.64 cm square); it made electrical contact with the plate on which it rested. Water was provided via nozzles located under the mesh in such a way that the nozzle was electrically equivalent to the mesh. The litter material was nylon (Antron III, DuPont); it was used only during the peri-natal period.

Mice were allowed to mate, gestate and deliver their offspring while continuously being exposed or sham-exposed to the electric field. Litters were reduced to 6 animals within 1 day after birth (except in MG-1 and the first generation of MG-2), to lessen the influence of litter size on development. The females remained with their offspring until weaning (when the offspring were separated by sex). At maturity, randomly selected individuals from the first generation were similarly allowed to produce and rear their

TABLE 1. Influence of 60-Hz Electric Field on Survival in Three Generations of Mice (MG-1). OR, odds ratio and 95% confidence limits for survival between birth and 35 days after birth. VE and HE respectively designate the groups exposed to vertical (15 kV/m) and horizontal (10 kV/m) fields. The expected survival was that observed in the control group. Data from Reference 3.

GENERATION	TREATMENT GROUP	OR (1–35 Days)
F_1	VE	0.79 (0.25–2.51)
	HE	* 0.11 (0.01–0.90)
F_2	VE	*12.44 (4.26–30.45)
	HE	0.58 (0.03–2.90)
F_3	VE	*26.06 (5.41–84.68)
	HE	* 9.60 (2.04–37.00)

*P <0.05

offspring while continuously being exposed; randomly selected individuals from the second generation then produced the third generation.

The effect of the electric field on survival was assessed by evaluating the odds ratio for survival in the exposed mice compared with that in the controls using the chi-square test. The effect on development was assessed by comparing the body weights of the exposed mice with the corresponding controls using the unpaired t test. The F test was used to evaluate differences in percent deviation between the exposed and control groups. In each instance, published (or in press) data was used for this analysis.

RESULTS

MG-1

In the first generation (F_1), survival between 1–35 days after birth was unaffected in the VE group, but increased in the HE group (Table 1). There was a respective 71% and 84% decrease in development at 35 days in females and males in the VE group, and corresponding 92% and 88% decreases in the HE group (Tables 2 and 3). Thus, both fields affected both sexes, but the decrease in weight (averaged over sex) associated with the VE was more than twice that seen in the HE (22.5 vs. 10%).

In the second generation (F_2), survival was reduced in the VE group but unaffected in the HE group; impaired development occurred in both sexes in both groups. The effect on weight was greater for both sexes and both fields, compared with the changes seen in the first generation. Again, the average (over sex) effect associated with the VE was more

TABLE 2. Influence of 60-Hz Vertical Field, 15 kV/m, on Development and Variance in Development (Percent Deviation) in Mice (MG-1). The average value ± SD (in grams) are listed at the indicated number of days after birth. VE and C respectively designate the field-exposed and control groups. Values of mean weight and SD that differed significantly from the corresponding controls are indicated by an asterisk. N is given in parentheses. Data from Reference 3.

			WEIGHT (Grams)	
			Day 35	Day 70
F_1	Male	VE	*22.8 ± *4.9 (22)	—
		C	27.0 ± 2.0 (25)	—
	Female	VE	*17.0 ± *3.2 (23)	—
		C	24.0 ± 1.5 (26)	—
			Day 35	Day 70
F_2	Male	VE	*15.4 ± *2.9 (15)	*29.2 ± 2.6 (15)
		C	27.5 ± 2.8 (44)	36.5 ± 2.9 (32)
	Female	VE	*13.6 ± *2.8 (17)	*27.5 ± 2.5 (16)
		C	23.6 ± 1.7 (42)	29.9 ± 2.5 (34)
			Day 35	Day 70
F_3	Male	VE	*17.3 ±*4.7 (16)	—
		C	21.7 ± 3.8 (49)	—
	Female	VE	17.6 ± 3.8 (19)	—
		C	19.2 ± 3.3 (47)	—

*P <0.05

than twice that seen with the HE. The magnitude of the effect on development in all four groups and the ratio of the effect in the vertical vs. horizontal field were reduced at 70 days compared with 35 days (Tables 2 and 3). Survival was decreased in both fields in the third generation (F_3), but only the males exposed to the vertical field exhibited altered weights at 35 days. Variance in development (VID) was altered by field exposure in each generation (Tables 2 and 3).

TABLE 3. Influence of 60-Hz Horizontal Field, 10 kV/m, on Development and Variance in Development (Percent Deviation) in Mice (MG-1). The average value ± SD (in grams) are listed at the indicated number of days after birth. HE and C respectively designate the field-exposed and control groups. Values of mean weight and standard deviation that differed significantly from the corresponding controls are indicated by an asterisk. N is given in parentheses. Data from Reference 3.

			WEIGHT (Grams)	
			Day 35	Day 70
F_1	Male	HE	*23.9 ± *34 (25)	—
		C	27.0 ± 2.0 (25)	—
	Female	HE	*22.0 ± 1.8 (21)	—
		C	24.0 ± 1.5 (26)	—
			Day 35	Day 70
F_2	Male	HE	*22.8 ± 2.8 (47)	33.7 ±3.0 (39)
		C	27.5 ± 2.8 (44)	36.5 ± 2.9 (32)
	Female	HE	*19.2 ± *27 (43)	27.2 ± 2.3 (28)
		C	23.6 ± 1.7 (42)	29.9 ± 2.5 (34)
			Day 35	Day 70
F_3	Male	HE	23.3 ± *2.9 (43)	—
		C	21.7 ± 3.8 (49)	—
	Female	HE	20.9 ± *2.5 (42)	—
		C	19.2 ± 3.3 (47)	—

*$P < 0.05$

MG-2

In F_1 the likelihood that the newborn animals would not survive to the 21st day after birth was significantly greater in the field-exposed animals in both the VE and HE groups (Table 4). In F_2 (where all litters were reduced to 6 animals within 24 hours of birth) the decrease in survival in the VE group between 21–108 days approached statistical significance. In F_3 long-term survival in the VE group (21–119 days) was significantly reduced. Field-induced changes in development and VID occurred in each generation in both the VE and HE groups (Tables 5 and 6).

TABLE 4. Influence of 60-Hz Electric Field, 3.5 kV/m, on Survival in Three Generations of Mice (MG-2). OR, odds ratio and 95% confidence limits for survival during the indicated interval after birth. VE and HE respectively designate the groups exposed to vertical and horizontal fields. The expected survival was that observed in the control group. Data from Reference 4.

GENERATION	TREATMENT GROUP	OR
		(1-21 Days)
F_1	VE	*2.33 (1.46–3.41)
	HE	*2.10 (1.27–3.52)
		(21-108 Days)
F_2	VE	**2.84 (0.78–11.12)
		(21-119 Days)
F_3	VE	*3.44 (1.27–9.62)

*P <0.05
**P<0.14

MG-3

Survival was not affected by either the vertical or horizontal electric field (Table 7). In both the VE and HE groups, 4 tests involving development in the males and 3 in the females were statistically significant (from 30 t tests in each group) (Tables 8 and 9). VID in the VE group was significantly different in 5 cases among the males and 7 cases among the females (Table 8). In the HE group, 5 differences in VID were found among the males and 2 differences among the females (Table 9).

MG-4

Enhanced survival occurred in F_1 (Table 10), accompanied by a consistent decrease in development in the exposed mice (Table 11). In F_2, survival was impaired and both increased and decreased body weights occurred (Tables 10 and 11). In F_3 survival was unaffected, but a consistent decrease in development occurred at 35–70 days (Table 11).

MG-5

In F_1 the field resulted in decreased development and increased VID, but did not alter survival (Tables 12 and 13). Survival was increased in F_2, and bidirectional changes in development were observed. In F_3 survival was unaffected (Table 12) but development and VID were altered following field exposure (Table 13).

TABLE 5. Influence of 60-Hz Vertical Electric Field, 3.5 kV/m, on Development and Variance in Development (Percent Deviation) in Mice (MG-2). The average value ± SD (in grams) are listed at the indicated number of days after birth. VE and VC respectively designate the field-exposed and control groups. Values of mean weight and SD that differed significantly from the corresponding controls are indicated by an asterisk. N=41 (minimum). Data from Reference 4.

			WEIGHT (Grams)			
			Day 21	Day 35	Day 49	Day 63
F_1	Male	VE	11.0 ± 2.5	23.6 ± 3.2	28.0 ± 3.0	30.1± 3.6
		VC	11.4 ± 2.9	24.6 ± 3.6	29.2 ± 3.1	31.6 ± 3.4
	Female	VE	*10.5 ± *2.0	19.9 ± *1.9	23.2 ± 2.2	25.6 ± 2.8
		VC	11.4 ± 3.2	20.7 ± 3.2	23.7 ± 2.3	25.3 ± 3.1
			Day 21	Day 48	Day 70	Day 108
F_2	Male	VE	14.1 ± 1.5	30.3 ± 3.0	35.5 ± 2.9	37.3 ± 3.9
		VC	13.8 ± 1.2	29.0 ± 5.0	35.0 ± 2.6	38.0 ± 3.4
	Female	VE	13.9 ± *1.7	24.8 ± 2.4	27.2 ± 2.4	29.0 ± *2.4
		VC	13.8 ± 1.3	25.6 ± 2.7	27.2 ± 2.3	29.6 ± 4.0
			Day 21	Day 49	Day 63	Day 119
F_3	Male	VE	*15.6 ± 2.0	30.4 ± *4.5	*34.5 ± 3.1	41.4 ± 5.5
		VC	14.6 ± 1.5	29.6 ± 3.1	32.7 ± 3.0	40.7 ± 4.8
	Female	VE	14.7 ± 1.4	25.9 ± 2.3	26.2 ± *3.1	31.5 ± 4.3
		VC	14.3 ± 1.5	24.9 ± 1.8	26.6 ± 2.2	31.8 ± 3.6

*P < 0.05

TABLE 6. Influence of 60-Hz Horizontal Electric Field, 3.5 kV/m, on Development and Variance in Development (Percent Deviation) in Mice (MG-2). The average value ± SD (in grams) are listed at the indicated number of days after birth. HE and HC respectively designate the field-exposed and control groups. Values of mean weight and SD that differed significantly from the corresponding controls are indicated by an asterisk. N=41 (minimum). Data from Reference 4.

			WEIGHT (Gram.)			
			Day 21	Day 35	Day 49	Day 63
F_1	Male	HE	10.5 ± *2.8	23.6 ± *3.6	28.9 ± 2.6	31.8 ± *4.3
		HC	10.2 ± 1.7	24.1 ± 2.3	28.2 ± 2.4	30.4 ± 2.3
	Female	HE	10.4 ± 2.8	20.2 ± *3.6	23.6 ± *3.0	25.7 ± 2.6
		HC	10.2 ± 2.0	21.1 ± 1.7	23.5 ± 1.5	24.8 ± 2.2
			Day 21	Day 48	Day 70	Day 108
F_2	Male	HE	13.9 ± *2.0	31.4 ± 2.6	*31.8 ± 3.4	36.8 ± *3.1
		HC	14.2 ± 1.6	31.0 ± 2.3	34.0 ± 4.1	35.5 ± 4.2
	Female	HE	14.2 ± 1.6	*25.8 ± *1.9	27.9 ± 2.4	29.6 ± *2.4
		HC	14.1 ± 1.4	24.8 ± 2.4	26.9 ± 2.3	29.1 ± 3.2
			Day 21	Day 49	Day 63	Day 119
F_3	Male	HE	*14.6 ± *1.6	*31.1 ± *2.5	32.3 ± 3.4	40.8 ± 5.3
		HC	14.0 ± 2.1	29.9 ± 3.1	32.4 ± 3.3	39.2 ± 4.9
	Female	HE	*14.5 ± 2.1	*25.7 ± 2.5	*26.9 ± 2.1	*30.9 ± *3.6
		HC	13.4 ± 1.8	24.6 ± 2.8	25.0 ± 2.3	28.6 ± 2.5

*P < 0.05

TABLE 7. Influence of 60-Hz Electric Field, 0.5 kV/m, on Survival in Three Generations of Mice (MG-3). OR, odds ratio and 95% confidence limits for survival during the indicated interval after birth. VE and HE respectively designate the groups exposed to vertical and horizontal fields. The expected survival was that observed in the control groups. Data from Reference 5.

GENERATION	TREATMENT GROUP	OR
		(1–105 Days)
F_1	VE	0.95 (0.30–3.02)
	HE	0.94 (0.26–3.47)
		(1-96 Days)
F_2	VE	0.56 (0.13–2.37)
	HE	1.78 (0.23–114.1)
		(1-98 Days)
F_3	VE	1.42 (0.39–5.35)
	HE	2.60 (0.43–28.5)

DISCUSSION

To reject the null hypotheses regarding development, VID, or survival in any of the experiments, it is necessary and sufficient to show that the observed results could not be due to chance. In MG-1, 7 of 8 t tests performed to compare the means of the VE and C groups were significantly different. The probability for x successes is $P(x) = (n!/x!(n-x)!)p^x(1-p)^{n-x}$, where n is the number of trials, p is the probability of success, and x is the number of successes. For 8 trials, each at a significance level of 0.05, the probability of finding three or more successes due to chance is less than 0.05. Since 7 successes were observed, the result cannot be attributed to chance, and it follows that electric field exposure affected development. Similar reasoning shows that VID was affected following exposure to the field because 5 of the 8 comparisons in the VE group were significantly different (Table 2). For 3 trials, the probability that 2 or 3 successes will occur due to chance is less than 0.05; since 2 of the 3 tests for survival were significantly different (Table 1), it follows that the field also affected survival. The null hypotheses regarding development, VID, and survival following exposure to a horizontal electric field of 10 kV/m must similarly be rejected (Table 3).

For series involving 24 or 30 tests as were made in MG-2 to MG-5, observation of 4 and 5 successes, respectively, requires rejection of the null hypothesis. Consequently, the null hypotheses regarding development and VID must both be rejected in MG-2, MG-3, MG-4, and MG-5 (Table 14). Additionally, the null hypotheses regarding survival must be rejected in several experiments (Table 14).

TABLE 8. Influence of 60-Hz Vertical Electric Field, 0.5 kV/m, on Development and Variance in Development (Percent Deviation) in Mice (MG-3). The average value ± SD (in grams) are listed at the indicated number of days after birth. VE and VC respectively designate the field-exposed and control groups. Values of mean weight and SD that differed significantly from the corresponding controls are indicated by an asterisk. N=39 (minimum). Data from Reference 5.

			WEIGHT (Grams)				
			Day 21	Day 35	Day 49	Day 63	Day 105
F_1	Male	VE	14.9 ± *1.3	28.6 ± 2.4	30.7 ± 3.6	*34.5 ± 3.1	43.1 ± 5.3
		VC	14.7 ± 1.0	29.0 ± 2.0	31.5 ± 3.0	35.8 ± 2.9	43.2 ± 4.5
	Female	VE	14.6 ± 1.3	23.0 ± *2.5	24.3 ± *2.6	26.2 ± 2.0	31.8 ± *4.9
		VC	14.4 ± 1.2	23.5 ± 1.4	24.5 ± 1.4	26.3 ± 1.7	31.6 ± 2.6
			Day 25	Day 41	Day 57	Day 78	Day 96
F_2	Male	VE	*20.6 ± 2.2	30.3 ± 2.7	34.0 ± 2.9	37.1 ± *5.8	*38.9 ± 3.8
		VC	19.5 ± 2.2	30.2 ± 3.3	34.2 ± 3.6	35.8 ± 4.1	36.4 ± 3.4
	Female	VE	18.2 ± 2.5	*23.1 ± 2.0	*24.6 ± *2.5	26.8 ± 2.8	*28.4 ± 3.2
		VC	18.8 ± 2.0	24.0 ± 1.7	26.0 ± 2.0	27.2 ± 2.6	26.7 ± 3.1
			Day 21	Day 42	Day 56	Day 84	Day 98
F_3	Male	VE	14.7 ± 1.6	*28.6 ± *2.3	32.3 ± *2.4	34.2 ± *3.4	37.6 ± *2.5
		VC	14.3 ± 1.8	27.2 ± 3.0	31.5 ± 3.2	33.1 ± 4.3	37.2 ± 4.6
	Female	VE	14.6 ± 1.4	23.2 ± *1.3	24.8 ± *1.5	26.2 ± *2.0	27.7 ± *2.3
		VC	14.3 ± 1.6	23.1 ± 2.2	24.7 ± 2.0	26.6 ± 3.9	28.5 ± 3.3

*$P < 0.05$

Convincing demonstration of mutagenic capability of moderate-strength EMFs has not appeared. If such EMFs don't produce genetic mutations, MG-1 may be viewed as consisting of an initial measurement of survival, development and VID (in F_1) and two replicate experiments (F_2 and F_3), with the qualification that the subsequent generations may have been affected by the field via a non-genetic mechanism. The field did not affect survival in the VE group in F_1, and thus the genome passed on to F_2 was not the result of a genetic selection process. In the HE group the field resulted in the survival of some animals that would otherwise have died. In F_2 there was a relatively high mortality in the VE group, suggesting that embryonic development was adversely affected in the offspring of the underdeveloped parents of F_1. In the HE group, exposure did not affect survival,

TABLE 9. Influence of 60-Hz Horizontal Electric Field, 0.5 kV/m, on Development and Variance in Development (Percent Deviation) in Mice (MG-3). The average value ± SD (in grams) are listed at the indicated number of days after birth. HE and HC respectively designate the field-exposed and control groups. Values of mean weight and SD that differed significantly from the corresponding controls are indicated by an asterisk. N=39 (minimum). Data from Reference 5.

			WEIGHT (Grams)				
			Day 21	Day 35	Day 49	Day 63	Day 105
F_1	Male	HE	14.9 ± *1.2	25.9 ± 3.0	30.4 ± 3.3	*33.4 ± 3.0	40.9 ± 4.4
		HC	14.6 ± 1.8	26.8 ± 2.8	30.5 ± 3.0	31.9 ± 3.6	40.8 ± 4.8
	Female	HE	14.8 ± 1.3	22.8 ± 2.2	24.8 ± 2.3	26.4 ± 2.0	32.0 ± 4.3
		HC	14.5 ± 1.7	22.5 ± 2.6	24.3 ± 1.9	26.0 ± 2.3	31.4 ± 3.8
			Day 25	Day 41	Day 57	Day 78	Day 96
F_2	Male	HE.	19.0 ± *2.0	30.5 ± *2.9	33.2 ± 2.5	35.2 ± *4.3	37.6 ± *4.3
		HC	19.6 ± 3.3	29.8 ± 4.0	33.4 ± 2.7	34.4 ± 3.2	37.4 ± 6.8
	Female	HE	17.8 ± *1.4	*22.5 ± 1.6	24.2 ± *2.0	26.3 ±2.4	*27.1 ± 3.9
		HC	18.1 ± 2.2	23.3 ± 1.5	24.4 ± 3.2	27.4 ± 3.0	29.3 ± 3.4
			Day 21	Day 42	Day 56	Day 84	Day 98
F_3	Male	HE	*15.0 ± 1.6	*29.5 ± *2.7	*31.9 ± 3.3	37.2 ± 4.0	38.7 ± 4.2
		HC	14.0 ± 1.3	27.7 ± 3.3	30.4 ± 3.4	35.8 ± 3.6	38.8 ± 4.2
	Female	HE	*14.6 ± 1.5	22.9 ± 2.3	24.4 ± 2.1	26.6 ± 3.2	28.9 ± 4.0
		HC	14.0 ± 1.6	23.3 ± 2.1	24.7 ± 2.3	27.5 ± 3.2	28.9 ± 4.1

*P < 0.05

TABLE 10. Influence of 60-Hz Vertical Electric Field, 100 kV/m, on Survival in Three Generations of Mice (MG-4). OR, odds ratio and 95% confidence limits for survival during the indicated interval after birth. The expected survival was that observed in the control group. Data from Reference 6.

GENERATION	TREATMENT GROUP	OR (1-70 Days)
F_1	VE	0.23* (0.06–0.99)
F_2	VE	4.04* (1.54–10.88)
F_3	VE	3.38 (0.78–15.84)

*P < 0.05

TABLE 11. Influence of 60-Hz Vertical Electric Field, 100 kV/m, on Development and Variance in Development (Percent Deviation) in Mice (MG-4). The average value ± SD (in grams) are listed at the indicated number of days after birth. VE and VC respectively designate the field-exposed and control groups. Values of mean weight and standard deviation that differed significantly from the corresponding controls are indicated by an asterisk. N is given in parentheses. Data from Reference 6.

			WEIGHT (GRAMS)				
			Day 1	Day 14	Day 28	Day 35	Day 70
F_1	Male	VE	*1.8 ± *0.2 (30)	*7.0 ± 0.8 (30)	*16.4 ± *2.4 (27)	23.8 ± 2.4 (27)	*34.6 ± 2.1 (27)
		VC	2.0 ± 0.2 (28)	7.5 ± 0.7 (28)	19.7 ± 1.9 (27)	24.8 ± 2.4 (27)	36.9 ± 2.1 (26)
	Female	VE	1.8 ± 0.2 (34)	7.1 ± 0.6 (34)	16.4 ± 1.9 (34)	*20.3 ± 1.6 (34)	*28.9 ± 1.4 (34)
		VC	1.9 ± 0.2 (28)	7.4 ± 0.6 (28)	17.1 ± 1.8 (27)	21.3 ± 1.5 (27)	29.9 ± 1.6 (26)
			Day l	Day 14	Day 28	Day 35	Day 70
F_2	Male	VE	*1.8 ± 0.2 (23)	7.5 ± 0.6 (22)	*20.4 ± *2.2 (22)	26.2 ± *1.6 (11)	35.9± 1.6 (22)
		VC	2.0 ± 0.2 (28)	7.3 ± 0.8 (28)	18.6 ± 3.0 (28)	25.8 ± 2.4 (24)	36.0 ± 2.1 (28)
	Female	VE	*1.8 ± *0.2 (22)	7.6 ± *0.4 (22)	*19.0 ± *1.7 (22)	23.6 ± 1.6 (11)	*29.2 ± 1.8 (22)
		VC	1.9 ± 0.1 (28)	7.2 ± 0.9 (28)	17.2 ± 2.8 (27)	22.9 ± 2.0 (23)	30.7 ± 1.9 (27)
			Day 1	Day 14	Day 28	Day 35	Day 70
F_3	Male	VE	1.9 ± 0.2 (33)	7.2 ± 0.7 (33)	19.0 ± 2.2 (33)	*25.0 ± 2.1 (31)	*34.2 ± 1.8 (32)
		VC	1.9 ± 0.2 (34)	7.4 ± 0.8 (32)	18.4 ± 2.6 (34)	26.2 ± 2.5 (32)	36.9 ± 2.7 (32)
	Female	VE	1.8 ± 0.1 (24)	7.0 ± 0.8 (24)	16.7 ± 2.4 (24)	*22.1 ± 1.8 (24)	*28.6 ± *1.1 (23)
		VC	1.8 ± 0.1 (30)	7.2 ± 0.8 (30)	17.5 ± 1.9 (29)	23.0 ± 1.4 (29)	29.9 ± 1.9 (28)

*P < 0.05

TABLE 12. Influence of 60-Hz Vertical Electric Field, 100 kV/m, on Survival in Three Generations of Mice (MG-5). OR, odds ratio and 95% confidence limits for survival during the indicated interval after birth. The expected survival was that observed in the control group. Data from Reference 6.

GENERATION	TREATMENT GROUP	OR (1-70 Days)
F_1	VE	1.15 (0.50–2.65)
F_2	VE	0.05* (10^{-2}–0.36)
F_3	VE	Not significant (no expected deaths)

*$P < 0.05$

but development was retarded. In F_3 in the VE group, the largest impact on survival and the smallest impact on development were observed, suggesting that a non-genetic selection process operated in the pre-weaning period to eliminate individuals incapable of coping with the exposure environment. A similar trend occurred in the HE group.

The exposure system used in MG-1, particularly the method of providing water to the exposed animals, resulted in the presence of a confounding variable that may have significantly influenced the observations. This possibility was discussed in the original report and we concluded that the confounding variable (the flow of weak electric currents (microcurrents) between the animal and the water reservoir which facilitated equilibration of the electric potential between the *2* conductors) was probably not the sole cause of the observations. The question subsequently became important because it determined the importance attached to the study in assessing possible human health risks due to exposure to power-frequency electromagnetic fields. When the possibility was viewed as significant, MG-1 was judged unsatisfactory for use in assessing the health influence of electric fields (11).

Uncertainty in MG-1 resulted both from confounding of independent variables (electric field and microcurrents), and from confounding of dependent variables (survival and development). We sought to eliminate these difficulties in MG-2. The exposure apparatus was redesigned, and the water-bottle was changed to make it nearly isopotential with the mouse's body. The field strength in MG-2 was reduced by about a factor of four (compared with MG-1), and that change alone was responsible for a decrease in microcurrents.

An effect of the field on survival, and a greater impact in the VE group (compared with the HE group) again occurred in MG-2. The changed conditions, however, resulted in a change in the nature of the effect on development; growth was significantly retarded

TABLE 13. Influence of 60-Hz Vertical Electric Field, 100 kV/m, on Development and Variance in Development (Percent Deviation) in Mice (MG-5). The average value ± SD (in grams) are listed at the indicated number of days after birth. VE and VC respectively designate the field-exposed and control groups. Values of mean weight and SD that differed significantly from the corresponding controls are indicated by an asterisk. N is given in parentheses. Data from Reference 6.

			WEIGHT (Grams)				
			Day 1	Day 14	Day 28	Day 35	Day 70
F_1	Male	VE	1.9 ± 0.2 (17)	7.4 ± 0.9 (17)	20.3 ± 1.8 (17)	27.3 ± 1.4 (17)	37.0 ± 2.1 (17)
		VC	1.9 ± 0.2 (28)	7.6 ± 0.7 (28)	20.4 ± 2.4 (28)	27.5 ± 1.7 (28)	36.7 ± 2.1 (28)
	Female	VE	1.8 ± 0.2 (23)	7.3 ± *1.0 (23)	*16.5 ± 2.1 (23)	*22.8 ± *1.5 (23)	29.5 ± *2.5 (23)
		VC	1.9 ± 0.2 (27)	7.6 ± 0.6 (27)	18.8 ± 1.8 (25)	23.7 ± 1.0 (25)	29.0 ± 1.6 (24)
			Day 1	Day 14	Day 28	Day 35	Day 70
F_2	Male	VE	2.0 ± 0.2 (28)	7.2 ± *1.2 (28)	19.3 ± *3.7 (28)	26.2 ± 2.8 (28)	*37.0 ± 2.3 (28)
		VC	2.1 ± 0.2 (23)	7.1 ± 0.6 (18)	19.5 ± 1.9 (21)	26.4 ± 2.0 (21)	35.4 ± 2.4 (20)
	Female	VE	*1.9 ± *0.2 (36)	7.1 ± 1.1 (36)	18.1 ± 2.3 (36)	23.2 ± 1.9 (36)	29.4 ± *1.3 (36)
		VC	2.0 ± 0.1 (30)	7.5 ± 0.9 (19)	17.9 ± 1.7 (29)	23.2 ± 1.9 (29)	29.3 ± 1.9 (28)
			Day 1	Day 14	Day 28	Day 35	Day 70
F_3	Male	VE	2.0 ± *0.1 (35)	*8.0 ± 0.7 (35)	19.8 ± *3.2 (34)	26.8 ± *2.6 (34)	*38.9 ± 2.3 (33)
		VC	2.0 ± 0.2 (30)	7.5 ± 0.6 (30)	19.6 ± 1.9 (30)	26.5 ± 1.6 (30)	36.4 ± 2.3 (30)
	Female	VE	*2.0 ± *0.1 (29)	*7.8 ± 0.6 (29)	18.0 ± *2.8 (27)	*22.3 ± *1.9 (27)	*29.9 ± 1.8 (27)
		VC	1.8 ± 0.2 (34)	7.3 ± 0.5 (34)	18.0 ± 1.5 (34)	23.3 ± 1.3 (34)	28.5 ± 1.8 (34)

*$P < 0.05$

TABLE 14. Summary of Results from MG-2 to MG-5 Regarding the Influence of Electric Fields (data from Tables 1–13). The last column indicates the decision regarding each null hypothesis (and its statistical basis). The number of successes ($P < 0.05$) and the number of tests performed are listed first and second, respectively.

Study Number	Strength (kV/m)	Field Direction	Null Hypothesis	DECISION		
				Development	VID	Survival
MG-1	15	Vertical	VC = C	Rejected 7/8	Rejected 5/8	Rejected 2/3
MG-1	10	Horizontal	HE = C	Rejected 4/8	Rejected 4/8	Rejected 2/3
MG-2	3.5	Vertical	VE = VC	Rejected 4/24	Rejected 7/24	Rejected 2/3
MG-2	3.5	Horizontal	HE = HC	Rejected 8/24	Rejected 13/24	Not rejected 1/3
MG-3	0.5	Vertical	VE = VC	Rejected 7/30	Rejected 14/30	Not rejected 0/3
MG-3	0.5	Horizontal	HE = HC	Rejected 7/30	Rejected 8/30	Not rejected 0/3
MG-4	100	Vertical	VE = VC	Rejected 15/30	Rejected 7/30	Rejected 2/3
MG-5	100	Vertical	VE = VC	Rejected 10/30	Rejected 13/30	Not rejected 1/3

in MG-1, but was generally increased in MG-2. The trend toward increased weight in the exposed animals in F_3 was consistent with the assumption of an epigenetic mechanism underlying the effects on survival and development. If genetic mechanisms mediated the effect, it would be difficult to visualize bidirectional changes in weight as a function of field dose. On the other hand, if the field-induced effect was mediated by the neuroendocrine system, there is ample evidence indicating that somatic stimuli can produce bidirectional changes in dependent parameters.

The mice made minimal use of the water bottle prior to weaning; consequently, microcurrents are not a plausible explanation for the elevated mortality in F_1 in the pre-weaning period in MG-2 (Table 4). Furthermore, the odds ratio in the VE group in MG-2 was 2.33 ($P < 0.05$), compared with 0.79 (not significant) in MG-1. Thus, an imputation of the increased mortality in MG-2 to the microcurrents would be equivalent to the untenable assertion that a reduction in the strength of the microcurrents (15 kV/m, water bottle outside the cage in MG-1 compared with 3.5 kV/m and the water bottle inside the cage in MG-2) resulted in decreased survival. The trend toward increased mortality in VE compared with HE may indicate that the vertically oriented field was more biologically active.

In MG-3 the electric field did not affect survival (Table 7). Thus, in distinction to MG-1 and MG-2, the effect of the field on development and VID could be directly assessed. Even so, the results of MG-2 and MG-3 were similar. In MG-2, 25% of the weight comparisons and 42% of the VID comparisons were significantly different; in MG-3 the corresponding values were 23% and 37%.

Two possible approaches existed to reduce the microcurrents present in MG-1. I placed the water bottle inside the mouse cage so that both were electrically floating, and were at essentially the same potential during drinking. The BNL investigators initially chose the same solution (2), but subsequently redesigned their watering system based on electric-field dosage considerations which led them to conclude that it was important to prevent the mice from rearing on their hind limbs (9). They reduced the cage height to 5 cm, and this made it impractical to place a water bottle inside the cage; water was therefore provided to the animals via a nozzle located at the cage floor. In my view the dosage argument was arbitrary (12), and we therefore adopted the bottle-in-the-cage solution. Both methods of providing water to the exposed animals apparently worked well.

In MG-4, significant effects on survival occurred in 2 of the 3 generations; 50% of weight comparisons and 23% of the VID comparisons were significantly different. In MG-5, a significant protective effect for survival occurred in F_2; 33% of the weight comparisons and 43% of the VID comparisons were significantly different.

In the six experiments in which the confounding role of microcurrents was eliminated (MG-2 to MG-5), 51 (out of 168) significant differences in weight were observed; the mean difference (± SD) was 6.6 ± 3.6%. Thus there was an *a posteriori* probability of 0.304 that exposure to 60-Hz electric fields of 0.5–100 kV/m would alter development. A total of 29 weight differences (6.7 ± 3.9%) were such that the exposed animals were heavier than the controls, and in 22 cases the reverse was true (6.5 ± 3.3%). In MG-2 to MG-5, 62 (out of 168) significant differences in VID were observed. Thus there was an *a posteriori* probability of 0.367 that exposure to 60-Hz electric fields of 0.5–100 kV/m would alter VID. In 31 cases, the exposed animals exhibited a VID greater than the controls; the mean (±SD) change in percent standard deviation was 60.3 ± 29.5%. In the remaining 31 cases the observed significant difference in VID was such that there was less variance in the exposed animals; the mean (±SD) in these cases was 35.4 ± 9.4%. Neither the magnitude nor the frequency of the field-induced effects on development and VID varied when the dose of electric field increased by a factor of 200 (0.5 to 100 kV/m). Thus, the multi-generation mice studies consistently showed that the physiological response to the electric field did not follow a DR relationship: The dependent variables were altered following presentation of the independent variable in only about 30% of the cases, and, the dependent variables did not increase proportionately with increases in the independent variable.

Since the impediments to the use of MG-1 in evaluating health risks were removed in MG-2 to MG-5. I think that the latter four studies are suitable for use in health-risk assessment. The average full-body background electric field at the location of the controls was probably about 2 V/m. Since effects occurred at 0.5 kV/m, it follows that an incremental dose no higher than 0.498 kV/m is sufficient to affect the growth of experimental animals.

The BNL investigators performed many studies (6-10), most of which were grounded on an assumption that if electric field effects in animals were real, they would exhibit a DE relationship. Myriad physiological parameters were measured, and a t test was typically used to compare the means of the exposed and control groups. If the test did not require rejection of the null hypothesis, the data was interpreted as indicating that electric fields caused no changes in the dependent variable. If the null hypothesis was rejected, the possibility that the effect was real (and hence merited consideration with respect to human health risks) was considered, and the study was repeated. If the replicate study yielded any result other than that observed initially, the studies were interpreted together as showing no real effect. A relatively frequent pattern consisted of an initial study that showed a significant increase in a particular variable, and the second study that showed a significant decrease; in these cases, the results were averaged and it was concluded that no effects had been observed. When the results of the replicate were identical to those of the initial study, the study was replicated again to insure the effect was not spurious. For example, when 60-Hz electric fields retarded fracture healing in 3 identical experiments, the BNL investigators reported that the effect was real (13).

BNL's approach was also clearly exemplified in the mice multi-generation studies. Because the BNL investigators did not observe a DE relationship regarding survival, development, or VID in all generations in both studies in both sexes, they added the results and concluded (6):

> There appear to be no differences in weights between the exposed and sham-exposed groups. Apparently, under the conditions of this experiment, the presence of a 60-Hz (100 kV/m) vertical electric field had no effect on mice conceived, born, and raised in the field for three successive generations with regard to body weight, breeding capacity, litter size, or mortality.

But this conclusion is based on their demand that an electric-field-induced bioeffect be of the DE type, as a condition precedent to their acceptance of the reality of the bioeffect. Not once in more than 500 publications and presentations between 1976 and 1990 have the BNL investigators rationalized this instance on absolute determinism by the field alone, irrespective of all host factors. Although the option apparently remained unconsidered, nature always reserved the possibility that non-DE field-induced bioeffects could be real and, as the multi-generational studies show, such effects do occur. Since there is no rationale for the BNL demand, and in view of the EMF pulpit the BNL investigators enjoyed, their analytical methodology was a serious historical error because it impeded use of their data (which was obtained under rigorous environmental controls), in conjunction with the data of other investigators, in assessing the significance of human health risks.

REFERENCES

(1) Becker, R.O.: Pre-filed Testimony on Behalf of the Staff of the New York Public Service Commission, Case 26559, *Application of RG&E Power Company to Construct a 765-kV Transmission Line,* 1974.

(2) Phillips, R.D.: *Biological Effects of High-strength Electric Fields on Small Laboratory Animals.* Battelle Pacific Northwest Laboratories, Richland, Washington, Proposal to the Energy Research and Development Administration, Washington, DC, 1975.

(3) Marino, A.A., Becker, R.O. and Ullrich, B.: The effect of continuous exposure to low frequency electric fields on three generations of mice: A pilot study, Experientia *32*, 565-566, 1976.

(4) Marino, A.A, Reichmanis, M., Becker, R.O, Ullrich, B. and Cullen, J.M.: Power frequency electric field induces biological changes in successive generations of mice, Experientia *36*, 309-311, 1980.

(5) Marino, A.A.: Low-strength power-frequency electric fields alter development in mice, Submitted, 1990.

(6) Phillips, R.D., Anderson, L.B. and Kaune, W.T.: *Biological Effects of High-strength Electric Fields on Small Laboratory Animals,* Pacific Northwest Laboratories, Richland, Washington, DOE/TIC-10084, Contract EY-76-C-06-1830, Energy Research and Development Administration, Washington, DC, 1979.

(7) Phillips, R.D., Anderson, L.E. and Kaune, W.T.: *Biological Effects of High-Strength Electric Fields on Small Laboratory Animals,* Battelle Pacific Northwest Laboratories, Richland, Washington, Dept. of Energy, Washington, DC, 1981.

(8) Phillips, R.D., Kaune, W.T., Decker, J.R. and Hjeresen, D.L.: *Biological Effects of High Strength Electric Fields on Small Laboratory Animals,* Battelle Pacific Northwest Laboratories, Richland, Washington, Energy Research and Development Administration, Washington, DC, 1976.

(9) Phillips, R.D. and Kaune, W.T.: *Biological Effects of High-strength Electric Fields on Small Laboratory Animals,* Battelle Pacific Northwest Laboratories, Richland, Washington, CONS/1930-2, Energy Research and Development Administration, Washington, DC, 1977.

(10) Phillips, R.D. and Kaune, W.T.: *Biological Effects of High-strength Electric Fields on Small Laboratory Animals,* Battelle Pacific Northwest Laboratories, Richland, Washington, HCP/T180-03, Contract EY-76-C-08-1830, Dept. of Energy, Washington, DC, 1978.

(11) Hastings, J.W., Adey, W.R., Dethier, V.G., Ely, T.S., Gardner, W.R., Gordis, L., Loftus, E.F., Michaelson, S.M., Miller, M.W., Novotny, D.W., Reeder, W.G., Schull, W.J., Schwan, H.P., Tukey, H.B., Jr., Wilkening, G.M. and Wu, T.T.: *Biologic Effects of Electric and Magnetic Fields Associated with Proposed Project Seafarer, Report of the Committee on Biosphere Effects of Extremely-Low-Frequency Radiation,* National Academy of Sciences, Washington, DC, 1977.

(12) Marino, A.A. and Reichmanis, M.: The Battelle studies: An analysis, *BEMS, Proceedings of the 6th Annual Meeting,* p.15, 1984.

(13) McClanahan, B.J. and Phillips, R.D.: The influence of electric field exposure on bone growth and fracture repair in rats, Bioelectromagnetics *4*, 11-19, 1983.