Biological Effects of High Strength Electric Fields

Second Interim Progress Report
September 1976 — March 1977

Date Published — May 1977

Work Performed under Contract No. AT(45-1)-1830

Battelle — Pacific Northwest Laboratories

**ENERGY RESEARCH AND
DEVELOPMENT ADMINISTRATION**

Division of Electric Energy Systems

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Battelle - Pacific Northwest Laboratories

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I. INTRODUCTION

Research was initiated on this project on March 9, 1976. The first interim report⁽¹⁾ described progress made during the first 6 months, through September 8, 1976. This interim progress report covers the period of September 9, 1976 to March 31, 1977.

A. NEED, SCOPE, AND OBJECTIVES

A comprehensive research study is in progress to assess possible biological effects of exposure to 60-Hz electric fields and to provide an improved data base for establishing safe exposure limits.

The major objective of this project is to determine the biological effects on small laboratory animals of exposure to 60-Hz electric fields. To achieve this goal a number of procedural and experimental objectives must be satisfied.

- Design, fabricate and test a prototype exposure system for exposing rats and mice to well-defined and relatively uniform 60-Hz electric fields.
- Design, fabricate and test a system for housing rats and mice during exposure to 60-Hz electric fields. The caging, watering and feeding systems must not alter electric field uniformity significantly and must adhere to good animal care practices.
- Conduct biological experiments in the prototype exposure system to determine the maximum field strength that does not

produce corona discharge and ozone formation, behavioral aversion, hair stimulation, or shocks when the animal eats or drinks.

- Design and fabricate systems for exposing large numbers of rats and mice to well-defined and relatively uniform 60-Hz electric fields.
- Conduct a broad and comprehensive series of screening experiments to investigate biological effects of exposure to 60-Hz electric fields.
- Establish the relationships between field strengths and durations of exposures for any effects observed in the screening experiments.
- Conduct dosimetry investigations to complement the animal studies.

B. SCHEDULE AND MILESTONES

Milestones that have been achieved in the first year of this project include:

- The prototype system for exposing rats and mice to uniform, 60-Hz electric fields has been designed, fabricated, and tested.
- Electric field strengths have been surveyed in the prototype exposure system, both on the surface of the lower electrode and in the space between the two electrodes. Effects on field uniformity of buswork, high-voltage cables, and grounded room structures have been quantified.

- A system for caging, feeding, and watering rats during exposure was designed and built. Biological experiments have established that the housing system does not have adverse effects on the animals. Measurements show the effect of the caging system on electric field uniformity is less than 5%.
- The exposure criteria studies have been completed. No evidence of shocks, hair stimulation, corona discharge or ozone formation have been found at field strengths of 130 kV/m or less. Behavioral tests indicate that rats chose to avoid exposure at field strengths ≥ 90 kV/m.
- A system for exposing large numbers of rats to 60-Hz electric fields has been designed and built. Field measurements inside the exposure cages indicate field strength does not vary more than 3% between cages.
- A system for exposing large numbers of mice has been designed. Fabrication is almost finished.
- All preliminary work and baseline measurements for the biological studies have been completed.
- Two experiments have been completed to determine the effect on heart rate of exposing rats to 80 kV/m electric fields for an 8-hr period and for 8 hr/day for 5 days. No significant differences were observed between exposed and sham-exposed animals.

Future milestones for this project are:

- Substantial harmonic distortion and line transients have been observed in the high voltage waveforms of the exposure system. Work is in progress to correct this by April 15, 1977.*
- Testing and checkout of the rat exposure system will be finished on April 1, 1977.*
- The first series of biological screening experiments using rats will begin April 5.*
- Construction, testing, and checkout of the mouse exposure system will be completed about May 15, 1977.
- Biological screening studies on rats and mice will be completed in FY 1978.
- Determination of dose-response curves of significant effects identified in the screening experiments will begin at the conclusion of the screening studies in FY 1978.
- In-depth studies of significant effects will be initiated as appropriate.
- Dosimetry studies to determine the relative dose to all animals in the exposure system will be completed in FY 1978.
- Dosimetry studies to characterize the surface electric fields and current distributions in the bodies of rats will be completed by the end of FY 1978.

* Completed on schedule

II. EXPOSURE SYSTEMS

Ultimately, three exposure systems will be in operation. The first, the prototype system, was completed in June, 1976; a large body of data relating to exposure system design was obtained with this system and was reported in the first interim report.⁽¹⁾ Some additional results will be given in this report. Having served its prototype function, this system has been redesignated the special studies exposure system and is being used for dosimetry and animal behavioral experiments.

The second system will be used for exposing large numbers of rats for the biological screening experiments. This system is complete and details of its design, construction, and initial testing will be given in this report. Construction of the mouse exposure system is in progress. Discussion of this system will be deferred to the next interim report.

A. PROTOTYPE AND SPECIAL STUDIES EXPOSURE SYSTEM

1. Effect of Electrode Shape

Details of the prototype exposure system were presented in the first interim report.⁽¹⁾ Partly because of discussions with scientists at the National Bureau of Standards, Drs. F. R. Kotter and M. Misakian, we decided to investigate the effects of altering one dimension of the prototype electrode, namely the dimension Δz shown in Figure 1. The electrode was originally built with $\Delta z = 1.9$ cm. Field scans done on the surface of this electrode showed a field strength which decreased

FIGURE 1. Cross section view of an exposure electrode showing the vertical dimension Δz . This dimension was varied to maximize electric field uniformity over the surface of the electrode.

FIGURE 2. The coordinate system used to describe field measurements on the lower electrode. A rectangular array of holes allowed electrical connection to the field probe.

FIGURE 3. Electric fields measured on the surface of the lower electrode for $\Delta z = 0.0, 0.6, 1.3$, and 1.9 cm. The prototype electrode was built with $\Delta z \approx 1.9$ cm. Future electrodes were built with $\Delta z \approx 0.6$ cm.

toward the electrode edge (Figures 10 and 11 of reference 1). The NBS researchers measured fields on a flat plate electrode ($\Delta z \approx 0$) which increased toward the electrode edge.⁽²⁾ It appeared likely that with a proper choice of Δz , these two effects could be offset to give a more uniform field near the edge of the electrode.

Electric field scans were made with $\Delta z = 1.9, 1.3, 0.6$, and 0.0 cm with a fixed electrode voltage of 27 kV. The electrode coordinate system used in describing these measurements is shown in Figure 2 and the results are shown in Figure 3. The measurements for each value of Δz have been normalized to a value of 1.00 at the electrode center ($x=0, y=0$). These data show an improvement in field uniformity as Δz is decreased. Even at $\Delta z = 0$ there is no major increase in field strengths as the electrode edge is approached. These results are in contrast to those by NBS, presumably because tubular conductors (8.9 cm diameter) were used at the edges of each electrode in this study and not in the NBS study. Based on these measurements, it was decided to select $\Delta z = 0.6$ cm in the design of the electrodes for the rat and mouse exposure system.

2. Voltage and Probe Calibration

The design of a surface field probe was described in the first interim report.⁽¹⁾ Also described were our difficulties in calibrating this probe. The high-voltage source used for these initial measurements was a 10-kV dielectric test set manufactured by Associated Research Inc., Model 422.

In October, 1976, a 100-kV supply manufactured by Hipotronics, Inc. (Model 7100-5) was received and installed. In November, 1976, a precision compensated voltage divider manufactured by Ross Engineering Corp. (Model VD-60-6.2-B-K-A) was received. With these tools, a new effort was made to calibrate the field probe.

a. Measurement of Electrode Voltage.

The Ross compensated voltage divider consists of a parallel resistor and capacitor ($R \approx 240 \text{ M}\Omega$, $C \approx 5.7 \text{ pF}$) connected in series with a second parallel combination ($R \approx 316 \text{ k}\Omega$, $C \approx 4960 \text{ pF}$). The voltage across the latter pair, when read by a voltmeter with an input impedance of $1 \text{ M}\Omega$ shunted by 20-50 pF and connected to the divider by 15 ft of RG 58-A coaxial cable, is 1/1000 of the voltage across the entire network. This ratio is frequency-independent. The accuracy of this unit is $\pm 0.5\%$.

In practice stray capacitive coupling across the 5.7 pF capacitance must be avoided; that is, the divider must not be placed too close to the high voltage electrode of the exposure system. This was accomplished by placing the divider on the floor under the lower grounded electrode, which acted as a shield.

The high-voltage waveform was displayed on an oscilloscope and photographed. Figure 4 shows these waveforms for electrode voltages of 11, 27.5, and 41.2 kV (the Ross divider is only rated to 60 kV peak voltage) and clearly indicates that appreciable harmonic distortion is present.

ELECTRODE VOLTAGE = 11 kV

ELECTRODE VOLTAGE = 27.5 kV

ELECTRODE VOLTAGE = 41.2 kV

FIGURE 4. Waveforms of electrode voltage on Special Studies Exposure System. Waveforms show substantial harmonic distortion.

The Ross divider output voltage was measured with a true-RMS responding voltmeter (Tektronix Model 213). This voltmeter was calibrated against a Fluke Model 8600 which is used as a calibration standard for meters in the laboratory. The Hipotronics high-voltage panel meter was found to read low by 10%.

b. Calibration of the Field Probe and Exposure System.

The principle of operation of the field probe was described in the first interim report.⁽¹⁾ It was shown that if the current in the probe is I , the field can be calculated as $E = I/\omega\epsilon_0 A$ where $\omega = 2\pi \times 60$ Hz, ϵ_0 is the permittivity of space, and A is the probe area. This formula only holds for pure sinusoidal fields; in fact, since the probe is capacitively coupled to the exposure system, harmonics can be very troublesome. The probe current waveform for an electrode voltage of 27.5 kV is shown in Figure 5. The harmonic distortion is clearly seen; comparison with Figure 4 demonstrates the enhancement of the harmonics because of the capacitive coupling. It should be noted that the current waveform induced in an animal placed in this exposure system would be the same.

In the presence of such waveforms, calibrating the field probe becomes more difficult. One could, in principle, extract the 60-Hz components either with filters or Fourier analysis. Instead, we have used a different approach.

If the exposure electrodes were very large in size, the field at the center of the lower electrode would simply be the parallel plate

ELECTRODE VOLTAGE = 27.5 kV

FIGURE 5. The current waveform in a field probe capacitively coupled to the Special Studies Exposure System. The higher harmonics shown in Figure 4 couple strongly to the probe because of the capacitive coupling.

field, V/d , where V is the applied voltage and d the plate spacing. This equation holds at 60 Hz and all higher harmonics (as long as the wavelength $\lambda \gg d$). However, because of the finite size of the plate, edge effects will cause the actual field to be slightly different from the parallel plate field. We corrected for this by introducing a geometry factor G which relates the RMS field strength at the center of the lower electrode and RMS electrode voltage by

$$E = G V/d. \quad (1)$$

To determine G , the probe was first calibrated in a known field of the same harmonic content as the exposure system field. The calibrated probe was then used to measure the field at the center of the lower electrode; V , the electrode voltage, was measured with the Ross divider and G was calculated from Eq. (1).

To produce a known field of the same harmonic content, the electrode spacing was reduced to 0.075, 0.152, and 0.229 m and measurements were made of the RMS electrode voltage and the RMS probe current. At these spacings and with an electrode size of 1.0 x 1.8 m, Eq. (1) applies with $G = 1.02$. Thus, a measurement of the RMS electrode voltage V , is sufficient to determine the RMS field strength E . The probe formula was corrected for the effects of harmonic distortion by the introduction of a factor C_h as follows:

$$E = C_h \frac{I}{\omega \epsilon_0 A}. \quad (2)$$

The presence of harmonic distortion would tend to make the probe read high, requiring that $C_h < 1$. Table I shows the observed results.

TABLE I. Probe Calibration Data
(Electrode Voltage = 25.5 kV)

Electrode Spacing d , (m)	$\frac{V}{d}$ (kV/m)	$\frac{I}{\omega \epsilon_0 A}$ ($\frac{kV}{m}$)	C_h
0.076	335	347	0.965
0.152	167	171	0.977
0.229	112	114	0.972

The thickness of the probe is 0.78 mm (1/32 in.) and will introduce the most uncertainty at the lowest value of d . Thus, for C_h we use the measured values for the two larger values of d , giving $C_h = 0.975$. It is important to note that C_h includes the effects of harmonic distortion and may be different for different values of the electrode voltage.

The next step was to measure the electric field with the full plate spacing of $d = 0.56$ m. This measurement was made at the same electrode voltage. One may argue that by changing d we are changing the transformer load and thus possibly the high-voltage harmonic distortion. However, most of the load is the capacitance of the cable connecting the transformer to the electrodes; the estimated load change in going from $d = 0.56$ m to $d = 0.152$ m is 12%. And, as Table I shows, changing d from 0.229 m to 0.152 m (a change in the transformer load of about 11%) produced very little change in C_h .

Knowing both E and V, the geometric factor G can be calculated. For the prototype exposure system, $G = 0.977 \pm 0.019$.

c. Harmonic Distortion.

In the course of the measurements described above, it became apparent that appreciable harmonic distortion was present in the high-voltage waveform. An animal capacitively coupled to this system would have an even more distorted current waveform in its body as shown in Figure 5. Any biological effects observed from exposure to such as electric field could be attributed to this harmonic content. Therefore, it is desirable to eliminate as much of the harmonic content as is practical, and we plan on doing this.*

3. Three-Dimensional Field Mapping

In the last interim report,⁽¹⁾ details were given of the mapping of the electric field on the surface of the lower electrode. It was also noted that a complete knowledge of the electric field on the surface of the lower electrode enabled one to extrapolate vertically from the lower electrode. However, a direct measurement of the fields between the two electrodes is desirable.

A small (14.0 x 8.9 x 4.4 cm) dipole fieldmeter was constructed by modifying a commercially available digital multimeter (Data Precision Model 245), following drawing provided by Bonneville Power Administration. Thin copper sheeting was used to form a Faraday cage for the electronics

* Harmonic distortion was eliminated shortly after this reporting period and will be described in the next semiannual report.

of the meter. A second sheet was placed on top of this cage and electrical connection was made between the two through the RC input stage of the meter.

Experiments established that plexiglass in close proximity to the meter exerted a measurable influence on the meter reading. Therefore, to support the meter, the arrangement shown in Figure 6 was used.

A gross calibration of the meter was made by positioning it midway between the two electrodes and assuming that the field was V/d , where V was the electrode potential and d the electrode spacing. A more accurate method of calibration was subsequently developed and is discussed below.

Only a preliminary set of measurements will be presented in this report. For these measurements the fieldmeter was positioned over the center of the lower electrode and a vertical scan was made. Figure 7a shows the observed data at an electrode voltage of 27.5 kV, where z is the vertical distance from the lower electrode to the center of the fieldmeter and E_z is the vertical electric field.

This type of fieldmeter will not be accurate when placed close to other conductors in the system, because the meter's own electric field will alter the charge distribution on nearby conductors and enhance the field at the meter's location. This behavior can evidentially be seen in Figure 7a, where a sudden increase in measured field is observed as $z \rightarrow 0$ (as the lower electrode is approached) and possibly as $z \rightarrow 0.57$ m (as the upper electrode is approached).

We wanted to know the field at all points between the two electrodes for two reasons: (1) the vertical field could be integrated from the

FIGURE 6. The small dipole fieldmeter and its plexiglass stand.

FIGURE 7. Electric field measurements in the Special Studies Exposure System were made with a small dipole fieldmeter. The vertical distance above the lower electrode surface is z ; the upper electrode is at $z = 0.58$ m. The top graph (a) shows uncalibrated data. The lower graph (b) shows the data after calibration and a theoretical fit to this data, Gauss's law requires that this fitted curve be horizontal at the two electrode surfaces.

lower to the upper electrode to obtain the voltage between the two electrodes and the fieldmeter could then be calibrated by comparison to the known voltage; and (2) the field extrapolated to the lower electrode could be compared to our direct measurement of the field with the surface probe.

Since the horizontal components of the field are zero at the electrode surfaces, it follows that $\partial E_z / \partial z = 0$ at those surfaces. This constraint means that the true field in Figure 7a must be a curve which is horizontal at $z = 0$ and $z = 0.57$ m. Since the measured field for $z < 0.13$ m and $z > 0.43$ m clearly does not lie on such a curve, these points have been deleted in the subsequent analysis. A quadratic form was fit to the remaining data, subject to the constraint that $\partial E_z / \partial z = 0$ at the two electrode surfaces. In particular, we used

$$E(z) = \alpha_0 + \alpha_1 z^2 \left(1 - \frac{z^2}{2d^2}\right)$$

where α_0 and α_1 are constants determined by the data and $z = d$ at the upper electrode. The measured fields were assumed to have an uncertainty of $\pm 0.25\%$ and a least square fit was made with the following results:

$$\alpha_0 = 40.71 \pm 0.06 \text{ kV/m}$$

$$\alpha_1 = 24.35 \pm 0.81 \text{ kV/m}^3$$

For this fit $\chi^2 = 7$ for 10 degrees of freedom indicating that the quadratic form provided a good description of the data.

This form for E_z was integrated to obtain the electrode voltage V .

$$V = \int_0^d E(z) dz = \alpha_0 d + \frac{7}{30} \alpha_1 d^3 = 24.5 \pm 0.1 \text{ kV}$$

The actual electrode voltage, as measured by the Ross voltage divider, was 27.5 ± 0.2 kV. This result establishes that the fieldmeter reads low and should be corrected by multiplying by a factor of 1.12 ± 0.01 .

Figure 7b shows the corrected data and curve for a least squares fit of the data. This curve extrapolated to the lower electrode yields a field strength of 45.6 ± 0.5 kV/m. This may be compared to the field measured on the lower electrode with the surface probe of 46.8 ± 0.9 kV/m. This value is also shown on Figure 7b and is in agreement with the extrapolated value.

A detailed vertical scan over the entire area of the electrodes is being made to obtain a complete field profile.

B. RAT EXPOSURE SYSTEM

The goal was to build a system suitable for the exposure of large numbers of rats to a uniform electric field. Our experience in building and using the prototype exposure system provided several guidelines that were adopted in the design of the rat exposure system.

- The basic exposure electrode design was sound. A change in the detailed electrode geometry was made to maximize the field uniformity over the area of the electrode.

- The electrode spacing was reduced to 0.41 m from the 0.57 m of the prototype exposure system on the basis of model calculations.⁽¹⁾
- To maximize field uniformity and minimize electrical interaction with grounded room walls it was decided to energize all electrodes with adjacent electrodes 180° out of phase.
- The minimum clearance between the electrodes and grounded room walls was maintained at about 0.45 m.
- A minimum clearance of about 0.45 m was maintained between the electrodes and the vertical, high voltage buswork to prevent local distortions in the exposure field.

1. Exposure Room

Figure 8 is a plan view of the rat exposure system. A 20 ft x 30 ft room was divided into four equal compartments by wood and 1 in. x 1 in. metal screen. An exposure rack capable of exposing or sham-exposing 72 rats is located in each compartment. All four racks have high voltage coaxial cables attached. These cables are connected from the electrodes, through the back wall of the building, to two high voltage transformers that are located in a small metal utility building. The control units for the high voltage transformers are mounted in the wall of the exposure room facing the building hallway.

The rat exposure system is divided into left and right sides, each consisting of two exposure racks and one high voltage supply. Any two

FIGURE 8. Plan view of the rat exposure system.

exposure racks may be energized by simply connecting the proper cables to the transformer. The unconnected cables are grounded. From inside the exposure room it is not possible to tell which systems are energized. It is planned that only the project manager and one engineer will know which racks are energized, to eliminate any possibility of experimental bias by the investigator knowing which are the exposed and the sham-exposed animals.

2. Exposure Rack

An exposure rack is shown in Figure 9. Four 1.0 m x 2.2 m electrodes are arranged vertically and supported by plexiglass stands at four corners. The vertical separation between electrodes is 0.41 m. Each of the lower three electrodes has positions for three animal modules, with each module holding eight rats. Water is supplied from beneath the cages through a manifold. The animal housing system will be discussed in detail later in this report.

Each of the lower three electrodes has two layers. The upper layer consists of three rectangular sections of 1/2-in. x 1/2-in. stainless steel mesh, on which is attached cage modules with nylon screws, and two small aluminum plates as end pieces (Figure 9). The lower layer of each electrode is a stainless steel door. Paper (cageboard) is placed on this layer to absorb excreta from rats standing on the upper layer.

Each electrode is equipped with a water bottle placed adjacent to the electrode on a separate plexiglass stand. This is shown in Figure 10.

FIGURE 9. A rack of four electrodes for exposing rats. The vertical separation between electrodes is 0.41 m.

Connection is made to the water manifold of each shelf with plastic tubing. Each bottle is placed 0.15 m higher than the electrode to attain a small pressure head.

3. High Voltage System

All four electrodes of each rack are energized, as shown in Figure 10. Alternate electrodes are connected together with 3/4-in. diameter metal tubing. The high voltage is supplied to each of these buses by shielded coaxial cable (Belden #8871) terminated at each end with stress cones. The connection of the center conductors of the cable to the bus is covered with a corona guard. This guard was made from a hardwood ball which was formed to join smoothly to the vertical bus and then covered with conducting paint. A piece of copper foil was placed on the ball and a wire soldered to it; the other end of this wire was attached to the bus to provide an electrical contact between the bus and the ball.

Two custom, high voltage power supplies were purchased from Hipotronics, Inc., of Brewster, New York. One of the transformers is shown in Figure 11. Each transformer has two outputs of equal voltage (30 kV maximum, 2 kVA) but of opposite phase. The control units for these power supplies are shown in Figure 12.

4. Safety

The safety aspects of the exposure system are based on experience gained from the prototype exposure system, as described in the first

FIGURE 10. The two high voltage coaxial cables connect to buswork which distributes the voltage to alternate electrodes. The water reservoirs for the three lower electrodes are shown in the rear.

FIGURE 11. One of the two high voltage transformers supplying the rat exposure system. The two bushings supply equal voltages out of phase with each other.

FIGURE 12. The two high voltage control units for the rat exposure system.

interim report.⁽¹⁾ All doors providing access to areas of high voltage are interlocked with limit switches. The door to the transformer enclosure is locked with a key that is kept in a lockbox. Only the project manager and chief engineer have access to the lockbox. A lock and tag procedure is used when entry to the transformer building is required.

The hall doors to the rat exposure system are equipped with key interlocks. The keys cannot be removed until the door is closed and locked, and neither high-voltage system can be energized until the keys from both hall doors are inserted into an interlock panel of the control units.

While work is in progress inside the exposure system, all four racks will have protective grounds applied. These can be seen in Figure 9. Designated people have been trained as operators of this system and will be responsible for the installation and removal of the protective grounds as well as the operation of the high-voltage controls. They are required to search the exposure compartments when they remove protective grounds and prior to energization of the high voltage supplies.

5. Animal Housing

The animal housing system was discussed in detail in our first interim report.⁽¹⁾ The system that was finally adopted is very similar; only changes that have occurred in the last 6 months will be described in this report.

Figure 13, taken from our first interim report, shows the eight-compartment cage module. The original roof design incorporated six

FIGURE 13. Drawing of rat housing module. The unit holds eight rats in lexan compartments for exposure to electric fields. Individual food pellets are placed in the slots along the front and rear edges. The ceiling height is 4 in. and prevents the rats from standing erect on their hind legs in order to minimize field enhancement.

1-in. diameter ventilation holes over each rat compartment. However, it was soon learned that rats were able to enlarge these holes by chewing until they could escape. The design was therefore changed to fifteen 1/4-in. diameter holes per compartment. This eliminated chewing but did not provide adequate ventilation as evidenced by a 2°C temperature rise over ambient in an occupied rat compartment. The final design incorporates twenty-one 3/8-in. diameter holes per compartment. The temperature rise with a rat in the compartment is 0.5-1.0°C, similar to that observed in a standard rat cage. Chewing of the cage by the rat is no longer a problem.

The design of the water system has undergone significant changes from that described in the first interim report. It was found that rats could lie down and have parts of their body on the nozzle. This would eventually drain the water reservoir and produce a mess. To combat this problem, the design shown in Figure 14 was adopted.

Two rats share a single nozzle. The nozzle tip is flush with the cage floor. A small notch was cut in the cage wall so animals in opposite cages have access to the nozzle. Small verticle plastic strips were glued to each side of the hole to form a 10-cm thick bumper to keep any part of the resting rat's body from lying on the nozzle. Results from tests demonstrated rats can easily obtain water and that water leaks are no longer a problem.

In order to provide access to the nozzle, a 1 x 1-inch hole was cut in the cage floor and the tip of the nozzle approximately centered in

the hole. The nozzle thus serves electrically as a part of the floor and the electrical current induced in it during field exposure will be the same as in the cage floor. The impedance between the nozzle and the electrode must be sufficiently low to prevent the development of a significant nozzle voltage.

The induced nozzle current has been measured. One nozzle was insulated and electrically attached to the electrode through a current measuring device. The nozzle current for an applied field of 1 kV/m is

$$\frac{I_n}{E} = 2.1 \text{ nA/(kV/m)}.$$

This represents an average value since the nozzle current is a function of the vertical height of the nozzle relative to the cage floor. This value represents about 1% of a rat's short circuit current.

The watering nozzles are inserted into a stainless steel water manifold (Figure 14). The nozzles are seated in rubber grommets so that the only electrical connection between the nozzles and the electrodes is through the water. We are using distilled water and estimate the resistance between the electrode and the nozzle is about 200-500 k Ω . The nozzle voltage at an applied field of 1 kV/m will be approximately 0.4-1.0 mV/(kV/m). Even at 100 kV/m the nozzle voltage should present no problem; in fact, it will actually tend to minimize interaction between the rat and the nozzle since the rat's body will also have a

FIGURE 14. Photograph of the animal watering system. The vertical tubes are demand-operated nozzles. Small plastic bumpers were installed around each nozzle to keep animals from lying on the nozzle.

small voltage to ground because of the finite feet-to-floor impedance. More work is planned in the future to better define these relationships.

6. Field Measurements

We are able to report only preliminary results on voltage calibration and exposure fields in one rack of the rat exposure system at this time. More detailed results will be reported in the next interim report.

The high voltage to alternate pairs of electrodes has been measured with the use of a Ross voltage divider and the control panel meters have been calibrated. Waveforms were viewed on an oscilloscope and showed significant harmonic distortion, but not as severe as that shown in Figure 4 for the Special Studies Exposure System.*

The surface field probes have been calibrated in this system at a voltage of 10 kV on each electrode. The same calibration technique was used as that described earlier for the prototype system.

The electric field was measured at the center of one electrode before insertion of the plastic animal cages and with the electrodes separated by the nominal 0.41 m. It was $0.98 \pm 0.02\%$ of the parallel plate field, V/d . A scan was made of the electrode surface and all measured values were within 5% of each other. The uniformity was significantly better than that observed in the prototype system. Spot checks of the other two electrodes on the rack showed identical fields.

The rat housing modules were then placed on all three lower electrodes and surface field measurements were made inside a few compartments. These measurements showed field uniformity was better than 3%.

* The harmonic distortion has subsequently been corrected and details will be given in our next progress report.

Field strength measurements will be continued. However, even on the basis of these preliminary measurements, it seems clear that the original goal of building a system capable of producing vertical fields uniform to within 10% has been met. These measurements have not addressed the problem of the interaction of an animal with the applied field and with other animals on an exposure electrode. Some preliminary measurements have been made and will be described in Section III of this report.

7. Present Status

As of the end of March, 1977, construction of the rat exposure system has been completed. In preparation for the first animal exposures, scheduled to start April 4, 1977, with a 2-week acclimation period followed by a 30-day exposure, preliminary field measurements have been completed. Testing for possible corona discharge and ozone formation is in progress.*

Significant amounts of harmonic discharge have been detected in the high voltage waveform and in the input voltage to the power supply. Steps will be taken to reduce this before the biological experiments begin to as low a value as practical and the residual distortion will be documented.*

* Completed on April 15

III. DOSIMETRY

The goals of the dosimetry portion of this project are:

- To assist in the development of a valid exposure system and to insure that the electric fields and other conditions are known and documented.
- To develop probes suitable for the measurement of parameters (surface fields, short-circuit currents, current distribution within the body) needed to quantify the exposure of animals to electric fields.
- To make measurements of the effects of animal cages, neighboring animals, and electrode separation on field strengths and field uniformity.
- To develop techniques for the measurement of electric fields and current densities inside the bodies of animals.

The greatest effort has gone into the first goal and is reported in Chapter II of this report. Significant progress has also been made on the second and third goals.

A. PROBE DEVELOPMENT

The first interim report⁽¹⁾ presented details of the development of a probe suitable for the measurement of fields on the surface of flat conductors. This probe produces a very small perturbation of the applied

electric field. On the other hand, an animal standing in a 60-Hz electric field produces major perturbations. The fields at the surface of the animal are in general quite different from the unperturbed field. It is particularly important to know these surface fields since body currents induced in animals is determined by the charge density on the surface of the animal.⁽²⁾ A probe suitable for dosimetric studies will be a three-dimensional probe which perturbs the field approximately the same as the body of a living animal. The most accurate probe is one that has the same geometry as that of the animal.

Because living tissue is almost a perfect conductor at 60 Hz,⁽³⁾ the perturbation of the applied field by an animal model with conducting skin will be the same as that produced by a living animal (except for small differences in geometry). The conducting skin can be divided into a number of segments to allow measurement of the electric field at the skin's surface and the distribution of currents between major sections of the body.

Figure 15 is a photograph of a rat model made in the form of a hemi-ellipsoid. This shape, similar to that of a rat, has the advantage that theoretical calculations of the short-circuit current and surface fields can be compared to empirical measurements. The model was constructed of 1/8-in. thick balsa sheets cut into ellipses and glued together. The resulting laminated structure was sanded smooth and painted with a sealer and a silver-based conductive paint. The measurements of the principle semi-axes are 8.23, 5.70 and 3.91 cm.

FIGURE 15. Hemi-ellipsoid used as a rat model for dosimetry. It is constructed from balsa wood and covered with conducting paint.

The total short-circuit current of the probe has been measured and is within 5% of the theoretical value. These measurements estimate the short-circuit current of a rat in a uniform field to be $0.2 \mu\text{A}/(\text{kV}/\text{m})$.

B. MULTIPLE-ANIMAL INTERACTIONS

In this project rats exposed to electric fields will be housed in individual cages but will live in close proximity to each other (Figure 13). Each rat will alter the electric field in its vicinity. Model calculations of field distortions produced by an animal were given in the first interim report.⁽¹⁾ If a second rat is placed close enough to the first so that the field perturbations caused by the two overlap, then one would expect this interaction to change the exposure parameters for both rats. In particular, one would expect the short-circuit currents in each of the animals to decrease and, probably, the distribution of current within their bodies to be altered.

To obtain quantitative measurements, the Special Studies Exposure System was fitted with two rat cage modules. Figure 16 shows a top view of the cage arrangement. The ellipsoidal probe described earlier was then positioned in one rat compartment, as shown and its short-circuit current was measured. Next, short-circuit currents of the model were measured with a rat placed sequentially in the other seven cages. Short-circuit currents of the model were reduced to 0.85 to 0.99 of normal, depending on where the rat was placed (Figure 16). The major effect, a reduction of 12-15%, was caused by a rat placed in a cage on either side of the ellipsoid.

FIGURE 16. Plan view of the lower electrode arrangement used to study rat-to-rat interactions. Valves give the fraction of normal short-circuit current of the ellipsoid model by placing a rat in one of the other seven cages. All valves refer to the short-circuit current of the model in the compartment shown in the drawing with the rat located where the valve is given.

Finally, nine rats were placed in the compartments neighboring the ellipsoid. About 40 measurements of the short-circuit current were made over time and for several ellipsoid positions within the cage. The short-circuit current was 0.65 ± 0.04 (S.E.) of the value measured without any rats in the cage, or a reduction in short-circuit current of about 35%. This experiment shows that multiple animal interactions are significant. One advantage of the individual housing of rats in small compartments is shown: the reduction, while large, is relatively constant and predictable. This would not be true for experiments housing more than one animal per cage where large differences in distances between animals would occur.

IV. CRITERIA STUDIES

As described in our last interim progress report⁽¹⁾, the criteria studies consist of experiments to validate the biological compatibility of the exposure systems, to identify and correct any potential sources of artifacts, and to establish the maximum electric field strength to be used for the biological screening experiments.

The system for housing animals during exposure to electric fields has been built and tested. Details were given in our last report. Several modifications were made to improve the drinking water system and housing ventilation, as described in Section II. Experiments have been completed that verify the system is reliable and consistent with good animal care practices. The housing system does not alter field uniformity significantly and it does not stress the animals.

Experiments have been completed to establish the maximum electric field strength to be used for the biological screening experiments. Described below are the experiments that were conducted to establish the upper limit on field strength that does not produce electric shocks to the animal from the housing system, corona discharge and ozone formation, hair stimulation, or other forms of field perception sufficient to cause the rat to prefer to move out of the electric field.

A. ELECTRIC SHOCKS

Induced voltages can result in discharge shocks to the animal as it touches other conducting objects in the electric field. The exposure

system has been designed to eliminate shocks by keeping the animal and all surrounding conducting objects (i.e., the watering nozzle) at the same potential. This method has been shown to work well in a previous study with swine.⁽³⁾

Experiments were conducted to confirm that rats do not receive shocks from any part of the system. A variety of configurations were used to deliberately produce shocks in order to observe the response of an animal to use as a reference for subsequent tests. All experiments were recorded on video tape, allowing later review and analysis.

Experiments were conducted with two standard cage modules placed on the grounded, lower plate of the Special Studies Exposure System. Standard food pellets were used in the feeding system and water was available through the grounded nozzles in each cage. Section II of this report gives details of the watering system. To facilitate video tape recording only the rear 8 cages of the two modules were used; these eight cages were numbered 1-8 from left to right. In the first test, the floors of cages 1, 3, 5 and 7 were covered with 1/16-in. plexiglass, effectively insulating the rats from ground.

Eight naive, male, Sprague-Dawley rats, 110 days of age, were tested in the numbered cages. Most tests were made with an electrode voltage of 80 kV, yielding an unperturbed field strength inside the cages of about 130 kV/m. Several minutes of video taping without an electric field was used to obtain baseline behavior. With the electrodes energized, rats in cages 1 and 3 showed clear recoil responses when

contact was made with the grounded watering nozzle, indicating the animals received shocks. The rat in cage 7 never made contact with the nozzle, and the rat in cage 5 made contact but exhibited no recoil response. Rats in cages 2, 4, 6, 8 (without an insulating floor) showed no evidence of shocks as they moved about and drank from the watering nozzles.

A single, naive rat was placed in the number 5 cage with all other cages empty. With an insulating floor of plexiglass in the cage, the animal received shocks at field strengths of 85 kV/m or higher. With the plexiglass floor removed, no evidence of shocks was observed at the highest field strength of 145 kV/m. This test was repeated with a second naive rat with the same result. These results, when contrasted with the results using eight animals, suggested a possible attenuation of the field due to interanimal shielding. These results led to the experiment discussed in Section III which describes the reduction in short-circuit current produced by mutual shielding of animals in the field.

These experiments confirm that rats will not be subject to shocks in the exposure system at field strengths of ≤ 145 kV/m.

B. CORONA DISCHARGE AND OZONE FORMATION

Techniques were developed and used to determine if significant corona discharge and ozone formation are present in the Special Studies Exposure System. The same techniques will be used in the rat and mouse exposure systems during their initial testing phases.*

* Experiments completed April 15; no corona or ozone was detected.

Very long photographic exposures (2-10 minutes) were made in a darkened room using Polaroid Type 57 film (ASA 3000) to detect the light emitted from regions of corona discharge. An initial series of tests was conducted to show that this method could indeed detect visible light from corona discharge. Several 6-in.-long, sharp-pointed wires were placed in a vertical position on the lower electrode to project towards the upper electrode. At a field strength of 40 kV/m, photographs showed light emission from corona discharge at the end of each wire (Figure 17). Although many objects were tested as potential corona producers, only sharp metal objects were effective. Several dielectric materials and a transected swine's ear did not show light emissions at field strength values as high as 150 kV/m.

The second phase of this study involved photographically mapping the exposure room to locate corona-emitted light from fixtures, wire screening, rat cages, electrode surfaces, and other possible sources. Three photographs of each area were taken: two with the lights off and one with the lights on. In this way, imperfections in the Polaroid film could be eliminated and the actual location of any observed corona discharge could be determined. No corona was observed.

Ozone (O_3) is produced as a result of corona. Two techniques were used to measure the O_3 concentration in the Special Studies Exposure System. In the first, a Bendix/GASTEC detection system was used. With this device, 100-ml air samples were drawn through detector tubes containing an organic dye (indigo) which decomposes in the presence of O_3 to produce

FIGURE 17. Corona at the tips of wires placed on the lower electrode of the Special Studies Exposure System. The unperturbed field strength was 40 kV/m.

a color change in proportion to the O_3 concentration. Two brands of tubes were used, one manufactured by Dräger (sensitivity 0.05-0.14 ppm) and the other by GASTEC (No. 18L, sensitivity 0.05-0.6 ppm). All measurements made with this system were negative, evidently because the O_3 concentration was always below 0.05 ppm.

With the other technique, a sampling pump (Mine Safety Appliances) was used to draw an air sample (typically 1.5 ℓ) through a midget impinger filled with potassium-iodide solution. These samples were drawn through 10 ft of tygon tubing so that the pump could be kept remote from the exposure system. The samples were analyzed colorimetrically at the Hanford Environmental Health Foundation laboratory. This method is reported to be capable of detecting O_3 concentration as low as 0.005 ppm.

Two series of measurements were made. In the first, measurements were made under four experimental conditions: (1) two normal rat housing modules positioned on the lower electrode; (2) a flat aluminum plate acting as the lower electrode surface; (3) a shuttlebox, to be described in the next section, positioned on the lower electrode; and (4) an array of 7-in. high wires to provide a known source of corona. In all four cases samples were drawn from two points about 10 in. above the surface of the lower electrode. For all but the last configuration, a 15-min (1.5- ℓ) background measurement was taken just before the system was energized. A second 15-min sample was then taken with the system energized to produce an electric field at the lower electrode surface of about 130 kV/m (80 kV electrode voltage).

The result of this series of measurements is shown in Table II. No significant ozone was produced with the electrode energized. Corona production from the wire points in the last run was audible and O_3 could be detected by smell, yet the concentration was very low. This result prompted questions about the validity of these measurements, and led to a second experiment.

TABLE II. Measured Ozone Concentrations

<u>Lower Electrode Configuration</u>	<u>Point of Sample</u>	<u>Time of Sample After System Energized (min)</u>	<u>Observed O_3 Conc. (ppm)</u>
Two rat modules	Two samples 5 in. above module roof	<0 0-15	0.010, 0.010 0.010, 0.013
Flat plate	Two samples 10 in. above plate	<0 0-15	0.006, 0.010 0.010, 0.017
Shuttlebox	Two samples 4-1/2 in. above roof	<0 0-15	0.012, 0.020 0.021, 0.028
7-in. high wires	13 in. from nearest wire tip	-- 0-15	--, -- 0.021, 0.028

The electrode configuration used in this series of measurements was the same as in the previous experiment. However, samples were drawn from inside the rat cages and shuttlebox. This is, of course, the most relevant place since this is where the animals will reside. Also, the samples were drawn after an extended period of operation to determine whether the concentration of O_3 increased with time in the animal cages.

TABLE III. Ozone Concentrations Measured Inside Animal Cages

<u>Lower Electrode Configuration</u>	<u>Point of Sample</u>	<u>Time of Sample After System Energized (min)</u>	<u>Observed O₃ * Conc. (ppm)</u>
Two rat modules	Inside rat cages	30-45	0.005, 0.005
		60-75	0.010, 0.010
Shuttlebox	Inside shuttlebox	0	<0.005, <0.005
		30-45	<0.005, <0.005
		60-75	<0.005, 0.007
Metal rod array	Rod tip inside sample tubing	10	0.10

The results are given in Table III. There were no significant increases in ozone levels with the system energized. There appears to be no significant accumulation of O₃ with time. In the last test, an array of metal rods was used to deliberately produce corona. The tip of one rod was inside the tygon sampling tube. Even in this configuration an O₃ concentration of only 0.10 ppm was observed.

The present Threshold Limit Value (TLV) for human exposure to O₃ is 0.10 ppm, as established by the American Conference of Governmental Industrial Hygienists (1976); this standard is accepted by OSHA. The O₃ concentration observed in the two experiments are all a factor of 5-10 below the TLV. Based on these measurements, significant concentrations of O₃ do not appear to be present.

However, we had reservations about the accuracy of these measurements. Another series of measurements were made using teflon rather than tygon tubing. In addition, a dynamic ozone detector (Monitor Labs, Model 8401)

was used to provide continuous readings over long time periods. The results were essentially the same as in the other experiments. No ozone is produced by the exposure system.

C. HAIR OSCILLATION

In previous work with swine⁽³⁾ it was shown that the threshold field strength for producing piloerection and hair oscillation is about 50 kV/m. The hair oscillation could readily be observed visually.

A similar series of experiments were conducted on rats to determine the threshold field strength for visible hair movement. A rat was anesthetized and placed on the lower electrode plate of the Special Studies Exposure System. A video camera with a 15 to 150 mm zoom lens and a 5 mm lens extension was used to document the experiments.

Six experiments were conducted. Electrode voltages to 80 kV (130 kV/m) yielded no evidence of movement of the vibrissae--the long, sensory hairs of the face. Similar negative results were obtained with the rat placed on a 1/4" lucite sheet which insulated it from the lower electrode. Hairs at the highest point on the dorsal surface of the animal were examined also and showed no evidence of visible hair movement.

The rat was then elevated on an insulated surface so that its ventral and dorsal surfaces were 6-1/2 and 9 in., respectively, above the lower electrode; a wire was used to electrically connect the rat to the lower electrode. At this elevated position above the lower electrode a clear threshold for hair movement and an ear flick response were observed at an electrode voltage of 55 to 60 kV. The estimated enhancement of

the electric field in this configuration was about three times that at the plate surface. Thus, to achieve visible hair movement an unperturbed field (i.e., before introduction of the animal) is estimated to be at least 300 kV/m. Such values are beyond the range of concern in this project.

D. BEHAVIORAL PREFERENCE

Experiments were conducted to determine how large electric fields had to be for rats to show a preference to remain shielded from the electric field. Although the particular behavioral paradigm used for the experiments does not allow one to make inference concerning any aversive characteristics of the field, it does provide useful information concerning perception and escape behavior. It is important to know the threshold field strength for perception and escape since it may represent perception of fields at the body surface by the animal, such as by hair stimulation or by some other unknown mechanism. It is conceivable that psychological and physiological changes could occur as a result of such perception and would be independent of currents induced in the body of the animals by the 60-Hz electric field.

The Special Studies Exposure System was used for the behavioral preference experiments. Briefly, the procedure was to place a rat in a long, narrow shuttlebox, one end of which was shielded from the electric field by metal screening, and record how much time the animal spent in the shielded side during a 45-minute test session. The threshold field strength that produced preference by the rat for the shielded side was determined by testing over a range of field strengths.

The shuttlebox and associated equipment used for the experiments are shown in Figures 18 and 19. The basic unit is a long, narrow lucite box

FIGURE 18. The shuttlebox system used in the behavioral preference experiments.

FIGURE 19. Photograph of the shuttlebox system.

40 in. long by 5 in. wide by 4 in. high. This box is enclosed within a larger lucite box. One end of the larger box is covered with aluminum window screen to form a Faraday shield; an animal located in this end of the box is shielded from the electric field. The other end of the larger box is covered with plastic window screen; it was established experimentally that the plastic screen has no significant effect on field strength or uniformity.

Field measurements were made inside the outer box on the lower electrode and it was found that the field ranged from 10% to 90% of full strength over an 8-in. horizontal distance under the edge of the aluminum screen. This span was not centered under the edge of the screen. Therefore, it was necessary to offset the edge of the aluminum screen 2-3/4 in. from the center of the box to obtain a 50% field strength at the center of the box. An aluminum rod was used at the edge of the screen to prevent corona. To obtain visual symmetry, the plastic screen edge was also offset 2-3/4 in. from the center of the box and its edge covered with a lucite rod. Finally, all parts were covered with a nonconducting paint so that the rat would have no visual cues as to what side of the box was shielded from the electric field.

Two light-beam detectors were used to provide information on the location of the rat in the shuttle box (Figures 18 and 19): left, right or center. Two lucite rods were used to transmit red light beams across the center region of the box. The beams were received by two rods on the other side of the shuttle box and transmitted to phototransistors.

Both the light sources and the phototransistors are located in Faraday shields located remote from the shuttlebox to prevent perturbation of the electric field.

The system was designed so that the animal could be scored as being in one of three areas of the box: right, center, left. The rat was scored as being in the center if either light beam was interrupted. Location of the rat in the right or left side was determined by the last light beam to be interrupted as the rat left the center region.

Information obtained from the phototransistors were interfaced with standard logic control modules and recording equipment. Data was obtained on the amount of time the animals spent in each side of the shuttlebox, by traverse and in 15-min intervals, and the number of traverses the animals made between compartments.

Several preliminary experiments were made using the shuttlebox to resolve any unexpected problems and to establish the duration of each behavioral run. Several minor equipment and procedural problems were encountered and appropriately corrected. It was found that most of the activity by the rat occurred during the first 45 minutes, after which the animals would typically curl up and go to sleep. Accordingly, a test duration of 45 minutes was selected for the first experiment.

Twenty-four male Sprague-Dawley rats, approximately 90 days of age, were distributed into 5 groups on the basis of equivalent means and variances in body weight. The groups were randomly designated for behavioral testing at 0, 50, 75, 100 or 130 kV/m. The run order was balanced over a 3-1/2 day period so that no two animals from the same group were tested consecutively,

and animals from each group were represented in each test day. Each rat was tested only once over a 45-minute session.

Control and recording equipment were located outside the exposure room to avoid auditory cues to the animals. Data was obtained on the amount of time each rat spent on the exposed and unexposed side, as well as in the center.

The percent of time in a 45-minute test session that rats spent in the exposed, unexposed and center region of the shuttlebox at different field strengths is shown in Table IV. Rats exposed to 100 and 130 kV/m spent significantly more time in the unexposed (shielded) compartment. No significant preferences for exposed or unexposed sides of the box were found for the groups exposed to 0, 50, or 75 kV/m.

TABLE IV. Mean Percent of Time ($\pm$ S.E.) in a 45-Minute Test Session that Rats Spent in Exposed, Center and Unexposed (shielded) Regions of a Shuttlebox During Exposure to Electric Fields.

Group (kV/m)	No. of Rats	Percent Residence Time**			Significance*
		Exposed Side	Center	Unexposed Side	
0	4	40.5 $\pm$ 6.4	8.3 $\pm$ 3.1	51.0 $\pm$ 8.6	
50	4	38.8 $\pm$ 7.4	9.8 $\pm$ 2.4	51.5 $\pm$ 6.3	n.s.
75	6	46.5 $\pm$ 12.6	4.7 $\pm$ 1.9	48.7 $\pm$ 12.6	n.s.
100	6	23.3 $\pm$ 5.4	6.5 $\pm$ 2.0	70.5 $\pm$ 6.9	<0.01
130	4	21.6 $\pm$ 5.2	5.1 $\pm$ 2.4	73.1 $\pm$ 6.4	<0.05

*2-tailed Mann-Whitney U Test

**Values may not add up to 100% due to rounding errors

The preference patterns of the five groups over each 15-minute interval during the 45-minute exposure session are shown in Figure 20. The preference for the unexposed (shielded) side of the shuttlebox by the rats exposed to 100 and 130 kV/m appears to develop over time within the 45-minute exposure session, with the full response evident during the last 30 minutes.

The behavioral experiment was replicated to obtain a more precise value of the field strength for producing the behavioral effect and to determine whether repeated exposures would alter the threshold for the effect. The designated exposure groups for this experiment were 0, 60, 75, 90 and 105 kV/m, with 8 male rats assigned to each group. The experimental procedure was essentially the same as that used in the preceeding experiment except the animals were retested at weekly intervals for an additional 3 weeks. Table V shows the run sequence for each day of exposure and behavioral testing.

TABLE V. Daily Sequence of Exposures and Behavioral Tests

<u>Day 1</u> <u>(kV/m)</u>	<u>Day 2</u> <u>(kV/m)</u>	<u>Day 3</u> <u>(kV/m)</u>	<u>Day 4</u> <u>(kV/m)</u>	<u>Day 5</u> <u>(kV/m)</u>
0	90	60	105	75
60	105	75	0	90
75	0	90	60	105
90	60	105	75	0
105	75	0	90	60
0	90	60	105	75
60	105	75	0	90
75	0	90	60	105

FIGURE 20. Mean percent of time ($\pm$ S.E.) rats spent in exposed, center and unexposed regions of a shuttlebox at electric field strengths of 0, 50, 75, 100 and 130 kV/m. Values shown for 45-minute test session and for 15-minute intervals within the session.

Each group was tested at least once each day and there were equal numbers of rats subjected to exposure on each side of the shuttlebox within each group. The run sequence was designed to balance for any possible diurnal rhythm effects or potential side preferences. In each subsequent week the run order was not altered although the side of exposure was alternated each week (e.g., rats exposed on the left side in weeks 1 and 3 were exposed on the right side in weeks 2 and 4).

The results of the first week (one 45-minute session per rat) closely parallel the results of the first experiment (Table VI). Rats exposed to 90 or 105 kV/m spent more time on the unexposed (shielded) side during the 45-minute session. Figure 21 shows the preference patterns of the sham-exposed and 4 exposed groups by 15-minute intervals during the 45-minute session. As was the case in the first experiment, the preference for the unexposed side seems to develop over the course of the exposure session.

TABLE VI. Mean Percent of Time ($\pm$ S.E.) in a 45-Minute Test Session that Rats Spent in Exposed, Center and Unexposed (shielded) Regions of a Shuttlebox During Exposure to Electric Fields

Group (kV/m)	No. of Rats	Percent Residence Time			Significance*
		Exposed Side	Center	Unexposed Side	
0	8	51.1 $\pm$ 7.9	10.3 $\pm$ 1.7	38.5 $\pm$ 7.8	
60	8	35.6 $\pm$ 9.0	13.5 $\pm$ 2.3	50.8 $\pm$ 9.6	n.s.
75	8	53.9 $\pm$ 10.2	9.4 $\pm$ 1.6	36.9 $\pm$ 10.0	n.s.
90	8	24.3 $\pm$ 3.9	9.9 $\pm$ 2.4	66.0 $\pm$ 5.8	<0.01
105	8	17.4 $\pm$ 2.8	10.1 $\pm$ 1.8	72.4 $\pm$ 4.3	<0.01

* 2-tailed Mann-Whitney U Test

FIGURE 21. Mean percent of time ($\pm$ S.E.) rats spent in exposed, center and unexposed regions of a shuttlebox at electric field strengths of 0, 60, 75, 90 and 105 kV/m. Values shown for 45-minute test session and for 15-minute intervals within the session.

Results for the second, third and fourth weeks of exposure were essentially the same as those observed during the first week (Table VII). Only rats exposed to 90 or 105 kV/m showed a significant preference for the unexposed side of the shuttlebox. There was no enhancement or attenuation of the behavioral response with repeated exposures.

Results of the two behavioral experiments demonstrated statistically significant preferences by rats to avoid electric field exposure at 90, 105, and 130 kV/m. At field strengths of 0, 50, 60, and 75 kV/m, no statistically significant preference to avoid exposure was found. Information derived from this experiment will be used to establish the highest field strength to be used for the biological screening experiments.

Two potential problems were identified after the behavioral experiments were completed. Significant harmonic distortion was found in the high voltage waveform. Also, field strength instability was noted inside the shuttlebox while field surveys were being made with the surface field probe.

The latter phenomenon was characterized by sudden shifts in field strengths by as much as 20% for no evident reason. The possibility existed that these two exposure problems could have been responsible for the behavioral results we obtained. Accordingly, both problems were corrected* and the first week of the second behavioral experiment was repeated. The results were essentially identical to those of the earlier experiment. Rats exposed to 90 and 105 kV/m spent significantly more time in the shielded side of the shuttlebox, while rats exposed at lower

* To be described in the next semiannual report.

TABLE VII. Mean Percent of Time (+ S.E.) Rats Spent in Exposed, Center and Unexposed (shielded) Regions of a Shuttlebox During Exposure to Electric Fields when Retested at Weeks 2, 3 and 4. (N = 8/group)

Group (kV/m)	Percent Residence Time			Significance*
	Exposed Side	Center	Unexposed Side	
<u>Week 2</u>				
0	39.1 $\pm$ 9.8	7.8 $\pm$ 1.9	53.1 $\pm$ 10.7	
60	33.0 $\pm$ 8.4	6.3 $\pm$ 1.0	60.8 $\pm$ 8.7	n.s.
75	40.0 $\pm$ 10.8	5.9 $\pm$ 1.9	54.1 $\pm$ 11.2	n.s.
90	43.6 $\pm$ 9.9	9.5 $\pm$ 1.5	46.9 $\pm$ 10.2	n.s.
105	25.6 $\pm$ 9.7	5.6 $\pm$ 1.4	68.7 $\pm$ 10.6	<0.05
<u>Week 3</u>				
0	45.8 $\pm$ 10.2	6.8 $\pm$ 1.8	47.5 $\pm$ 9.8	
60	36.6 $\pm$ 11.9	4.8 $\pm$ 1.3	58.6 $\pm$ 12.0	n.s.
75	38.5 $\pm$ 11.1	5.5 $\pm$ 1.3	56.0 $\pm$ 10.6	n.s.
90	38.6 $\pm$ 10.7	6.1 $\pm$ 1.5	55.3 $\pm$ 11.1	<0.05
105	22.5 $\pm$ 10.5	5.8 $\pm$ 1.1	71.8 $\pm$ 10.1	<0.05
<u>Week 4</u>				
0	52.8 $\pm$ 10.8	5.1 $\pm$ 0.8	42.1 $\pm$ 10.4	
60	31.5 $\pm$ 10.0	5.5 $\pm$ 0.8	63.0 $\pm$ 9.7	n.s.
75	39.5 $\pm$ 9.4	5.3 $\pm$ 0.9	55.1 $\pm$ 9.6	n.s.
90	23.0 $\pm$ 7.2	8.7 $\pm$ 1.3	68.7 $\pm$ 7.3	<0.01
105	26.0 $\pm$ 10.2	5.9 $\pm$ 1.1	68.1 $\pm$ 10.2	<0.01

* 2-tailed Mann-Whitney U Test.

field strengths showed no preference for the shielded side. It was concluded that the results of the first two experiments were not confounded by the harmonic distortion or field strength instability.

V. BIOLOGICAL STUDIES

The initial series of biological experiments will screen for possible effects from electric field exposure over a broad range of biological systems. Since the literature is often contradictory and in general inconclusive,⁴ this approach has been chosen to provide data on a wide variety of biological parameters to identify sensitive organs or functional systems. The major effort of these studies will commence in April, 1977. To date, progress has consisted of literature work, setting up equipment, development and refinement of techniques, accumulation of baseline data, and two experiments to measure the effects of electric field exposure on heart rates of rats.

Once sensitive parameters have been identified, additional research will be performed to elucidate dose-response relationships by varying the strength of the electric field and/or the duration of exposure. In-depth investigations will then be made to determine the significance of any observed biological effects. Ultimately an understanding of the mechanisms by which the electric field are able to produce particular bioeffects will be sought.

The following discussion presents details of current progress and future plans for the ten major areas of the biological studies. A short introduction is presented for each of these areas to help the nonbiologist to understand some of the reasons for the particular line of investigation and choice of biological parameters to be measured.

A. HEMATOLOGY AND SERUM CHEMISTRY - Dr. H. A. Ragan in charge

There is a paucity of information in the literature on the hematopoietic and clinical chemistry effects of high voltage electric fields.⁴ That which is available is conflicting, particularly when comparing animal studies to clinical examinations of people working in high-strength electric fields.

Studies have been planned to broadly screen different portions of the hematopoietic system and other organ systems for possible changes after exposure to electric fields greater than those which humans are likely to experience. This approach should maximize the opportunity of detecting changes which may then be examined in greater detail using lower field strengths.

Clinical pathology parameters for initial studies of electric field effects were selected because of their broad specificity in evaluating the functional capacity of the hematopoietic and other organ systems.

The formed elements of blood, i.e., red cells, white cells and platelets, are produced from progenitor cells in the bone marrow. This production and release of cells to the blood is controlled by homeostatic mechanisms designed to maintain strict equilibrium. Alterations in this balance may occur following insults to the bone marrow with death of cells before release to the blood. More subtle cellular alterations may occur with normal release of cells, but a shortened life span once these cells are in the circulating blood. Hematologic evaluations will be made to determine if electric field exposure perturbs the hematopoietic

system, and if so, whether changes are occurring in the marrow or after release of cells to the blood.

Chemical assays of serum constituents permit evaluation of specific organ systems. Accumulation of metabolic products may indicate alterations in the functional capacity of organs, e.g., elevation of blood urea nitrogen concentrations may be indicative of kidney lesions. Elevations of serum enzymes may indicate damage or death of cells with release of these constituents to the serum, e.g., transaminase, alkaline phosphatase, lactic dehydrogenase. Changes in concentrations of elements, e.g., calcium, phosphorus, or iron, may be indicative of direct alterations in metabolic rates of specific tissues or indirectly through damage to endocrine organs. A number of serum biochemical parameters will be evaluated following electric field exposures to determine if specific organ systems are perturbed.

1. Progress to Date

While waiting for the animal exposure facilities to be completed, efforts have been directed toward improving and/or modifying existing methods for use with rodents. Although heart blood from anesthetized rats is frequently used for hematologic evaluations, it is a less desirable source and subject to more variability than free-flowing venous blood. As shown in Table VIII the leukocyte concentration of heart blood is much less than that of venous blood; this is also true for erythrocyte concentration and volume of packed red cells (VPRC).

TABLE VIII. Hematologic Values from Heart Blood vs. Venous Blood (N=20)

	<u>Leukocytes</u> <u>($\times 10^3/\text{mm}^3$)</u>	<u>Erythrocytes</u> <u>($\times 10^6/\text{mm}^3$)</u>	<u>VPRC</u> <u>(ml/100 ml)</u>
<u>Heart Blood</u>			
Mean	2.5	5.97	32.5
S.D.	1.0	0.40	2.0
<u>Venous Blood</u>			
Mean	7.17	7.58	40.2
S.D.	1.5	0.24	1.3

Duplicate blood samples for hematologic examinations were collected from free-flowing venous blood of unanesthetized rats to evaluate variability between the two replicate samples. Results were compared from a group of 40 rats (Table IX).

TABLE IX. Comparison of Hematologic Values from Two Venous Blood Samples of Rats (N=40)

	<u>Leukocytes</u> <u>($\times 10^3/\text{mm}^3$)</u>		<u>Erythrocytes</u> <u>($\times 10^6/\text{mm}^3$)</u>		<u>VPRC</u> <u>(ml/100 ml)</u>		<u>MCV</u> <u>(μ^3)</u>	
	<u>Sample</u>		<u>Sample</u>		<u>Sample</u>		<u>Sample</u>	
	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>
Mean	13.8	13.7	7.46	7.50	35.5	35.7	47.7	47.6
S.D.	6.6	7.1	0.95	1.00	6.9	7.2	6.0	6.1

Existing methods for preparing bone marrow smears are inadequate for precise evaluation of mitotic indices with and without colcemid treatment. Several techniques were examined to reduce the number of

smudge cells (e.g., those damaged beyond classification) to a minimum. Marrow smears of excellent cytologic quality are now achieved by using a modified squash preparation and siliconized slides. To further evaluate these techniques, eight rats were given two intraperitoneal injections of 2.5 µg colcemid/gm of body weight 2 hours apart and then were killed 2 hours after the last injection. The mean mitotic index was $9.9 \pm 2.6\%$. Some necrotic cells were observed, so the colcemid dose will be reduced in future evaluations. Mitotic indices from mouse marrow were similar to those of rats.

2. Future Plans

Young adult Swiss-Webster mice and Sprague-Dawley rats will be used in the initial studies of both acute and subacute exposures at the electric field strength established by the criteria studies for 15 and 30 days. There will be one exposed, one sham-exposed, and one cage control group, with blood and bone marrow taken from 15 mice and 10 rats killed in each group following this schedule. These studies will be integrated with those evaluating immunologic and histologic effects in order to correlate observed changes.

Peripheral blood examinations will include determination of leukocyte, erythrocyte, and hemoglobin concentrations, volume of packed red cells (VPRC) and the red cell corpuscular constants (MCV, MCH, MCHC), all of which will be performed using a Coulter Counter (Model S).

Blood smears for leukocyte differential cell counts will be made, stained in Wright-Giemsa, and the differential count based on 200 cells

(100 cells counted by each of two medical technologists). Changes in red blood cell size and osmotic fragility may indicate subtle changes produced by either intra- or extracorporeal damage. Red cell osmotic fragility determinations will be performed with an instrument which employs an electronically integrated microdialysis membrane, a photometer and a strip chart recorder. Reticulocyte counts, an excellent indicator of bone marrow response to various insults, will be determined on peripheral blood stained with new methylene blue, and the enumerations will be performed by the same technologist in order to enhance the reliability of the test. Platelet concentrations will be determined in duplicate from blood collected in EDTA and counts made using phase microscopy. Femoral bone marrow will be evaluated from smears stained in Wright-Giemsa stain. The marrow mitotic index will be determined after colchicine administration in 50% of the animals.

Screening for effects of electric field exposure on various organ systems will include the following serum chemistry parameters: urea nitrogen, triglycerides, serum glutamic pyruvic transaminase, and total protein. These tests will be performed using an automated chemistry analyzer (CLINICARD). In addition, protein electrophoresis using cellulose acetate membranes will evaluate any possible alterations in α , β , or γ globulins. All the above tests will be performed on sera from the individual animals. Remaining sera from each group will be pooled to assay for calcium, phosphorus, glucose, sodium, and potassium concentrations. Although less desirable, this pooling is necessary because of

the small blood volume of rodents; it will permit evaluation of those parameters which might indicate trends for later in-depth studies.

From the results of these screening studies the most sensitive parameters will be selected to evaluate the relationship of any effect to field strength and duration of exposure. Throughout this project, correlation of hematologic effects will be determined in rats and mice, and ultimately compared to results of concurrent studies in miniature swine exposed to electric fields in EPRI Project RP-799. Such comparative evaluations are important to assure that any observed effects are not unique to rodents.

Additional tests will be considered after completion of the screening and dose-effect experiments. These will include evaluation of the hematopoietic stem-cell pool using both in vitro and in vivo methods. The determination of red cell life span and iron kinetic studies may indicate alterations not observed by less sensitive methods. Spontaneous and mitogen-induced lymphocyte transformations may indicate changes in lymphocyte populations not discernable by routine evaluations. Cytochemistry of circulating lymphocytes could reveal shifts in T and B lymphocyte populations and will be included in the second phase of the study. To examine leukocyte function, the ability of peripheral white cells to phagocytize and reduce foreign material can be determined using a nitro blue tetrazolium assay in vitro.

A high-leukemia strain of mice (AKR/J) may also be utilized. Such a test system could be valuable for detecting subtle changes induced by

electric fields that are not manifested in normal animals because of a long latent period. An alteration in the latent period in a high-leukemia strain could be extremely important for evaluating possible electric field effects on populations at risk.

B. IMMUNOLOGY - Dr. J. E. Morris in charge

The objective of the immunological studies is to describe the effects (if any) of high strength electric fields on the immune system of exposed animals. The importance and significance of determining any alterations that may occur in the immune mechanism can be understood by noting the two major functions of the system which are to prevent infection and disease produced by bacteria, viruses and fungi and to survey the tissue cells of individuals for immunocompetence.

In general, the immune system can be divided into two major functional categories, the humoral and cellular. Table X lists the major components and general functions of the humoral and cellular categories.

Our approach to assess any possible immunological deficiency that may occur as a result of exposure to electric fields will be to measure biological parameters which indicate the state of key humoral and cellular components of the immune system. We plan to evaluate: 1) T and B cell populations and distribution in the peripheral blood, 2) serum immunoglobulins, 3) complement levels and activity, 4) specific antibodies to known antigens, and 5) specific assays that correlate with cell-mediated immunity. The first three measurements provide a quantitative assessment of humoral and cellular components and the last two assess the functional aspects of the two components.

TABLE X. Major Components and Functions of the Immunological System

	Major Components	General Function
Humoral Arm	B lymphocytes (thymus-independent)	Production of antibodies
	Immunoglobulins (proteins produced by differentiated B cells)	Effector molecules for neutralizing foreign cells
	Complement (amplifying mechanism in immune response)	Destruction of bacteria viruses and tumor cells
Cellular Arm	T lymphocytes (thymus-dependent)	Cell-mediated immunity
	Lymphokines	Trigger proteins produced by T cells to stimulate and direct other cells
	Macrophages (phagocytic cells)	Engulf foreign cells and particles

1. Progress to Date, Humoral Studies

Serum levels of immunoglobulin G(IgG), immunoglobulin-M(IgM), complement and peripheral B lymphocytes have been quantified. A double antibody radioimmune assay was set up for measuring the serum immunoglobulins in mice. The assay uses goat anti-mouse IgG or IgM as the primary antibody and horse anti-goat as the second antibody. The assay measures IgG or IgM by inhibition of binding of ^{125}I tagged antigen and is capable of detecting IgG or IgM as low as 50 nanograms per milliliter. The development of the radioimmune assay for measuring serum immunoglobulin in rats is in progress.

For the in vitro studies of peripheral B lymphocytes, mouse lymphocytes were isolated with a ficoll-isopaque density gradient. With this system we are able to recover approximately 80% of the lymphocytes per unit volume with 95% or better of the isolated cells being lymphocytes. We are able to label about 25-30% of the isolated lymphocytes with fluorescent-labeled goat antibody to mouse immunoglobulins. This in vitro study provides a quantitative measure of cells associated with antibody synthesis (B cells).

The assay adapted for measuring complement activity (hemolysin titers) uses sheep red blood cells (SRBC) as the indicator or target cells, rabbit anti-SRBC as the standard hemolysin (antibody to SRBC) and guinea pig serum as a standard control for comparison of complement activity in mice and rats. This assay has been standardized to use a manually operated system, and is now being adapted to an automated technique.

In humoral functional studies the emphasis is on the effects of electric field exposure on the primary and secondary immune responses of animals. Preliminary work in this area has included range finding experiments for optimum immunization of mice by intraperitoneal injection with keyhole limpid hemocyanin. The optimum immunization dose appears to be 10 mg. This gives us the capability to assess suppression or stimulation of antibody production as a result of exposure to electric fields.

2. Progress to Date, Cellular Studies

This component of the immune system is being studied with an in vitro assay for T-lymphocytes in peripheral blood and in vivo measures to correlate with cell-mediated immunity.

An assay employing spontaneous rosette formation by T lymphocytes with sheep red blood cells (SRBC) has been adapted for quantitating T cells in peripheral blood. Lymphocytes were separated with a ficoll-isopaque density gradient and washed extensively in a balanced salt solution. The wash-separated cells were then incubated with SRBC and placed in an ice bath. The rosettes are quantitated in a white blood cell counter after gentle mixing. Approximately 60-70% of the isolated lymphocytes formed rosettes, a value in good agreement with data reported in the literature.

Procedures for assessing intact cell-mediated immune function have been evaluated and standardized. The test for rats uses the response to topically applied DNCB and the delayed hypersensitivity to BSA, as determined by macrophage migration inhibition and increased skin thickness. Cellular immune function in mice will be evaluated by delayed hypersensitivity to BSA and DNCB.

The delayed hypersensitivity analysis serves as a general measure of T-cell function and production of lymphokines and macrophage activity. The specific lymphokine being studied is the macrophage migration inhibition factor.

3. Future Plans

In the first series of screening experiments, studies will be conducted to evaluate the effects of electric field exposure on the primary and secondary response of the humoral immune system. Serum levels of immunoglobulin-G(IgG), immunoglobulin-M(IgM) and complement will be measured in exposed and sham-exposed mice.

These experiments will be followed by a study involving groups of mice immunized with a specific antigen complex (hemocyanin-dinitrophenol) before and after exposure to the electric field to evaluate specific antibody activity.

The cellular component of the immune system will be investigated initially by lymphocyte counts of exposed and control mice. This will be followed by in vivo and in vitro quantitation of cell mediated immunity. These studies will be augmented in FY 78 with differentiation studies of peripheral lymphocytes, spleen and lymph nodes with rosette formation techniques, and by fluorescent labeling studies with anti-mouse immunoglobulin antibodies.

C. PATHOLOGY - Dr. J. E. Lund in charge

A recent Russian publication⁵ has reported significant pathological effects from electric field exposure in tissues of brain, myocardium, liver, kidneys, adrenals, and thyroid. Insufficient information is presented in the report to allow evaluation of the findings. A detailed gross and microscopic examination will be made of various organs, including those reported to be damaged in the Russian report, to document any possible effects of electric field exposure on tissue morphology.

1. Future Plans

Ten rats from the first chronic exposure group (scheduled to come off exposure in May, 1977) as well as 10 sham-exposed and 10 cage control rats will be sacrificed and examined for possible tissue damage at the gross and light microscopic level. If these results are negative, no further pathology is planned unless deemed necessary from results of other investigations. If indicated, a search for ultrastructural lesions will be initiated.

D. PHYSIOLOGY - Dr. R. D. Phillips in charge

Several reports in the literature indicate possible effects of electric field exposure on the cardiovascular system^{5,7} and on body growth.^{6,8} Several experiments will be conducted in the initial biological screening studies to assess these reported effects. Electrocardiograms will be made and heart rates determined on rats after exposure to electric fields for 8 hours, 40 hours (8 hours/day for 5 days) and approximately 630 hours (21 hours/day for 30 days). In another experiment, metabolic status of rats will be evaluated both during and following 30 days of exposure to electric fields by making daily measurements of body weight, food consumption and water consumption. At the end of the exposure period, metabolic rates will be determined and weights recorded of the brain, liver, heart, testis, pituitary gland and adrenal glands. Subsequent experiments on cardiovascular and metabolic functions will be contingent on the results of these initial studies.

1. Progress to Date

The technique for measuring oxygen consumption rate ($\dot{V}_{O_2}$) and carbon dioxide production rate ($\dot{V}_{CO_2}$) of rats has been completed and standardized. The determinations are made in an open system in which $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ are calculated by measuring the amount of oxygen the animal removes from an air sample and the amount of carbon dioxide it adds to the air sample. For these measurements, the animal resides in a modified Bollman holder (Figure 22) with its head extended through the hole in the endplate into the cone. By means of a vacuum pump in the system, ambient air is pulled into the cone through four 1/8-inch holes in the endplate, past the animal's head, then out through a tube attached to the tip (outlet) of the cone. The air is passed through a water absorber, filter, flowrator and then through a paramagnetic oxygen analyzer and an infrared carbon dioxide analyzer. By maintaining a constant flow rate and measuring the amount of O_2 removed and CO_2 added to the air sample, one can calculate $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$. The respiratory quotient, or R.Q., can be calculated by simply dividing $\dot{V}_{CO_2}$ by $\dot{V}_{O_2}$.

Mean values obtained for $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ of five rats well adapted to the Bollman holder were 0.021 ± 0.001 ($\pm$ S.E.) and 0.019 ± 0.001 ($\pm$ S.E.) ml/g, respectively. The calculated R.Q. is 0.91. These values are consistent with those given in the literature for a nonstressed rat.

Two experiments were conducted to determine the effects of electric field exposure on heart rates of rats. In one experiment, 12 rats were exposed to 80 kV/m for 8 hours and heart rates were measured for 1 hour

immediately after the completion of exposure. Another 12 rats were sham-exposed and measured the same way. In the second experiment 12 rats were sham-exposed (controls) and 12 were exposed to 80 kV/m for 40 hours (8 hours/day for 5 days). At the end of the last sham-exposure or exposure session, heart rates were determined for 1 hour.

All heart rate determinations were made on unanesthetized rats contained in Bollman holders (Figure 22). Electrocardiograms were recorded from two surface electrodes located on the lateral surfaces of the thorax. The electrodes were attached to and held in place by a soft rubber belt (15 x 2 cm) placed around the thorax of the animal and secured with an adjustable fastener. Heart rates were determined electronically using tachograph units that convert the beat-to-beat interval into a linearly displayed rate function.

The animals were extensively adapted to the Bollman holder, ECG belt, and electric field exposure cages for 2 weeks prior to exposure or sham exposure. A brief outline of the acclimation schedule for the first experiment is shown in Table XI.

TABLE XI. Acclimation Schedule for First Experiment to Determine the Effect of Electric Field Exposure (80 kV/m for 8 hours) on Heart Rates of Rats

<u>Days</u>	<u>Procedure</u>
1-2	1 hour Bollman holder acclimation, 2 times/day
3-6	2 hours Bollman holder acclimation, 2 times/day
7-10	2 hours Bollman holder and ECG belt acclimation, 2 times/day
11-12	8 hour exposure room and exposure cage acclimation with heart rates measured for 1 hour at the end of the 8 hour period.
13	Exposure or sham-exposure for 8 hours with heart rates measured for 1 hour at the end of the 8 hour period.

FIGURE 22. Modified Bollman holder used to contain rats for respiratory gas determinations. The same type of holder, without the head cone, was used to contain the rats for heart rate measurements.

In preliminary experiments it was found that this amount of acclimation was sufficient to insure the animals would not be stressed by confinement in the Bollman holders or by the other experimental procedures.

Heart rate determinations made on the 11th and 12th days of acclimation for the groups designated for exposure or sham exposure on day 13 are shown in Figures 23 and 24. There are no significant differences between groups on the 2 days prior to exposure.

On day 13, one group was exposed to 80 kV/m for 8 hours and the other group was sham-exposed in the Special Studies Exposure System in standard exposure cages. Heart rates were measured for 1 hour after the end of exposure. The results are shown in Figure 25. Exposure to 80 kV/m has no significant effect on heart rate of rats.

In the second experiments, 12 rats were exposed and 12 rats sham exposed to 80 kV/m for 8 hours/day for 5 consecutive days. Heart rates were measured for 1 hour after the completion of the last exposure session. The results are shown in Table XII. Exposure to 80 kV/m for 40 hours (8 hours/day for 5 days) has no significant effect on the average heart rate over the 60 minute measurement period (t-test). A full statistical analysis of this data within the 60-minute measurement period will be given in the next semiannual report.

Experiments are now in progress to determine whether exposure to 60-Hz electric fields alters the time characteristics of the ECG as reported by Blanchi.⁷

FIGURE 23. Mean heart rates ($\pm$ S.E.) of rats two days prior to exposure or sham-exposure to electric fields at 80 kV/m for 8 hours.

FIGURE 24. Mean heart rates ($\pm$ S.E.) of rats one day prior to exposure or sham-exposure to electric fields at 80 kV/m for 8 hours.

FIGURE 25. Mean heart rates ($\pm$ S.E.) of rats after 8 hours of exposure or sham-exposure to 60 Hz electric fields at 80 kV/m. Measurements made for 1 hour immediately following exposure or sham exposure.

TABLE XII. Mean Heart Rate ($\pm$ S.E.) of Rats After 40 Hours Exposure (8 hours/day for 5 days) to 60 Hz Electric Fields at 80 kV/m. Measurements made for 1 hour immediately following the last 8-hour exposure. N = 12/group.

Elapsed Time (min)	Mean Heart Rate $\pm$ S.E.	
	<u>Exposed</u>	<u>Sham Exposed</u>
5	431 $\pm$ 16	415 $\pm$ 10
10	416 $\pm$ 16	393 $\pm$ 14
15	383 $\pm$ 9	361 $\pm$ 11
20	368 $\pm$ 9	343 $\pm$ 13
25	366 $\pm$ 10	362 $\pm$ 15
30	365 $\pm$ 14	334 $\pm$ 10
35	355 $\pm$ 9	336 $\pm$ 9
40	382 $\pm$ 14	344 $\pm$ 15
45	361 $\pm$ 13	340 $\pm$ 10
50	352 $\pm$ 11	356 $\pm$ 17
55	351 $\pm$ 10	360 $\pm$ 16
60	<u>362 $\pm$ 12</u>	<u>359 $\pm$ 20</u>
1-hour MEAN	376 $\pm$ 27	359 $\pm$ 27

2. Future Plans

Experiments to investigate possible effects of electric field exposure on metabolic rate are planned. Ten exposed and 10 sham-exposed rats from the first group of chronically exposed rats will be used to investigate possible effects of 60 Hz electric fields on metabolic rate. This exposure will be for 30 days at the highest field strength consistent with the findings of the criteria studies. During the 3-hour period each day when the rat exposure system is turned off for animal care and other maintenance, the animals will be acclimated to the Bollman holder. At the end of the 30-day exposure, O_2 and CO_2 measurements will be made on all animals. The respiratory quotient and the metabolic rate will then be computed for each animal. These measurements will be supplemented by daily measures of body weight, food consumption and water consumption on 48 rats (24 exposed and 24 sham-exposed) during the 30-day exposure period to provide a complete picture on the metabolic status of animals exposed to electric fields.

In a future study, a gradient layer calorimeter will be used to measure metabolic rates directly. Animals will be measured immediately after exposure.

A study will be performed to investigate possible effects of chronic exposure to electric fields on ECG and heart rates of rats. Animals will be exposed for 30 days (~ 21 hours/day) with ECG and heart rate measurements made after exposure. A complete evaluation of the ECG tracings also will be made.

summation of the combined action potentials occurring in all the individual axons can be recorded by electrodes placed on the outside of the nerve. The electrical activity thus recorded is called the compound action potential. It is possible to detect many forms of altered nerve cell function by examining characteristics of the compound action potential.

The point at which one nerve cell makes functional contact with another nerve or muscle cell is called a synapse. The propagation of action potentials between nerve cells is called synaptic transmission. Synaptic transmission is a complex chemically-mediated event. When an action potential arrives at a synapse it causes the nerve cell to release a chemical substance (neurotransmitter) which rapidly diffuses across the space between the two nerve cells. Upon contacting the membrane of the second cell, an action potential is generated. Therefore, information, coded in the form of action potentials, can be transmitted from one point to other points in the nervous system. The complex process of synaptic transmission is very susceptible to external influences such as nutritional status, drugs, and environmental "pollutants". A detailed examination of synaptic function should provide a very sensitive indicator capable of revealing subtle changes in nervous system function as a result of exposure to electric fields.

Muscle cells contract in response to action potentials synaptically transmitted from associated motor nerve cells. The specialized synapse between a nerve cell and a muscle cell is called the neuromuscular junction. Thus the strength of muscle contraction as well as many other

In the event no changes in baseline values are observed in the initial screening experiments, studies will be made to evaluate possible effects of electric field exposures on physiological reserve capacities. Various stress tests will be used for these studies, such as the metabolic response to cold exposure to determine metabolic reserves

An attempt will be made to study the effects of electric fields on cardiovascular and metabolic functions during exposure. Animals will be monitored for ECG, heart rate, O_2 consumption and CO_2 production while they are being exposed to electric fields. Many of the difficulties associated with obtaining these measurements (electrical interference and artifacts) will need to be resolved before these experiments are started.

E. NEUROPHYSIOLOGY - Dr. R. A. Jaffe in charge

All living cells exhibit a resting potential difference across their outer membrane. In mammalian nerve and muscle cells this voltage difference ranges between -50 and -70 mV, the inside of the cell negative with respect to the outside. When a nerve or muscle cell is stimulated, an abrupt change in the membrane potential occurs, the so-called action potential. An action potential occurring in one region of the cell membrane is capable of stimulating adjacent regions of the cell membrane, and thus is able to propagate itself from one end of the cell to the other (a distance that may exceed 1 meter).

Any nerve consists of many thousands of individual nerve cell processes called axons. When the whole nerve is stimulated the electrical

characteristics of muscle function are not simply dependent upon properties of the muscle itself. Effects on the motor nerve, the neuromuscular junction, or the muscle itself can all be manifested by changes in the characteristics of muscle contraction. In order to assess the effects of any treatment (e.g., exposure to electric fields) on muscle function, therefore, it is necessary to examine the intact nerve-muscle preparation.

1. Future Plans

The neurophysiology screening experiments have been divided into three principal areas to provide a broad coverage of the fundamental components of mammalian nerve and muscle systems.

- a. **Peripheral Nerve Function:** To investigate peripheral nerve function, an autonomic nerve (vagus) and a general somatic nerve (sciatic) will be studied in order to assess any possible effects of electric field exposure on the ability of specific nerve fiber types to generate and conduct impulses. Specific parameters to be examined include: conduction velocity, characteristics of the compound action potential, refractory period, and strength-duration relationships.
- b. **Neuromuscular Function:** Possible effects of electric fields on neuromuscular function will also be investigated. The gastrocnemius muscle and tibial nerve preparation will be used. Specific parameters to be examined include: strength of contraction, contractile properties such as fusion frequency and tetanus, post-tetanic potentiation, and fatigue-induced changes in muscle function.

- c. **Synaptic Transmission:** The ability of one nerve cell to transmit information to other nerve or to muscle cells is a fundamental property of a normally functioning nervous system. In order to assess the possible effects of electric field exposure on synaptic transmission, the rat superior cervical sympathetic ganglion will be studied. Specific parameters include: properties of the post-ganglionic compound action potential, frequency response, conditioning-test response, conduction velocity and synaptic delay, and fatigue-induced changes.

F. CENTRAL NERVOUS SYSTEM - Dr. J. C. Hampton in charge

Effects of electric fields on humans that have appeared in the literature are mostly subjective and suggest possible effects on the central nervous system (CNS).⁴ Other functional impairments that have been reported,⁵ such as loss of libido, also may be mediated by disturbances in the CNS. Accordingly, this study is intended to evaluate vascular permeability in the CNS and to examine the microstructure of brain tissue to look for possible structural changes in the nerve tissue itself.

1. Future Plans

Sixteen mice will be exposed for 30 days to the largest electric field consistent with the findings of the criteria studies. At the end

of the exposure these animals will be injected with the tracer horseradish peroxidase and then sacrificed. The brain tissue will be examined with an electron microscope to document any submicroscopic changes in the structure of nerve cells and fibers and any alterations in vascular permeability to low molecular weights tracers. Results will be compared to those obtained from population of sham-exposed control animals. If effects are observed, further experiments will be made to investigate the relationship between field strength and the nature of the observed effects.

G. ENDOCRINOLOGY AND MALE REPRODUCTION - Dr. M. J. Free in charge

Previous research has reported exposure to electric fields has an adverse effect on libido in the male,⁵ produces stress,⁶ and has an effect on thyroid function.⁵ Studies will be made to investigate endocrine glands related to the reported effects.

1. Future Plans

Possible effects of electric field exposure on general endocrine function will be investigated in the first series of screening experiments. Forty animals will be divided into two experimental groups. Twenty animals will be exposed for a period of 30 days. The other 20 will be sham exposed. An additional group of 10 cage controls will be used.

At the end of the exposure period, each animal will be quickly guillotined and organ weights will be made of the pituitary, adrenals,

thyroid, thymus, testes, epididymides, seminal vesicles, and coagulating glands. Histologic examination will be made on the adrenals, thyroid, and testis; the thymus and pituitary will be fixed and stored for possible future study. In addition, an assay will be made of the following circulating hormones: glucocorticoids, thyroid hormones, androgens, and gonadotrophins.

The emphasis of the second experiment will be the male reproductive system. Two groups of 10 rats will be exposed and sham exposed for a 30-day period. The experiment will also include a group of 10 cage controls. Exposures will be staggered so that only a few animals will be removed from the system each day.

At the completion of the 30-day exposure period, each animal will be anesthetized with halothane/O₂/NO₂. A testicular vein and a femoral and right carotid artery will be catheterized. Plasma samples (200 µl) will be taken over 10 minutes from the testicular and femoral artery for multiple androgen analysis. When sampling is completed ¹³¹I-labeled microspheres will be injected into the left ventricle of the heart via a carotid catheter. A reference sample will be drawn from the femoral artery and the testis will be quickly excised for assay of radioactivity. Testicular blood flow and androgen secretion rates will be calculated for each animal. Androgens assayed will include testosterone, dihydro-testosterone, dehydroepiandrosterone and androstane diols.

Additional experiments will be performed as indicated by the results of these first screening experiments.

H. BONE GROWTH AND STRUCTURE - Dr. B. J. McClanahan in charge

A number of investigators⁴ have shown bone growth and structure is affected by DC electric currents, and perhaps AC currents, in the range of 5-10 μ A. Since significant current densities will occur in the limbs of rats during exposure to large electric fields, it was deemed important to examine for possible effects on bone growth and structure.

1. Future Plans

An experiment will be made to examine several parameters related to possible effects of electric field exposure on bone growth and structure. Two groups of 10 weanling rats will be exposed or sham exposed for a 30-day period. A third group of 10 will serve as cage controls. On days 27 and 28 of exposure each rat will be injected intraperitoneally with Terramycin; on day 29 each will be injected with ⁴⁵Ca. At the end of the 30-day exposure period all animals will be sacrificed.

A number of parameters will then be examined. The ⁴⁵Ca content of the liver, kidney, tibia, lumbar vertebra, serum, and urine will be measured. On samples of tibia and lumbar vertebra the following measurements will be made: length of periosteal, endosteal, and trabecular surfaces; area of cortical and trabecular bone and marrow space; and the length of active surfaces from Terramycin labeling. The concentration of calcium and phosphorous in blood serum obtained at the time of necropsy will also be measured.

Further experiments will be designed on the basis of the results of this first experiment.

I. GROWTH AND DEVELOPMENT - Dr. M. R. Sikov in charge

Growth and development have been found to be sensitive indications of the deleterious effects produced by a variety of physical insults. Yet, previously published experiments have not looked at possible effects of electric field exposure on reproduction, prenatal, and postnatal growth and development. In this project several measures will be used to screen for possible effects. If effects are found research will be performed to establish dose-response relationships and sensitive periods of development.

1. Progress to Date

As the design of the exposure facility progressed, it became apparent that there might be conflicts between the animal husbandry requirements for the studies of growth and development and the engineering requirements. These related to the 4" cage height and grounding requirement and evolved into three primary questions:

1. Could rats mate with the reduced cage height?
2. Would parturition and rearing progress normally with the reduced ceiling height?
3. Would rats rear their litters successfully without the bedding materials usually used for nesting?

Preliminary studies, reported earlier, suggested that parturition and rearing were not adversely affected by the lowered cage height but

that the absence of nesting material had a deleterious effect. Subsequent studies have been performed using lexan cages (4" height) in a prototype module identical to those to be used in the exposure system. These experiments demonstrated that mating frequency in the prototype cages was the same as for rats in a standard caging arrangement. Other experiments confirmed the preliminary observations, so that subsequent efforts have centered on identifying a litter material which would satisfy the two primary criteria:

1. The material must be electrically conductive so that there would be no marked potential differences between the cage floor, the mother, and the pups.
2. The material must be satisfactory for nesting and must be nontoxic to the pups.

Three materials were evaluated and eliminated on physical basis:

1. Moistened San-i-cel (the routine litter material) - Difficulties in providing uniform and consistent moistening and the increased humidity.
2. Metal-coated Christmas tree tinsel - Electrically conductive but migrated upwards in the electric field.
3. Brunslon (Bigelow Carpet), spun nylon with 2% fine stainless steel fibers used for antistatic carpeting - insufficient conductivity. Although additional stainless steel fibers could be added, it would be anticipated that they would migrate and serve as a focus for corona.

Two additional materials, which were physically suitable, have been found to be less than adequate in the biological sense:

1. Ball bearings satisfy the maternal nesting instincts but do not appear to provide the necessary thermal insulation needed in the neonatal period.
2. Carbon granules tentatively seemed to meet all criteria although it was unaesthetic. On necropsy of the offspring, moderate amounts of carbon were noted in the gastrointestinal tract although the lungs appeared clear. Preliminary microscopic examination of sections of the lungs suggest that there may be carbon-containing histiocytes, which might lead to undesirable consequences. This is being studied further before the use of carbon is definitely ruled-out.

Accordingly, the search for an appropriate material has continued.

1. Antron-III, a Dupont carpet fiber in which some fibrils contain a carbon core has been subjected to preliminary test and thus far appears satisfactory.*
2. Herculon, a conductive sheeting has been obtained but not yet tested.
3. Vitreous carbon filaments - arrangements are being made to obtain samples for evaluation.

* Experiments have been completed and it appears Antron-III meets the primary criteria; it is sufficiently conductive to eliminate spark discharge problems and it is biologically compatible.

4. Stainless steel or copper scouring pads - an unlikely, but possible, solution which has been suggested.

2. Future Plans

The experiments described here have been designed to screen for potential effects of electric fields on several measures of reproduction and prenatal and postnatal development. These results will provide a basis for design of subsequent studies to establish dose-response relationships and sensitive periods of development. Each experiment will use an incremental design, consisting of multiple overlapping replicates, to maximize the use of the special cages required and to accommodate the needs of other investigators.

Experiment I - Reproductive Behavior and Embryotoxicity

Within each replicate, two male and eight female rats will be exposed to the field, or sham exposed, for 6 consecutive days, while housed in their individual home cages. They will then be randomly assigned and transferred to the mating cages where they will cohabitate for 5 additional days; the animals will continue to be exposed to electric fields during this time. Daily vaginal smears will be obtained to determine the occurrence of mating within this period. It is estimated that four to five increments will be required to obtain the desired 20 mated females in the exposed and sham-exposed groups.

At the end of this 5-day mating period, all rats will be returned to their single cages for continued exposure. The males will be exposed

for an additional 20 days (30 days total exposure) and then allowed to cohabit with normal females to determine effects on libido, ejaculate volume, and sperm counts. The animals will be killed and the testes preserved for histological examination if alterations in sperm count or gross morphology are noted.

The female rats which mate will receive exposure until 20 days of gestation, when they will be killed for study of embryotoxicity. Prenatal mortality will be established and variations in the quality or color of amniotic fluid or in the size, shape, and color of the placenta will be determined. All fetuses, live or dead, will be weighed, sexed, and examined for external malformations. The live fetuses will be examined for internal abnormalities by dissection and examination of serial cross-sections. The other fetuses will be cleared and stained by the alizarin-red technique and examined for skeletal defects.

The nature of any morphologic abnormalities should allow for tentative identification of effects and will permit design of subsequent studies during more narrowly defined periods of gestation, to specifically identify the developmental stage affected and the relevant dose-response relationships.

Experiment II - The Postnatal Sequelae of Prenatal Exposure

The postnatal sequelae of prenatal exposure will be studied in the second experiment which again uses an incremental design. Within each replicate, four female rats will be mated and placed in individual cages

in the field or sham field. At 18 days of gestation they will be transferred to the larger littering cages, to permit delivery and rearing of the pups. The rats and their litters will be removed from the field when the pups reach 8 days of age, yielding a total of 30 days exposure. A total of 20 sham exposed and 20 experimental females will be used.

The litters will be examined immediately following parturition to establish the number of living and stillborn offspring as well as the subsequent survival of the offspring. The offspring will be weighed and examined for gross anomalies or pathology at birth, on removal from the field, and at weekly intervals through 7 weeks of age. They will be observed to determine the time of the opening of the vagina or descent of the testes and of eye opening.

Reflex development and general neuromuscular development will be tested in neonates, and animals of several sequential ages, to determine whether there are any deficiencies and if so, whether they are transient or reflect more permanent effects.

Evaluation of development by the neonates will include reflexive grasping of a fine wire laid against the palmar surface of the paw and the retraction of the paw when electrically stimulated. After removal from the field, the pups will be evaluated by recording the occurrence of spontaneous movement, such as departure from a designated area, head lifting, grooming, and attempts and success at escape from a test box, and the elicited startle response using a brief flash of light. The upright response; and performance on the inclined plane (a measure of

negative geotropism), and the presence of the righting reflex will be determined. The animals will be examined for the placing and hopping reactions, for evidence of ataxia or spasticity of the limbs, and for gait defects.

At 7 weeks of age, one male and four females will be randomly selected from each litter for study of reproductive integrity. These animals will be returned to the field and reproduction and development studied utilizing the protocol defined for Experiment I. This will also establish whether or not prenatal exposure had sensitized the animals to subsequent exposure. The remaining animals will be tested for their susceptibility to audiogenic seizures to assess for possible effects of exposure on central nervous system excitability.

Experiment III - The Effects of Neonatal Exposure

The effects of neonatal exposure will be studied in a third experiment, which will allow evaluations when the central nervous system and the male gonads mature. This study will be initiated following completion of Experiment II, again using multiple replications to yield a total of 20 exposed and 20 sham-exposed rats. In this manner, the littering cages constructed for the earlier study can be reutilized. For this study, however, pregnant rats maintained in the animal quarters will be introduced into the littering cages in the electric field or sham field at 17 days of gestation. They will be maintained there for 26 additional days, (21 days of age) at which time the offspring will be weaned. The offspring will be separated into exposed or sham exposed cages for an additional 4 days to evaluate postweaning survival.

The test procedures to be employed will be similar to those used in Experiment II; minor modifications related to evaluations which cannot be performed while the animals reside in the field may be required. It is also recognized that other modifications suggested by earlier findings may be desirable.

J. ANIMAL BEHAVIOR - Mr. D. L. Hjeresen in charge

Behavioral studies are in progress as part of the criteria studies to establish the maximum electric field strength that does not produce behavioral avoidance of electric fields by rats. Current results indicate rats spend more time in an unexposed (shielded) compartment of a shuttlebox than in the exposed (unshielded) compartment at a field strength of >90 kV/m. Another study has been completed that shows the threshold does not change with repeated exposures; the animals do not acclimate or become more sensitive with repeated exposures to electric fields.

1. Future Plans

Behavioral studies scheduled for the remainder of FY 77 and for FY 78 include:

1. Experiments to determine the motivating qualities of electric fields to allow a definition of any possible aversive characteristics of the field.
2. Threshold detection experiments to determine possible differences in detection and avoidance thresholds.

3. Experiments to evaluate changes in work related activity due to electric field exposure.
4. Experiments to determine whether exposure to electric fields produces a nonspecific stress reaction by using electric field exposures as a stimulus in a learning task for avoidance behavior.
5. Experiments to determine the modality of electric field perception.

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