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BIOLOGICAL EFFECTS OF HIGH STRENGTH ELECTRIC FIELDS
ON SMALL LABORATORY ANIMALS

Interim Progress Report March 9, 1976—September 8, 1976

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MASTER

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Battelle Pacific Northwest Laboratories
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ENERGY RESEARCH AND
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Division of Electric Energy Systems

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SEPTEMBER 1976

Prepared by

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TABLE OF CONTENTS

	<u>Page</u>
LIST OF FIGURES.	v
LIST OF TABLES	vii
 I. INTRODUCTION	 1
A. NEED	1
B. SCOPE.	1
C. OBJECTIVES	2
D. SCHEDULE AND MILESTONES.	3
E. PROJECT ORGANIZATION AND PRINCIPAL INVESTIGATORS.	4
II. EXPOSURE SYSTEMS	7
A. DESIGN CONSIDERATIONS AND GOALS.	7
1. Choice of Field Configuration.	7
2. Electrode Spacing.	8
3. Control of Dosing.	13
4. Maximum Field Strength	14
B. PROTOTYPE EXPOSURE SYSTEM RESULTS.	15
1. Exposure Room Design	17
2. Exposure Electrodes.	19
3. High Voltage Supply and Distribution	21
a. Charging Current Requirements.	21
b. High Voltage Distribution.	25

4. Electric Field Uniformity.	25
a. Measurement Procedures	26
b. Electric Field Measurements.	33
c. Effect of Room Walls	40
d. Effect of High Voltage Distribution Bus Work . .	43
e. Conclusions.	46
5. Animal Housing	46
a. Basic Design	46
b. Feeding and Watering Systems	49
c. Effects of Animal Cage on Electric Field	52
6. Safety	57
III. CRITERIA STUDIES	61
A. ANIMAL HOUSING SYSTEM.	62
B. BREEDING STUDIES	67
C. CORONA AND OZONE TESTING	69
D. HAIR STIMULATION, BEHAVIORAL AVERSION, AND SPARK DISCHARGE (SHOCK) EXPERIMENTS.	71
E. EFFECTS OF CAGING CONFIGURATION ON BEHAVIOR AND FIELD STRENGTH	72
IV. BIOLOGICAL SCREENING EXPERIMENTS	75
A. HEMATOLOGY AND SERUM CHEMISTRY	75
B. IMMUNOLOGY	76
1. Humoral Studies.	76
2. Cellular Studies	77

C. METABOLISM AND GROWTH.	77
D. CARDIOVASCULAR FUNCTION.	78
E. ENDOCRINOLOGY.	78
F. CNS STRUCTURE AND FUNCTION	79
G. NEUROPHYSIOLOGY.	79
H. REPRODUCTION, GROWTH AND DEVELOPMENT	79
V. REFERENCES	81

LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
1.	The distortion of an uniform 10 kV/m field caused by a conducting ellipsoidal model of a rat's body. . .	11
2.	The maximum vertical displacement of the equipotential caused by the animal's body.	12
3.	Plan view of the prototype exposure system	16
4.	The prototype exposure room.	18
5.	The prototype exposure electrodes and lucite supports	20
6.	The circuit used to measure the components of the charging current to the electrodes	23
7.	The surface electric field probe	28
8.	The circuit used to measure the electric field at the surface of the lower electrode.	29
9.	The frequency response of the amplifier used in the field measuring system	31
10.	The coordinate system used to describe the field measurements on the lower electrode.	34
11.	The surface electric fields measured at an electrode potential of 5.0 kV.	36
12.	Outlines of the animal cages are superimposed on the lower electrode to show the field strengths at selected points and the average field in each cage.	37
13.	The horizontal components of the electric field at a height of 4 in. above the lower electrode, as estimated from the measurements of the vertical component at the lower electrode surface	39
14.	Electric field plots showing the measured effect of a grounded wall located at 7 in. and 14 in. from the narrow end of the electrode	41

LIST OF FIGURES (Continued)

<u>Figures</u>		<u>Page</u>
15.	a) One way to reduce interactions with the walls is to energize both electrodes symmetrically with respect to ground. In b) is shown a method to simulate this with a single transformer.	42
16.	In a) a wall is simulated by a 32 in. by 50 in. piece of wire mesh. In b) are shown measured plots of field strength when the simulated wall is grounded or energized	44
17.	This arrangement was used to investigate the effects on field uniformity of electrical bus connected to the upper or lower electrodes	45
18.	Measured field plots show the effect of bus connected to the upper or lower electrodes	47
19.	This unit holds eight rats in a lexan cage for exposure to electric fields.	48
20.	This photograph shows a cage module mounted on 1/2-in. stainless steel mesh.	50
21.	The measured effect of a 3/16-in., 4-in. high roof on the surface field strength is shown.	54
22.	The measured surface field strength in the proximity of the center of a 20-in. long, 4-in. high, 3/16-in. thick lucite wall	55
23.	The circled numbers are the measured surface field strengths in the corners and the center of the indicated cage arrangement which included a 4-in. high roof.	56
24.	This block diagram shows the various safety systems which have been incorporated into the final prototype system design.	59

LIST OF TABLES

	<u>Page</u>
I. Prototype Electrode Charging Currents at a Voltage of 5.0 kV.	24
II. Calibration Data for the Surface Electric Field Probe.	32
III. DC Resistivities and AC Resistances per Inch of 1/4-inch Diameter Polyethylene Water Line.	51
IV. Daily Body Weight Gain, Food Consumption and Water Consumption of Rats (N=5/group) Housed for 4 Days in Standard Cages with 7-in. or with 4-in. High Ceilings.	64
V. Average Daily Body Weight Gain, Food Consumption and Water Consumption of Rats (N=10/group) Individually Housed for 30 Days in Standard Cages with 7-in. or 4-in. High Ceilings.	64
VI. Hematologic Profiles and Adrenal Weights of Rats Housed for 30 Days in Standard Cages or Cages with the Ceiling Height Lowered to 4-in.	65
VII. Mean Oxygen Consumption Rates, Carbon Dioxide Production Rates and Respiratory Quotients of Two Groups of Rats (N=5/group) Per Unit Body Mass After 9 Days of Acclimation in a Bollman Holder or a Lucite Tube.	78

I. INTRODUCTION

Authorization to initiate research on this project was received March 9, 1976. This report gives a detailed description of progress made during the first 6 months, through September 8, 1976. Included in this chapter are brief statements of Needs, Scope, Objectives, Schedule and Milestones, and Project Organization and Principal Investigators. Progress made on the Exposure Systems, Criteria Studies and Biological Screening Experiments is described in Chapters II, III and IV, respectively.

A. NEED

The National Electric Safety Code has established criteria for the problems of clearance, flashover, and contact accidents, but it does not consider the possibility of electric field effects on living systems. Available data are inadequate to establish reliable criteria for safe electric field exposure standards. A well-planned and comprehensive research study is needed to establish an improved data base by which safe exposure limits may be better defined.

B. SCOPE

A broad and comprehensive series of biological experiments will be made under strictly controlled laboratory conditions to screen for possible effects of exposure to 60-Hz electric fields. Electric field strengths

comparable to and exceeding those under existing and anticipated transmission line designs will be used. Dosimetry studies will complement the animal studies to establish the relationship between tissue dose and any observed biological effects. Information derived from this project will provide a better basis for evaluating potential hazards of exposure to 60-Hz electric fields and help define parameters to be studied in clinical evaluations on humans.

C. OBJECTIVES

The overall 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 system 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 mice and rats to well-defined and relatively uniform 60-Hz electric fields.
- Conduct a broad and comprehensive series of experiments to investigate the effects of exposure to 60-Hz electric fields.
- Establish the relationship between effect, field strength and duration of exposure for any effects observed in the screening experiments.
- Conduct dosimetry investigations to complement the animal studies.

D. SCHEDULE AND MILESTONES

The project schedule for FY 1976 was outlined in the original proposal⁽⁵⁾ and restated in our first monthly progress report for March, 1976.

Milestones achieved during the first 6 months of the project include:

- The prototype system for exposing rats and mice to uniform, 60-Hz electric fields has been designed, fabricated and tested.
- The electric field has been measured in the prototype exposure system and is acceptably uniform ($\pm 5\%$).
- A system for caging, feeding and watering rats during exposure to electric fields has been designed and fabricated. Preliminary tests indicate the effects of this system on electric field uniformity is small ($<5\%$).
- Biological experiments are in progress to insure the exposure housing system meets good animal care practices and that it does not have any adverse effects on the animals.

- The exposure criteria studies have been started.
- Work has been initiated on many of the biological screening experiments to establish methodologies and baseline data for the biological experiments scheduled to begin late this fall when the rat and mouse exposure systems are ready for use.

E. PROJECT ORGANIZATION AND PRINCIPAL INVESTIGATORS

Given below is an outline of the organizational structure of the project and the names of the principal investigators at Battelle-Northwest who are responsible for accomplishing the Tasks and Subtasks.

Project Manager - R. D. Phillips

Assistant Project Manager - W. T. Kaune

Task A - Exposure System Design and Testing - Task Leader, W. T. Kaune

Subtask 1 - Exposure System Design - W. T. Kaune

Subtask 2 - Electrical Engineering - R. L. Richardson

Subtask 3 - Animal Housing System - J. R. Decker

Subtask 4 - Mechanical Engineer - C. H. Allen

Subtask 5 - Field Measurements - W. T. Kaune

Subtask 6 - Drafting - J. W. Sadler

Subtask 7 - Facility Engineering - O. M. Lyso

Task B - Exposure Criteria Studies - Task Leader, D. L. Hjeresen

Subtask 1 - Behavioral Aversion - D. L. Hjeresen

Subtask 2 - Animal Housing - D. L. Hjeresen

Subtask 3 - Breeding Experiments - M. R. Sikov

Subtask 4 - Hematology and Serum Chemistry - H. A. Ragan

Subtask 5 - Corona, Ozone and Shocks - D. L. Hjeresen

Task C - Dosimetry - Task Leader, W. T. Kaune

Task D - Biological Screening Experiments - Task Leader, R. D. Phillips

Subtask 1 - Hematology and Serum Chemistry - H. A. Ragan

Subtask 2 - Immunology - J. E. Morris

Subtask 3 - Metabolism and Growth - R. D. Phillips

Subtask 4 - Bone Structure and Growth - B. J. McClanahan

Subtask 5 - Cardiovascular Function - R. D. Phillips

Subtask 6 - Endocrinology - J. E. Lund

Subtask 7 - Pathology - J. E. Lund

Subtask 8 - Reproduction, Growth and Development - M. R. Sikov

Subtask 9 - Male Reproduction - M. J. Free

Subtask 10 - CNS Structure and Function - J. C. Hampton

Subtask 11 - Neurophysiology - R. A. Jaffe

Subtask 12 - Animal Behavior - D. L. Hjeresen

We have retained the services of four consultants to assist us on the project:

Mr. C. C. Diemond - Electrical Engineer

Dr. A. W. Guy - Electrical Engineer

Dr. W. A. Lewis - Electrical Engineer

Dr. R. H. Lovely - Physiological Psychologist

II. EXPOSURE SYSTEMS

In the six month period of March 9, 1976 through September 8, 1976, work on the design and construction of exposure systems has gone from general considerations and definition of goals through the construction and testing of a prototype system.

In this chapter our work on design considerations and goals is reported first, followed by a presentation of results obtained from the prototype system. These results are now being applied to the design of the rat exposure system. This work is just beginning and will be covered only briefly here.

A. DESIGN CONSIDERATIONS AND GOALS

A number of decisions were required before a design for a prototype system could be completed. In this section we briefly review these decisions.

1. Choice of Field Configuration.

As most other investigators, we have chosen to produce in the laboratory a fairly uniform, vertical, single-phase, 60-Hz electric field (before insertion of the animals). This is an approximation to the situation found under a high voltage transmission line where, close to the ground, the field is fairly vertical.

The body of an experimental animal, such as a rat, is a sufficiently good conductor of electricity to extensively modify the field in its vicinity. For example, the field at the top of one particular swine's back has been measured to be 6.7 times larger than the field before the swine was introduced.⁽¹⁾ It is the fields at the surface of the body which are responsible for inducing currents in the body of an animal or causing surface effects such as hair stimulation. The interaction between the animal and the field is very important, and little is known at present about this interaction.

The present practice of expressing exposure dose is to specify the field strength without the animal in the field. Actually the electric field at the surface of the animal's body depends not only on the magnitude of the unperturbed field, but also on the geometry of the exposure system and on the animal's shape and orientation. The practice of using uniform fields as produced in parallel plate systems facilitates the comparison of results between various experiments.

To produce a fairly uniform field in the laboratory is relatively easy with parallel metal plates. Unless the plates are very large (usually impractical), effects from the edges will be present. We have chosen as a design goal a parallel plate exposure system that produces a field uniform to 10% in the region between the plates.

2. Electrode Spacing.

If two parallel plates are very far apart, the animal standing on the lower plate will exert no influence on the upper plate. That is,

the charge density distribution on the upper plate will remain unchanged. At the other extreme, if the two plates are very close together, the animal's body will exert a strong influence on the upper plate and will modify the field to which the animal is exposed. In this latter case the interaction between the animal and the field will be more complicated, and will depend not only on the field strength before introduction of the animal but also on the plate spacing.

It is desirable, therefore, to separate plates sufficiently so that the animal's presence on the lower plate only weakly affects the upper plate. Little information has been published about plate spacing in small animal exposure systems. Illinois Institute of Technology Research Institute recently conducted a literature survey and analysis for the Electric Power Research Institute.⁽²⁾ In the report it is stated "The plate spacing must be three times greater than the height of the rat when standing erect," but no reasons for this statement are given.

There are reasons to keep the plate spacing as small as possible. The high voltage needed to attain a given field strength is directly proportional to the plate spacing. Significant savings in power supply costs can be made by decreasing the plate spacing. Smaller plate spacing also reduces edge effects and results in a more uniform field for a given plate size. To put the plate spacing question on a more quantitative basis, we have estimated the potential field in the vicinity of the rat by approximating the body shape by half of an ellipsoid. For parallel electrodes far apart, this problem can be solved.⁽³⁾ For a rat model we used an ellipsoid with a length of 6.5 in., a width at the

center of 2.8 in., and a height at the center of 2.25 in. Figure 1 shows this shape and the calculated lines of equal potential. Sections through the center of the rat as viewed from the side and from the front are shown.

Before the introduction of the rat's body, the equipotential lines are, for a uniform field, horizontal. The rat's body is an effective conductor of electricity at 60 Hz,⁽¹⁾ and therefore forces the equipotential surfaces to be displaced upwards over its back. As shown in Figure 1, the equipotentials again become horizontal several body heights above the rat, such that a horizontal metal plate could be inserted at this height with little change in the field structure.

In Figure 2 we show the maximum vertical displacement of the equipotential lines from the uniform field position as a function of height above the animal. This figure shows the very quick return to the uniform field situation above the animal. For example, four animal heights above the lower plate (9 in.) the equipotential is only displaced 2% by the animal's body. Placing a metal plate here, which would force the equipotential to be perfectly horizontal, would represent only a 2% perturbation of the situation with the plates very far apart. This seems negligible and would suggest a plate spacing of 9 in. is adequate. However, the rat can move and change his height. In the extreme, it could stand on its rear legs and attain a height over 6 in. For reasons to be described in the next section, we have decided to prevent this by placing a 4-in. high roof on each rat cage. Using the factor of 4, as deduced above, would suggest the plate spacing should be 16 in.

Figure 1. The distortion of an uniform 10 kV/m field caused by a conducting ellipsoidal model of a rat's body. The body's influence on the equipotential curves becomes small several body heights above the ground.

Figure 2. The maximum vertical displacement of the equipotential caused by the animal's body. The vertical coordinate is z , the animal's height h , and the vertical displacement of the equipotentials from the uniform field location Δz .

The plate spacing originally proposed was 24 in. Most of our prototype work so far has been done with this spacing; however, the advantages appear to justify a reduction of plate spacing to 16 in. in the rat exposure system.

3. Control of Dosing.

As stated previously, we plan to house the small animals in cages with a restricted ceiling height. For rats, this height will be 4 in., and for mice 2 in. This decision was made as we became aware of the importance to dosimetry of the animal's shape and orientation with respect to the electric field.

It is well known that the electric field enhancement near objects depends on the sharpness of the object. A rat standing erect is a much sharper protruberance than a rat lying prone. A similar situation exists when comparing the situation for a man standing erect to that of a swine. Simple estimates using measured short circuit currents for men and pigs show that the average current density (current per unit area) produced in a man is approximately 10 times greater than the average current density produced in a pig by the same "uniform" field, and that the field enhancement at the top of a man's head is two to three times greater than the enhancement at a pig's back.

On the basis of these considerations, it was decided to investigate the feasibility of placing a roof on the cages sufficiently low to keep the animals from rearing up. Several experiments, to be described

later in this report, have been done to identify any possible biological effects associated with a lower roof. No such effects have been found, and the decision has been taken to do animal exposures in special cages with low roofs.

The feeding and watering of animals is another area in which great care must be exercised. The conventional rat watering system uses an inverted water bottle with a sipper tube. This bottle would be directly in the electric field and would, at the minimum, produce a substantial local enhancement of the electric field because of the large dielectric constant of water. The consequences could be serious if the bottle or water should become even slightly conducting. The water system, could shock the rat whenever it tried to drink, or, if the rat could drink, the total current induced in the bottle would be shunted to ground through the animal's body. To avoid this problem, a somewhat novel watering system was devised. Water will be introduced from beneath the floor of the cage and will be dispensed by nozzles which will protrude only slightly above the floor level. These will be maintained near or at the floor potential. The system to be used will be described in detail later in this chapter.

4. Maximum Field Strength.

In order to design the exposure system, it was necessary to make a decision concerning the maximum field strength to be used in the project. We have chosen a value of 160 kV/m for initial work. The small animal

study is intended first to reveal biological systems most sensitive to electric fields, excluding effects from direct field perception such as through arc discharges, shocks, or hair stimulation. Therefore, it is necessary to determine the threshold field strength above which direct field perception occurs in rats. It is believed, based on our experience with swine and on other investigators' published work, that a field strength of 160 kV/m will be sufficient to produce field perception by rats. One of the first biological experiments to be performed will be a determination of the threshold field strength for field perception by rats; this threshold value will then be used in specifying maximum field strengths for the rat and mouse exposure systems.

B. PROTOTYPE EXPOSURE SYSTEM RESULTS

The prototype exposure system was built to determine how and to what degree the design goals listed in Section A could be met, and to determine the feasibility and practicality of the various mechanical and electrical components that were selected for the system.

A simplified drawing of the prototype system is shown in Figure 3. Two electrodes are connected to a 100-kV power supply, as shown. The lower plate is operated at ground potential. For reasons to be covered below the high voltage transformer is placed outside of the building in a metal weather shield. Connection through the building wall to the upper electrode is made by a short length of high voltage coaxial cable.

Figure 3. Plan view of the prototype exposure system. The 100-kV high voltage transformer is located outside of the building.

This system has been installed in a laboratory at the BNW Biology Annex. A 11.5-ft by 20-ft room was subdivided into two sections by a partition constructed of wood and chicken wire, as shown in Figure 3. The interior walls in the electrode section were lined with screen (chicken wire) to a height of 8 ft. All of this screen was grounded to form a partial Faraday cage about the exposure apparatus.

All of the electrical measurements to be reported here were made using a 10-kV dielectric test set manufactured by Associated Research, Inc. (Model 422). This was done because delivery of the 100-kV supply was somewhat delayed; it is now on hand and being installed.

1. Exposure Room Design.

Figure 4 is a photograph of the exposure room and shows the screen partition and walls. The walls were screened to provide additional attenuation of the electric field outside the exposure room. The screened partition divides the room into an exposure and an observation area. The partition also represents a safety guard, in the sense used in the ANSI National Electrical Safety Code.⁽⁴⁾ The recommended clearance between live parts and guards at 100-kV line-to-ground is 5 ft and is maintained in this system.

The effectiveness of the wall and partition screening as an electrostatic shield was determined by experiment using a commercially available

Figure 4. The prototype exposure room.

Polytek electric fieldmeter.* With a plate spacing of 24 in. and a plate voltage of 8.0 kV, the electric field between the plates was about 13 kV/m. The electric field in the observation area was about 3.0 V/m, and in an adjacent room about 0.04 V/m. Scaling these numbers to a maximum 100-kV electrode potential, we would estimate that a 37-V/m field would be present in the observation area and a 0.5-V/m field would be produced in the next room. These field levels are very small and are comparable in size to the electric fields found in the vicinity of home appliances.⁽²⁾

2. Exposure Electrodes.

The electrodes made for the prototype system are similar to those described in our original proposal⁽⁵⁾ and are shown in Figure 5. The electrodes are ringed on the outside by 3-in. diameter aluminum tube. This tube supplies the structural strength for the electrode and also serves to limit the concentration of electric field strength at the edge of the plate. Computer simulation of this system estimated that the maximum field strength at the edge of the electrode will be less than 10% of the corona inception voltage gradient of 3000 kV/m.

The interior of the electrodes consists of two layers separated by about 1.5 in. The upper layer, of 1/2-in. by 1/2-in. stainless steel mesh, supports the animals and their cages and allows biological wastes and other debris to fall onto absorbent paper placed on the lower layer.

* Model FBM 100-7, Polytek Materials, Inc., Scotia, New York.

Figure 5. The prototype exposure electrodes and lucite supports.

This lower layer, an aluminum sheet, is supported on one side by hinges and can be lowered from the other side for changing the absorbent papers. The space between the layers is field-free.

The prototype electrodes were generally satisfactory except for the stainless steel mesh and the lower aluminum plate, which were too flimsy. The lower aluminum plate can be stiffened by incorporating flanges in the design. This approach is harder to use for the upper mesh layer, since the region under the cages cannot be blocked. We are pursuing two approaches presently. In one, flanges are fixed around the outer edge of the mesh to stiffen it. In the other, the animal cage modules, to be described shortly, are fastened to the mesh so as to provide structural support.

The exposure electrodes are supported at each corner by a lucite stand. Figure 5 shows the assembled system. The plate spacing can be varied easily by moving the support peg position. This system has worked well in practice and a similar one will be used for both the rat and mouse exposure systems.

3. High Voltage Supply and Distribution.

a. Charging Current Requirements.

The charging requirements of the prototype system were determined to provide data for the specification of power supplies for the rat and mouse exposure systems. In the prototype system the lower plate is grounded and the upper plate is energized. There are two electrode capacitances of importance: the coupling between the two electrodes; and the coupling between the upper energized electrode and the grounded room walls, light fixtures, and so forth.

Figure 6 shows the circuit used to measure the charging requirements of the exposure system. As mentioned earlier, a small 10-kV power supply was used to energize the system for these tests. The output voltage of this supply was measured with a simple resistance voltage divider made of ten 1-meg Ω , 2-watt carbon resistors in series for R_1 , and a single 10-k Ω , 2-watt carbon resistor for R_2 . This divider was directly calibrated to 700 volts and, in this range, had a linear response; subsequent measurements, to be described later in this section, have led us to question its behavior and we now consider the uncertainty in its reading at 5.0 kV to be about 5% and at 10 kV to be in excess of 10%.

Three different connections were used in the low side of the high-voltage output as shown in Figure 6. The total low-side current is the sum of the current to the lower electrode plus the current flowing through the room ground to the wire screen on the walls, light fixtures, and other grounded objects. This total current, I_{SUM} , was measured by the upper connection shown in Figure 6. The middle connection was used to measure the component of current, I_{PLATE} , to the lower electrode, and the lower connection measured, I_{GND} , the current to the room ground.

Data were taken at a voltage of 5.0 kV with and without the electrodes connected (Table I). Note that even without the electrodes connected, an appreciable current to ground exists. This current is evidently through a leakage impedance between the high voltage winding of the supply transformer and the grounded metal parts of the transformer and the high voltage supply cabinet. Table I also shows 4 μA of current

Figure 6. The circuit used to measure the components of the charging current to the electrodes.

towards the disconnected lower electrode. This current is required to charge the capacitance between the wires leading to the (disconnected) upper and lower electrodes (2 pF). This component, I_{PLATE} , of the total current, I_{SUM} , is capacitive and the fact that $I_{\text{PLATE}} + I_{\text{GND}} \approx I_{\text{SUM}}$ shows that the leakage impedance in the power supply through which I_{GND} flows is largely capacitive. This leakage capacitance is about 80 pF.

TABLE I. Prototype Electrode Charging Currents at a Voltage of 5.0 kV.

<u>Exposure Electrodes Connected</u>	<u>I_{PLATE}</u>	<u>I_{GND}</u>	<u>I_{SUM}</u>
No	4 μA	153 μA	157 μA
Yes	81 μA	278 μA	363 μA

The data with the exposure electrodes connected are also given in Table I. Again notice that $I_{\text{PLATE}} + I_{\text{GND}} \approx I_{\text{SUM}}$. Assuming all the currents are capacitive, the currents with the exposure electrodes disconnected can be subtracted from the electrode-connected currents to give currents typical of the exposure electrodes alone. In this way we deduce that the capacitance between the two electrodes is 41 pF, and between the upper electrode and all the grounded items in the vicinity, 68 pF. The total capacitance of the exposure electrodes is 109 pF.

If one simply treated this system as a parallel plate capacitor and neglected edge effects and coupling to ground, a capacitance of 27 pF would result, much less than the measured total.

The leakage capacitance in this particular supply appears in parallel with the electrode capacitance. This additional load must be considered when specifying supplies for the rat and mouse exposure systems.

b. High Voltage Distribution.

For reasons which will be discussed later, the oil-insulated 100-kV transformer will be placed outside of the laboratory building and connected through the wall to the exposure electrodes by high-voltage shielded coaxial cable. We were able to obtain a short piece of special cable made for Hewlett Packard by Belden Cable.*

High voltage cables are normally terminated by stress cones. The outer shield is tapered to a sufficiently large diameter so that it can be brought into air without corona inception. The inner conductor is carried beyond this point in a dielectric sheath for several feet before being brought into the air. Stress cones have been wound on the ends of the cable mentioned above and will be tested as soon as the 100-kV supply is installed.

4. Electric Field Uniformity.

The ultimate objective of the electrodes is to provide a sufficiently uniform field(5%) for the exposure of small laboratory animals. It

* Belden Corp., Y013181; Hewlett Packard Part II 8120-1988, McMinnville, OR.

must be shown not only that the basic electrode design is sound but also that the field uniformity is not disturbed by grounded room walls, by high voltage distribution bus work, or by the animal cages. In this section the results from our prototype system concerning all but the last of the above questions are presented; data concerning the effects of cages is given in Section 5.

a. Measurement Procedures.

Two methods of approach to this problem can be followed. The usual way is to use some sort of fieldmeter which is inserted in the field. An alternating current is then induced in the meter which is proportional to field strength and can be readily measured. This works as long as the fieldmeter is kept rather far, compared to its dimensions, from any conductors or dielectrics in the system.

The plate spacing in the prototype system was 24 in., and may be reduced to 16 in. for the rat exposure system. No fieldmeter whose size is much less than these dimensions is commercially available. Furthermore, the rat resides on the lower plate so measurements close to the plate are particularly important.

A second approach is to measure the field at the surface of the exposure plate. This turns out to be fairly easy to do. An additional advantage of this approach is that all three components of the electric field can be estimated from Maxwell's equations in the region near the surface of the plate where the animals will be located.

The best approach is to combine both techniques. Measurements on the surface of the plate will define the field near the plate and measurements with fieldmeters will determine the field in the region midway between the plates.

The Bonneville Power Administration (BPA) regularly uses a field-meter which is built around a small (3-in. x 5-in. x 2-in. high) commercially available voltmeter. BPA has kindly given us the plans for this unit and we are presently building one for this project. Its small size may permit its use in the rat exposure system.

To measure the field at the surface of the lower electrode, a simple probe was designed and fabricated (Figure 7). This probe is placed on the lower electrode with the outer copper ring connected directly to the plate and the inner copper disc connected to the plate by a resistor (10 k Ω was used for the prototype measurements). Figure 8 shows this arrangement.

The probe, because of its thin construction, represents a small perturbation on the surface of the plate. The perturbation in field will be at the edge of the probe. By using conformal transformations, one can show that the edge effects will be less than 2% at a distance equal to 15 times the thickness of the probe. Thus, the edge effects will only affect the charge distribution on the outer 1-in. wide "guard" ring. The inner copper disc, which is separated from the guard ring by a very thin gap, is exposed to the unperturbed field.

Figure 7. The surface electric field probe.

Figure 8. The circuit used to measure the electric field at the surface of the lower electrode.

By using Gauss's Law one can relate the average field, E , over the probe area, A , to the current, I_E , induced in the probe. If $\omega = 2 \pi \times 60$ Hz is the angular frequency, then $I_E = \omega \epsilon_0 EA$. In our case, the voltage developed across R_E was small (typically 0.5 mV) and was amplified by a simple operational amplifier, as shown in Figure 8. If g is the gain of the amplifier at 60 Hz and V_E the measured voltage, then $V_E = g \omega \epsilon_0 EA R_E$.

It was found that the amplifier with a 10-k Ω resistor across the input produced an appreciable high frequency noise signal. To reduce this, a capacitor was included in the feedback loop of the operational amplifier to produce a controlled roll-off in gain at higher frequencies. The measured frequency response of this unit is shown in Figure 9. The points represent measured data and the solid curve a calculation based on the known components of the amplifier feedback network. The gain at 60 Hz was measured to be 93.1 ± 0.5 .

To test and calibrate this probe, the spacing of the electrodes was reduced to 3.13 in., and the probe was placed directly on the surface of the lower, grounded electrode at its geometric center. The lower electrode for this test was a section of 1/4-in. thick, aluminum plate with a small hole drilled through it for the probe lead.

Since the plate spacing was much less than the plate dimensions, the field at the probe location was calculated as $E_{PAR} = V_{ELEC}/d$, where V_{ELEC} is the applied potential and d the plate spacing. This value we

Figure 9. The frequency response of the amplifier used in the field measuring system. A roll-off in gain above 100 Hz was incorporated to reduce high frequency noise.

call the parallel plate field. Next the measured field, E_{MEAS} , was determined from the probe data. The ratio of $E_{\text{MEAS}}/E_{\text{PAR}}$ then expresses the degree of agreement between the two field values.

The data is shown in Table II. The agreement between the probe and the calculated field strength is good at the lowest electrode voltage. At higher electrode voltages the probe appears to read low. That is, the field strength as measured with the probe is a slightly nonlinear function of the electrode voltage.

TABLE II. Calibration Data for the Surface Electric Field Probe

<u>Electrode Voltage (kV)</u>	<u>Calculated Field Strength (kV/m)</u>	<u>Ratio of Measured to Calculated Field $\frac{E_{\text{MEAS}}}{E_{\text{PAR}}}$</u>
2.49	31.3	1.015
4.94	62.1	0.986
7.44	93.6	0.962
10.10	127.0	0.936

At this time we do not fully understand the nonlinear behavior. However, this is not critical since all the following measurements of electric field uniformity were made at a fixed electrode voltage of 5.0 kV. It now seems clear that the carbon resistor voltage divider did

not function well and exhibited a nonlinear behavior of its own. We are considering buying a capacitive voltage divider of laboratory quality for subsequent work. Secondly, direct observation of the waveform of the 10-kV supply at the output side of the resistive voltage divider indicated that substantial distortion was present in the supply at the higher voltages. High frequency distortion will introduce errors in the field measurements since the frequency enters directly into the probe formula.

b. Electric Field Measurements.

The basic prototype system is shown in Figure 3. The exposure electrodes are in the middle of a large room and are reasonably distant from the grounded room walls (minimum clearance of 32 in.). The lower plate is 36 in. above the floor and the upper, energized plate is 24 in. higher, with a clearance to the room ceiling of about 40 in.

High voltage to the upper plate is supplied through a wire run from the floor directly up one of the lucite stands. Field measurements were made at the other end of the lower electrode.

The surface of the lower electrode for these tests consisted of a 1/4-in. thick aluminum plate. A series of holes were drilled in this plate to allow positioning of the probe for a rapid field survey. This arrangement is shown in Figure 10. The coordinate system used to describe these measurements is also shown in Figure 10.

Figure 10. The coordinate system used to describe the field measurements on the lower electrode. A rectangular array of holes allowed electrical connection to the field probe.

The field was surveyed with the surface probe at a fixed electrode voltage of 5.0 kV. The resulting data is shown in Figure 11. Notice that in both of these graphs the zero of the vertical scale is suppressed.

The upper graph shows the electric field strength as a function of y for various values of x . No difference in E_z is seen between $y = 0$ and $y = 8$ indicating that any end effects from the edge along the narrow dimension of the electrode are insignificant. As we get closer to the edge along the long dimension of the electrode (larger x) a reduction in field strength was seen. Note that the shapes of all four curves are similar.

The lower graph shows E_z as a function of x for various y values and is a restatement of the data in the upper graph. Again, all four curves appear similar in shape.

Figure 12 shows a view of one quadrant of the lower electrode with the 5-in. by 10-in. animal cages superimposed. The field strength at the corners and halfway along the 10-in. cage sides are shown. In addition, the field strength averaged over the floor area of each cage is shown. Values given in this figure were normalized to 100 kV/m at the electrode center. The variation in average field strength between cages near the middle and the ends of the exposure electrode is 5.8%, well within our goal of 10%. Within an individual cage, the maximum variation in field strength occurs in the outer unit, and is about 7%.

Using Maxwell's equations of electromagnetism, all three components of the electric field can be estimated at locations close to the electrode

Figure 11. The surface electric fields measured at an electrode potential of 5.0 kV. The closest wall was 32 in. from the electrodes.

Figure 12. Outlines of the animal cages are superimposed on the lower electrode to show the field strengths at selected points and the average field in each cage. These data are normalized to 100 kV/m at the center of the electrode.

from a knowledge of the field at the surface of the plate. Gauss's Law in differential form states that in a charge-free region,

$$\frac{\partial E_x}{\partial x} + \frac{\partial E_y}{\partial y} + \frac{\partial E_z}{\partial z} = 0.$$

At the surface of the plate, $E_x = E_y = 0$, so that $\frac{\partial E_z}{\partial z} = 0$. To first approximation, the vertical component of the electric field is constant as one moves vertically from the lower plate. Since magnetic effects will be negligible in this system, $\vec{\nabla} \times \vec{E} = 0$ and

$$\frac{\partial E_x}{\partial z} = \frac{\partial E_z}{\partial x}, \quad \frac{\partial E_y}{\partial z} = \frac{\partial E_z}{\partial y}.$$

Thus, a knowledge of the behavior of the vertical electric field as one moves horizontally along the electrode enables one to estimate the horizontal components as one moves vertically off of the plate.

Figure 11 gives the behavior of E_z as a function of x and y . It is easily seen that $\partial E_z / \partial x$ is almost independent of y , and $\partial E_z / \partial y$ almost independent of x . In Figure 13, the horizontal components of the field are estimated at a height of 4 in. above the lower electrode, that is at the height of the top of the rat cage. The horizontal components are given as a fraction of the vertical component which, as we have seen, is to first order independent of z , the vertical coordinate.

Figure 13. The horizontal components of the electric field at a height of 4 in. above the lower electrode, as estimated from the measurements of the vertical component at the lower electrode surface.

The horizontal components are less than about 5% of the total field within the volume occupied by the rats. The situation with regard to both the vertical and horizontal fields seems satisfactory and is substantially better than our target goal of field uniformity to within 10%. However, this is a relatively isolated and simple prototype system, considerably simpler than when we start placing many more trays in a room and must distribute much higher voltages to all of these trays.

c. Effect of Room Walls.

A 32-in. by 50-in. piece of 1/2-in. metal screen was placed 7 and then 14 in. from the narrow end of the exposure electrodes to simulate a grounded wall. A survey was taken of the surface field strength as described earlier in this section. Some of these data are shown in Figure 14 and clearly show that the wall has a considerable and undesirable influence on field uniformity. The maximum variation in field strength with a wall 7 in. from the electrodes is over 20%, compared to 11% with no wall (i.e. with only the room wall 32 in. away).

One way to minimize this interaction with the walls is to energize both plates at equal voltages but out of phase with one another. This technique is being used in an EPRI-sponsored study at Westinghouse Electric Corporation.⁽⁶⁾ Figure 15 shows how this would be done with either a single center-tapped transformer or with two single-ended transformers connected out of phase. For the prototype tests only a

Figure 14. Electric field plots showing the measured effect of a grounded wall located at 7 in. and 14 in. from the narrow end of the electrode.

Figure 15. a) One way to reduce interactions with the walls is to energize both electrodes symmetrically with respect to ground. In b) is shown a method to simulate this with a single transformer.

single transformer was available. The scheme in the lower part of Figure 15 was devised to simulate the connection with both plates energized.

The 32-in. by 50-in. screen was connected to both plates by 4.7-meg Ω resistors and therefore attained a voltage halfway between the two (checked by direct measurement). The screen was positioned as shown in Figure 16. This figure also shows (for $y = 8$ in.) data with no wall, a grounded wall, and a wall energized to half of the upper electrode voltage. The energized screen slightly raises the field at the electrode edge, and produces a field almost independent of x .

Based on these results, we plan to build the rat and mouse exposure systems with all plates energized. This technique has the additional desirable feature that the voltage-to-ground only needs to be half of the potential difference between the electrodes, which reduces the insulation problems with the high voltage supply and distribution systems.

d. Effect of High Voltage Distribution Bus Work.

The exposure systems will consist of multiple stacked plates. To energize every plate, either many cables will have to be run from the power supply, with a substantial cost in charging current, or alternate plates will have to be tied together with tubular bus work. To investigate the effects of bus work on the electric field between plates, the system shown in Figure 17 was improvised.

Figure 16. In a) a wall is simulated by a 32 in. by 50 in. piece of wire mesh. In b) are shown measured plots of field strength when the simulated wall is grounded or energized as shown in Figure 15.

Figure 17. This arrangement was used to investigate the effects on field uniformity of electrical bus connected to the upper or lower electrodes.

A piece of aluminum tube was connected to either the upper or lower plate to simulate bus feeding each plate. Figure 18 shows the measured field profiles for values of $y = 0$ and $y = 24$ in. The bus work connected to the lower plate (at ground potential) had only a small effect on the field. Bus work connected to the upper energized plate tended to raise the field near the edge of the plate.

e. Conclusions.

The following conclusions can be made from the results of the measurements made using the prototype exposure system. The basic electrode design appears sound and capable of providing a sufficiently uniform field for the project. The adverse effects of the room walls on field uniformity can be minimized by energizing both plates out of phase with one another. In fact, the walls can actually cause the surface electric field at the lower electrode to be more uniform. However, these walls will introduce larger horizontal field components above the surface of the lower plate. It appears to be preferable to minimize the interaction with the walls and we have decided to maintain a minimum 18-in. clearance between the exposure electrodes and any wall or voltage distribution bus work. Animal cages affect field uniformity; this subject will be discussed in the section on animal housing.

5. Animal Housing.

a. Basic Design.

The basic cage module for rats, shown in Figure 19, will house eight animals. Each module will have its own mesh floor and each

Figure 18. Measured field plots show the effect of bus connected to the upper or lower electrodes.

Figure 19. This unit holds eight rats in a lexan cage for exposure to electric fields. Individual food pellets are placed in the slots along the front and rear edges. The roof height is 4 in. and prevents the rats from standing erect on their hind legs.

exposure electrode will support three of these modules. It is anticipated that these modules will be cleaned at least weekly, not only for general hygiene but also to prevent accumulation of body oils and hair on the sides of the cages which may alter the electrical properties of the cage materials. The cages will be made of lexan instead of lucite to withstand the high temperatures of the cage washing process.

b. Feeding and Watering Systems.

Each of the five slots at the lower edge of each cage (Figure 20) accommodates a food pellet. In this way an adequate daily supply of food is provided for each rat while the pellets are kept low to minimize any interaction with the electric field. Experiments will be conducted to identify any field effects from the food pellets. As discussed in Section A, Part 3, drinking water will be supplied to the animals from nozzles that will come from below the mesh floor and will protrude only slightly above floor level. Figure 20 shows the watering arrangement. Special small valves that can be operated by a rat are screwed into a water manifold that is pressurized to about 3 psi. These nozzles will protrude slightly above the floor level but are nicely rounded to minimize field enhancement. We have constructed and are testing a prototype watering system.

One difficulty with this approach is how to supply a day's supply of water under pressure. A reservoir contained entirely on the electrode would have to fit in the limited space between the two layers of the

Figure 20. This photograph shows a cage module mounted on 1/2-in. stainless steel mesh. The watering nozzles are seen near the center partition.

electrode and still allow room for the absorbent paper to collect animal wastes. This appeared quite difficult in practice and so it was decided to try a different approach, to bring the drinking water in from a distant pressurized container by passing a water line from a grounded reservoir to each energized plate.

Distilled water, a poor conductor, will be distributed via a water line of 1/4-in. polyethylene tubing, a good insulator. To examine the feasibility of this approach, we have made measurements of the DC resistivity of the actual distilled water available to us and also direct measurements of the leakage currents in a prototype water line when subjected to 60-Hz electric fields of about the strength that would be experienced in our exposure systems. Table III contains our measured result for the DC water resistivities and the measured AC resistances per inch of water line. Data is given for empty, dry, 1/4-in. polyethylene tubing and tubing filled with tap and distilled water.

TABLE III. DC Resistivities and AC Resistances per Inch of 1/4-inch Diameter Polyethylene Water Line

<u>Type of Water Filling Line</u>	<u>DC Resistivity</u>	<u>AC Resistance Per Inch of Water Line</u>	
		<u>$E \approx 10 \text{ kV/m}$</u>	<u>$E \approx 100 \text{ kV/m}$</u>
None	--	$4 \times 10^8 \text{ ohms/in.}$	$4 \times 10^8 \text{ ohms/in.}$
Tap	5.3 k Ω -cm	$4 \times 10^5 \text{ ohms/in.}$	$4 \times 10^5 \text{ ohms/in.}$
Distilled	880 k Ω -cm	$6 \times 10^7 \text{ ohms/in.}$	$5 \times 10^7 \text{ ohms/in.}$

The electrode impedance will be about 15 meg Ω . The resistance of the water lines should be much greater than this. For distilled water this is no problem at all. Even 1 in. of line has a resistance of about 50 meg Ω and the line length will be much larger than this.

The only other difficulty that we might encounter would be the gradual formation of conducting tracks on the tubing. At the worse, this may require the changing of the lines periodically, but with the line length we are planning to use, this may not be necessary.

c. Effects of Animal Cage on Electric Field.

As far as we are aware, no investigation has been made on the effects of lucite, lexan, or other cage materials on electric field uniformity. It seems to be generally assumed that these materials, since they are insulators, are completely transparent to the electric field. Yet insulators have significant electrical properties. Effects from lucite can be demonstrated merely by resting a conventional field meter on a small lucite sheet. The indicated field will rise appreciably.

We have measured the effects of a lucite roof, a lucite wall, and a lucite cage assembly on the surface field strength. Figure 21 shows the results of our measurements of the effect of a lucite roof.

The surface field probe was placed at $x = 0$, $y = 0$, at the center of the lower electrode. A 12-in. by 20-in. by 3/16-in. lucite roof 4 in. above the electrode was then moved in 1-in. steps so as to gradually obscure the probe. The horizontal scale was defined so that at a probe

position of $x = 0$, the center of the probe was directly under the middle of the 20-in. edge of the roof. The measured field was normalized to the field measured with the roof not present. Figure 21 shows that the effect of the roof is rather slight and vanishes once the roof is 3 or 4 in. beyond the probe. The field is intensified by about 1% under the roof.

Figure 22 shows the same type of an experiment using a lucite wall. The horizontal scale is such that at a probe position of 1 in., the outside edge of the 2-in. diameter probe is just touching the edge of the 20-in.-long wall. This figure shows that near to the wall the field is reduced by about 2.5%. Seven inches from the wall the field is still 1% less than what it would have been with no wall at all.

In Figure 23, the effect of a more elaborate cage assembly was examined. The test module was constructed of lucite strips, 4-in. high, and consisted of four animal compartments 6 in. x 10 in. This assembly was then moved to position the probe in the four corners and in the middle of the cage unit, as shown in Figure 23. Data was taken both with and without a roof. Only small differences were noted. Figure 23 gives the data with the roof. The field strength is reduced 4 to 5% at all points in the cage except at the outer corner.

The results of these experiments demonstrate that the effects of this cage on electric field uniformity at the surface of the electrode are small. However, caution must be exercised in making conclusions. If we did the same set of measurements with a metal roof instead of a

Figure 21. The measured effect of a 3/16-in., 4-in. high roof on the surface field strength is shown. $E_{NO\ CAGE}$ is the field before introduction of the roof. The horizontal coordinate measures the position of the field probe relative to the center of the 20-in. edge of the lucite roof.

Figure 22. The measured surface field strength in the proximity of the center of a 20-in. long, 4-in. high, 3/16-in. thick lucite wall. $E_{NO\ CAGE}$ is the field strength with no wall present. The horizontal coordinate measures the position of the center of the 2-in. diameter field probe relative to the edge of the lucite wall.

Figure 23. The circled numbers are the measured surface field strengths in the corners and the center of the indicated cage arrangement which included a 4-in. high roof. The data has been normalized to the field strength with no cage present.

lucite roof (but with lucite sides), the results would be similar. But the situation for an animal in this field would be very different because his interaction with the field would be altered by the presence of a horizontal equipotential, established by the metal roof, only a few inches above his back. We are now building metal models of ellipsoids and of rats for the purpose of studying the effects of the cages on total short circuit currents. If we can show that the short circuit current is only reduced by a small amount by the cage, we will then have good evidence that the animal's interaction with the field is not significantly altered by the cage.

After the above measurements have been made, a technique will be developed to measure the electric field at the surface of the animal's (or model's) body.

6. Safety.

This subject, while perhaps not as intrinsically interesting as some others, has created a great deal of interest locally within both Battelle and ERDA. These various discussions have led to the following design aspects for the prototype system.

The 100-kV transformer is mineral-oil insulated and is considered, therefore, a fire hazard. In accordance with the National Electrical Code, we have installed this transformer outside of the laboratory building. This same procedure will probably be required for the rat

exposure system but not for the mouse system where the voltage is low enough to be supplied by an air insulated transformer.

Figure 24 is a block diagram showing the various interlocks, indicating lights, power disconnect switches, and protective grounds. Both the partition door to the exposure room and the door to the high voltage transformer enclosure will be equipped with limit switches which allow energization of the exposure system only when both doors are closed. The door to the exposure room, which will be used regularly in the course of experiments, will have a special lock designed so that its key can only be removed when the door is closed and locked. This same key must be locked into a key switch in the primary power circuit to the high voltage systems before energization of the exposure system can occur. Lights by both doors indicate whether the power to the high voltage transformer is on.

A manual disconnect switch is located close to the high voltage control unit for easy implementation of lock and tag procedures as will be used when work on the high voltage transformer or extended work on the exposure electrodes is required. Hot-stick applied protective grounds will be used whenever work involving contact with the exposure electrodes is required.

As an additional safety procedure, the key to each transformer enclosure will be retained by the project manager in a locked key box. Only authorized personnel with an approved work clearance may obtain the key, and then only when they have turned in the interlock key in exchange.

Figure 24. This block diagram shows the various safety systems which have been incorporated into the final prototype system design.

The project manager will retain and be responsible for the key to the lock box and will maintain control over the keys to the transformer enclosures.

III. CRITERIA STUDIES

The main purpose of this research project is to conduct an integrated series of experiments covering a broad range of organ systems to determine whether exposure to electric fields have any biological effects. Before such experiments can be conducted, a number of experimental prerequisites need to be satisfied. First, we must design, fabricate and test an exposure system that is well understood and free from potential sources of artifact. Second, we need to design, fabricate and test a cage system for housing animals during exposure to electric fields that does not alter field uniformity or stress the animals; the system must incorporate a reliable system for providing adequate food and water to the animals during exposure without having a significant effect on field uniformity. Third, we have to determine the maximum electric field strength to be used for the main body of biological screening experiments that does not produce corona and result in ozone formation, that does not result in the animal being shocked from the feeding or watering systems, and that does not produce hair stimulation or some other, as yet unidentified, perceptual effect.

The engineering aspects of the first two prerequisites are nearing completion. We now have a prototype exposure system and a prototype housing system, as described in the previous chapter. A series of experiments have been initiated to determine the biological compatibility of these systems and to identify any potential sources of artifact. We have termed these experiments "criteria studies" to differentiate them from the

biological screening experiments to be made when the rat and mouse exposure systems are operational in several months. Some of the criteria studies on animal caging have been completed, and the initial results will be presented here. Experiments to be conducted to establish the maximum field strength to be used for the biological screening experiments will be started when the 100-kV transformer for the prototype exposure system has been installed in early September, and are described briefly in this report.

A. ANIMAL HOUSING SYSTEM

Dosimetry and engineering requirements in the design of the small animal housing system demanded a cage configuration that would not allow rats to rear up during exposure. Such rearing would alter the local electric field and make it difficult to obtain a reasonable degree of control over dosing. The goal was to limit ceiling height in the rat cages to 4 in., provided it produced no biological effects.

Two experiments have been completed and a third is in progress to determine if any effects occur by housing rats in cages with a ceiling height of 4 in. In the first two experiments standard stainless steel cages with wire mesh floors were used. While all cages were 7-3/4-in. wide and 8-1/4-in. long, the ceiling height in the experimental group was restricted to 4 in. with a lucite insert, while the ceiling height for controls was kept at the standard 7 in. height. The experiments were conducted in an animal room with a 12-hr on/12-hr off (7 a.m. on) lighting schedule with all measurements taken during the early portion of the "lights on" period. Standard rat pellet food was provided in a

crushed form to prevent spillage due to rats dropping partially consumed pellets through the mesh floor. This mash food was served in a 2-in. diameter, 2-in. deep tube with an overhanging lip on the inside to minimize digging and spillage by the rat. This was done in order to obtain accurate measures of food consumption. Water was provided in calibrated tubes with nonspill spouts.

The first experiment was a pilot study using 10 male Wistar rats (about 120 days of age at the start of the experiment) to determine if the lower than normal ceiling height produced acute changes in animal irascibility or metabolism. Five rats were housed in cages with 4-in. high ceilings and the remaining five in cages with standard 7-in. high ceilings. Food and water intake were measured daily, as was body weight, during the duration of the 4-day experiment. After 48 hr in the cages the rats were individually tested using a system designed to quantify irascible behavior in rats.⁽⁸⁾ The procedure incorporates six behavioral parameters, each rated on a 1 - 6 scale. Two investigators rated the animals independently using a double-blind design. The results gave no indication of irascibility differences between experimental groups. No significant differences in body weight or in food and water consumptions were apparent between groups (Table IV). It was clear, however, that a longer duration test with a larger number of animals would be required.

A second experiment was conducted to investigate in more depth any potential adverse effects of housing rats in cages with a lower than standard ceiling height. Two groups of male, Wistar rats (N=10/group) were housed individually for 30 days in cages with either a 4-in. or

7-in. high ceiling. In addition to making daily measurements of body weight, food consumption and water consumption, a complete hematologic analysis was made on all animals at the end of the 30-day period.

TABLE IV. Daily Body Weight Gain, Food Consumption and Water Consumption of Rats (N=5/group) Housed for 4 Days in Standard Cages with 7-in. or 4-in. High Ceilings. Rats were 120 days of age at the onset of the experiment.

	<u>7-in. Ceiling</u>		<u>4-in. Ceiling</u>	
	<u>Mean</u>	<u>S.E.</u>	<u>Mean</u>	<u>S.E.</u>
Average Daily Body Weight Gain (g)	5.9	5.3	8.2	1.9
Average Daily Food Consumption (g)	24.6	6.7	27.0	3.0
Average Daily Water Consumption (ml)	45.0	4.6	40.9	1.8

No significant differences in body weight or in food and water consumptions were observed between the groups over the duration of the experiment (Table V).

TABLE V. Average Daily Body Weight Gain, Food Consumption and Water Consumption of Rats (N=10/group) Individually Housed for 30 Days in Standard Cages with 7-in. or 4-in. High Ceilings. Rats were 74 days of age at the onset of the experiment.

	<u>7-in. Ceiling</u>		<u>4-in. Ceiling</u>	
	<u>Mean</u>	<u>S.E.</u>	<u>Mean</u>	<u>S.E.</u>
Average Daily Body Weight Gain (g)	7.7	1.2	9.2	1.1
Average Daily Food Consumption (g)	33.4	1.8	33.1	1.7
Average Daily Water Consumption (ml)	45.5	2.2	44.1	2.7

At the end of 30 days the rats were sacrificed and blood samples were drawn for analysis. The adrenal glands were removed, weighed and values for the two groups were compared. The results indicate no significant differences between groups in any of the hematological parameters examined, nor in the weights of the adrenal glands (Table VI).

TABLE VI. Hematologic Profiles and Adrenal Weights of Rats Housed for 30 Days in Standard Cages or Cages with the Ceiling Height Lowered to 4-in. (N=10/group).

Blood Samples	7-in. Ceiling		4-in. Ceiling	
	Mean	S.E.	Mean	S.E.
Mean Corpuscular Volume, μm^3	54.0	0.7	54.0	0.5
Mean Corpuscular Hemoglobin, μg	20.0	0.2	20.1	0.4
Mean Corpuscular Hemoglobin Concentration, g/dl	37.5	0.1	37.9	0.7
Reticulocyte $\times 10^3/\text{mm}^3$	288.0	15.7	288.0	17.3
Hematocrit, ml/dl	40.3	0.5	41.1	1.0
Hemoglobin, g/dl	15.0	0.2	15.5	0.2
Red Blood Cells $\times 10^6/\text{mm}^3$	7.6	0.1	7.8	0.2
White Blood Cells $\times 10^3/\text{mm}^3$	7.7	0.8	7.4	0.7
Segmented Neutrophils $\times 10^3/\text{mm}^3$	0.65	0.12	0.67	0.14
Lymphocytes $\times 10^3/\text{mm}^3$	6.7	0.8	6.6	0.5
Monocytes $\times 10^3/\text{mm}^3$	0.25	0.03	0.10	0.02
Eosinophils $\times 10^3/\text{mm}^3$	0.10	0.05	0.05	0.02
Adrenal Gland Weights, mg	57.0	3.7	51.0	3.0

Experiments are in progress to insure that the rat exposure cages (including the watering and feeding systems), as described in the previous section of this report, meet design criteria. The cages must not alter electric field uniformity significantly, and they must not introduce biological artifacts. Experiments related to the interaction of the cages with the electric field were discussed previously. Studies concerned with the biological aspects of the cage system have been started to make sure that animals receive adequate food and water and are not stressed when housed in the smaller than normal size cages.

Two rat housing modules, each containing 8 rat cages per module, with attached feeding and watering systems, have been fabricated (Figure 20). The system is now being tested with rats housed in each type of cage in order to make sure the system works and to determine what, if any, modifications are needed. As soon as we are satisfied that the system is operating reliably, we will conduct an experiment comparing metabolic and hematologic parameters of rats housed in the exposure cages (each cage measures 5-in. wide x 10-in. long x 4-in. high) to those housed in standard rat cages (each measuring 7.75-in. wide x 8.25-in. long x 7-in. high). Specifically, the experiment will consist of 32 male rats, about 60 days of age, with 16 animals housed, individually, in each type of cage for 30 days. Measurements of body weight, food consumption and water consumption will be made daily during the 30-day period. On the 30th day, blood samples will be drawn and a complete hematologic examination will be made on all rats, similar to that described in the previous experiment.

Based on our own experience and from reports in the literature, we do not anticipate the smaller than normal size cages will introduce any problems. However, this must be and will be documented.

B. BREEDING STUDIES

Several of the screening experiments will involve the breeding of rats and the subsequent development of the pups in the electric field.⁽⁵⁾ Two questions need to be dealt with before such research can be undertaken: 1) What effects will the low ceiling height cages have on mating behavior, parturition and raising of the pups? 2) How to maintain the pups electrically grounded and at the same potential as the mothers to prevent the pups from being shocked when they nurse?

A study was conducted to determine whether a lowered ceiling height would interfere with normal parturition and rearing of the newborn. Ten adult female Wistar rats were housed in 9-1/2-in. x 18-in. cages with either 7-in. or 4-in. high ceilings (N=5 rats/group). Presumably pregnant rats were placed in the cages 8 days after mating and kept there through gestation, parturition and rearing of the pups to weanling age, 21 days. Absorbent litter was put in all cages and was changed on a regular basis. All rats had access to food and water (ad libitum) and the lighting control of the room was as described for the previous ceiling height experiments. Three rats in low-ceiling cages and 2 rats in standard cages were pregnant and delivered litters. The lowered ceiling had no apparent effect on parturition, litter size, infant mortality, maternal behavior, or development of the pups.

Due to the relatively few pregnant females available for observation in the first experiment, the study was replicated to verify the results and to determine the importance of providing litter in the cages. Fifteen adult female Wistar rats were mated, as confirmed by a positive vaginal smear, and placed into one of three caging conditions. The first group of five were housed in cages with the 7-in. high ceilings, and the second group was housed in cages with 4-in. high ceilings. Both of the first two groups had absorbent litter in the cages. The remaining five rats were housed in cages with 7-in. high ceilings but without any litter material in the cages. All mated females were pregnant and delivered.

As found in the previous experiment, the lowered ceiling height had no adverse effect on parturition, litter size, infant mortality or development and growth of the pups. One observer who weighed the pups daily noted somewhat more activity among the mothers housed in the 4-in. cages than in the 7-in. cages. However, this subjective finding needs to be verified and quantitated.

A marked effect was observed in the rats housed in 7-in. high cages without litter. Some of the mothers did not care for the young and there was a high infant mortality rate. Although the surviving pups in the group without litter were comparatively normal in size, they had a semiliquid coating from the various waste materials in the cage. If the cages were not cleaned daily, some of the females would construct a nest for the pups from small bits of food pellets and fecal boli, demonstrating

a strong nest-building and maternal instinct. It was obvious from the results of this study that we must use litter in cages for the breeding experiments. We cannot use the standard absorbent litter since it is a poor electrical conductor, and the newborn would likely get shocked when they try to nurse. We need to find a good conductor that can serve as a litter material.

Further experiments in the breeding study will evaluate various conducting materials as litter for the cages. Studies are planned to examine the breeding capability of rats housed in 4-in. ceiling height cages. Also, an investigation will be made to determine the upper limit on field strength that does not interfere with mating. Exposure cage modules, similar to those described earlier in this report but with fewer and larger cages per module (4 cages/module, each 10 in. x 10 in. x 4 in., and 2 cages/module, each 20 in. x 10 in. x 4 in.) have been ordered and will be used for testing the caging system in subsequent breeding experiments.

C. CORONA AND OZONE TESTING

To be confident that the experiments are free from corona and ozone formation, we will determine the threshold field strength for their inception and operate the exposure system below that level during the biological screening experiments. Once the prototype exposure system is outfitted with the 100-kV source, scheduled for mid-September, tests will be made to detect corona and quantify any possible ozone by following several procedures.

The initial corona tests will be made using a photographic technique previously described in a study we conducted for the Electric Power Research Institute.⁽¹⁾ In this method a Polaroid camera is mounted at a fixed position relative to the electrode system and a set of reference photographs are taken. The room is then completely darkened and the electrode system energized. A long-exposure photograph is taken of the electrode system and examined for any light-emitting sources of corona. By comparing the experimental photos with the reference photos, using transparency overlays, we can determine the location of any visible corona. If corona is observed at relatively low electrode voltages, necessary corrective action will be taken to improve the situation.

A second procedure will involve the use of a radio frequency (rf) meter (Wescore Model #NP-21). This hand-held device is widely used in the power industry to locate sources of corona in switchyards and along transmission systems. The Wescore unit operates in the broadband and is sensitive in a frequency range from 1.75 MHz to 260 MHz.

We have arranged with the Hanford Environmental Health Foundation to use two of their ozone detection units. One is a Mast Ozone Meter which operates via oxidation reduction of potassium iodide in solution and is sensitive to 2 parts per hundred million of ozone. The other is a REM chemoluminescent ozone meter sensitive to 1 part per billion. The two meters will be used over the range of field strengths being tested to document no ozone is present.

D. HAIR STIMULATION, BEHAVIORAL AVERSION, AND SPARK DISCHARGE (SHOCK)
EXPERIMENTS

Experiments have been designed to determine the maximum field strength that can be used for the biological screening experiments without causing unwanted phenomena such as hair stimulation,* behavioral aversion, or shocks from the feeding, watering, or caging systems. These experiments will be conducted using the special studies exposure system,** scheduled to be operational by about September 15. A description of the planned experiments was included in the original proposal,⁽⁵⁾ and only progress will be given in this interim report.

The equipment and apparatus needed for the behavioral aversion experiments has been designed and fabricated. The behavioral paradigm requires the use of a shuttle-box that can be placed in high-intensity electric field without altering field uniformity significantly. Such a device was built. It consists of a shuttle-box in conjunction with photo-electronic detectors (to be located in the electric field) and remote logic circuits to provide a nonambiguous indication of the position of a rat in the box. The photo-electronic detector system can discriminate between three positions in the box: left, right or center. TTL logic, as well as outputs from relay closures, are provided to operate behavioral control modules or for

* While hair stimulation could result in a form of avoidance behavior, we will consider hair stimulation and behavioral aversion as different phenomena:⁽¹⁾ hair stimulation will be defined as visible piloerection and/or hair movement (oscillation), and behavioral aversion as escape from the electric field by the rat when it is given the contingency to escape.

** The special studies exposure system will be the redesignated title of the prototype exposure system once the 100-kV transformer has been installed and the system is operational.

further processing of the position data. The shuttle-box is constructed of nonconducting material (lucite) and includes 12-foot, electrical-nonconducting light cables leading to and from the photo-electronic detectors.

This apparatus will be used in a behavioral paradigm that will allow the rat to communicate to us the electric field strength it perceives and finds aversive. A detailed description of the paradigm has been published⁽⁹⁻¹¹⁾ and will not be included in this report.* Tests are in progress to characterize and validate the reliability of the apparatus. Also, baseline data on the behavior of rats in the shuttle-box is being obtained in preparation for the behavioral aversion experiments.

Experiments to determine the threshold for hair stimulation and shocks from the caging system will be initiated as soon as the special studies exposure system has been assembled and tested. A video technique, similar to that used in an earlier study for EPRI,⁽¹⁾ will be used to determine the field strength that produces visible piloerection and hair oscillation. The tests for spark discharge (shocks) from the feeding, watering and caging system will be observational, and will be documented photographically either on video tape or 8 mm film.

E. EFFECTS OF CAGING CONFIGURATION ON BEHAVIOR AND FIELD STRENGTH

During the course of the study, hundreds of rats and mice will be housed in lexan cages and exposed to high strength electric fields. Rats will be housed individually in 5-in. wide x 10-in. long x 4-in. high cages with self-contained feeding and watering systems, as described in a previous

* We have used this paradigm in another project at Battelle-Northwest⁽¹¹⁾ to test for the detection and avoidance by rats of low intensity microwave radiations.

chapter of this report. Two rows of 4 adjacent cages make up an exposure module, (Figure 20). This design is such that as many as four rats could group close to each other by moving to a corner common to four cages. Such a grouping might cause mutual shielding from the field and reduce the actual exposure of each animal in the group. While steps can be taken, if necessary, to reduce such an effect, we must first determine the degree of interaction, if any, created by grouped animals. A further, strictly behavioral, question is whether rats are capable of recognizing the benefits of such a grouping and take advantage of the situation whenever possible.

To investigate this potential problem, time-lapse photographs will be made while animals are exposed to electric fields in the special studies exposure system. We will use two Kodak Analyst Super-8 movie cameras with 13- to 28-mm f/1.9 zoom lenses capable of operating from one frame every 1-1/4 sec to one frame every 90 sec. A preliminary set of tests have been made to establish lighting, camera locations and film development procedure. A series of experiments will be conducted to determine if there is any correlation between animal positions in the cages and field strength, or, if animals group in a common corner at high field strengths.

We plan to operate the cameras at one frame every 10 sec, yielding 2880 frames per eight-hour session. Included in each frame will be the positions of the rats in their cages, their specific activity (grooming, sleeping, etc.), a visual display of the field strength at a location of interest, and a time reference. To assist in the evaluation of the data each of the cages will be divided into quadrants. For each frame evaluated

the location and activity of each rat can be correlated with the observed field strength. Since there are obviously a great many possible combinations and many frames to be evaluated, a program will be set up with biostatistical and computer staff members to process the data. A previous study at Battelle on the movement patterns of pocket gophers used such a program, and it is possible that it can be adapted to our purposes. If this is not possible, we will analyze randomly selected frames from the data to score behavior and the relationship between relative position and field strength.

IV. BIOLOGICAL SCREENING EXPERIMENTS

Most of the research effort on this project during the past 6 months has been directed to the design, fabrication, and testing of the exposure systems (Chapter II) and, more recently, to the criteria studies (Chapter III). Activity on most of the biological screening experiments is just getting started, as scheduled. An exception is the immunology studies, started in May, in which a great deal of progress has been made.

Work was initiated on most of the subtasks about August 1, in preparation for the main body of biological experiments scheduled to begin late this fall when the rat and mouse exposure systems are ready for use. Current activities include: 1) procurement of materials and equipment; 2) fabrication, assembly and calibration of test systems; 3) development and refinement of experimental procedures and 4) establishment of baseline data. A brief summary of work and plans on each subtask is given below.

A. HEMATOLOGY AND SERUM CHEMISTRY

Preliminary efforts have been directed to establishing a methodology for microtitration of hemagglutination titers to sheep red blood cells in mice. Other work in progress includes: 1) developing a method to automate complement titration; 2) setting up ion specific instrumentation for serum sodium and potassium assays; 3) developing a method for lymphocyte transformation assays; 4) performing pilot studies on methodologies for red cell lifespan on iron kinetics in mice; 5) performing hematology and serum

chemistry determinations to establish any effects of housing mice and rats in smaller than normal cages (see Chapter III of this report for correct results).

B. IMMUNOLOGY

The immunology studies are designed to analyze for potential effects of electric field exposures on the humoral and cellular components of the immune system. Work is in progress to adapt, develop and standardize assays for determining alterations in the immune system.

1. Humoral Studies.

We have purified immunoglobulin-G (IgG) and immunoglobulin-M (IgM). These isolated proteins are used for production of antisera in goats, components in the radioimmune assay, and titering antibody levels in the immunized goats. High titer antibody should be obtainable in the goats within the next month, permitting the use of the sera in the radioimmune assays for serum levels of immunoglobulins and specific antibody to a known immunizing antigen. In the the interim, we have obtained a small amount of antisera to these immunoglobulins. This antisera will be used for preliminary experiments in the development of the radioimmune assays.

The second part of this study relating to possible effects on humoral immunoglobulins is the analysis of primary and secondary response to a known antigen. We have ordered the needed reagents and have initiated preparation of the immunizing antigen.

2. Cellular Studies.

The first phase of the in vitro cellular studies has been the development of techniques and conditions for the isolation and purification of peripheral lymphocytes from mice. We will continue to work on improving bleeding techniques and recovery of lymphocytes with a separating system.

The next step in the study will be the characterization of the peripheral lymphocyte population with fluorescent labeled antibody to mouse immunoglobulins and with the rosette assays. All of these tests relate to markers on lymphocytes and are used to discriminate the two major populations, T-lymphocytes and B-lymphocytes. Preliminary experiments have been initiated using commercially available antibody. The data obtained indicates that approximately 30-40% of the cells isolated from peripheral blood are labeled. Our next step is to standardize this assay and initiate preparatory work needed for setting up the rosette assays.

C. METABOLISM AND GROWTH

Instrumentation has been assembled and calibrated for making metabolic measurements on rats. A comparison was made between two different methods for measuring respiratory gas exchange. In one, the animal is held in a modified Bollman holder and gas exchange is measured using an open system.⁽¹²⁾ In the other, the animal is restrained in a closed lucite tube while measurements are made.⁽¹³⁾ The results are summarized in Table VII.

TABLE VII. Mean Oxygen Consumption Rates, Carbon Dioxide Production Rates and Respiratory Quotients of Two Groups of Rats (N=5/group) Per Unit Body Mass After 9 Days of Acclimation in a Bollman Holder or a Lucite Tube. Values shown are means based on measurements made during a 30-minute period.

	Body Weight (g)		O ₂ Consumption (ml/min/g)		CO ₂ Production (ml/min/g)		Respiratory Quotient ($V_{CO_2} \div V_{O_2}$)	
	$\bar{X}$	S.E.	$\bar{X}$	S.E.	$\bar{X}$	S.E.	$\bar{X}$	S.E.
Bollman Holder	305	6	0.021	0.001	0.019	0.001	0.91	0.07
Lucite Tube	310	9	0.029	0.001	0.026	0.001	0.91	0.07

Values obtained using the Bollman holder were consistent with those given in the literature for a rat in a "resting" state, indicating no undue stress was imposed on the rat by the measurement technique. Accordingly, this procedure will be used in the experiments for the biological screening studies.

D. CARDIOVASCULAR FUNCTION

Instrumentation required for the cardiovascular studies has been assembled and calibrated. Baseline measurements will be started in September.

E. ENDOCRINOLOGY

Materials have been ordered and animals received for preliminary studies on T₃ and T₄ levels in rats and time parameters for TSH response. A study will be initiated in September to determine the response curve for ACTH stimulation.

F. CNS STRUCTURE AND FUNCTION

Work is in progress using tracer techniques to reveal permeability characteristics of the microvasculature of the CNS. Baseline data on mice is being collected through electron microscopic examination of brain tissue following injection of horseradish peroxidase (HRP, molecular weight 40,000) and histochemical techniques to localize the HRP reaction product. Currently, the cortex, area postrema, hypothalamus and cerebellum are being studied. Thus far it appears that the blood brain barrier in each of these portions of the brain is impermeable to HRP in normal animals.

G. NEUROPHYSIOLOGY

Acquisition of materials and equipment for the neurophysiological experiments is continuing. As soon as the instrumentation has been assembled and calibrated, methodologies and baseline measurements will be started. The first phase of the research will be concerned with the technical aspects of peripheral nerve, electromyographic and electroencephalographic studies. Specifically, control experiments will be performed on normal, unexposed animals with the goal of obtaining baseline data and characterizing the nature and magnitude of individual variations. The problems associated with noninvasive technique development and electrode placement will be studied during this phase.

H. REPRODUCTION, GROWTH AND DEVELOPMENT

Most of the activity on this subtask has been concerned with the housing system for mothers and pups, and was discussed in Chapter III of

this report. Research will be continued to insure the exposure cage system is adequate for housing pregnant female rats and mothers with newborn. The major problem to be solved is to locate an electrically conducting litter to put in the bottom of the exposure cages during the first week or so after delivery of the pups.

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