

A proposal for

THE EFFECTS OF VERY LOW FREQUENCY ELECTROMAGNETIC IRRADIATION UPON PRE-AND POST-NATAL SEQUENTIAL GENERATIONS IN THE ALBINO RAT

**Submitted to: Biomedical and Environmental Research
Division of Atomic Energy Commission
Deputy Director, W. W. Burr, Jr. , M.D.
Mail Station E-201 Room 215
Washington, D. C. , 20545**

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I. INTRODUCTION

Each day an increasing number of individuals are exposed to electromagnetic fields produced by intrusion and portal detection systems - frequently without the individual being aware of the exposure. These detection systems make use of waves described as X-ray, microwave and active magnetic fields. Some of the individuals exposed to these systems are in the early stages of pregnancy; therefore, these individuals are exposed to energy from these waves that penetrate the individual by induction or radiation. Many of these detection techniques are quite effective in their surveillance and detection functions, and can be expected to be placed into more widespread use. Added to the field of these portal detection systems, electromagnetic energy emitted from equipment at permanent facilities already operational (I. L. S. transmitters, radar-search, weather, air traffic control, emergency equipment T. V. and radio stations, etc), make up the broad exposure spectrum to individuals.

Biological effects of most intrusion devices have not been unequivocally determined. Reports found in the literature are exceedingly difficult to interpret, due both to the substantial qualitative variation encountered among the experimental approaches and frequent ambiguity of reported results. Consequently, a program designed to seek out potentially significant interactions, both transitory and long-term, between the human biological system and the emanations involved is needed. With proper experimentally derived evidence, sound decisions can be made regarding widespread deployment and use of detection devices.

Acquisition and documentation of valid, convincing data will demand an interdisciplinary team of biological scientists, medical instrumentation specialists, intrusion techniques, device development engineers, and experts in practical and theoretical aspects of electromagnetic radiation. Southwest Research Institute has this special team and has demonstrated competence in all phases of the work proposed herein.

II. RESEARCH OBJECTIVES AND WORKSCOPE

The objectives of this program are to expose groups of rodents to a 25 kHz RF field for varying time periods and varying powers before mating and to observe the effects of this relative high power density field upon the reproductive performance of the male and female. Secondly, to observe the growth performance of the neonates born within each varying power and exposure time period. This growth observation period of the neonates will be during and up to puberty. Thirdly, the neonates will be randomly selected within treatment groups (power and time), and subjected to the same treatment as their sires and dams. After the exposure period they will be mated and allowed to produce the next generation. This procedure will proceed through three generations. From each generation a randomly selected number of animals will be utilized for gestation period studies. These animals will be selected within treatment group, power and time of exposure and mated. On the predetermined specified day during pregnancy, they will be exposed to the appropriate radiation treatment for that day only. On day twenty of their gestation period, they will be sacrificed to evaluate effects of the treatment upon pre-natal development. This will continue through three generations.

The exposure levels utilized in this experiment are defined as follows:

Full Power exposure level will be 15000 v/m and 7.5 A/m. Half power exposure level will be 10,600 v/m and 5.3 A/m.

In a previous experiment in our laboratory, utilizing these exposure levels, the effects of the thermal response was measured. The assessment of the energy deposition was that there were no significant body temperature changes. One very important objective here is to examine fetal development exposed to electromagnetic irradiation at a level that a thermal response will not be encountered. Therefore, any biological deviation from the control groups in this experiment will not be attributed to a thermal response.

In addition to examining the physiological aspects of pre- and post-natal reproduction, neonatal growth and development, the histopathological and hematological parameters will also be investigated. Therefore, the data content from this study should be of significant value to the scientific community.

III. V. L. F. IRRADIATION APPARATUS

A very low frequency (VLF) high intensity electromagnetic field generator using the concept of independently generating the electric and magnetic field components by means of parallel plate electrodes and Helmholtz coils will be used to irradiate the experimental animals. The generator operates at 25 kHz and can generate a maximum electric field intensity of 15,000 V/m and a maximum magnetic field intensity of 7.5 V/m. Figure I, illustrates the design concept of the generator. The parallel plates and the Helmholtz coils are positioned on a common axis in such a manner that the electric and magnetic field intensity vectors are orthogonal. They are energized by amplifiers driven from a common source. A phase shift network is used to adjust the current flowing through the Helmholtz coils so that it is in time phase with the voltage applied to the parallel plates.

Conventional methods of generating controllable high intensity electromagnetic fields at low frequencies utilize rectangular coaxial structures, parallel strip transmission lines, or resonant antenna structures. The rectangular coaxial structure and the parallel strip transmission line both use real power flow from a source at one terminal to a load at the other terminal. Both provide orthogonal electric and magnetic field components and an exposure volume of known uniform field intensity in which one may place test samples. Use of a resonant antenna relies on the use of an existing transmitter installation. Most of the source power in these systems is dissipated in the load or radiated from the antenna and only a very small percentage is absorbed by the test sample. Large power sources are required to generate high intensity fields with these systems. The Southwest Research Institute VLF exposure facility generates high intensity fields which have all the essential characteristics of the fields generated by conventional means but does not require high driving power.

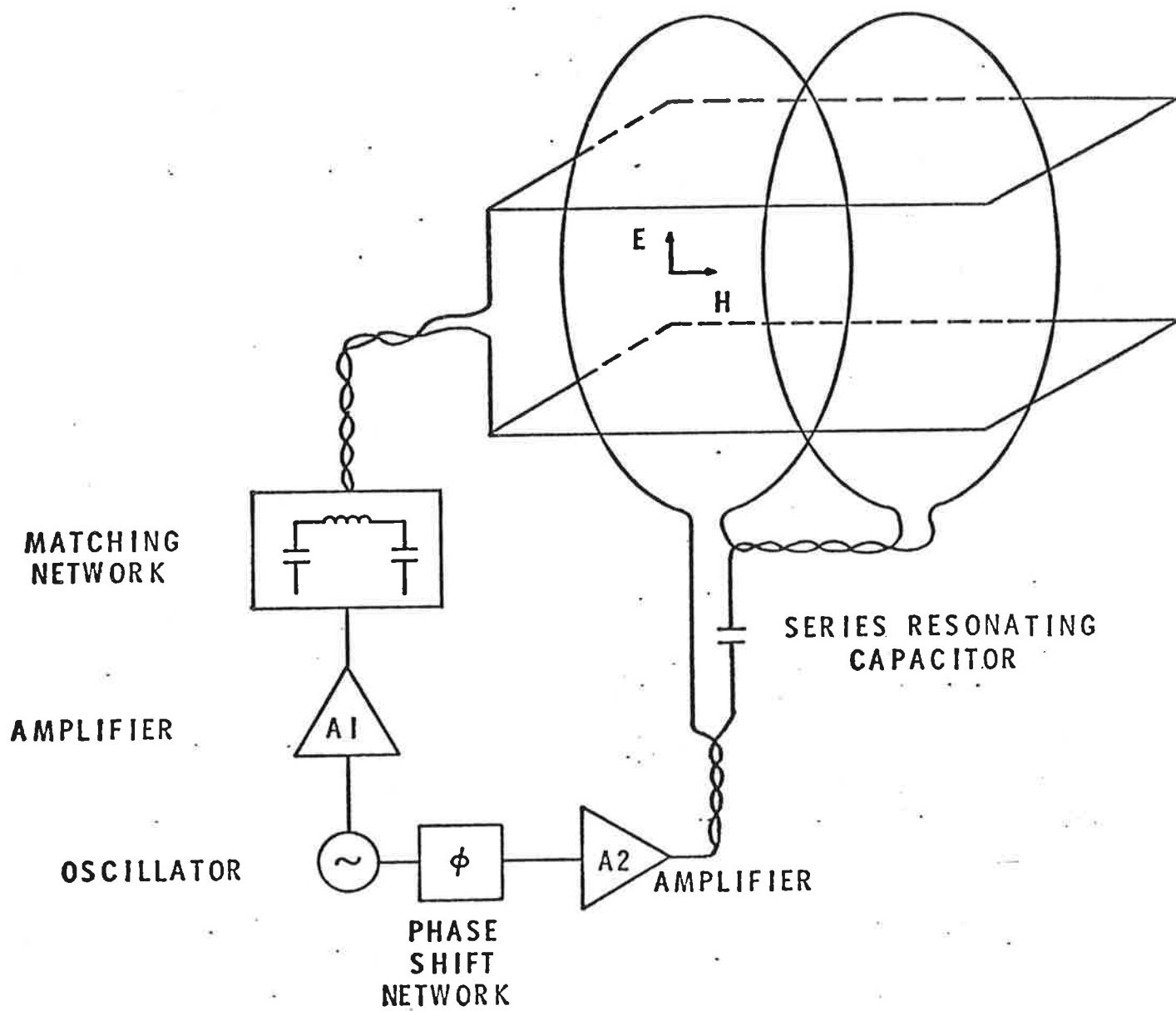


FIGURE 1

CONCEPT OF VLF GENERATOR

Figure 2, is a photograph of the generator as seen through the door of the wooden building in which it is housed. A cubical wooden frame approximately two meters on a side forms the supporting structure. Two circular wooden rings, each two meters in diameter, serve as the forms for the Helmholtz coils. The wooden rings are held in position by the supporting structure. The parallel plates which generate the electric field may be seen in edge view in the center of the photograph. The plates are spaced one third of a meter apart and are centered on the axis of symmetry of the Helmholtz coils. The plates consist of strips of aluminum foil sandwiched in between two layers of fiberglass material. In the photograph, the fiberglass support and several containers for experimental animals may be seen in place on the lower of the parallel plates. The wooden box seen in the lower left of the figure contains a pi-network for matching the amplifier to the parallel plates.



Figure 2.

V. L. F. GENERATOR

Figure 3, is a photograph of the interior of a trailer used as the instrumentation and control room for the VLF generator. The trailer is located 25-feet from the generator well out of the maximum electromagnetic field. The McIntosh 200 AB Amplifier, which is used to drive the electric field elements, is shown mounted in a rack in the left of the photograph. A spare McIntosh 200 AB Amplifier is mounted in the same rack but is not visible in the photograph. On top of the rack is the Hewlett Packard 200ABR oscillator which serves as the common frequency determining element for the electric and magnetic fields. On the left end of the bench is located the EICO HF-89 Amplifier used to drive the Helmholtz coils. Next to the EICO HF-89 Amplifier is the Hickok 3200 counter used to monitor the frequency of operation and next to the counter is the Tektronix 5103N oscilloscope which is used to adjust and monitor the relative phase of the electric and magnetic fields.

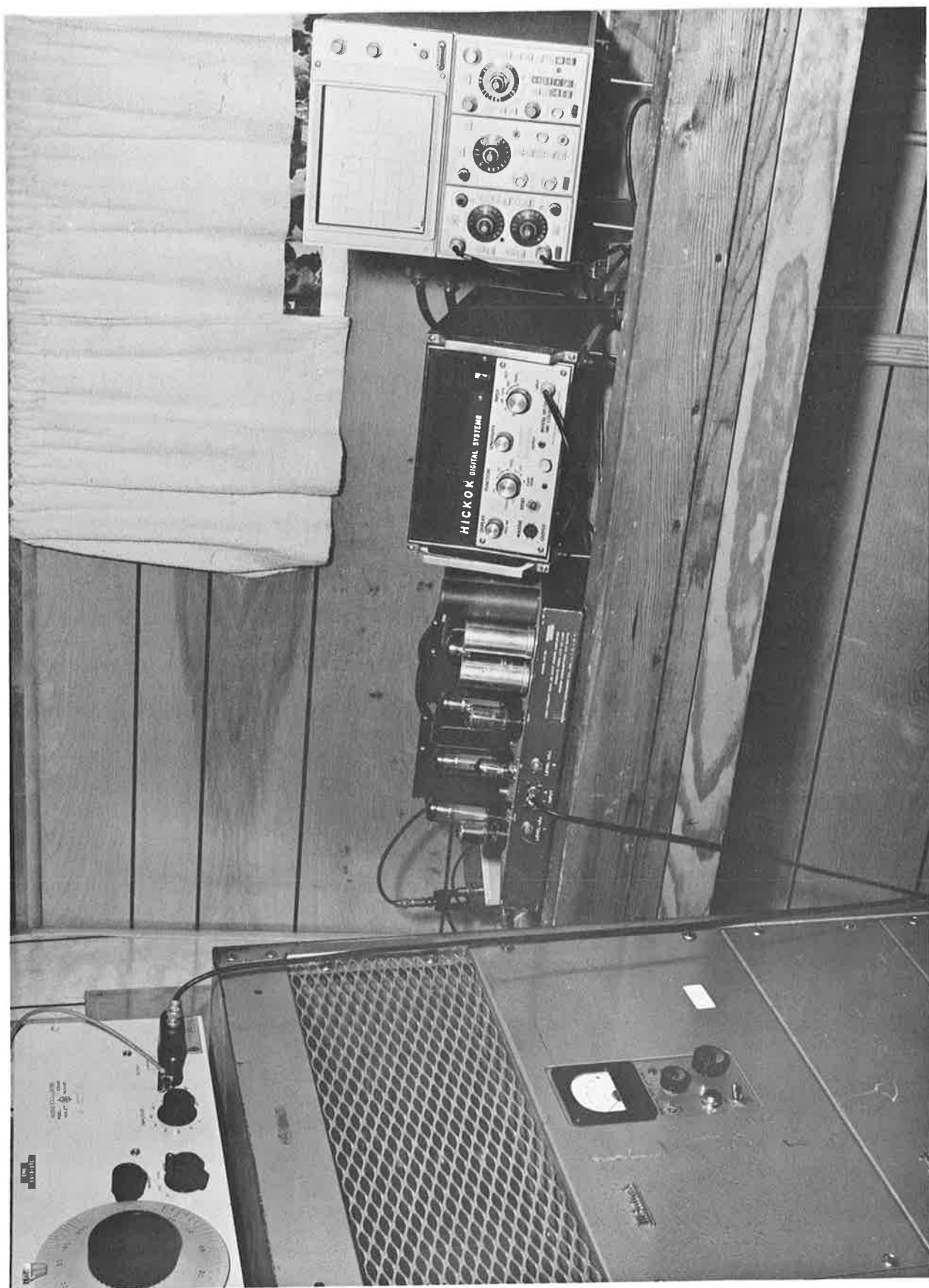


Figure 3.
INSTRUMENTATION AND CONTROL ROOM

A block diagram of the VLF generator is shown in Figure 4. An HP200CDR Oscillator is used as the frequency determining element for the generator. A Hickok Model DMS 3200 Counter with DP 150 Plug In is used to set the frequency of operation.

To simulate a plane wave condition in the exposure volume, the electric and magnetic fields must be synchronized. A phase adjust network following the HP200CDR Oscillator permits compensation for the difference in phase shift which occurs between the electric field and magnetic field driving channels and thus enables the fields to be synchronized.

The phase adjust network has outputs for the electric field driving circuits and for the magnetic field driving circuits. These outputs are applied to level adjusting potentiometers associated with meter indicators in the control panel. From the level adjusting potentiometers on the control panel and enable the attending operator to easily set the electromagnetic field components to the proper level.

The Helmholtz coils are driven by an EICO Model HF-89 100 watt power amplifier and the parallel plates by a McIntosh 200AB 200 watt power amplifier. The Helmholtz coils are series tuned by a 0.47 uF capacitor to reduce the impedance as seen by the driving amplifier. The parallel plates are coupled to the output of the McIntosh 200AB amplifier by a UTC LS-6L1 transformer and a pi-network. The transformer is used in a step-up configuration. It transforms the amplifier impedance from 4 ohms to an estimated 10,000 ohms and steps-up the voltage to approximately 1200 volts. The pi-network transforms the impedance to a still higher level (estimated 200,000 ohms) and steps-up the voltage to 5,000 volts.

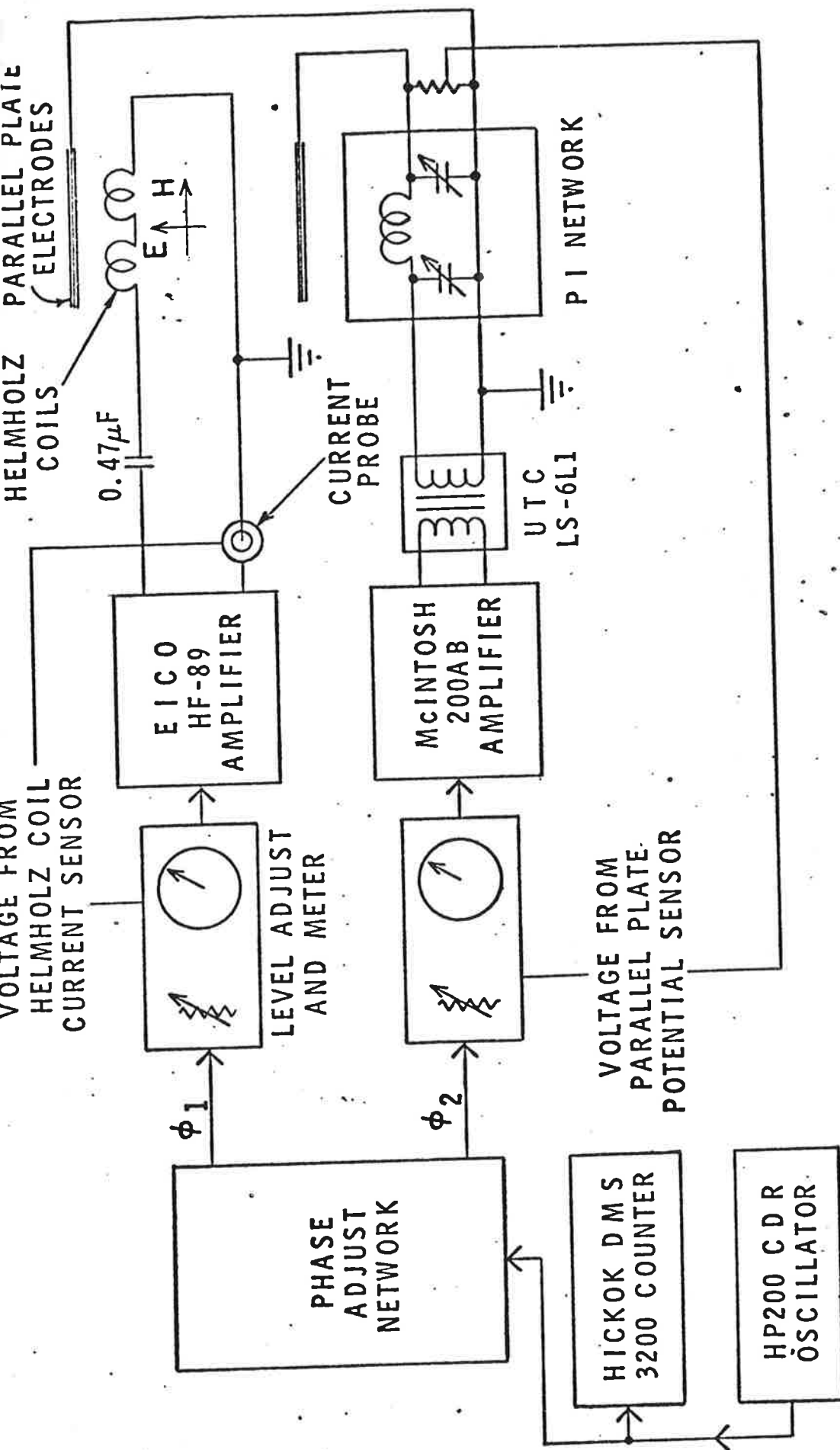
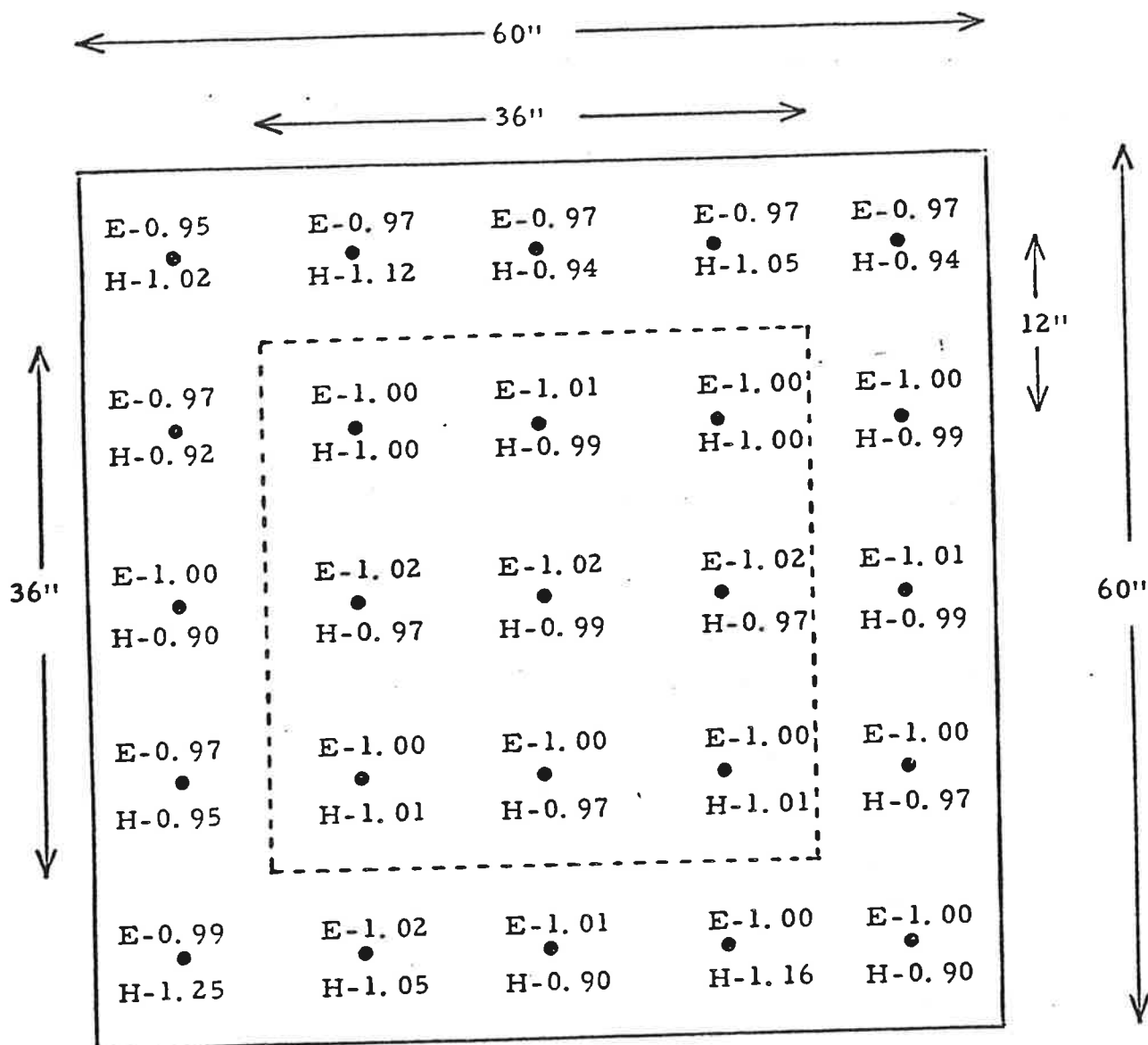


Fig. 4

BLOCK DIAGRAM OF VLF ELECTROMAGNETIC FIELD GENERATION

The degree of uniformity of the electric and magnetic fields established over the plane centered between the parallel plate electrodes is illustrated in Figure 5. In the 5' x 5' area, 25 points on 12" centers are indicated. At each point the normalized electric field intensity and normalized magnetic field intensity is recorded. Within a 3' x 3' area centered on the 5' x 5' area, the electric field intensity is uniform to $\pm 1\%$ and the magnetic field intensity is uniform to $\pm 2\%$. Over the 5' x 5' area, the electric field intensity is uniform to $\pm 3.5\%$ and the magnetic field intensity is uniform to $\pm 17\%$. Experimental animals will be placed within the 3' x 3' area for exposure.



E-0.95 denotes normalized electric field intensity
H-1.02 denotes normalized magnetic field intensity
Dashed line encloses area used for exposure of experimental animals.

FIGURE 5
UNIFORMITY OF ELECTRIC AND MAGNETIC FIELD INTENSITIES

IV. PROCEDURES AND EXPERIMENTAL DESIGN.

The procedures listed below will be used throughout this study.

A. Criteria for Ascertaining Effects of Irradiation.

1. Ability of irradiated animals to continue to reproduce.
2. Litter sizes produced.
3. Weight of litters produced.
4. Appearance of gross abnormalities in litters produced.
5. Histological manifestations of gonadal and/or adrenal injury.
6. Gross manifestations of damage to accessory reproductive organs.
7. Amount of sperm in the epididymides.
8. Ovarian follicular development.

B. Method of Animal Maintenance.

The animals used in this experiment will be albino rats of the Sprague-Dawley Strain. These animals will be maintained throughout the experiment on Wayne Laboratory Chow and water supplied ad libitum. A mean temperature of 78° Fahrenheit, a relative humidity of 50% and a twelve-hour light-dark cycle in the colony room will be maintained automatically. All animals are to be maintained in filtered "Health Guard System" cages, and a high standard of prophylaxis will be maintained to prevent epidemics and to promote survival to the termination of the experiment.

C. Method of Determining Actual Known Time of Mating (Conception).

Known stages of gestation will be obtained by placing "teaser" males with mature females between the hours of 8 and 12 p.m. From the observed behavioral response, females in early estrus will be determined. These females will be removed and placed overnight with males of proven fertility. This procedure, which has been shown to be 95% effective in determining time of conception, will be repeated daily to produce sufficient matings for this investigation. The morning following conception (day 1 of the gestation period), the females will be removed from the cages housing the males and will be maintained in the control colony until the appropriate day of the irradiation as described in the experimental design.

The estrus cycle will be further checked by microscopic observations of vaginal smears taken day to day prior to mating. Examination of these vaginal smears under the microscope at low power will show that during the anestrus portion of the cycle the smear will consist predominantly of leucocytes, with an occasional cornified epithelial cell. The first appearance of estrus is marked by mucification of the vagina, followed quickly by the complete, or nearly complete, disappearance of leucocytes and their replacement by large numbers of round, nucleated, epithelial cells. These cells are about three times as large as the leucocytes and, once seen, cannot be mistaken. At this time and for about twelve (12) hours afterward, the animal will breed, but at no other time. Desquamation of the epithelial cells now occurs, and white, cheesy masses of disintegrating squamous cells are found on the smear. Leucocytes now again make their appearance, and the cycle is repeated. The whole cycle in rats lasts about five (5) days, being divided roughly into three (3) days of anestrus and two (2) spent in the manifestation of estrus just described. The cycle makes its appearance at puberty, which is reached in the rat at an age of sixty (60) - ninety (90) days.

D. Post-natal and Growth Studies

1. Males

Fifty (50) sexually mature male albino rats of the Sprague-Dawley Strain will be utilized. Forty (40) rats will be randomly placed in the experimental groups and exposed to radiation as shown in the chart below, (5 days per week, for 12 weeks). A control group of ten animals will be placed on the same experimental regime (but without exposure) for 4 hours per day.

<u>Number of Animals</u>	<u>Treatment</u>	<u>Group</u>	<u>Exposure Time</u>
10	Control (none)	I	None
10	1/2 power	II	2 hrs.
10	Maximum Power	III	2 hrs.
10	1/2 power	IV	4 hrs.
10	Maximum Power	V	4 hrs.

2. Females

Fifty (50) sexually mature female albino rats of the Sprague-Dawley Strain will be utilized in the same experimental paradesign as for the males. The combined male and female experiments are summarized below.

<u>Number of Animals</u>		<u>Treatment</u>	<u>Group</u>	<u>Exposure Time</u>
<u>Females</u>	<u>Male</u>			
10	10	Control (none)	I	None
10	10	1/2 power	II	2 hrs.
10	10	Maximum Power	III	2 hrs.
10	10	1/2 power	IV	4 hrs.
10	10	Maximum Power	V	4 hrs.

The F₀ males and females will receive irradiation treatments twelve (12) weeks prior to mating. Then they will be allowed to litter to produce the F₁ generation. At sexual maturity males and females from the F₁ neonates will be randomly selected and placed on a twelve (12) week irradiation treatment and then mated and allowed

to litter to produce the F₂ neonates. At sexual maturity the F₂ Neonates will be selected in the same manner and kept within treatment groups to produce the F₃ neonates.

The three generations F₀, F₁, and F₂ will each have received twelve (12) weeks of treatment prior to mating. The F₃ generation will have received a total of 36 weeks of post-natal accumulative irradiation in their genetic background.

E. Pre-natal

After the initial 12 weeks of irradiation males and females from the original F₀ group will be mated. After mating, males will be retained for post-mortem evaluations.

Females will be allowed to litter to produce the F₁ Neonates. The F₁ male and female neonates from this group (within treatment) will be utilized to produce the F₂ generation as previously stated. These matings will be selected at random from within treatment groups as follows:

MATINGS

	Groups	Male	Female	Treatment	Exposure Time	Within		Generation
						Groups	Matings	
F ₀	I (Control)	10	10	Control (none)	None	C X C		C Males, C Females
	II	10	10	1/2 power	2 hrs	II X II		II Males, II Females
	III	10	10	Maximum power	2 hrs	III X III		III Males, III Females
	IV	10	10	1/2 power	4 hrs	IV X IV		IV Males, IV Females
	V	10	10	Maximum power	4 hrs	V X V		V Males, V Females
F ₁	I (Control)	10	10	Control (none)	None	C X C		C Males, C Females
	II	10	10	1/2 power	2 hrs	II X II		II Males, II Females
	III	10	10	Maximum power	2 hrs	III X III		III Males, III Females
	IV	10	10	1/2 power	4 hrs	IV X IV		IV Males, IV Females
	V	10	10	Maximum power	4 hrs	V X V		V Males, V Females
F ₂	I (Control)	10	10	Control (none)	None	C X C		C Males, C Females
	II	10	10	1/2 power	2 hrs	II X II		II Males, II Females
	III	10	10	Maximum power	2 hrs	III X III		III Males, III Females
	IV	10	10	1/2 power	4 hrs.	IV X IV		IV Males, IV Females
	V	10	10	Maximum power	4 hrs	V X V		V Males, V Females

Male and female F_2 neonates produced from F_1 generation will be mated from within treatment groups in the same manner to produce the F_3 generations. The progeny from each generation will be observed for any abnormalities that might occur from the radiation insult. F_1 , F_2 , and F_3 neonates will be weighed daily, beginning on the 4th day post-partum until the 31st day, then once a week for the duration of the experiment as previously described. Sexes will be segregated at the 21st day. Litter size and time of occurrence of resulting pregnancies are to be observed. Ten animals from each group of the F_1 , F_2 , and F_3 neonates will be used for growth studies and ten animals from each group will be sacrificed at maturity for post-mortem evaluations.

The following table shows the time periods for this phase of the study:

Time Periods of Work Scope

Post-natal

F_1 Generation

Pre-mating Irradiation Treatment	Mating Gestation	Neonates Growth	
(12 weeks ----	5 weeks -----	12 weeks)	= 29 weeks

F_2 Generation

Pre-mating Irradiation Treatment	Mating Gestation	Neonates Growth	
(12 weeks ---	5 weeks -----	12 weeks)	= 29 weeks

F_3 Generation

Pre-mating Irradiation Treatment	Mating Gestation	Neonates Growth	
(12 weeks ----	5 weeks -----	12 weeks)	= 29 weeks
			<u>87 weeks</u>

The following chart shows the irradiation schedule for this portion of the study:

Irradiation Schedule
(Generation Studies)

Generation	1/2 power		Maximum Power		Daily Time			# Weeks (5 days)
	Male	Female	Male	Female	1/2	Max	Tot	
F ₀	10	10	10	10	2	4	6	12
F ₁	10	10	10	10	2	4	6	12
F ₂	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>2</u>	<u>4</u>	<u>6</u>	<u>12</u>
Totals	30	30	30	30				36 weeks

A total of sixty (60) animals will receive irradiation at 1/2 power and sixty (60) animals at maximum power.

F. Gestation Period Irradiation (F₁, F₂, and F₃ Generations)

Based on the critical period of fetal development in the human the first 90 days of pregnancy, the first seven days of the albino rats' gestation period will be observed more extensively than the 2nd and 3rd tri-mesters. The F₁, F₂ and F₃ groups previously outlined will receive irradiation treatment of two or four hours duration each exposure period during days 3, 5, 7, 11, 15 and 17 of the gestation period. Treatments will be consistent within groups as per the previous groups tested in the original F₀ groups of the post-natal design. The females will be allowed to litter to produce the next generation and will be sacrificed on day twenty (20) of the gestation period. The pre-natal observations will be explained under the section: Embryogenesis.

The known stages of gestation will be determined (as previously described) by placing "teaser" males with mature females between the hours of 8 p.m. and 12 p.m.

The experimental design for each gestation period observation is shown below (6 different periods).

Gestation Period Pre-Natal Matings

<u>Number of Animals</u>	<u>Treatment</u>	<u>Group</u>	<u>Exposure Time</u>
5	Control (none)	I	none
5	1/2 power	II	2 hrs.
5	Maximum power	III	2 hrs.
5	1/2 power	IV	4 hrs.
5	Maximum power	V	4 hrs.

The following table shows the time table of this phase of the experiment:

Time Table for the Gestation Period Studies

F₀ Generation

F₁ Mating period
to day 20

F₁ Neonate
Growth to
Puberty

(8 weeks ----- 12 weeks) = 20 weeks

F₂ Mating period
to day 20

F₂ Neonates
Growth to
Puberty

(8 weeks ----- 12 weeks) = 20 weeks

F₃ Mating period
to day 20

F₃ Neonates
Growth
to puberty

(8 weeks ----- 12 weeks) = 20 weeks

60 weeks

The following tables show the F₁ generation gestation irradiation periods. Twenty five (25) animals will be utilized for day three. Each gestation period groups of animals will have their respective control group.

<u>F₁ Generation</u>		<u>Day three (3) Gestation Period</u>	
<u>Group</u>	<u>Treatment</u>	<u>Exposure time-hrs.</u>	<u>Number animals</u>
I	Control	None	5
II	1/2 power	2	5
III	Max. power	2	5
IV	1/2 power	4	5
V	Max. power	4	5
	Total		25 animals

A second group of different animals are to be utilized for day five (5) gestation period studies as follows:

<u>Day Five (5) Gestation Period</u>			
<u>Group</u>	<u>Treatment</u>	<u>Exposure time-hrs.</u>	<u>Number animals</u>
I	Control	None	5
II	1/2 power	2	5
III	Max. power	2	5
IV	1/2 power	4	5
V	Max. power	4	5
	Total		25 animals

A third group of different animals will be utilized for day seven (7) gestation period studies as follows:

<u>Day Seven (7) Gestation Period</u>			
<u>Group</u>	<u>Treatment</u>	<u>Exposure time-hrs.</u>	<u>Number animals</u>
I	Control	None	5
II	1/2 power	2	5
III	Max. power	2	5
IV	1/2 power	4	5
V	Max. power	4	5
	Total		25 animals

A fourth group of different animals will be utilized for day eleven (11) gestation period studies as follows:

Day Eleven (11) Gestation Period

<u>Group</u>	<u>Treatment</u>	<u>Exposure time-hrs.</u>	<u>Number animals</u>
I	Control	None	5
II	1/2 power	2	5
III	Max. power	2	5
IV	1/2 power	4	5
V	Max. power	4	5
	Total		25 animals

A fifth group of different animals will be utilized for day fifteen (15) gestation period studies as follows:

Day Fifteen (15) Gestation Period

<u>Group</u>	<u>Treatment</u>	<u>Exposure time-hrs.</u>	<u>Number animals</u>
I	Control	None	5
II	1/2 power	2	5
III	Max. power	2	5
IV	1/2 power	4	5
V	Max. power	4	5
	Total		25 animals

A sixth group of different animals are to be utilized for day seventeen (17) gestation period studies as follows:

Day Seventeen (17) Gestation Period

<u>Group</u>	<u>Treatment</u>	<u>Exposure time-hrs.</u>	<u>Number animals</u>
I	Control	None	5
II	1/2 power	2	5
III	Max. power	2	5
IV	1/2 power	4	5
V	Max. power	4	5
	Total		25 animals

In summary on the F₁ generation: There will be a total of (6 x 25 = 150) one hundred and fifty animals utilized and a total irradiation time for the facility of (6 x 6 = 36) thirty six (36) hours.

The F₂ and F₃ generation group of animals will follow the same procedure as previously outlined for the F₁ generation, gestation study animals.

Note: All animals selected for treatment group and exposure will be selected from that treatment and exposure only. This will be followed constantly down through the generations. Therefore, for example: the F₃ generation animals will have (within their respective treatment groups) an accumulated irradiation of thirty six (36) weeks exposure plus the gestation day exposure to electromagnetic irradiation.

The total number of animals utilized in this portion of the study will be (F₁ generation = 25; F₂ = 25; F₃ = 25), one hundred and twenty five (125) females. The males from the generation studies will be utilized as sires for these studies before they are sacrificed.

G. Growth Studies (F₀, F₁, F₂, and F₃ Neonates)

F₀ Generation will be weighed weekly. Their offspring, the F₁ neonates will be weighed on day four (4) post-partum and daily body weights will be taken through weaning (31 days old). Thereafter body weights will be weekly. (Weaning at 21 days + 10 days = 31 days daily weights).

The F₂ and F₃ generation will be handled in the same manner as the F₁ neonates. Ten neonates from each generation and group will be carried through maturity for these growth evaluations.

At day four (4) post-partum all neonates will be marked for identification. At weaning age, permanent identification will be tattooed in each ear as per animal number, groups, treatment, and generation.

The following chart shows the research program on the generation, growth, embryogenesis and generation sacrifice studies.

RESEARCH PROGRAM

eration Studies, Embryogenesis Terata Studies, Growth Studies and Generation Sacrifice)

Generation	Activities	Time-Weeks	#Animals on Experiments
F ₀ Generation	Pre-Experiment	4	100
	Irradiation 12 weeks	12	100
	Mate	2	50
	Sacrifice F ₀ Males *		
F ₁ Generation	Wean F ₁ Generation	3	420
	Sacrifice F ₀ Females *		
	F ₁ Neonates - Select		
	Generation Terata		
	60 males + 60 females 60 males + 60 females		
	F ₁ Growth to puberty studies	12	420**
	Irradiation - 12 weeks	12	420
	Mate Generation and Terata		
	Neonates	2	420
	Sacrifice F ₁ Males*		
F ₂ Generation	Wean F ₂ Generation	3	370
	Sacrifice F ₁ females *		
	F ₂ Neonates - Select		
	Generation Terata		
	60 males + 60 females 60 males + 60 females		
	F ₂ Growth to puberty studies	12	420**
	Irradiation - 12 weeks	12	420
	Mate - Generation and Terata		
	Neonates	2	420
	Sacrifice F ₂ Males *		
F ₃ Generation	Wean F ₃ Generation	3	370
	Sacrifice F ₂ females *		
	F ₃ Neonates - Select		
	Generation Terata		
	60 males + 60 females 60 males + 60 females		
	F ₃ Growth to puberty studies	12	420**
	Sacrifice Generation		
	Study F ₃ Neonates	5	420
	Mate terata neonates		
		96	

*There will be extra animals on the experiment for a short period during the postmortem intervals.

** There will be two animals from each generation and treatment group carried over on the growth to puberty studies for postmortem evaluation. A total of forty (40) animals each growth period.

H. Clinical (Hematology)

Initial blood samples will be taken from the males and females on all generation study groups prior to treatment. Samples will be taken at the end of the irradiation period. These blood samples will be evaluated as follows:

Red blood cell count, white blood cell count, hematocrit, hemoglobin, and differential counts.

The following table shows the bleeding schedules for this study, five treatment groups, including control groups:

Generation	# animals Initial bleeding		# animals Bleeding at		# animals Termination of	
	<u>Pre Treatment</u> <u>Males</u> <u>Females</u>		<u>end of Irrad.</u> <u>Males</u> <u>Female</u>		<u>Growth Period</u> <u>Males</u> <u>Females</u>	
F ₀	50	50	50	50		
F ₁	50	50	50	50	50	50
F ₂	50	50	50	50	50	50
F ₃	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u>50</u>	<u>50</u>
Totals	150	150	150	150	150	150

A total of nine hundred (900) blood samples will be evaluated.

I. Histo-Pathological

Organ weights will be taken at the postmortem sacrifice and histo-pathological evaluations will be made on the following organs or tissues:

<u>Males and Female</u>	<u>Males</u>	<u>Females</u>
Liver	Epididymis	Uterine Tissue
Kidney	Seminal Vesicles	examination*,
Adrenals	Ventral lobes of	Ovaries
	prostate; testes.	Vagina and cervix
	Epididymis	

At the termination of each test group, a representative number of animals from each test group will be sacrificed for postmortem examination (5 males and 5 females).

The following table shows the post mortem sacrifice.

Postmortem Schedule for Each Treatment Group: (5 Groups)

Pre-natal and growth studies.

	<u>Sires & Dams at Weaning</u>		<u>At Termination of Growth Period</u>	
	Male	Female	Male	Female
F ₀ Generation	5	5	5	5
F ₁ Generation	5	5	5	5
F ₂ Generation	5	5	5	5
F ₃ Generation	5	5	5	5
	20	20	20	20

A total of eighty (80) animals will be sacrificed, forty (40) males and forty (40) females. Eleven (11) organs from the males and eleven (11) from the females will be examined. Two (2) slides from each organ will be sectioned for these examinations. A total of four hundred and forty (440) slides from the males and four hundred and forty (440) from the females will be evaluated.

*Uterine tissue will be examined for implantation and resorption sites as described under teratological evaluations.

The male slides will be examined using the following criteria to assess radiation damage:

1. Total activity of the seminiferous tubules (active inactive, regeneration) per testes cross-section, by microscopic examination.
2. Activity of the germinal cells in the tubules. The epididymides will be examined for the presence of sperm (amount) and also for the presence of abnormal sperm and other degenerating cells.

The female slides will be examined using the following criteria for determining any deviation from the control groups.

1. Activity of the ovaries.
2. Uterine tissue.
3. Uterus and vaginal sections.

All tissue taken will be fixed in formal acetic alcohol (FAA) and histological sections will be prepared where significant variation occurs from the control group. The tissue will be prepared by employing standard staining techniques. A formal report will be submitted at the end of each 12 month period. The final report will document the protocol, test results, analysis of the results and a summary attesting to the existence or absence of any potential hazard imposed by this electromagnetic irradiation regime.

J. Embryogenesis: The Developing Embryo.

The biological effects of the relative low-level fields on the developing embryo have not been determined. Pregnant women or women of childbearing potential are frequently exposed to these fields even without their knowledge. We, therefore, propose to examine the uterine tissue and embryos as generally done in teratogenic evaluations. This will give us an indication of any damage that might occur during fetal development.

a. Teratogenic evaluations:

Teratology refers to the study of congenital malformations which are anatomic abnormalities resulting from aberrant morphogenesis of specific tissues, organs, and organ systems. Although teratologic malformations are a frequent manifestation of

inherited and acquired genetic lesions, they may also be due to environmental alterations occurring during critical periods of development. Organs and specific tissues are particularly susceptible during periods of accelerated growth.

All pregnant animals will be subjected to the irradiation treatment as previously shown and, on day twenty (20) a caesarean section will be performed. Since this species has a consistent gestation period, the particular day will not be a serious problem. However, some variations do exist and this will be taken into consideration in determining the days of pregnancy. The animals will be administered ether as a general anesthetic. Under anesthesia a laparotomy will be performed on each animal. The following studies and observations will be made at this time according to guidelines put forth by the World Health Organization and Food and Drug Administration. Refer to Appendix I.

These observations to be made are as follows:

1. Number and position of resorption sites and fetuses (dead and alive).
2. Number of implantation sites.
3. Observations of corpora lutea.
4. Weights.
5. Sex.
6. External examination of each fetus for abnormalities.
7. Number of normal and abnormal fetus.

A consistent system of numbering and indicating the relative positions of fetuses will be adopted and recorded along with the other information mentioned above. A standard protocol sheet similar to that designed by Wilson will be utilized for this purpose. Refer to Appendix I.

After external examination, weighing, etc., has been completed, 1/3 of the fetuses (randomly selected), will be placed in Bouin's fixative for later free hand razor blade dissection (Wilson Technique). Any grossly anomalous fetuses will be dissected. The remaining 2/3 of the fetuses will be fixed in 95% alcohol prior to being subjected to clearing-alizarin red methods for skeletal examinations. Refer to Appendix I.

The following table shows the schedule for the embryo analysis.

Table for Embryo Analysis
(Five Groups on Six separate treatment times)

<u>Pre-natal exposure:</u>	<u>Per Generation</u>
5 animals x 5 groups = 25	Day 3 exposure
5 animals x 5 groups = 25	Day 5 exposure
5 animals x 5 groups = 25	Day 7 exposure
5 animals x 5 groups = 25	Day 11 exposure
5 animals x 5 groups = 25	Day 15 exposure
5 animals x 5 groups = 25	Day 17 exposure

Total animals 150 per generation will be examined teratologically. An estimated total of ten (10) fetuses per animal and one hundred and fifty (150) females per generation to be utilized, gives an estimated fifteen hundred (1500) fetuses per generation. Fifteen hundred (1500) fetuses per generation, and there are three generations, yield an approximate total of forty five hundred (4500) evaluations that will be considered in this phase of the study. The following is an approximate breakdown of the evaluations. These fetuses used will be randomly selected for these evaluations.

Two animals per treatment examined by free hand razor techniques. (Wilson method).

2 animals x 5 groups x 6 exposure periods = 60 animals.
60 animals x 10 (est.) fetus = 600 fetuses.

Two animals per treatment will be subjected to clearing-alizarin red method for fetal skeletal examination.

2 animals x 5 groups x 6 exposure periods = 60 animals.
60 animals x 10 (est.) fetus = 600 fetuses.

K. Analysis of Data

The basic experimental design is a randomized block design. The results of the experiments will be statistically analyzed with analysis of variance, chi-square, and regression analysis, as appropriate.

Statistically the following comparisons can be made on the

F₀, F₁, F₂ and F₃ generations;

1. Body weight of adult control, vs., 1/2 power vs., maximum power groups.
2. Growth of neonate control, vs., 1/2 power vs., maximum power groups.
3. Litter size from control, vs., irradiated dams and sires at the various powers.
4. Histological organ evaluations, control, vs., 1/2 power vs., maximum power groups.
5. Teratological evaluations control, vs., 1/2 power, vs., maximum power groups.
6. Hematological evaluations control vs., 1/2 power, vs., maximum power groups.

The above parameters will also be evaluated with respect to the length of exposure time: zero, two or four hours per day.

A variety of packaged, statistical analysis programs (IBM 360 A Scientific Subroutine Package, BMD programs, SSPS), are available at Southwest Research Institute for use on large computers. Southwest Research Institute is linked to a large computer time-sharing network via the cyber 74 system and has terminal connections to other computers in addition. Several minicomputers and programmable desk calculators are also available for data analysis.

L. Reports

Any change in test protocol will be formulated and presented to the sponsor for their concurrence.

A formal report will be submitted at the end of each 12 month period. The final report will document the protocol, test results, analysis of the results and a summary attesting to the existence or absence of any potential hazard imposed by this electromagnetic irradiation test regime.

V. SELECTED LITERATURE SURVEY

1. Cardiovascular Effects: Several investigators, including Gordon (1960), have reported exposures of animals or man to microwave system. Some investigators (e.g., Gorodetskaia, 1964) also suggest that exposure to microwaves at intensities that do not produce appreciable thermal effects may lead to functional changes, which are observed with acute as well as chronic exposures. Cooper (1962) reports increased heart rate after exposure to power densities of 50-130 mW/cm.² for variable periods of time ranging from 10-140 minutes. Tyagin (1957) reports slowing of the heart rate with low, or what the Soviets consider nonthermal, levels of microwaves, although there have been some Soviet reports of increased heart rate with low-level microwave exposure over the dorsal aspect of rabbits.

Tolgskaya and Gordon (1960) suggest that receptors of the reflexogenic zone of the curve of the aorta, the carotid sinus, and all layers of the auricular wall are highly sensitive to microwaves. They report morphological modification of receptors after one exposure to microwaves with these changes decreasing with repeated exposures.

2. Central Nervous System Effects: It has been known for some time that an RF field can be reinforced in the region of peripheral nervous tissue causing temperature elevation, even though nearby muscle and skin show no measurable temperature effect, and that when peripheral nerves are heated above a minimum level they may trigger spontaneously. McAfee (1961) presents convincing evidence that it is thermal stimulation of the peripheral nervous system which produces the neurophysiological and behavioral changes observed. Thus, the interaction between the peripheral nervous system and the central nervous system may account for observed effects on heart rhythm, blood chemistry, etc., as reported by the Soviets and alluded to previously. Frey (1962), reports that an auditory response occurs at power levels below 1 mW/cm.² of 200-300 MHz. microwaves and is believed to be a direct auditory nerve response to microwave stimulation. It is known that significant (though extremely inefficient) rectification of microwave energy may be possible in vivo, an effect which may explain some of the nonthermal neurologically related observations reported - especially where one hears pulse repetition frequency.

Presman (1965), a leading Soviet Spokesman on biologic effects of microwaves, states that many changes in the functions of the nervous system produced by microwaves are not specific, and some writers have argued that the action of microwaves is due to stimulation or variation of the excitability of neural tissues. However, clarification of the physical and chemical mechanisms of microwaves on nervous system structures involves considerable difficulties because the physical-chemical mechanisms of excitability of living tissue in general are still far from being clearly understood.

3. Lens of the Eye Effects: The eye is sensitive to many types of electromagnetic radiation, and microwaves have been shown to produce cataracts in a variety of experimental animals (Carpenter, 1962). Certain parts of the eye are more susceptible to particular wavelengths than others. Longer wavelengths are readily transmitted through the globe of the eye with little if any absorption and do not give rise to deleterious effects in the lens. In contrast, Cogan (1950) reports that shorter waves, such as those in the centimeter range, can be harmful to the anterior half of the eye because of absorption and consequent heating effects.

Barron et al. (1955) and Daily (1943) failed to find changes in the eyes of individuals working with radar. However, Hirsch and Parker (1952) report a case in which a technician developed bilateral cataracts while operating a radar unit in the 1500-3000 MHz. range, with exposures up to 100 mW/cm.², several months elapse prior to appearance of posterior capsular opacification; at this level, several years are required for production of formed cataracts.

4. Testes Effects: The effect of microwaves on the testes is indicated in several studies, such as the one reported by Ely et. al. (1957). These studies suggest that exposure of the scrotal area results in varying degrees of testicular damage such as edema, enlargement of the testis, atrophy, fibrosis, and coagulation necrosis of seminiferous tubules in rats, rabbits, and dogs exposed to 2,450, 3,000, 10,000 or 24,000 MHz. at power densities of about 10 to 15 mW/cm.². Imig et. al. (1948) reports experimental production of testicular effects such as degeneration, and reduced sperm generation at power densities sufficient to produce a measureable temperature rise (250 mW/cm.²).

5. Embryonic Development Effects: There is relatively little experimental data available reporting on the effects of electromagnetic irradiation on embryonic development. VanUmmersen (1961) investigated the effect of microwaves on development of the chick embryo using a 2,450 MHz. CW source (at 200-400 mW/cm.²) and found interference with cellular differentiation in the absence of effects on cellular proliferation.

6. Alteration in Electrophoretic, Immunologic and Enzymatic Activity of Proteins by Irradiation:
Changes in the electrophoretic and antigenic reactivity of human gamma globulin and reduced reactivity of alpha-amylase have been reported by Bach et. al. (1961). In a study on the effects on human gamma globulin, exposures were performed by using RF energy in a range of 10 to 200 MHz. (primarily in the range from 10 to 40 MHz.). While distinct effects attributed to harmonics of 6 MHz. were noted, they were not explored. A systematic search for effects was performed at 10 kilocycle intervals in the range of 13.04 to 13.39 MHz. Pronounced effects on antigenic reactivity were found at a power density of 13.4 mW/cm.² using short duty cycle pulses in order to produce high electric field strengths. The investigators noted that the effect was highly frequency dependent, in the sense that a strong effect could readily be found 10 to 20 kc away from a frequency at which no effect was observed. The marked change in the antigenic reactivity could characteristically be observed at field strengths in the order of 25V/m.

7. Genetic Effects: There is little direct evidence of possible genetic aberrations in experimental animals due to exposure to microwaves. However, Heller and Teixeira-Pinto (1959) reported RF-induced chromosomal abnormalities in garlic root tips growing in water when exposed for 5 minutes to 27 MHz. radiation. However, Schwan (1968) criticizes these results since thermal effects were not convincingly ruled out, leaving the possibility that the chosen parameters of the applied field caused biologically significant field induced-force effects. Finally, Sigler et al. (1965) noted that a higher incidence of children with Down's Syndrome among fathers with prior occupational exposure to radar.

VI PROGRAM ORGANIZATION

This proposed program will be conducted under the Section of Biomedicine, Department of Bioengineering. This section is under the Direct supervision of C. William Hall, M.D., Director, and H. Haskell Ziperman, M.D., Assistant Director. Dr. Ziperman is also Section Manager of the Biomedicine Section responsible for this research.

Dr. Rommon Lawson, Senior Research Physiologist, will be the principal investigator responsible for conducting this research. Dr. Lawson has had extensive research experience in the field of Reproductive Physiology. His previous experience at Texas A&M University, Research Foundation, as Manager of the Radiation Testing Laboratory, has included the effects of radiation on pre- and post-natal evaluations on various species of animals. While in charge of the day-to-day management of the testing laboratory, the experiments included toxicological, mutagenic, histological, and hematological evaluations of varied experiments.

Dr. Dennis M. Boenig will supervise the pathological evaluations of the tissue. Dr. Boenig received his D.V.M. from the Texas A&M College of Veterinary Medicine in 1968. He trained for four years at the same institution in the Department of Veterinary Pathology prior to coming to Southwest Research Institute. He has completed requirements for the Ph.D. degree, and expects to be awarded the degree during the 1974 academic year.

Mr. Russell E. Stevens, III, Research Scientist in the Department of Bioengineering will assist Dr. Lawson in conducting the proposed program. Because of Mr. Stevens' formal education and laboratory experience while at Texas A&M University and while at Southwest Research Institute, he is qualified to perform such a task. Mr. Stevens' expert evaluations on fetal brain development will add support to the overall scope of this project.

Messrs. Richard B. Schulz and Walter C. Dolle of the Department of Electronic Systems Research will be responsible for the functioning of the microwave irradiation apparatus. Mr. Schulz will provide the special technical expertise required to calibrate and monitor the levels of RF energy absorbed by the experimental organisms. Internationally recognized in the field of Electromagnetic Compatibility, Mr. Schulz is best known for his RF shielding work

and was cited for such in his designation as an IEEE Fellow. Several of his patents concern improvements in RF shielding. In addition to advancements in this area, he has developed EMC-type measurement and calibration instrumentation. Mr. Schulz has worked on cable coupling, filtering, and transient-reduction techniques. His broad technical grasp is evidenced by his activity as editor of the IEEE. Transactions on EMC and his responsibility is evidenced as a registered professional engineer (Pennsylvania). Mr. Dolle has over ten years of experience in the measurement and analysis of electromagnetic radiation from equipment items and in susceptibility (irradiation) testing of electronic equipment. He has designed the VLF electromagnetic field generator to be used for this experimental work.

The statistical examination of the rats will be under the supervision of Dr. George Barnwell, Senior Research Biomathematician. Dr. Barnwell has considerable experience in the statistical assessment of Biological data. He has served as a consultant for experimental design and data analysis for a variety of biological studies, including research in biochemistry, neurophysiology, behavior, teratology, and toxicology.

VII. FACILITIES, CAPABILITIES, AND

RELATED EXPERIENCE

Physical facilities for the housing, care, and maintenance of research animals to be utilized in the proposed investigation are presently available in the Institute's research and housing complex for small animals.

Southwest Research Institute has been actively engaged for sixteen years in research and development work related to the measurement, analysis, and suppression of unintentional electronic emanations from equipment and systems and, in the determination of the susceptibility of such installations to electromagnetic emanations of their operating environment. This experience has included the generation and control of relatively high intensity electromagnetic fields. The technical concepts, instrumentation, and measurement techniques therein utilized are directly related to the design, construction, and operation of the irradiation facilities to be used and described elsewhere in this proposal.

Prior research experience directly related to the investigations herein proposed stems from contract work concerned with the assessment of biological effects of electromagnetic radiation under USAF Contract F41609-70-C-0025. The latter project concerned the evaluation of certain biological effects of high frequency electromagnetic radiation on Macaca Mulatta monkeys and was conducted for the School of Aerospace Medicine, Brooks AFB, Texas. Mr. Walter C. Dolle of the Southwest Research Institute Professional Staff assisted and advised the project manager on matters related to exposure facilities, the coupling of electromagnetic energy into test specimens, and biomedical instrumentation used for monitoring EKG and deep body temperature during periods of high intensity irradiation. In this connection Mr. Dolle designed a unique filter system which permitted observation and recording of the low-level, low-frequency EKG and temperature data without interference from the high intensity irradiation field.

USAF Contract F41609-73-C-0035 is concerned with selected biological effects of high intensity, very low frequency (VLF) electromagnetic radiation. Mr. Walter C. Dolle of the Southwest Research Institute staff designed and constructed the exposure facility for this project. This system is capable of generating an electric field of 15,000 v/m and a magnetic field of 7.5 A/m so oriented in space and

phased in time as to stimulate a wave front of 2,000 ohms characteristic impedance. This same exposure facility will be used for the experimental work of this proposal.

USAF Contracts F41609-72-C-0027 and F41609-73-C-0026 performed for the School of Aerospace Medicine, Brooks AFB, Texas were concerned with expansion of the capabilities of the School of Aerospace Medicine's existing 40KW, 2-30 MHz exposure facility for experimental investigation of the biological effects of electromagnetic radiation. Mr. Richard B. Schulz of the Southwest Research Institute staff designed and supervised the modification of this system to add capabilities for exposure at 20-400 MHz and 1.0-3.5 GHz at power levels of 1KW and 400W, respectively.

GEORGE M. BARNWELL
Senior Research Biomathematician
Department of Bioengineering

B.S. in Mathematics, University of Arkansas, 1962
M.S. in Psychology, University of Georgia, 1966
Ph.D. in Biomathematics, North Carolina State University, 1971

In addition to his Ph.D. in biomathematics, Dr. Barnwell has the course equivalent of a Ph.D. in experimental psychology. He has had extensive training in biology, psychology, mathematics, and statistics.

After completing course work for his M.S. in psychology, Dr. Barnwell worked at Civil Defense Research at the University of Georgia. He supervised the writing and testing of a programmed instruction Fallout Shelter Handbook for untrained people. Writing and testing this handbook became a primary task of the Civil Defense Research Project.

Dr. Barnwell's Ph.D. thesis research concerned a mathematical model of the effects of varying spatio-temporal patterns of dendritic synaptic inputs on neuronal somatic potentials. The mathematical model developed in his thesis allows theoretical investigation of any spatio-temporal dendritic activation pattern and has several theoretical implications for learning and memory mechanisms and neuronal function. Thesis research included construction of the mathematical model and calculations of the theoretical effects of various input patterns on somatic potentials, using an IBM 360/75 computer.

Upon completion of his Ph.D., Dr. Barnwell received a National Research Council Postdoctoral Research Associateship to develop mathematical models of the neural control of food intake, at the USAF School of Aerospace Medicine, Brooks AFB, Texas. The systems theory framework and modeling techniques permit the construction of models which can encompass all factors known to affect food intake. Phases of research include development of a general theoretical framework, construction of logical-connective models specifying the interconnections among neural centers and other subsystems in a given species, quantification of the logical-connective models, and computer simulation studies based on the mathematical models. Dr. Barnwell recently presented papers based on this research at the IV International Biophysics Congress in Moscow, and the International Congress of Cybernetics and Systems in Oxford, England.

At SwRI, Dr. Barnwell has continued his research on neural models and food intake. He is also working on mathematical models of the effects of ionizing radiation on the neuromuscular system. As part of this research effort, he has developed a neural network model capable of tracking. He is also interested in mathematical models and computer simulation studies of the effects of various drugs, fatigue, and other variables on driver behavior, and plans further investigations in this area. In addition, he has been extensively involved in establishing methods for collecting, processing, and analyzing traffic accident data from several countries participating in the CCMS/NATO program.

PROFESSIONAL CHRONOLOGY: Administrative coordinator, Civil Defense Research, University of Georgia, 1965-6; biomathematics graduate student, North Carolina State University, 1966-70; National Research Council Postdoctoral Research Associate, USAF School of Aerospace Medicine, Brooks AFB, Texas, 1970-2; Southwest Research Institute, 1972-(senior research statistician, department of automotive research, 1972-3; senior research biomathematician, department of bioengineering, 1973-).

Rev Mar/73

DENNIS M. BOENIG
Senior Research Veterinarian
Department of Bioengineering

B.S. in Veterinary Science, Texas A&M University, 1967
D.V.M., Texas A&M University, 1968

Dr. Boenig graduated from Texas A&M College of Veterinary Medicine in August 1968. Following graduation, he spent approximately one year in private small animal practice in Houston, Texas. He then accepted a National Institute of Health Fellowship at Texas A&M in order to pursue a Ph.D. degree in veterinary pathology. He lacks only the completion of the dissertation, which has been submitted for approval in order to fulfill the requirements of the degree.

Dr. Boenig's research for his dissertation was performed on dogs that were naturally and experimentally infected with canine distemper virus. This study was undertaken primarily to determine the pathogenesis of the demyelinating lesion of the central nervous system which is often a sequel to infection with this virus, and to also shed light on the pathogenesis of similar demyelinating lesions which accompany several central nervous system diseases of man. While completing his research he has become proficient in immunofluorescent microscopic procedures, electron microscopy and light microscopy. Aside from his research, duties in the necropsy laboratory for the large and small animal clinics has given Dr. Boenig additional experience in interpretation of gross and microscopic lesions. Also, through formal graduate courses and weekly departmental seminars Dr. Boenig gained a working knowledge of areas closely allied to pathology such as toxicology, immunology, teratology, virology, etc.

Since arriving at SwRI in September of 1973, Dr. Boenig has been involved in various projects which have required gross and histopathological evaluation of tissues from various laboratory animals, including primates.

PROFESSIONAL CHRONOLOGY: Private practice, Houston, Texas, 1968-9; National Institute of Health Ph.D. Fellowship at Texas A&M University Department of Veterinary Pathology, 1969-73; Southwest Research Institute, 1973-(senior research veterinarian, department of bioengineering, 1973-).

Memberships: American Veterinary Medical Association, Phi Zeta Honor Society.

Rev Apr/74

WALTER C. DOLLE
Manager, Electromagnetic Compatibility Evaluations Section
Department of Electronic Systems Research

B. S. in Military Engineering, U. S. Military Academy, 1939
M. S. in Electronics Engineering, University of Michigan, 1949
Graduate Work in Electronics, University of California, 1950-2

Mr. Dolle's experience includes 20 years as a regular officer in the U. S. Army Signal Corps. From this he has acquired a broad technical background in communications-electronics with specialized experience in installation, operation, and maintenance of military communications-electronics systems during extended field operations and technical staff work at Department of the Army and International Joint Service Theater levels. His industrial experience prior to joining Southwest Research Institute includes transmitter instrumentation for experimental double frequency shift teletypewriter transmissions; work as a field engineer for the installation and operation of a tropospheric scatter link with associated special instrumentation to record the effects of a nuclear explosion on propagation of scatter signals; and supervisory responsibilities related to the construction of a passive satellite communications link involving 30- and 60-foot steerable antennas, the development of low-noise parametric amplifiers, the development of special antenna feed systems, the development of a 10-kilowatt 2-gigacycle transmitter, and the development of special-purpose phase lock receivers. His experience at Southwest Research Institute includes RFI and TEMPEST measurements and suppression, study of shielding materials and techniques, analysis of near field attenuation effects, study of shielded enclosure resonance effects on measurement accuracy, development of an Isolated Antenna System for improved electric field measurements in shielded enclosures, development of a system for RF impedance measurement on active powerlines, development of instrumentation for monitoring body temperature and ECG traces of test animals during exposure to high intensity RF fields, design, and development of unique special purpose instrumentation for TEMPEST measurement and analysis, and design and construction of irradiation facilities for experimental investigation of the biological effects of electromagnetic radiation.

PROFESSIONAL CHRONOLOGY: Commissioned officer, U. S. Army Signal Corps, 1939-60; National Electronics Laboratories, Inc., Washington, D. C., 1960 (head research section); Page Communications Engineers, Inc., Washington, D. C., 1960-2 (staff engineer, assistant project manager and project manager); Southwest Research Institute, 1962-(senior research engineer, department of electronic systems research, 1965-7; manager, electromagnetic compatibility evaluations section, department of electronic systems research, 1967-).

Memberships: Institute of Electrical and Electronics Engineers, IEEE Professional Groups on Electromagnetic Compatibility, American Radio Relay League.

Rev Feb/74



S O U T H W E S T R E S E A R C H I N S T I T U T E

C. WILLIAM HALL
Director
Department of Bioengineering

A.B., Kansas University, 1950
M.A., Kansas University, 1952
M.D., Kansas University Medical School, 1956

Dr. Hall is internationally recognized as one of the leaders in artificial organs and biomaterials research. Prior to coming to the Institute, he directed the Artificial Heart Program at Baylor University College of Medicine in Houston. He was instrumental in establishing the contract mechanism for the National Heart Institute and acted as the first project officer of the National Artificial Heart Program. He is certified by the American Board of Surgery, the Kansas Board of Medical Examiners, and the Texas Board of Medical Examiners. Dr. Hall is the author of over sixty papers and contributions in seven books, most of which are in the field of artificial organs research and biomedical materials.

PROFESSIONAL CHRONOLOGY: Project officer, National Artificial Heart Program, and assistant to the associate director, National Heart Institute, 1964; project director, Artificial Heart Program, Baylor University College of Medicine, 1964-8; assistant professor of surgery and assistant professor of physiology, Baylor University College of Medicine, 1964-8; Professor Honoris Causa, Catholic University of Cordoba, Argentina, 1965-; Southwest Research Institute, 1968- (manager, artificial organs research, department of bioengineering, 1968-70; director, department of bioengineering, 1970-).

Appointments: Bexar County Hospital district, San Antonio, Texas (active clinical staff, 1969-); and The University of Texas Medical School at San Antonio (clinical assistant professor of surgery, 1969-), Clemson University National Advisory Board on Biomaterials Research, 1971-).

Memberships: American Association for the Advancement of Science; American Burn Association; American Association for Laboratory Animal Science, Texas Branch, 1972-; American College of Cardiology (Fellow); American College of Chest Physicians; American Heart Association; American Heart Association, Texas Affiliate (Board of Directors, Chairman of Scientific Sessions Committee); American Heart Association, San Antonio Chapter (President, Chairman of Research Committee); American Medical Association; American Society for Artificial Internal Organs; Association for the Advancement of Medical Instrumentation; Bexar County Medical Society; Biomedical Engineering Society; Instrument Society of America; International Cardiovascular Society; Journal of Biomedical Materials Research, Editorial Board; New York Academy of Sciences; Scientific Research Society of America; Sigma Xi; Society of Plastics Engineers; Society of Surgery of Rosario (honorary), Rosario, Argentina; and Texas Medical Association. (Further biographical data can be found in *American Men of Science: Dictionary of International Biography*, 1971 Edition; *Director of Medical Specialists*; *Personalities of the South*; *U.S. Medical Directory*; *Who's Who in America—World Notables*; *World Who's Who*; and *World Who's Who in Science*.)

Rev Sept/73

ROMMON L. LAWSON
Senior Research Scientist
Department of Bioengineering

A. A. in Pre-Medical, San Angelo College, San Angelo, Texas, 1953

B. S. in Animal Science, Texas Agricultural and Mechanical College of Texas
College Station, Texas, 1961

M.S. in Physiology of Reproduction, Texas A&M University, College Station, 1964

Ph. D. in Physiology of Reproduction, Texas A&M University, College Station, Texas, 1969

Dr. Lawson has had extensive experience in the field of Radiation Biology. He was employed at the Radiation Biology Laboratory at Texas A&M University from 1960-1971. During his tenure there, he was in charge of mice, rat, and hamster colonies involving pre- and post-natal radiation effects upon reproduction. These studies involved complete hematological evaluations and the preparation of histological tissue.

During the last few years of his employment with the Radiation Biology Laboratory, he had the responsibility of the Radiation Testing Laboratory. This involved the responsibility of conducting research for various contractual agencies of the Federal Government involving various other species.

Dr. Lawson has an extensive background in animal research. While at the Texas A&M Radiation Biological Laboratory, he assisted in experiments with laboratory animals in testing various compounds and their physiological effects. These studies were conducted under USA-SGO Contract DA 49-007-MP-597 on protective action of Drug WR361 on chronically irradiated male rats; evaluation of the toxicity WR361 on albino rats; toxicity studies of MEA and AET; the metabolic pattern of albino rats maintained on diets of different caloric levels and exposed to chronic gamma irradiation; feeding schedule as a factor in radiation resistance of the albino rat; wound and bone healing in adult male rats fed vitamin deficient diets; and the effect of biotin and pantothenic acid deficiency on survival of mice following thermal trauma, as well as many other related studies in the biological field. These toxicity studies were conducted by the compound control fed in the animals' diet and/or by intramuscular injection and/or intraperitoneal injection and/or oral administration. Routine evaluations are done on these animals to determine histopathological effects and the facilities to do these studies are available here at Southwest Research Institute.

PROFESSIONAL CHRONOLOGY: Texas A&M University, Radiation Biology Lab, 1960-9 (laboratory assistant, 1960-1; research assistant, 1961-4; research associate, 1964-9); research consultant in chinchilla reproduction, self-employed, 1970; research associate, marine biomedical research, Texas A&M University, 1971; Southwest Research Institute, 1972- (reproductive physiologist, department of bio-engineering, 1972) extensive affiliation with Chinchilla Breeders Association 1953-72.

Memberships: Sigma Xi, Society for Study of Reproduction, Texas Association for Radiation Research, Society for Study of Fertility.

June/73.

SOUTHWEST RESEARCH INSTITUTE

RICHARD B. SCHULZ
Staff Engineer
Department of Electronic Systems Research

B.S. in Electrical Engineering, University of Pennsylvania, 1942
M.S. in Electrical Engineering, University of Pennsylvania, 1951
Graduate Work in Electrical Engineering, University of Pennsylvania, 1951-3

Internationally recognized in the field of Electromagnetic Compatibility, Mr. Schulz is best known for his RF shielding work and was cited for such in his designation as an IEEE Fellow. Several of his patents concern improvements in RF shielding. In addition to advancements in this area, he has developed EMC-type measurement and calibration instrumentation. Also, he has worked on cable coupling, filtering, and transient-reduction techniques. Recently, he has been in charge of programs to develop automatic instrumentation to measure, record, and plot the RF attenuation of building walls; to design and install a high-intensity RF exposure system for biological effects; to measure automotive ignition RF emanations using an amplitude probability distribution approach, etc. His broad technical grasp is evidenced by his activity as editor of the IEEE Transactions on EMC and his responsibility is evidenced as a registered professional engineer (Pennsylvania).

PROFESSIONAL CHRONOLOGY: Radio engineer, Hazeltine Service Corporation, summer 1942; research associate, University of Pennsylvania, 1942-5; partner, Schulz and Weisbecker Company, 1945-7; owner, Electro-Search, 1947-55; program development coordinator, Armour Research Foundation, 1955-61; chief of electro-interference, United Control Corporation, 1961-2; chief of electrocompatibility, The Boeing Company, 1962-70; Southwest Research Institute, 1970-(staff engineer, department of electronic systems research, 1970-).

Memberships: Institute of Electrical and Electronics Engineers (Fellow), IEEE Group on Electromagnetic Compatibility (formerly Chairman, presently editor of Transactions and member of Administrative Committee, various subcommittees), Tau Beta Pi, Sigma Xi, Eta Kappa Nu, Sigma Tau, active on various industry and professional committees (Society of Automotive Engineers AE-4, Electronic Industries Association Committee G-46, American National Standards Institute Committee C-63, EMC Figure of Merit Committee, etc.).

Feb/74



S O U T H W E S T R E S E A R C H I N S T I T U T E

RUSSELL E. STEVENS, III
Neurobiologist
Department of Bioengineering

B. A. in Biology, Lehigh University, 1966
M. S. in Biology, Texas A&M University, 1972

While at Texas A&M University, Mr. Stevens, as a graduate student in biology, received extensive training in physiology, electrophysiology, neurobiology, and animal surgery. He specialized by studying the changes in the electroencephalogram (EEG) and in integrated multiple-unit activity (action potentials from a small pool of neurons) at several brain sites in rats and rabbits after chemical or electrical stimulation of the brain. At the College of Veterinary Medicine at Texas A&M, he was formally trained to do aseptic surgery on dogs and rats. In addition, he became experienced in the use of standard toxicological instruments such as gas-liquid, thin-layer, and paper chromatography as well as ultraviolet, infra-red, and atomic absorption spectroscopy. While a graduate teaching assistant, Mr. Stevens taught physiology and neurophysiology laboratories where students were taught blood and urine analysis, abdominal and thoracic surgery, and stereotaxic surgery.

Mr. Stevens is more than knowledgeable in the field of electronics through formal courses and through the daily use of many complex electronic components used in electrophysiology and neurobiology. The following are some of the electronic equipment with which he is familiar: E & M and Grass physiograph transducers, Grass model 78 polygraph, Grass model 7P3 preamplifier and integrator, Kopf R/C spike filter, Grass SU4 Stimulator, Grass S4 Stimulus Isolation Unit, and Grass Constant Current Unit.

Mr. Stevens wrote a master's thesis on the Electrographic Evaluation of the Neurotoxicity of Mirex. His research was designed to compare the neurotoxicity of mirex with that of DDT to see if mirex like DDT specifically affected the Ascending Reticular Activating System to result in a spontaneously aroused rat. Hippocampal theta rhythm, nuchal muscle tone, and the multiple-unit activity in the reticular formation were monitored for changes.

After receiving his Master of Science Degree, Mr. Stevens was employed at Texas A&M University as a research technician to determine which brain structures were primarily affected by alcohol by means of simultaneously monitoring changes in EEG and multiple-unit activity at seven different brain sites. All experiments were acute utilizing tracheal intubation, stereotaxic electrode placement, and a tubocurarine recording situation.

Memberships: Phi Sigma, AAAS, and Toastmasters International.

Rev Jan/73

H. HASKELL ZIPERMAN, M.D.

Senior Research Physician
Department of Bioengineering

A.B., Indiana University, cum laude, 1937
M.D., Indiana University School of Medicine, 1941

Internationally recognized for his contributions in trauma surgery of combat wounds, and management of vascular injuries, Dr. Ziperman has made substantial contributions in wound ballistics; management of hiatus hernia; frostbite; cardiac arrest; and gastrointestinal physiology. He is the author of some fifty papers and has acted as technical advisor and director for four professional medical films produced by the U.S. Army Medical Department on the subjects of Debridement, Tracheotomy, Management of Mass Casualties and Teaching of Combat Surgery.

Prior to joining the Institute, Dr. Ziperman was the Commander of Martin Army Hospital and all U.S. Army Medical Department Activities at Fort Benning, Georgia. While in that position, he was directly responsible for innovations designed to improve health care delivery systems. He inaugurated and supervised the first Family Practice Residency Training Program in the Army Medical Department, establishing an affiliation with Emory University School of Medicine to support the academic requirements of this program. He also inaugurated the first Ambulatory Care Nurse-Clinician Training Program in the Army Medical Department and pioneered in establishing the Family Practice Ambulatory Care System. Under Dr. Ziperman's guidance, the Problem Oriented Medical Record was introduced at Martin Army Hospital as a means of improving the level of health care afforded both military personnel and their dependents. Dr. Ziperman was Health Director for the Canal Zone Government and, as principal physician of that government, was the medical advisor to the Governor on health matters and international medical affairs. He directed and supervised four Canal Zone hospitals and activities of four divisions of the Canal Zone Health Bureau—Sanitation, Veterinary, Preventive Medicine, and Mental Health. He is certified by the American Board of Surgery, the Indiana Board of Medical Examiners, and the Texas Board of Medical Examiners.

PROFESSIONAL CHRONOLOGY: Director, Department of Medicine & Surgery and director, Wound Studies Laboratory, Medical Field Service School, Ft. Sam Houston, Texas, 1954-9; chief, General Surgery, Letterman General Hospital, San Francisco, California, 1959-61; chief, Surgery, and chief, Professional Services, 97th General Hospital, Frankfurt, Germany, 1961-3; chief professional consultant and surgical consultant, U.S. Army Europe, 1963-5; chief, Department of Surgery and director, Medical Education, William Beaumont General Hospital, El Paso, Texas, 1965-7; health director, Canal Zone Government, 1967-70; Commander, U.S. Army Medical Department Activity and Martin Army Hospital, Fort Benning, Georgia, 1967-73; Southwest Research Institute, 1973-(senior research physician, department of bioengineering, 1973-).

Appointments: Associate Professor of Clinical Surgery, Baylor University Graduate School of Medicine, 1957-9; Clinical Associate Professor of Surgery, Emory University School of Medicine, Atlanta, Georgia, 1972; Clinical Professor of Surgery, University of Texas Medical School at San Antonio, 1973.

Memberships: Diplomate of American Board of Surgery, 1950; Fellow, American College of Surgeons, 1951; Fellow, American Association for Surgery of Trauma, 1960; Association of Military Surgeons; Marion County Medical Society (IND); Indiana State Medical Association; American Medical Association; Isthmian Medical Society (CZ); Canal Zone Heart Association (President), 1969-70; Kiwanis International; Association of the U.S. Army; Nominee, American College of Hospital Administrators; Armed Forces Management Association.

Rev Apr/73

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DEPARTMENT OF DEFENSE CONTRACT PRICING PROPOSAL (RESEARCH AND DEVELOPMENT)				Form Approved Budget Bureau No. 22-R100	
This form is for use when (i) submission of cost or pricing data (see ASPR 3-807.3) is required and (ii) substitution for the DD Form 633 is authorized by the contracting officer.				PAGE NO.	NO. OF PAGES
NAME OF OFFEROR Southwest Research Institute HOME OFFICE ADDRESS (Include ZIP Code) 8500 Culebra Road San Antonio, TX 78284		SUPPLIES AND/OR SERVICES TO BE FURNISHED The Effects of Very Low Frequency Elec- tromagnetic Irradiation upon Pre- and Post- Natal Sequential Generations in the Albino Rat SUMMARY			
DIVISION(S) AND LOCATION(S) WHERE WORK IS TO BE PERFORMED		TOTAL AMOUNT OF PROPOSAL		GOVT SOLICITATION NO.	
		\$ 175,390			
DETAIL DESCRIPTION OF COST ELEMENTS					
1. DIRECT MATERIAL (Itemize on Exhibit A)			EST COST (\$)	TOTAL EST COST ¹	REFER- ² ENCE
a. PURCHASED PARTS See Attached List			5,658		
b. SUBCONTRACTED ITEMS					
c. OTHER - (1) RAW MATERIAL					
(2) YOUR STANDARD COMMERCIAL ITEMS					
(3) INTERDIVISIONAL TRANSFERS (At other than cost)					
TOTAL DIRECT MATERIAL				5,658	
2. MATERIAL OVERHEAD ³ (Rate % X \$ base =)					
3. DIRECT LABOR (Specify)			ESTIMATED HOURS	RATE/ HOUR	EST COST (\$)
Technical Supervision			200	14.34	2,868
Senior Professional			2060	9.55	19,673
Technical			1480	4.46	6,601
Clerical			80	3.98	318
Laboratory Assistants			9360	3.27	30,607
Total Salaries & Wages					60,067
Burden to Cover Fringe Benefits 28%					16,819
TOTAL DIRECT LABOR					76,886
4. LABOR OVERHEAD (Specify department or cost center) ³			O.H. RATE	X BASE =	EST COST (\$)
Provisional Overhead			103%	76,886	79,193
TOTAL LABOR OVERHEAD					79,193
5. SPECIAL TESTING (Including field work at Government Installations)			EST COST (\$)		
TOTAL SPECIAL TESTING					
6. SPECIAL EQUIPMENT (If direct charge) (Itemize on Exhibit A)					
7. TRAVEL (If direct charge) (Give details on attached Schedule)			EST COST (\$)		
a. TRANSPORTATION Air & Ground (to Washington, DC)			550		
b. PER DIEM OR SUBSISTENCE 4 man days			140		
TOTAL TRAVEL			690		
8. CONSULTANTS (Identify - purpose - rate)			EST COST (\$)		
TOTAL CONSULTANTS					
9. OTHER DIRECT COSTS (Itemize on Exhibit A)			1,491		
10. TOTAL DIRECT COST AND OVERHEAD			163,918		
11. GENERAL AND ADMINISTRATIVE EXPENSE (Rate % of cost element Nos.)			Included in Overhead		
12. ROYALTIES ⁴					
13. TOTAL ESTIMATED COST			163,918		
14. FEE OR PROFIT			11,472		
15. TOTAL ESTIMATED COST AND FEE OR PROFIT			175,390		
This proposal is submitted for use in connection with and in response to (Describe RFP, etc.)					
SwRI Proposal No. 13-613-9					
and reflects our best estimates as of this date, in accordance with the instructions to offerors and the footnotes which follow.					
TYPED NAME AND TITLE			SIGNATURE		
S. H. Birgel, Manager Contract Administration					
NAME OF FIRM			DATE OF SUBMISSION		
Southwest Research Institute			August 15, 1974		

[illegible]

☒ YES ☐ NO *If yes, identify below.*

TELEPHONE NUMBER/EXTENSION

(512) 344-6351

Defense Audit Agency, 7077 San Pedro, San Antonio, TX (512) 344-6331

☐ YES ☒ NO If yes, identify on a separate page

III. DO YOU REQUIRE GOVERNMENT CONTRACT FINANCING TO PERFORM THIS PROPOSED CONTRACT?

DO YOU REQUIRE GOVERNMENT GUARANTEE? ☐ YES ☒ NO If yes, identify: ☐ ADVANCE PAYMENTS ☐ PROGRESS PAYMENTS OR ☐ GUARANTEED LOANS

IV. DO YOU NOW HOLD ANY CONTRACT (or, do you have any independently financed (IR & D) projects) FOR THE SAME OR SIMILAR WORK CALLED FOR BY THIS PROPOSED CONTRACT? ☐ YES ☒ NO If yes, identify

V. DOES THIS COST SUMMARY CONFORM WITH THE COST PRINCIPLES SET FORTH IN ASPR, SECTION XV (See 3-807.2 (c) (2))?

☒ YES ☐ NO *If no, explain on a separate page.*

1. The purpose of this form is to provide a standard format by which the offeror submits to the Government a summary of its current estimated cost *(as attached supporting information)* suitable for detailed review and analysis. Prior to the award of a contract resulting from this proposal the offeror shall, under the conditions stated in ASPR 3-807.3, be required to submit a Certificate of Current Cost or Pricing Data (see ASPR 3-807.3(e) and 3-807.4).

2. As part of the specific information required by this form, the offeror must submit with this form, and clearly identify as such, cost or pricing data (that is, data which is verifiable and factual and otherwise as defined in ASPR 3-807.3(e)). In addition, he must submit with this form any information reasonably required to explain the offeror's estimating process, including:

- explain the offeror's estimating process, including:
- a. the judgmental factors applied and the mathematical or other methods used in the estimate including those used in projecting from known data, and
 - b. the contingencies used by offeror in his proposed price.

3. When attachment of supporting cost or pricing data to this form is impracticable, the data will be specifically identified and described (with schedules as appropriate), and made available to the contracting officer or his representative upon request.

4. The format for the "Cost Elements" is not intended as rigid requirements. These may be presented in different format with the prior approval of the contracting officer if required for more effective and efficient presentation. In all other respects this form will be completed and submitted without change.

5. By submission of this proposal, offeror, if selected for negotiation, grants to the contracting officer, or his authorized representative, the right to examine, for the purpose of verifying the cost or pricing data submitted, those books, records, documents and other supporting data which will permit adequate evaluation of such cost or pricing data, along with the computations and projections used therein. This right may be exercised in connection with any negotiations prior to contract award.

1 Enter in this column those necessary and reasonable costs which in the judgment of the offeror will properly be incurred in the efficient performance of the contract. When any of the costs in this column have already been incurred (e.g., on a letter contract or change order), describe them on an attached supporting schedule. Identify all sales and transfers between your plants, divisions, or organizations under a common control, which are included at other than the lower of cost to the original transferor or current market price.

2 When space in addition to that available in Exhibit A is required, attach separate pages as necessary and identify in this "Reference" column the attachment in which information supporting the specific cost element may be found. No standard format is prescribed; however, the cost or pricing data must be accurate, complete and current, and the judgment factors used in projecting from the data to the estimates must be stated in sufficient detail to enable the contracting officer to evaluate the proposal. For example, provide the basis used for pricing materials such as by vendor quotations, shop estimates, or invoice prices; the reason for use of overhead rates which depart significantly from experienced rates (reduced volume, a planned major rearrangement, etc.); or justification for an increase in labor rates (anticipated wage and salary increases, etc.). Identify and explain any contingencies which are included in the proposed price, such as anticipated costs of rejects and defective work, or anticipated technical difficulties.

3 Indicate the rates used and provide an appropriate explanation. Where agreement has been reached with Government representatives on the use of forward pricing rates, describe the nature of the agreement. Provide the method of computation and application of your overhead expense, including cost breakdown and showing trends and budgetary data as necessary to provide a basis for evaluation of the reasonableness of proposed rates.

4 If the total royalty cost entered here is in excess of \$250 provide on a separate page (or on DD Form 783, Royalty Report) the following information on each separate item of royalty or license fee: name and address of licensor; date of license agreement; patent numbers, patent application serial numbers, or other basis on which the royalty is payable; brief description, including any part or model numbers of each contract item or component on which the royalty is payable; percentage or dollar rate of royalty per unit; unit price of contract item; number of units; and total dollar amount of royalties. In addition, if specifically requested by the contracting officer, a copy of the current license agreement and identification of applicable claims of specific patents shall be provided.

5 Provide a list of principal items within each category indicating known or anticipated source, quantity, unit price, competition obtained, and basis of establishing source and reasonableness of cost.

SOUTHWEST RESEARCH INSTITUTE
Materials and Supplies Breakdown - Summary

Rats 120@\$4.10 each	\$ 492
Lab Chow - Wayne Lab Block 12,000 lbs. @\$147.50/thou.	1,770
Bedding, San-I-Cel a corn cobb material 14,600 lbs. @\$75.00/thousand	1,095
20 Gal. Rocal-D Disinfectant @\$11.05/gal.	221
Ether (Surgical Grade) 24-1/4lb. cont. @\$.68 Helco, pg. 106 #MCW 0804-02	16
600 Specimen Bottles @\$.15	90
10 gal. formaldehyde @\$22.72/5 gal. Helco, pg. 118, MCW 5016-5 gal.	45
Buffer Salts Mono-Basic 1 lb. pg. 230 #7892-1	4
Helco Anhydrous - 1lb. pg. 230 #7917-1	5
6 gal. Glycerine (Lab Grade) @\$29.20/gal. Fisher, pg. 1083 G33	175
1 case Hemoglobin unopette pipet SP B4065-2 pg. 98	163
Accovette Vials, coulter, 8 pkgs/144 @\$9 SP B3157-7 vial pg. 93	72
5 gal. Acetone @\$21.12, Helco, pg. 2@ 2440-5GL	106
10 gal. (Reagent Alcohol) @\$6.75/gal. Helco, pg 6 C4305	68
10 gal. Xylene @\$24.30/5 gal., Fisher, pg. 1268, X5	49
Phosphomolybdic acid 1/4 lb. Helco, pg. 191, MCW 2772-01	16
Glass Slides 10 gross @\$4.25/gross, SP, pg. 660, M6155	43
Cover Slips 10 oz @\$3.25/oz. AP, Pg. 658 #1 M6155	33
10 lbs. Paraplast @\$3.20/lb. SP, Pg 681 M7370	32
Alizarin Red - S Stain 100 gr. Helco, Pg. 7 HARL 116-100	10
KOH Clearing Agent. 12 bottles @\$2.52/bottle, Helco, Pg. 201, MCV 6984-1	30
Wrights Stain 200 gr., Helco Pg. 263, #376-200	50
5 gal. Methyl, Alcohol, Helco, Pg. 157, #3016-5 GL	16
1 Microtome Knife SP, Pg. 670, M7226-1	39
8 gal. Ethyl Alcohol @\$6.75/gal. Helco, Pg. 6 #C4305	54
1 Bottle Sharpening Abrasive SP, Pg. 682 M7436-3	14
1000 Embedding Ring Plastic - Rings 1000/case SP, Pg. 677 M7320	40
10 Slide Boxes (Plastic 1-100) 12 @\$2.95/each SP, Pg. 664 M6300	35
1 Gal. Isoterge, Coulter SP, Pg. 92 B3145-16 Isoterge	15
5 Gal. Isoton Solution, Coulter SP, Pg. 94 B3157-11 Isoton	17
Vacutainers 15% EDTH SP, Pg. 82 B3023-12A 2 ml/1000	67
12 pr. Disecting Scissors @\$4.40 ea. VWR Pg. 434, #25878-106	53
12 pr. Forceps @\$1.82 VWR Pg. 430 #25712-001	22
12 Boxes disposable gloves @\$10/box of 300	120
24 Boxes disposable Masks @\$5/box of 50	120
250 plastic irr. containers @\$1.89	223

40 Poloroid Pack @\$5
30 Pack Flash Cubes @\$1.25

200
38

Total

\$ 5,658

DEPARTMENT OF DEFENSE CONTRACT PRICING PROPOSAL (RESEARCH AND DEVELOPMENT)				Form Approved Budget Bureau No. 22-R100	
This form is for use when (i) submission of cost or pricing data (see ASPR J-807.3) is required and (ii) substitution for the DD Form 633 is authorized by the contracting officer.				PAGE NO.	NO. OF PAGES
NAME OF OFFEROR Southwest Research Institute		SUPPLIES AND/OR SERVICES TO BE FURNISHED The Effects of Very Low Frequency Electromagnetic Irradiation upon Pre- and Post-Natal Sequential Generations in the Albino Rat Year 1			
HOME OFFICE ADDRESS (Include ZIP Code) 8500 Culebra Road San Antonio, TX 78284					
DIVISION(S) AND LOCATION(S) WHERE WORK IS TO BE PERFORMED		TOTAL AMOUNT OF PROPOSAL \$ 85,415		GOVT SOLICITATION NO.	
DETAIL DESCRIPTION OF COST ELEMENTS					
1. DIRECT MATERIAL (Itemize on Exhibit A)			EST COST (\$)	TOTAL EST COST ¹	REFER. ² ENCE
a. PURCHASED PARTS			3,075		Exh. A
b. SUBCONTRACTED ITEMS					
c. OTHER - (1) RAW MATERIAL					
(2) YOUR STANDARD COMMERCIAL ITEMS					
(3) INTERDIVISIONAL TRANSFERS (At other than cost)					
TOTAL DIRECT MATERIAL				3,075	
2. MATERIAL OVERHEAD ³ (Rate % X \$ base =)					
3. DIRECT LABOR (Specify)		ESTIMATED HOURS	RATE/HOUR	EST COST (\$)	
Technical Supervision		100	14.34	1,434	
Senior Professional		900	9.55	8,595	
Technical		600	4.46	2,676	
Clerical		20	3.98	80	
Laboratory Assistants		5040	3.27	16,481	
Total Salaries & Wages				29,266	
Burden to Cover Fringe Benefits 28%				8,195	
TOTAL DIRECT LABOR				37,461	
4. LABOR OVERHEAD (Specify department or cost center) ³		O.H. RATE	X BASE =	EST COST (\$)	
Provisional Overhead		103%	37,461	38,585	
TOTAL LABOR OVERHEAD				38,585	
5. SPECIAL TESTING (Including field work at Government Installations)			EST COST (\$)		
TOTAL SPECIAL TESTING					
6. SPECIAL EQUIPMENT (If direct charge) (Itemize on Exhibit A)					
7. TRAVEL (If direct charge) (Give details on attached Schedule)		1 man trip		EST COST (\$)	
a. TRANSPORTATION Air & Ground (to Washington, DC)				275	
b. PER DIEM OR SUBSISTENCE 2 man days				70	
TOTAL TRAVEL				345	
8. CONSULTANTS (Identify - purpose - rate)			EST COST (\$)		
TOTAL CONSULTANTS					
9. OTHER DIRECT COSTS (Itemize on Exhibit A)				362	Exh. A
TOTAL DIRECT COST AND OVERHEAD				79,828	
11. GENERAL AND ADMINISTRATIVE EXPENSE (Rate % of cost element Nos.)			Included in Overhead		
12. ROYALTIES ⁴					
TOTAL ESTIMATED COST				79,828	
14. FEE OR PROFIT				5,587	
TOTAL ESTIMATED COST AND FEE OR PROFIT				85,415	
This proposal is submitted for use in connection with and in response to (Describe RFP, etc.)					
SwRI Proposal No. 13-613-9					
and reflects our best estimates as of this date, in accordance with the instructions to offerors and the footnotes which follow.					
TYPED NAME AND TITLE S. H. Birgel, Manager Contract Administration			SIGNATURE 		
NAME OF FIRM Southwest Research Institute			DATE OF SUBMISSION August 15, 1974		

COST EL NO	ITEM DESCRIPTION (See footnote 5)	EST COST (\$)
I. a.	Purchased Parts Materials and supplies purchased in Year I are approximately 1/2 of total for program with the exception of rats which are to be purchased in Year I (See Summary for detail)	\$3,075
g.	Other Direct Costs	
	Report Services	100
	Telephone Tolls and Telegraph 18 calls@\$3.00/call	54
	Computer Use Charge 1/4hr@ \$750/hr. Cyber 74	188
	Freight	20
	Total Other Direct Costs	\$ 362
I. HAVE THE DEPARTMENT OF DEFENSE, NATIONAL AERONAUTICS AND SPACE ADMINISTRATION, OR THE ATOMIC ENERGY COMMISSION PERFORMED ANY REVIEW OF YOUR ACCOUNTS OR RECORDS IN CONNECTION WITH ANY OTHER GOVERNMENT PRIME CONTRACT OR SUBCONTRACT WITHIN THE PAST TWELVE MONTHS?		
☐ YES ☒ NO If yes, identify below.		
NAME AND ADDRESS OF REVIEWING OFFICE (Include ZIP Code)		TELEPHONE NUMBER/EXTENSION
II. WILL YOU REQUIRE THE USE OF ANY GOVERNMENT PROPERTY IN THE PERFORMANCE OF THIS PROPOSED CONTRACT?		
☐ YES ☒ NO If yes, identify on a separate page.		
III. DO YOU REQUIRE GOVERNMENT CONTRACT FINANCING TO PERFORM THIS PROPOSED CONTRACT?		
☐ YES ☒ NO If yes, identify: ☐ ADVANCE PAYMENTS ☐ PROGRESS PAYMENTS OR ☐ GUARANTEED LOANS		
IV. DO YOU NOW HOLD ANY CONTRACT (or do you have any independently financed (IR & D) projects) FOR THE SAME OR SIMILAR WORK CALLED FOR BY THIS PROPOSED CONTRACT?		
☐ YES ☒ NO If yes, identify		
V. DOES THIS COST SUMMARY CONFORM WITH THE COST PRINCIPLES SET FORTH IN ASPR, SECTION XV (See 3-807.2 (c) (2))?		
☐ YES ☒ NO If no, explain on a separate page.		

INSTRUCTIONS TO OFFERORS

- The purpose of this form is to provide a standard format by which the offeror submits to the Government a summary of incurred and estimated costs (and attached supporting information) suitable for detailed review and analysis. Prior to the award of a contract resulting from this proposal the offeror shall, under the conditions stated in ASPR 3-807.3, be required to submit a Certificate of Current Cost or Pricing Data (see ASPR 3-807.3(e) and 3-807.4).
- As part of the specific information required by this form, the offeror must submit with this form, and clearly identify as such, cost or pricing data (that is, data which is verifiable and factual and otherwise as defined in ASPR 3-807.3(e)). In addition, he must submit with this form any information reasonably required to explain the offeror's estimating process, including:
a. the judgmental factors applied and the mathematical or other methods used in the estimate including those used in projecting from known data, and
b. the contingencies used by offeror in his proposed price.
- When attachment of supporting cost or pricing data to this form is impracticable, the data will be specifically identified and described (with schedules as appropriate), and made available to the contracting officer or his representative upon request.
- The format for the "Cost Elements" is not intended as rigid requirements. These may be presented in different format with the prior approval of the contracting officer if required for more effective and efficient presentation. In all other respects this form will be completed and submitted without change.
- By submission of this proposal, offeror, if selected for negotiation, grants to the contracting officer, or his authorized representative, the right to examine, for the purpose of verifying the cost or pricing data submitted, those books, records, documents and other supporting data which will permit adequate evaluation of such cost or pricing data, along with the computations and projections used therein. This right may be exercised in connection with any negotiations prior to contract award.

FOOTNOTES

- Enter in this column those necessary and reasonable costs which in the judgment of the offeror will properly be incurred in the efficient performance of the contract. When any of the costs in this column have already been incurred (e.g., on a letter contract or change order), describe them on an attached supporting schedule. Identify all sales and transfers between your plants, divisions, or organizations under a common control, which are included at other than the lower of cost to the original transferor or current market price.
- When space in addition to that available in Exhibit A is required, attach separate pages as necessary and identify in this "Reference" column the attachment in which information supporting the specific cost element may be found. No standard format is prescribed; however, the cost or pricing data must be accurate, complete and current, and the judgment factors used in projecting from the data to the estimates must be stated in sufficient detail to enable the contracting officer to evaluate the proposal. For example, provide the basis used for pricing materials such as by vendor quotations, shop estimates, or invoice prices; the reason for use of overhead rates which depart significantly from experienced rates (reduced volume, a planned major rearrangement, etc.); or justification for an increase in labor rates (anticipated wage and salary increases, etc.). Identify and explain any contingencies which are included in the proposed price, such as anticipated costs of rejects and defective work, or anticipated technical difficulties.
- Indicate the rates used and provide an appropriate explanation. Where agreement has been reached with Government representatives on the use of forward pricing rates, describe the nature of the agreement. Provide the method of computation and application of your overhead expense, including cost breakdown and showing trends and budgetary data as necessary to provide a basis for evaluation of the reasonableness of proposed rates.
- If the total royalty cost entered here is in excess of \$250 provide on a separate page (on DD Form 783, Royalty Report) the following information on each separate item of royalty or license fee: name and address of licensor; date of license agreement; patent numbers, patent application serial numbers, or other basis on which the royalty is payable; brief description, including any part or model numbers of each contract item or component on which the royalty is payable; percentage or dollar rate of royalty per unit; unit price of contract items' number of units; and total dollar amount of royalties. In addition, if specifically requested by the contracting officer, a copy of the current license agreement and identification of applicable claims of specific patents shall be provided.
- Provide a list of principal items within each category indicating known or anticipated source, quantity, unit price, competition obtained, and basis of establishing source and reasonableness of cost.

[illegible]

☐ YES ☐ NO If yes, identify below:		TELEPHONE NUMBER/EXTENSION
NAME AND ADDRESS OF REVIEWING OFFICE (Include ZIP Code)		

III. DO YOU REQUIRE GOVERNMENT CONTRACT FINANCING TO PERFORM THIS PROPOSED CONTRACT?

III. DO YOU REQUIRE GOVERNMENT CONTRACT FINANCING TO BE DISBURSED IN THE FOLLOWING MANNER:

☐ YES ☐ NO If yes, identify: ☐ ADVANCE PAYMENTS ☐ PROGRESS PAYMENTS OR ☐ GUARANTEED LOANS

IV. DO YOU NOW HOLD ANY CONTRACT (or, do you have any independently financed (IR & D) projects) FOR THE SAME OR SIMILAR WORK CALLED FOR BY THIS PROPOSED CONTRACT? ☐ YES ☐ NO If yes, identify

V. DOES THIS COST SUMMARY CONFORM WITH THE COST PRINCIPLES SET FORTH IN ASPR, SECTION XV (See 3-807.2 (c) (2))?

1. The purpose of this form is to provide a standard format by which the offeror submits to the Government a summary of incurred and estimated cost *(and attached supporting information)* suitable for detailed review and analysis. Prior to the award of a contract resulting from this proposal the offeror shall, under the conditions stated in ASPR 3-807.3, be required to submit a Certificate of Current Cost or Pricing Data (see ASPR 3-807.3(e) and 3-807.4).
2. As part of the specific information required by this form, the offeror must submit with this form, and clearly identify as such, cost or pricing data (that is, data which is verifiable and factual and otherwise as defined in ASPR 3-807.3(e)). In addition, he must submit with this form any information reasonably required to explain the offeror's estimating process, including:
 - a. the judgmental factors applied and the mathematical or other methods used in the estimate including those used in projecting from known data, and
 - b. the contingencies used by offeror in his proposed price.
3. When attachment of supporting cost or pricing data to this form is impracticable, the data will be specifically identified and described *(with schedules as appropriate)*, and made available to the contracting officer or his representative upon request.
4. The format for the "Cost Elements" is not intended as rigid requirements. These may be presented in different format with the prior approval of the contracting officer if required for more effective and efficient presentation. In all other respects this form will be completed and submitted without change.
5. By submission of this proposal, offeror, if selected for negotiation, grants to the contracting officer, or his authorized representative, the right to examine, for the purpose of verifying the cost or pricing data submitted, those books, records, documents and other supporting data which will permit adequate evaluation of such cost or pricing data, along with the computations and projections used therein. This right may be exercised in connection with any negotiations prior to contract award.

- 1 Enter in this column those necessary and reasonable costs which in the judgment of the offeror will properly be incurred in the efficient performance of the contract. When any of the costs in this column have already been incurred (e.g., on a letter contract or change order), describe them on an attached supporting schedule. Identify all sales and transfers between your plants, divisions, or organizations under a common control, which are included at other than the lower of cost to the original transferor or current market price.
- 2 When space in addition to that available in Exhibit A is required, attach separate pages as necessary and identify in this "Reference" column the attachment in which information supporting the specific cost element may be found. No standard format is prescribed; however, the cost or pricing data must be accurate, complete and current, and the judgment factors used in projecting from the data to the estimate must be stated in sufficient detail to enable the contracting officer to evaluate the proposal. For example, provide the basis used for pricing materials such as by vendor quotations, shop estimates, or invoice prices; the reason for use of overhead rates which depart significantly from experienced rates (reduced volume, a planned major rearrangement, etc.); or justification for an increase in labor rates (anticipated wage and salary increases, etc.). Identify and explain any contingencies which are included in the proposed price, such as anticipated costs of rejects and defective work, or anticipated technical difficulties.
- 3 Indicate the rates used and provide an appropriate explanation. Where agreement has been reached with Government representatives on the use of forward pricing rates, describe the nature of the agreement. Provide the method of computation and application of your overhead expense, including cost breakdown and showing trends and budgetary data as necessary to provide a basis for evaluation of the reasonableness of proposed rates.
- 4 If the total royalty cost entered here is in excess of \$250 provide on a separate page (or on DD Form 783, Royalty Report) the following information on each separate item of royalty or license fee: name and address of licensor; date of license agreement; patent numbers, patent application serial numbers, or other basis on which the royalty is payable; brief description, including any part or model numbers of each contract item or component on which the royalty is payable; percentage or dollar rate of royalty per unit; unit price of contract item; number of units; and total dollar amount of royalties. In addition, if specifically requested by the contracting office, a copy of the current license agreement and identification of applicable claims of specific patents shall be provided.
- 5 Provide a list of principal items within each category indicating known or anticipated source, quantity, unit price, competition obtained, and basis of establishing source and reasonableness of cost.

SOUTHWEST RESEARCH INSTITUTE
Cost and Pricing Information
Proposal No. 13-613-9

The total estimated cost to perform the proposed program is \$175,390 including a fee of \$11,472.

Manpower allocations are engineering estimates based upon a detailed evaluation of program requirements. In consultation with key technical personnel to be assigned responsibility for accomplishment of this project, engineering estimates of man hours required were obtained. The man hours thereby identified with specific skill levels were then grouped into appropriate labor classifications as shown on the DD Form 633-4 (Cost Summary).

The labor rates used in the cost estimate were based on the following:

<u>Dept.</u>	<u>Labor Classification or Individual</u>	<u>Dept. Avg. Hrly Rate (6-22-74)</u>	<u>Proposed Hourly Rate</u>	<u>Man-Hours</u>
13	Technical Supervision	\$13.68	\$14.98	100
14	Technical Supervision	12.52	13.70	100
Technical Supervision Weighted Avg. Rate is \$14.34/hour.				
13	Senior Professional	8.72	9.55	2060
13	Technical	4.07	4.46	1480
13	Clerical	3.64	3.98	80
13	Laboratory Assistants	3.12	3.42	7560
14	Laboratory Assistants	2.40	2.63	1800
Laboratory Assistants Weighted Avg. Rate is \$3.27/hour.				

An allowance has been included for salary increase based on seven percent annual increase.

Prices for materials are engineering estimates and established from phone quotations, vendors, price sheets and/or past purchase orders on file in our Purchasing Department.

Report reproduction cost includes composition, proofreading and press, and bindery to be used in connection with processing the report requirements of this program. The amount is based on a cost estimate prepared using standard rates currently in effect.

Cost and Pricing Information Continued:

In conduct of this program 2 man trips to Washington, DC will be required. The cost of this travel was computed in the following manner:

<u>Destination</u>	<u>No. of Trips</u>	<u>No. of Days</u>	<u>Air Fare</u>	<u>Grnd. Trans.</u>	<u>Sub.</u>	<u>Total</u>
Washington, DC	2	4	\$450	\$100	\$140	\$690

The attached contractual information sheets provide more information on Government Administrative Services, Salary rates and ranges, provisional overhead, rates, financial statements, type of contract desired, travel policy, basis for fixed fee, and representatives authorized to conduct negotiations.

In the event of contract award, it should be noted that the performance period is based on a two year program. However, due to the fact that the Government may elect to fund subject proposal on an incremental funding basis, an estimate for Year 1 and Year 2 was made.

SOUTHWEST RESEARCH INSTITUTE
CONTRACTUAL INFORMATION
COST-PLUS-FIXED-FEE PROPOSAL

SwRI Proposal No. 13-613-9
Purchase Request No. _____

Southwest Research Institute is a nonprofit corporation organized in the public interest and existing under the laws of the State of Texas, with its general offices at 8500 Culebra Road, San Antonio, Texas 78284. Laboratories are maintained at San Antonio, 3600 Yoakum Boulevard, Houston, Texas 77006, and 406 Belden, Corpus Christi, Texas 78403. The Institute presently employs approximately 1100 full time scientists, engineers, technicians, and service personnel.

The Defense Supply Agency, Defense Contract Administration Services Office, 7071B San Pedro, San Antonio, Texas 78216, has been assigned responsibility for administration of Department of Defense contracts. The agency having cost cognizance on all Government contracts awarded this Contractor is the Defense Contract Audit Agency, San Antonio, 7077 San Pedro, San Antonio, Texas 78216.

Contractor's current financial statements are filed quarterly with the Defense Supply Agency, the audit agency, and the Directorate of Procurement Headquarters, Air Force Systems Command, Andrews Air Force Base, Washington, D. C. 20331, who has been assigned cognizance under the program for the coordinated negotiation of overhead rates.

The accounting policy and procedures of the Institute are reviewed and approved on a current basis as acceptable for Government cost-type contracts by the Defense Contract Administration Services Office of the Defense Supply Agency.

The Government-approved policy of the Institute with regard to reimbursement for transportation and other travel expenses is limited to the actual reasonable cost incurred. Subsistence expenses are limited to the actual cost of lodging and related tips plus the actual cost incurred, which are reasonable for the geographic location of travel, for meals, related tips and other subsistence expenses. Transportation by personal and/or Institute-owned automobiles is reimbursed at 12¢ per mile as representing the actual cost of such transportation.

It is desired that a cost-plus-fixed-fee contract be provided with cost determination in accordance with the Armed Service Procurement Regulations, Section XV, Part 2. In accordance with current approved procedures, direct labor cost includes a burden to cover all fringe benefits. This method of accounting for fringe labor costs has been approved by the cognizant Administrative Contracting Officer, DCASR. A final negotiated

Contractual Information - Continued
Cost-Plus-Fixed-Fee Proposal

overhead rate of 99.13 percent of regular staff direct labor cost has been established for fiscal year ended September 29, 1973. Direct labor cost includes a burden to cover fringe benefits at 28 percent of direct salaries and wages. Based upon data applicable to last fiscal year and projections for the ensuing year, the Government approved provisional overhead rate is 103 percent of direct labor cost as applicable to regular staff direct labor.

Government financing to the extent of current payments on account of allowable costs as provided in the clause entitled "Allowable Cost, Fee and Payment" in accordance with Paragraph 203.4 of Section VII of the Armed Services Procurement Regulations is requested.

The fixed fee, in the case of the Institute is paid not only for the "know-how," which it is in a position to furnish, but for the growth and expansion of the organization which has been set up primarily for the public good through scientific progress and as a specific service to the Government, industry, and the public generally. The Institute, a nonprofit organization, does not have the capital structure to provide for expansion outside of the fee received for work performed, and nominal contributions from interested individuals and organizations. Experience has proven that funds must be available to expand facilities, and also procure new and replace obsolete equipment, in order for the Institute to keep abreast with the latest in scientific development. The fixed fee proposed in this instance has been determined with due consideration given to factors set forth in ASPR, Section III, Par. 808.

This proposal shall remain in effect no longer than 90 days from date of presentation. This proposal constitutes an offer and, if accepted by a Notice of Award placed in the mail addressed to Southwest Research Institute, will form a binding contract on the terms covered by this proposal. It is agreed that any such Notice of Award will be replaced at a later date by a definitive contract bearing the same date as the Notice of Award and containing the details of the agreement between the parties.

Personnel to be contacted for any negotiations required on this procurement:

Contractual:	Mr. R. B. Kalmbach, Contract Administrator, Area Code 512, 684-5111, Ext. 2235
	Mr. John F. Sprencel, Assistant Manager, Contract Administration, Area Code 512, 684-5111, Ext. 2238;
	Mr. S. H. Birgel, Manager Contract Administration, Area Code 512, 684-5111, Ext. 2230;
	Mr. D. D. Belto, Assistant Treasurer and Director, Business Operations, Area Code 512, 684-5111, Ext. 2233.

Contractual Information - Continued
Cost-Plus-Fixed-Fee Proposal

Technical: Dr. R. Lawson, Sr. Research Physiologist, Area Code 512,
684-5111, Ext. 2601
C. W. Hall, M.D. Director, Department of Bioengineering,
Area Code 512, 684-5111, Ext. 2670

Contingent Fee Statement

Bidder represents: (a) That he has not employed or retained any company or person (other than a full-time bona fide employee working solely for the bidder) to solicit or secure this contract, and (b) that he has not paid or agreed to pay to any company or person (other than a full-time bona fide employee working solely for the bidder) any fee, commission, percentage or brokerage fee, contingent upon or resulting from the award of this contract, and agrees to furnish information relating to (a) and (b) above as requested by the Contracting Officer. [For interpretation of the representation, including the term "bona fide employee," see Code of Federal Regulations, Title 41, Subpart 1-1.5 (April 1966)(August 1967)].

SOUTHWEST RESEARCH INSTITUTE

By 
S. H. Birgel, Manager
Title Contract Administration
Date August 15, 1974

APPENDIX I.

GESTATION PERIODS OF DEVELOPMENT

- A. Pre-differentiation period - usually not susceptible to teratogenesis. (Cleavage and blastula stages).
- B. Period of early differentiation - highly susceptible to teratogenesis. (Before the 8th, 9th days rat).
- C. Period of advanced organogenesis - increasingly resistant to teratogenesis. (12th - 14th rat) with increasing age.

HEMATOLOGY

List of hematological measurements that may be utilized.

<u>Measurement</u>	<u>Method</u>	<u>Reference</u>
<u>Erythropoietic System</u>		
Hemoglobin concentration.	Measured as cyanmethaemoglobin using a Klett-Summerson photoelectric colorimeter. ^a	Wintrobe, 1961
Hematocrit (packed cell volume)	Drummond microhematocrit apparatus. ^b	
Erythrocyte counts	Coulter counter. ^c	Grant, Britton & Kurtz, 1960
Erythrocyte glucose-6-phosphate dehydrogenase.	Spot test. ^d	Fairbanks & Beutler, 1962.
Reticulocyte counts	Number per 1,000 erythrocytes	
Sedimentation rate	Citrated blood in Wintrobe Hematocrit tubes for 1 hour.	
<u>Leukopoietic System</u>		
Leukocyte counts	Coulter counter. ^c	Richar & Breakell, 1959
Differential counts	Conventional counts	
<u>Coagulation System</u>		
Clotting time		Lee & White, 1913
Platelet counts	Levy-Hausser counting chamber	Bjorkman, 1959
Fibrinogen		Goodwin, 1961
Prothrombin time	Quick's test using Simplastin reagent ^e with an Oxford Prothrometer ^f .	Cartwright, 1963

-
- a. Klett Manufacturing Co., New York.
 - b. The Drummond Scientific Co., Philadelphia.
 - c. Coulter Electronics, Inc., Hialeah, Florida.
 - d. Calbiochem, Box 54282, Los Angeles, California.
 - e. Warner-Chilcott Laboratories, Morris Plains, New Jersey.
 - f. Oxford Laboratories, San Mateo, California.

HISTOLOGY

Summary of Histological Quantification Methods that may be done.

<u>Procedures</u>	<u>Histological Substrates</u>	<u>Comments</u>
Counts	Bile plugs, giant cells, mast cells, calcification sites, mitoses, etc.	Expressed as average number/field; total number/section, etc.
Linear measurements	Thinning of femoral epiphyseal disc; hyperplasia of zona glomerulosa in adrenal cortex; reduction in height of spermatogenic epithelium; decrease of tubular diameter of testes.	Calibrated micrometer, results in micrometer units or μ .
Area measurements	Muscles: necrosis. Uterus: atrophy of mucosa and myometrium. Brain: hydrocephalus.	Components expressed in planimeter values obtained from tracings or projections.
Nuclear volume	Hyperplasia of thyroid gland; dystrophy of adrenal cortex; atrophy of liver due to starvation.	Two perpendicular measurements using computerized formula of rotation ellipsoid for calculating μ^3
Composition of organs	Hyperplasia of thyroid gland: epithelium colloid, open capillaries, etc. Atrophy of ovaries: ratio of primary follicles to corpora lutea. Cirrhosis of liver: density of reticulum fibres. Composition of submaxillary glands: ratio of mucous to serous terminal portions.	Point sampling ^a Components expressed in percentage of organ volumes.
Nuclear density	Dystrophy in zona fasciculata of adrenal cortex	
Internal surface measurements	Alveolar surface in lungs altered by inflammation, fibrosis, or emphysema. Trabecular surface in femoral epiphysis altered by osteodystrophy.	Line sampling ^a Results expressed in mm^2/mm^3 of organ volume.

a. For methodology and examples see Henning (1958) and Benitz & Dambach (1964).

EXAMINING RAT FETUSES FOR MALFORMATIONS

A. External Examination:

Rats and mice tend to eat their defective offspring - therefore, accurate evaluations of the effects of a particular drug or insult to the developing animal (embryo), must be done prior to parturition, 12-24 hours before delivery.

The general procedure is as follows:

1. Kill the mother on day 20 of gestation period with an overdose of ether.
2. Quickly without a time lapse, open the abdominal wall to the full extent, exposing both uterine horns.
3. Carefully count both fetal swellings and "metrial glands." Metrial glands are highly vascularized yellowish nodules - found along the mesometrial margin of the uterine horns - they mark any original implantation site, whether the embryo or fetus survived at that site or not. There is a metrial gland at the base of each placental attachment. These sites represent resorption of embryos or fetuses.
4. Open the uterus without delay, and determine which fetuses are alive and which are dead - but intact. This is done by observing: (a) spontaneous movement (b) a more ruddy color than dead tissue (fetuses), (c) in case of doubt - gentle pressure applied with forceps at head or neck will elicit reflex movement.
5. Number and record position of fetuses, i. e., begin at left ovary down the left horn and across the cervix and up the right horn to the right ovary. List all living, dead, and resorbed fetuses and sites within the uterus, and within each horn - draw a line or arrow indicating the position of the cervix. See following chart.
6. Remove the living young. Removal is done by clamping a small hemostat on the umbilical cord, flush with the abdominal wall, cut or break the cord distal to the clamp (this serves as a handle), lift the fetus out onto blotting paper.
7. Weigh the fetuses - after blotting the excess amniotic fluid and blood. (without clamp) (Weights of 0.1 gram and under is a waste of time).

Gestational age (s)

ment Wt. _____

Date _____

Treatment Details:

Leukocytes

Epithelial

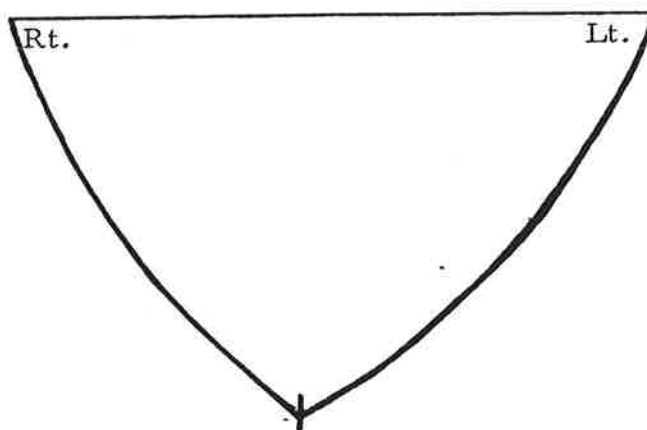
Cornified

Sperm

Bred	Male	Present	Wt.
1900	1900	1900	1900
1901	1901	1901	1901
1902	1902	1902	1902
1903	1903	1903	1903
1904	1904	1904	1904
1905	1905	1905	1905
1906	1906	1906	1906
1907	1907	1907	1907
1908	1908	1908	1908
1909	1909	1909	1909
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1919	1919	1919	1919
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1921	1921	1921	1921
1922	1922	1922	1922
1923	1923	1923	1923
1924	1924	1924	1924
1925	1925	1925	1925
1926	1926	1926	1926
1927	1927	1927	1927
1928	1928	1928	1928
1929	1929	1929	1929
1930	1930	1930	1930
1931	1931	1931	1931
1932	1932	1932	1932
1933	1933	1933	1933
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1971	1971	1971	1971
1972	1972	1972	1972
1973	1973	1973	1973
1974	1974	1974	1974
1975	1975	1975	1975
1976	1976	1976	1976
1977	1977	1977	1977
1978	1978	1978	1978
1979	1979	1979	1979
1980	1980	1980	1980
1981	1981	1981	1981
1982	1982	1982	1982
1983	1983	1983	1983
1984	1984	1984	1984
1985	1985	1985	1985

[illegible]

Laparotomy Date:



Autopsy, date _____ Wt. _____

Remarks:

[illegible]

8. External examination of fetus (from head to tail - possible under 2-3X scope) for malformations.

a. Malformations:

1). Contour of cranium - profile and face-on view. (Compare with normal control).

2). Examine the limbs. Count the toes. Check the depth of the digital furrows. Obvious defects are:

- a). Syndactyly = having united or webbed fingers or toes.
- b). Polydactyly = more than the usual number of fingers or toes.
- c). Adactyly = lacking fingers or toes.
- d). Club foot = distortion of distal parts of an extremity.

Note: Leaving the fetuses too long in an unopened uterus after killing the mother may distort extremities.

- e). Micromelia = abnormal smallness of limbs.
- f). Phocomelia = a fetus with hands and feet but no arms or legs.

3. Dome-shaped configuration - typical hydrocephaly. Note: Cranium normally rises proportionally higher than in more mature animals.

4. Eye bulges - equal in size and position.

- a). Anophthalmia = congenital absence of eyes.
- b). Microphthalmia = abnormal smallness of the eyes.

5. Some forms of frank ocular malformations may be recognized externally.

6. Cranial and brain defects

a). Exencephaly = brain exposed, cranium and skin are absent, usually everted on surface of head.

b). Meningoencephalocele = skin intact, but brain or some part of it, together with the meninges protruding through a cranial defect to cause an irregular mass beneath the skin.

c). Simple meningocele = skin intact, translucent and elevated by fluid filled vesicles of meninges which protrude through a midline defect in the cranium.

b. Examine face or muzzle for gross distortions, notches or furrows that indicate cleft lip, cleft mandible, or oblique facial cleft. Cleft palate, if extensive, can be seen by inserting forceps in mouth, forcing jaws apart. (Should section to confirm).

c. Examination of the trunk: Abnormalities of the trunk are usually quite conspicuous, e.g., umbilical hernia = a few loops of intestine protrude through the umbilical opening. Gastroschisis = most of abdominal viscera are outside the defective abdominal wall.

d. Defects along the dorsal midline. They are easily identified. Myelomeningocele = bubble-like bulge spina bifida, usually bloody depression or raw.

e. Short trunk = missing vertebrae above caudal level. Scoliosis = abnormal vertebral curvatures. Note: Caudal vertebrae are relative often subject to variation, resulting in short, curly, angular, or absent tail. (Render these animals to skeletal examination for further details and confirmation of the anomalies).

f. Sex = distance between anus and the genital tubercle. (About twice as great in the males, e.g., two (2)mm in new born male rats, one (1)mm in new born female rats).

After external observations are made and recorded, animals should be preserved or fixed for further study. The type of preservation depends on the type of further study to be undertaken.

B. Internal Structure Examination:

The three types of internal structure examinations are (1) free hand razor blade sections utilizing the dissecting microscope, (2) observation of the skeletal structure after it is made visible by clearing soft tissues and staining with alizarin red, and (3) histological serial sections.

1. Free-hand razor blade sections.

a. One third (1/3) of the fetuses preserved in Bouin's (this allows for infrequent anomalies of soft tissue to be missed one time in three).

b. Fetuses placed (as soon as possible), in about 25cc Bouin's fluid (Allen's modification preferred), per animal and allowed to remain for one (1) week.

c. After fixation - remove or clip off with scissors, legs and tail.

d. Holding the neck firmly with large forceps place the fetus in a supine (dorsal) position on a flat paraffin pan. Insert a sharp, single-edge razor between the jaws and with firm, even, back-and-forth strokes, cut off the upper part of the head in a plane just above the ears.

e. Examine the under side of the palate by removing the body of the tongue which may have remained with the upper part of the head.

f. Examination of the Head

1) Place the cut surface of the head down on the paraffin block and begin making one (1)mm transverse slices through the head beginning just in front of the eyes and proceeding slice-by-slice backward to the region of the ear. This will yield 5 or 6 one (1)mm slices. Place slice of tissue in a spot-test dish covered with 70% alcohol.

2) Examine the previous sections in the spot dish under the dissecting microscope at powers of 5X to 7.5X.

Note: The head should reveal abnormalities of the palate, nasal cavities, eyes, and abnormalities of the brain.

g. Examination of the trunk.

Place trunk on paraffin block in supine position and make transverse one (1)mm-thick sections beginning in region of shoulder joint and continue caudally until the kidney region is passed.

Note: With forceps, hold the trunk just caudal to the plane of each section. Care should be exercised as to pressure on the trunk. Too much pressure will cause displacement of the viscera.

Malformations that can be identified in this section through the trunk are:

In the thoracic sections interventricular (between ventricles) septal defects, transpositions of the great arteries, tracheoesophageal fistulae, herniated abdominal viscera resulting from diaphragmatic defects.

Abdominal sections have shown - diaphragmatic herniae, situs inversus of the abdominal viscera, ectopic kidneys, fused kidneys, hypoplastic kidneys, hydrocephrosis, and renal agenesis.

h. Examination of pelvic cavity.

1) Without sectioning one may observe such abnormalities as hydroureters, cryptorchid testes and various degrees of hermaphroditism.

SKELETON CLEARING STUDIES

A. Skeleton clearing studies - 2/3 fetuses preserved in 95% alcohol to be used in clearing procedures. (In skeletal anomalies a possible miss of two times out of three due to selection of 2/3 of the fetuses to be sectioned).

1. Fixative - 95% ethanol (4-7 days) remove all of the abdominal and thoracic organs and as much skin as possible at the time of sacrifice, or after a few days of fixative.

2. Clearing - Clear in 2% KOH for 6-8 hours until bones are clearly visible through the surrounding tissue. Avoid excessive temperature. Small specimens must be watched carefully to avoid disintegration.

3. Staining - Transfer directly to Mall's solution containing alizarin red (approximate proportion 100ml of Mall's with 2-3 drops of saturated aqueous solution of alizarin red). Leave specimen overnight in stain.

Note: Mall's Solution (3 liters):

Distilled water	2370 ml
Glycerine	600 ml
4 percent KOH	7.5 ml
Distilled water	22.5 ml

4. Differential decolorization - Transfer stained specimens to Mall's next morning. Make frequent changes to hasten the process, and observe until the bones are clearly seen through the muscles.

5. Upgrading in glycerine - Transfer through several changes of 50, 70, 80, and 90 percent glycerine in water. Examine and store in 100 percent glycerine. Add a few crystals of thymol to prevent fungal growth.