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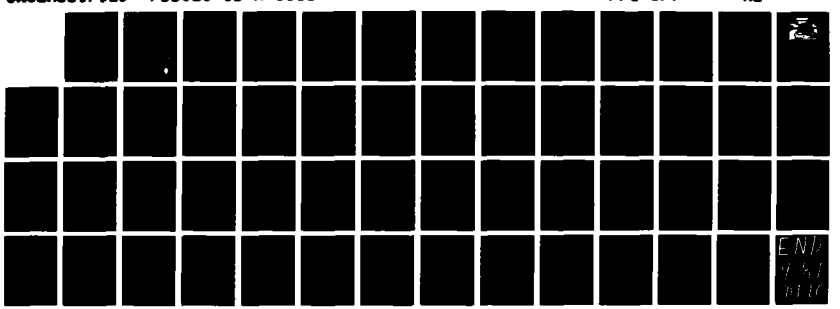

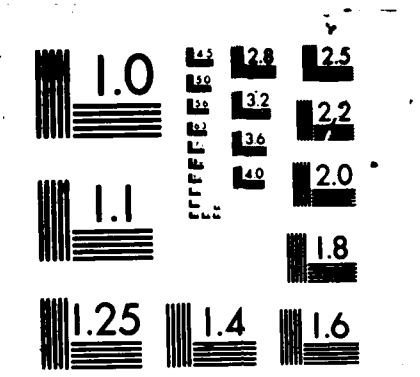

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LONG-TERM BIOEFFECTS OF 435-MHz RADIOFREQUENCY RADIATION ON SELECTED BLOOD-BORNE ENDPOINTS IN CANNULATED RATS

Volume 3. Plasma Prolactin

Vojin P. Popovic, Ph.D.
James C. Toler, M.S.
Stephen J. Bonasera, B.S.
Pava P. Popovic, Ph.D.
Clegg B. Honeycutt, M.S.
Demetrios S. Sgoutas, Ph.D.

Georgia Institute of Technology
Atlanta, GA 30332

June 1987

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Prepared for
USAF SCHOOL OF AEROSPACE MEDICINE
Human Systems Division (AFSC)
Brooks Air Force Base, TX 78235-5301

NOTICES

This final report was submitted by Georgia Tech Research Institute, Georgia Institute of Technology, Atlanta, Georgia, under contract F33615-83-R-0600, job order 7757-01-78, with the USAF School of Aerospace Medicine, Human Systems Division, AFSC, Brooks Air Force Base, Texas. James H. Merritt (USAFSAM/RZP) was the Laboratory Project Scientist-in-Charge.

When Government drawings, specifications, or other data are used for any purpose other than in connection with a definitely Government-related procurement, the United States Government incurs no responsibility nor any obligation whatsoever. The fact that the Government may have formulated or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication, or otherwise in any manner construed, as licensing the holder, or any other person or corporation; or as conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources-National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

JAMES H. MERRITT, B.S.
Project Scientist

JEROME H. KRUPP, D.V.M.
Supervisor

JEFFREY G. DAVIS, Colonel, USAF, MC
Commander

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Two hundred adult male white rats (Sprague-Dawley, CAMM Labs) with chronically implanted aortic cannulas were randomly divided into two groups. Animals in the first group were exposed to low-level (1.0 mW/cm^2) pulsed-wave 435-MHz radiofrequency radiation for approximately 22 h daily, 7 days a week, for 6 months. Animals in the second group were maintained under identical conditions but were not radiated. The chronic cannulas were used to draw 0.3 mL of aortic blood from the unrestrained, unanesthetized rats on a cyclic schedule. Plasma prolactin concentrations were determined by radioimmunoassays. Statistical analysis of the results showed no significant difference in plasma prolactin concentration between exposed and sham-exposed animals. Exposure to this low-level radiofrequency environment did not induce stresses that resulted in an alteration of plasma prolactin concentrations.

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Volume 3. Plasma Prolactin

I. INTRODUCTION

Throughout the developed world, and particularly in the United States, the 20th century has marked a period of tremendous progress in communication, information, and electronic sciences. Many of the major technological advances during this period involved transmitting energy over vast distances using electromagnetic waves. This progress had the side effect of altering the planet's electromagnetic environment. Radio, radar, and television transmissions have increased the ambient electromagnetic radiation level by several orders of magnitude. At this time, despite many studies performed in this field, the biological effects of this omnipresent electromagnetic environment on organisms are not well understood.

This report presents results of plasma prolactin levels measured in 200 male Sprague-Dawley rats chronically exposed to a 1.0 mW/cm^2, 435-MHz pulsed-wave (1.0 μs pulse width, 1-kHz pulse rate) electromagnetic environment for a 6-month duration. The exposure group consisted of 100 cannulated rats housed in Plexiglas cages arrayed on the tiers of a stacked, parallel-plate circular waveguide. Engineering aspects of this waveguide and the exposure environment it generated have been previously reported [1]. The sham-exposure group consisted of 100 cannulated rats housed in an identical, but unenergized, collocated facility. The biological effects of this radiofrequency radiation (RFR) exposure on plasma adrenocorticotropic hormone (ACTH) and plasma corticosterone concentrations in the same animals have already been reported [2].

Prolactin was identified 50 years ago as a lactogenic hormone secreted by the anterior pituitary. Recently developed sensitive and specific radioimmunoassay methods have led to knowledge of the physiology and pathophysiology of prolactin secretion. Prolactin is apparently released in a pulsatile fashion [3]. The pulses are small, except during sleep when marked rises in prolactin concentrations have been noted. Plasma prolactin level in undisturbed intact male rats is about 10-15 ng/mL [4]. In male animals, plasma prolactin levels rapidly increase from 4 to 10 times the basal level in response

to various stressors [5,6]. Some known stressors include surgery or anesthesia (increase or decrease [7]), feeding [8], and brief handling or mild ether exposure [4,9]. Elevated ambient temperature (36 °C for 20 to 360 min) associated with body hyperthermia evokes increases in circulating levels of prolactin [10]. During stress, prolactin is released in a quantitative fashion [11]; thus, the level of plasma prolactin can be used to measure the level of stress [12].

Intraventricular brain injection of β-endorphin (βLPH_{61-91}) in urethane-anesthetized male rats leads to a dose-dependent increase of plasma prolactin levels [13]. Thus, the plasma prolactin level corresponds to the level of stress in a fashion similar to plasma ACTH and plasma corticosterone. Male rats acutely exposed to visual or audiogenic stimulation exhibit rapid and marked prolactin secretory responses [14]. This suggests that the response to an acute exposure of neurogenic stress in the male rat is elicited via a neural pathway impinging upon the medial basal hypothalamus from the rostral direction. Midbrain lesions slightly alter the level of plasma prolactin in adult male rats [15], but the integrity of the amygdala is not essential for the normal basal and diurnal hormone profile of prolactin [16].

Plasma prolactin increase is observed 2 min after initiation of stress; 15 to 20 minutes after the stress, the concentration of plasma prolactin returns to the basal, resting level. Prolactin release is also under the influence of catecholamine levels [12].

Although the functional importance of prolactin release remains obscure (essential actions of prolactin are mammotrophic and lactogenic), it is known that this hormone is released during stress and the release is mediated by the hypothalamus [17].

II. MATERIALS AND METHODS

For this study, the concentration of plasma prolactin was chosen as a sensitive indicator of possible environmental stresses induced by RFR. To detect and quantitatively evaluate possible increases in plasma prolactin levels induced by long-term exposure to RFR, blood (0.3 mL) was periodically sampled from 62 exposed and 64 sham-exposed animals. Analysis of the data obtained from blood sample assays determined whether there were any RFR-induced changes in plasma prolactin concentration.

Animals. Male Sprague-Dawley rats were used in this study. All experimental animals were obtained from the same building and room at CAMM Research Labs, Wayne, New Jersey. The animals, weighing approximately 60 g, were delivered to Emory University where they were caged singly and given water and food (Purina Rat Chow) ad libitum. Temperature in the animal rooms was maintained at 24 ± 1 °C and the photo period was 12 hours/12 hours, with the lighted phase occurring between 8 AM and 8 PM.

Experimental Facility. The Georgia Tech Research Institute's Radiation Facility [18] consisted of 8 collocated rooms on the basement floor of the Baker Building on the main campus. These 8 rooms provided a closed, complete facility for long-term biceffects studies involving rodents.

The 100 exposure and 100 sham-exposure animals were housed in two identical, collocated rooms in the Radiation Facility. Each room contained a stack of circular, parallel-plate waveguides fed by a slotted-cylinder antenna system for radiating the animals. The stacks of parallel waveguides consisted of five 3.6-m (12 ft) diameter plates that made up 4 sets of circular waveguides. Twenty-five individually housed rats were positioned around the circumference of each waveguide set. The walls of both rooms were lined with anechoic absorbing material and shielded with aluminum foil to prevent excessive microwave leakage radiation.

The circular, parallel-plate waveguide assembly provided a 1.0 mW/cm^2 exposure field around the circumference of the plates. The 45.7-cm (18 in.) plate separation distances permitted propagation of a TE_{10} mode wave with horizontal polarization. The result was an electric field vector oriented parallel to the rat's longitudinal axis, thereby maximizing the coupling between the electric field and the rat. The power density displayed a cosine-squared dependency between the plates, with the maximum power density occurring midway between each set of plates.

A slotted-cylinder antenna with the proper diameter, thickness, slot length, and slot width dimensions fed the stack of circular waveguides in a manner that provided an essentially constant electric field intensity in the azimuth plane.

Cages. The animal cages were constructed of Plexiglas to facilitate visual observation of the rats and provide radiofrequency (RF) transparency. Each cage was 22.9-cm (9 in.) long by 12.7-cm (5 in.) wide by 17.8-cm (7 in.) tall. These dimensions complied with recommended caging requirements [18] for long-term housing of rats. The food hopper and water bottle were placed on the distal side of the cage to minimize their interaction with the exposure field. The glass floor rods in the cage were oriented perpendicular to the cage's long axis to encourage the rats to preferentially align themselves parallel to the electric field vector. Sipper tubes for the water bottles were made of glass to be nonperturbing in the field. Evaluations of the cages conducted in the circular, parallel-plate waveguide assembly showed field scattering from the Plexiglas and water to be below the range of detection.

The Radiation Facility also contained a data acquisition system for storing and processing experimental data, an electronic balance for weighing the rats during the study, and rooms for transmitter operation, blood sampling, cage washing, and materials storage.

The entire Radiation Facility was locked to avoid unauthorized entry. This step significantly reduced the introduction of noise that otherwise could have caused artifacts in the study results. Only the animal caretaker and the technician who sampled blood from the animals were permitted uncontrolled entry to the Facility.

Cannulation. To use each animal as its own control, arterial blood was sampled by means of implanted aortic cannulas. Cannulation provided a simple, inexpensive technique that permitted remote, stress-free blood sampling in conscious, unrestrained, and resting rats [2,20]. Arterial blood drawn from the chronically implanted aortic cannulas was assayed for plasma prolactin. Venous blood was not sampled because the blood flow in veins is laminar and, therefore, flows in discrete layers that do not mix. Only physiologically minute amounts of arterial blood (up to 0.3 mL) were withdrawn from resting rats approximately every 2 weeks.

The carotid artery of each rat was cannulated using a PE-10 cannula 8 to 10 days before the animals entered the study. The surgery, which required about 8 min, was performed using ketamine-xylazine anesthesia (1:1 mixture; ketamine 100 mg/mL, xylazine 20 mg/mL, i.m. 0.1 mL/100 g of body weight). The cannulas were filled with slightly heparinized saline* and their distal ends were sealed with nylon plugs. Stress hormone levels returned to the basal values about 3 days after implantation of the chronic arterial cannulas (Table 1). The first blood sampling occurred 10 days after aortic cannulation.

Blood Sampling. Restraint and handling increase stress hormone levels in rats, as confirmed during the study (Table 2). However, the animals had to be handled upon removal from their exposure cage and placement in the "sampling box" in preparation for blood withdrawal. To avoid the undesired effects of handling and stress on hormone levels, blood from the aortic cannula was sampled 30 min after the animal was placed in the sampling box. This procedure permitted the altered plasma prolactin level sufficient time to return to its basal value (Table 2). Each animal was previously preconditioned for the sampling box through a regime of several 30-min-long experiments conducted during a 1-week period before entering the study.

After acclimating for 30 min in the sampling box, the rat's cannula was positioned through the slot in the top of the box (Fig. 1). The heparinized saline was then removed from the cannula, and a 0.3-mL blood sample was taken from the resting rat. The withdrawal of larger amounts of blood from the cannulated rats would have altered the level of stress hormones. Using a sterile 1-cm^3 tuberculin syringe fitted with a 30-ga needle, the blood sample was taken from the cannula. The syringe and the needle were rinsed with ethylenediaminetetraacetate (EDTA) before sampling. The blood sample was placed in an EDTA-treated 0.3-mL capillary blood collection container (Walter Sarstedt Co., Princeton, New Jersey), shaken, and then placed on ice. The blood sampling procedure required about 2 min for each rat.

Blood Sampling Schedule. Figure 2 shows the sampling schedule designed for the experiment. Note that the 200 rats were introduced into the study in 4 groups of 50 animals each. The groups entered in a staggered manner to facilitate the process of logging in and establishing the new animals. Each group contained 25 exposure and 25 sham-exposure animals. Of the 25 exposure

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* 0.5 cm^3 heparin sodium (from beef lung), 1000 units/mL per 30 cm^3 saline.

TABLE 1. PLASMA PROLACTIN VALUES (ng/mL) ±SD OBTAINED IN RESTING RATS SEVERAL DAYS AFTER IMPLANTATION OF THE CHRONIC AORTIC CANNULA FOR BLOOD SAMPLING (0.3 mL)*

Arterial Blood Sampled After Days of Aortic Cannulation				
1 day	3 days	5 days	7 days	14 days
58	16	4	11	30
32	21	23	22	7
47	21	15	12	7
30	7	12	6	12
12	19	19	21	10
52	46	14	13	18
38	29	16	10	9
18	41	9	3	15
27	7	7	18	17
49	8	21	3	9
36 ± 15	22 ± 14	14 ± 6	12 ± 7	13 ± 7

*Each group of rats consisted of 10 animals (1,3,5,7, and 14 days). The animals were adapted to their cages for 3 weeks before the cannulas were implanted.

TABLE 2. PLASMA PROLACTIN VALUES (ng/mL) ±SD IN 10 RESTING RATS AND IN THE SAME RATS 7 MIN AFTER PLACEMENT INTO NEW CAGES*

Animal	Resting Rats	7 min After Placement into New Cages
1	25	29
2	12	21
3	8	16
4	11	43
5	14	29
6	12	11
7	17	38
8	18	29
9	7	26
10	12	31
	14 ± 5	27 ± 10

*Sampling through chronic aortic cannula while the animal rests in its home cage.

Figure 1. Sampling 0.3 mL of blood from the chronically implanted aortic cannula of a resting rat.

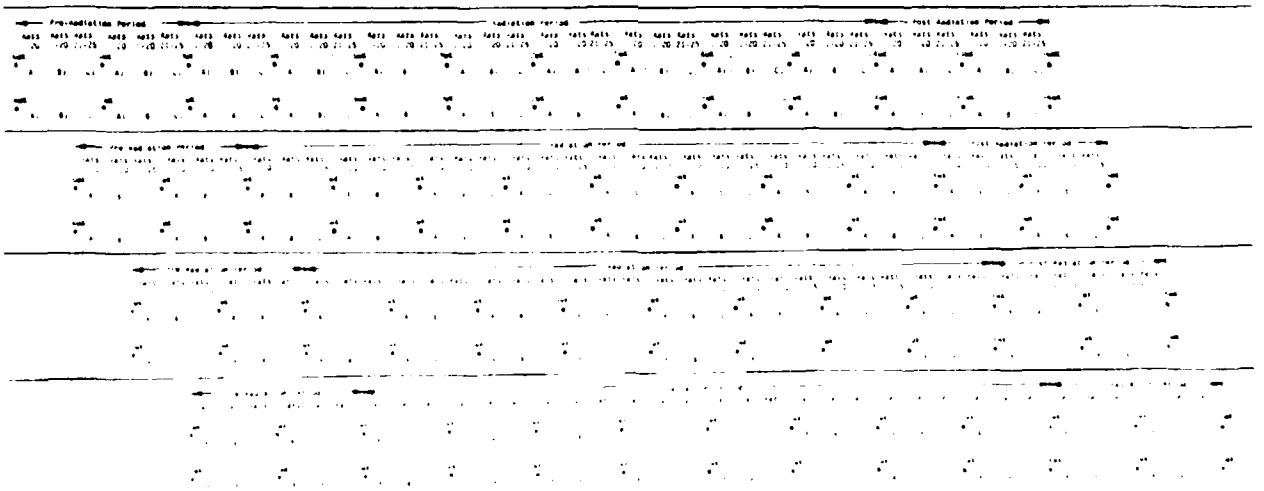

Figure 2. Sampling and exposure timetable.

(or sham-exposure) animals, 20 were sampled for plasma stress hormones, while the remaining 5 were used for hematology studies.

The sampling duration was 36 weeks long, including a 6-week preexposure adaptation period, a 24-week exposure period, and a 6-week postexposure period. With allowing for group staggering, the experiment duration was 42 weeks long (since the 4 groups entered 2 weeks apart from one another). Plasma prolactin was sampled for all periods marked (A) in Figure 2. Therefore, each animal should have been sampled for plasma prolactin at weeks -6, -3, 0, 3, 6, ..., 27. This schedule was rather rigorous, and therefore could tolerate slight fluctuations in protocol without ill effects.

Prolactin Determination. Rapid, sensitive, and specific radioimmunoassays that require a minimum quantity of blood were used in this study. These qualities were especially important because repetitive sampling was required and small laboratory animals were used. Plasma prolactin from individual plasma samples was measured in duplicate by double antibody radioimmunoassays for rat prolactin [21] using the NIAMD* reagents. Results are expressed as ng/mL, and the reference standard was Rat Prolactin RP-2.

Resting Value of Plasma Prolactin. At the initiation of the study, preliminary experiments were performed to determine the basal value of plasma prolactin in the cannulated resting rats (see Table 1). Plasma stress hormones in the rat follow circadian rhythm, increasing during evening hours and decreasing to the lowest level between 9 AM and 1 PM [22,23]. To avoid the effects of circadian rhythm on the study results, blood sampling occurred only between 9 AM and 1 PM when prolactin concentration was at its lowest (true resting) level [9].

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*National Institute of Arthritis, Metabolic & Digestive Diseases, Bethesda, Maryland.

III. RESULTS AND ANALYSIS

Appendix A contains the data collected during the course of the pre-exposure and radiation periods for both exposure and sham-exposure animals. Over the entire blood sampling period, there was considerable variance in the data, suggesting animal activity at the time of blood sampling. Since the sampling boxes had opaque walls, the physical activity of each animal immediately prior to sampling was not recorded; however, each animal had sufficient time (30 min or more) to return to basal hormonal level after the stimulation of being placed into the sampling box.

Figures 3 and 4 present the data of Appendix A in scatter form (one plot corresponds to sham-exposure animals, the other to exposure animals). The dotted line passes through the mean hormone response at each week. In general, plots of both exposure and sham-exposure hormone concentration versus time were essentially linear (although there was some curvature present at the exposure onset and conclusion). Furthermore, the trend of the data suggested that plasma prolactin concentrations in both exposure and sham-exposure groups began somewhat high, declined into the study, and then rose slightly toward the end of the exposure. There was little variation in the two plots when they were overlaid and compared (Fig. 5). This was preliminary evidence indicating that 435-MHz RFR did not increase resting plasma prolactin concentrations. To attach numerical probabilities to this conclusion, the data were statistically analyzed.

The plasma prolactin data were analyzed with linear regression model-building techniques. A quadratic model (hormone concentration as a function of time) was constructed to fit the data. Terms of the quadratic model were then tested to determine whether or not there were significant microwave-induced effects on hormone concentration. Appendix B contains a detailed discussion of the methodology, procedure, and results of the statistical analysis.

Results of the analysis indicated that, if there were any RFR-induced effects on plasma prolactin concentration, these effects were within the range of $\pm$ 3.32 ng/mL from the estimated normal resting value of 17.05 ng/mL. Since this range was within the normal range of plasma prolactin concentration variability in unstressed male rats, there was, from a practical standpoint, no indication of RFR-induced stress affecting animal resting plasma prolactin concentrations.

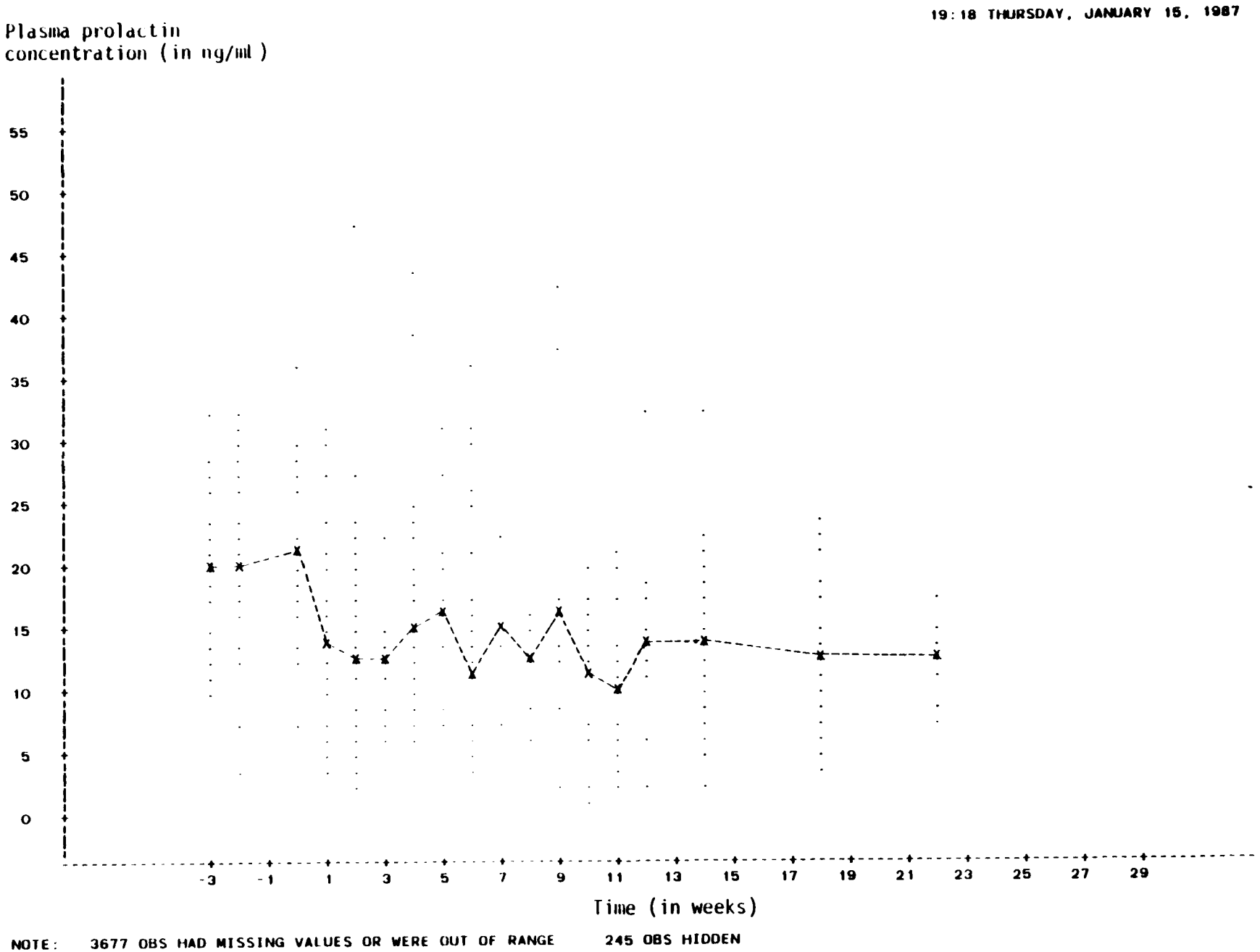

Figure 3. Prolactin concentration data scatter diagram (sham-exposure group).

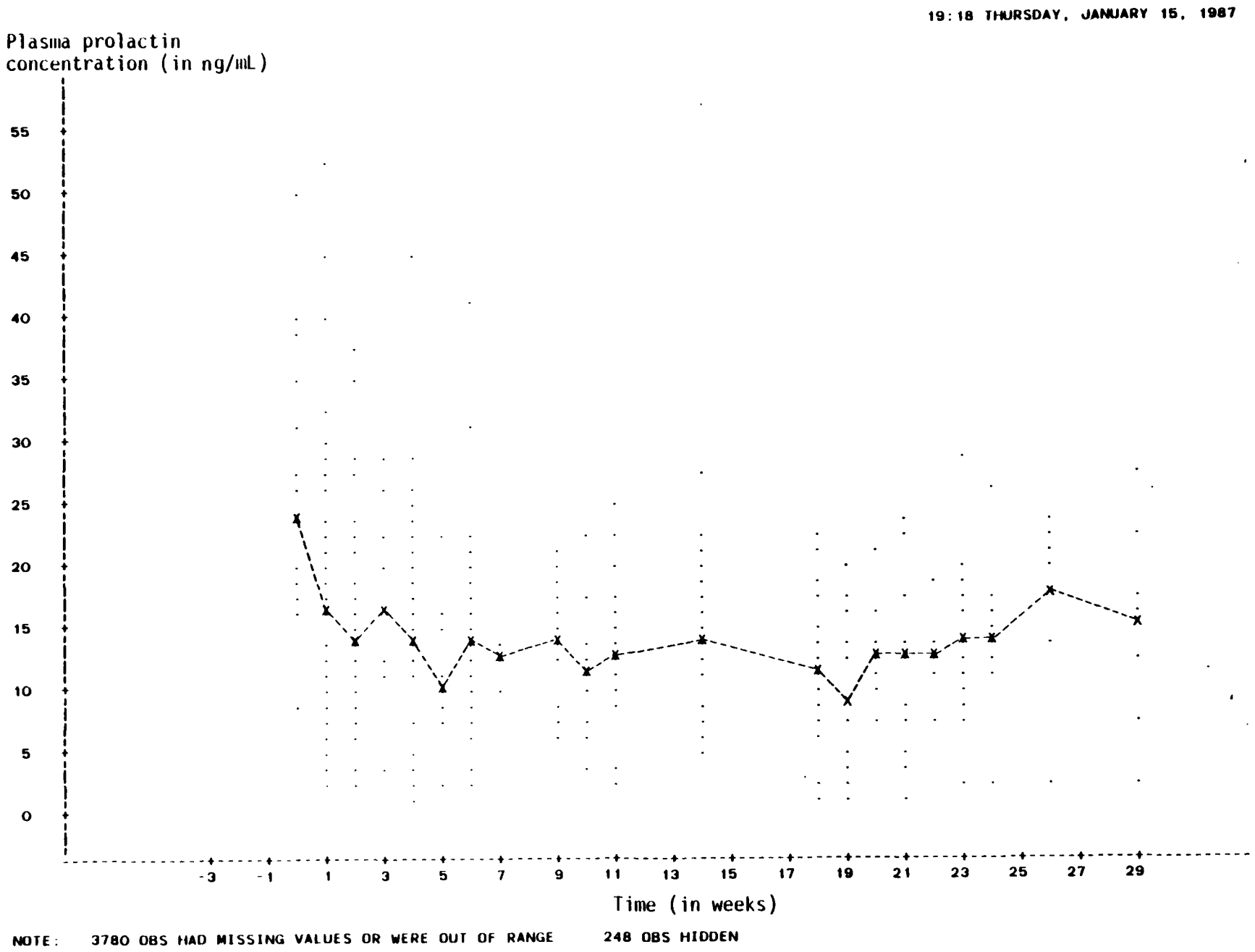

Figure 4. Prolactin concentration data scatter diagram (exposure group).

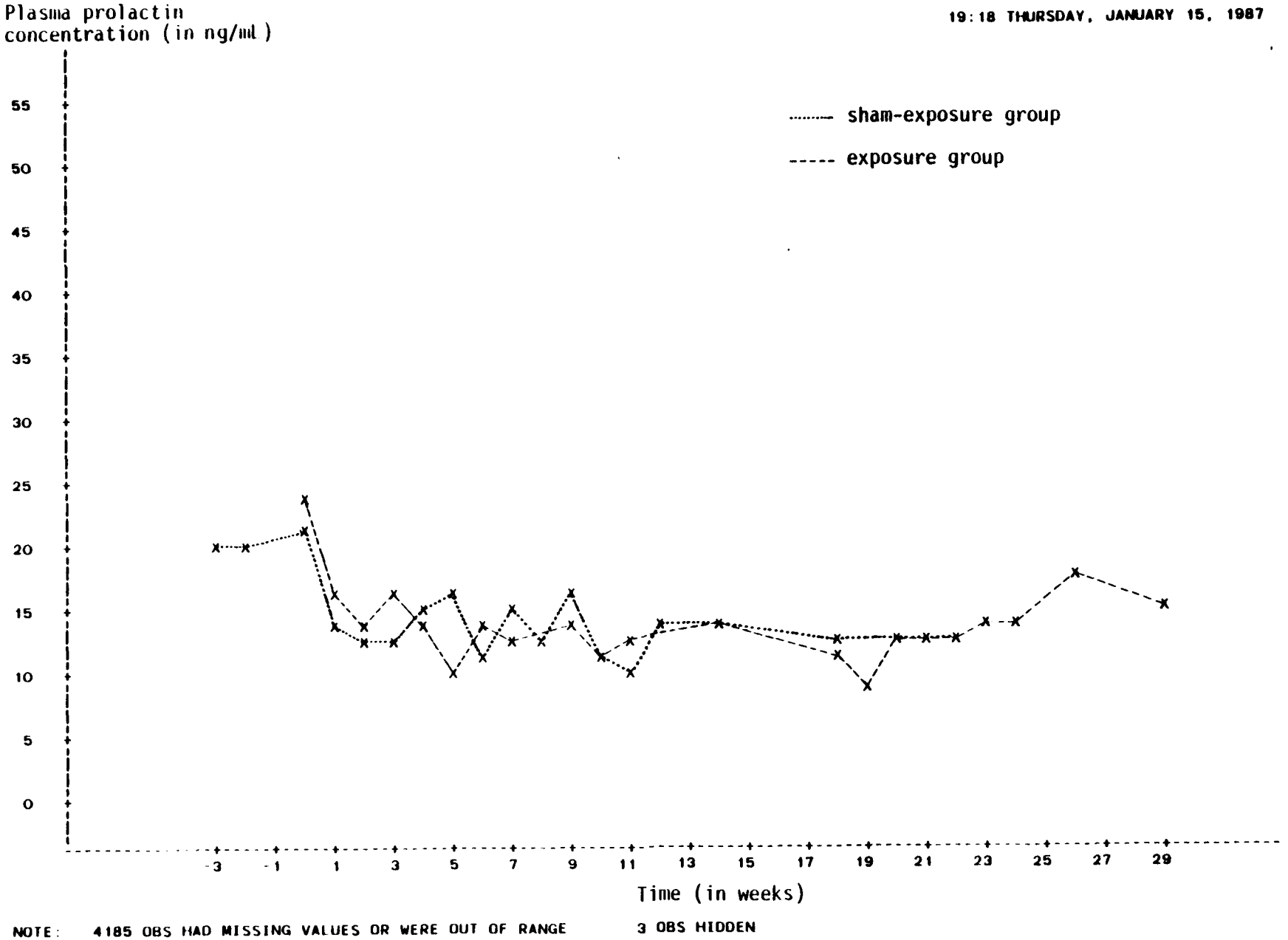

Figure 5. Mean plasma prolactin concentrations versus time.

IV. DISCUSSION

It is known that stress increases the level of plasma stress hormones. Thus, handling of the animals [24], exercise [25], immobilization [26], withdrawal of large volumes of blood [27], exposure to new or unfamiliar housing [28], noise, hypoxia [29], cold or heat exposure [30], and many other environmental factors increase the plasma concentration of stress hormones. Both neurogenic (emotional) stimuli and systemic (somatic) stimuli are effective in evoking increased secretion of stress hormones in animals (and in man), and these stimuli had to be avoided in the Radiation Facility used for this study.

Handling and removing a rat from its cage also induces an increase in plasma stress hormones even if the stimulus is removed immediately. The increase in plasma stress hormones was observed for 20 to 30 min [31].

The plasma prolactin increase in response to stress [4,5,12] can be quantified. The degree of plasma prolactin increase is related to the type and intensity of stress to which the animal is exposed [32] as well as to the duration of stress. The physiological importance of increased prolactin release in response to stress remains poorly understood.

There are few studies that deal with the effects of long-lasting stress. Burchfield et al. [33] demonstrated that the resting plasma corticosterone levels in chronically stressed rats had elevated as much as in control animals during acute stress, but plasma ACTH levels remained unchanged. In another study, it was shown that adaptation to stress did not result in an increased rate of adrenocortical response and "an overall increased responsiveness of the pituitary-adrenal system" [34].

The high sensitivity of the brain-pituitary-prolactin system observed during stress demands that blood sampling be done remotely. Repeated sampling of blood from the same cannulated rat provided reliable resting patterns of prolactin secretion that would reveal any increases induced by a long-term, low-level RFR environment. Apparently, even the smallest environmental perturbation, such as low-level RFR, would be detectable if it had any significant influence on the release of this hormone. Although relocation of a rat from the cage into the sampling box 30 min before blood sampling slightly disturbed the environment of the rat, such perturbations did not alter resting plasma prolactin levels at the time of sampling (Table 2).

Results of a study concerning plasma ACTH and corticosterone concentrations in rats exposed for 6 months to the same RFR environment used in this study were reported previously [2]. These results showed that plasma corticosterone and plasma ACTH concentrations were not changed in rats exposed to low-level, pulsed RFR fields for a 6-month duration. In this report, plasma prolactin levels in the same animals are reported. These 3 hormones were studied because every stress does not release all stress hormones. While in certain cases, associations are observed in the release of some stress hormones (for instance, corticosterone and ACTH, [35]), multiple hormone release is not always observed. Furthermore, while corticosterone is released in a pulsatile fashion, the release of prolactin, though also pulsatile, induces smaller variations from the mean and thus might provide a better method for measuring the resting level of stress hormones.

It has already been shown that short-term exposure to low-level microwave radiation does not change the plasma level of some stress hormones in rats [36,37]. Johnson and associates [38] found an elevation of plasma corticosterone the first time the blood was sampled from microwave-exposed rats in their long-term study. In the same study, plasma corticosterone returned to resting control levels throughout the remaining 2-year period.

As previously mentioned, plasma prolactin is a sensitive indicator of various types of environmental stress in mammalian systems. Stressors lead to increased prolactin release and an increased plasma prolactin concentration. This increase depends on the intensity and duration of stress, and can reach 8 to 10 times the normal resting plasma concentration. Our results show that low-level RFR does not change plasma prolactin levels in rats. The statistical analysis indicates that any RFR-induced effects on rat resting prolactin concentration would lay within a range of $\pm$ 3.32 ng/mL from an estimated resting concentration of 17.05 ng/mL. These values are not typical of rats exposed to stress. Therefore, this study concludes that a 1.0 mW/cm^2 435-MHz pulsed-wave (1.0 μs width, 1 kHz pulse rate) RFR environment did not induce any detectable increase in stress, as measured by resting prolactin concentration, in the exposure group of 62 cannulated male Sprague-Dawley rats when compared to a sham-exposure group of 64 cannulated male Sprague-Dawley rats.

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APPENDIX A

RAW PROLACTIN DATA SPREADSHEETS

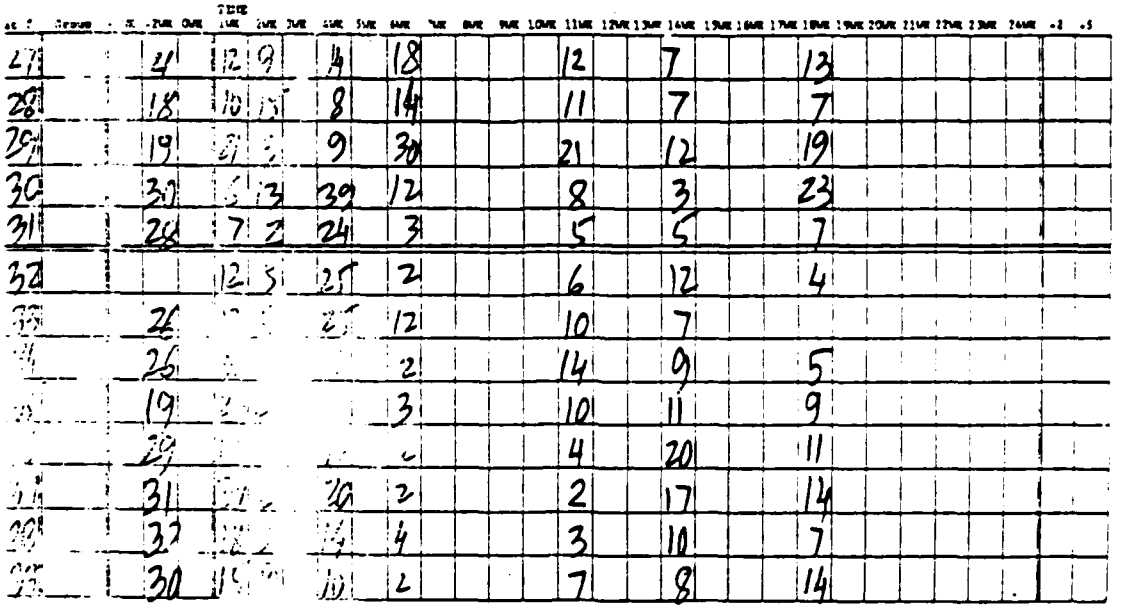
Prolactin
Control I

		TIME																														
		-3HR	-2HR	-1HR	1HR	2HR	3HR	4HR	5HR	6HR	7HR	8HR	9HR	10HR	11HR	12HR	13HR	14HR	15HR	16HR	17HR	18HR	19HR	20HR	21HR	22HR	23HR	24HR	+2	+3
1					11	13	14	6		5		9	9			11		12				14				12					
2			22	21	8	11	15	9		14		16	38			3		10				12				15					
3			26	14	15	12	13	12								6		8				15				7					
4			17	26	4	19	22	13		6		9	12			13		9				7									
5			19	21	11	14	6	11		13		6	3					6				9				9					
C			19	17	12	14	14	15		12		16	43			6		33				8				17					
7				18	11	7	11	12		7																					
8	e		24	20	12	12	9	15				15	9			11		14				7				14					
9					18	21	7																								
10			19	22	20		12			14		13	12			15		12				8				13					
11			24	16	16	20	12						16			17															
12			13		18					11						14		16				11				11					
13			19	18	16	17	15	44		7			14			33		17				14				11					

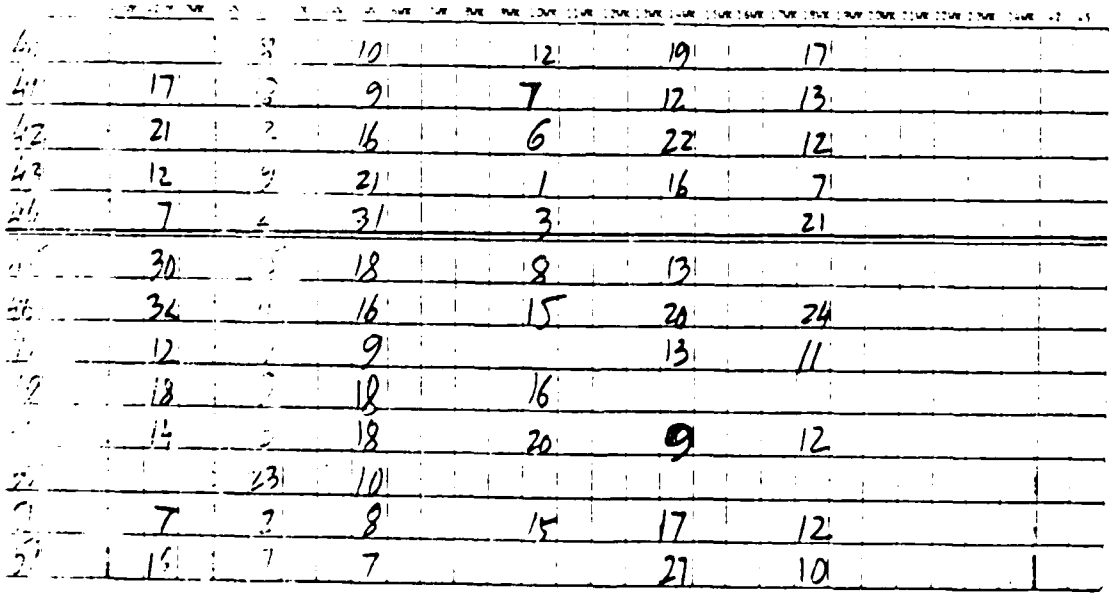
Prolactin
Control II

		TIME																														
		-3HR	-2HR	-1HR	1HR	2HR	3HR	4HR	5HR	6HR	7HR	8HR	9HR	10HR	11HR	12HR	13HR	14HR	15HR	16HR	17HR	18HR	19HR	20HR	21HR	22HR	23HR	24HR	+2	+3
14		18	23	27		22										13		21				12									
15		19	21	18		15		24		7		14		13		14		7				17									
16			18	18		21				6				18		19		14													
17		23	16	26				11		5		22		14		12		12				8									
18		26	19	20		12		3		5		8		14		14		18				14									
19		27		26		18		6		25						10		15				14									
20		29		13		18		11		21						17		9													
21		13		29		24		10		26						12		20				15									
22		33		36		4		19		15						7		7				7									
23		19		30		4		7		36						17		19				6									
24		18		18		10		11		16						11						21									
25		10		7		10				31						7		14													
26		11		19		5		22		7						20		14				12									

Prolactin
Contrql III



Prolactin
Control IV



Prolactin
Control V

at #	Group	-7HR	-2HR	0HR	TIME 1HR	2HR	3HR	4HR	5HR	6HR	7HR	8HR	9HR	10HR	11HR	12HR	13HR	14HR	15HR	16HR	17HR	18HR	19HR	20HR	21HR	22HR	23HR	24HR	+2	+5
53		29			20			28						7				12				12								
54		20			13			20																						
55		11			12			16						17				14				8								
56		20			4			20						14				12				17								
57		15			13			16						14																
58		18			19			17																						
59		24			47			9						12				7				18								
60		21			27			17						8				18				18								
61		14			11			14						16				17				24								
62		19			6			17						16				9				7								
63																														
64																														
65																														

Prolactin
MW I

at #	Group	-7HR	-2HR	0HR	TIME 1HR	2HR	3HR	4HR	5HR	6HR	7HR	8HR	9HR	10HR	11HR	12HR	13HR	14HR	15HR	16HR	17HR	18HR	19HR	20HR	21HR	22HR	23HR	24HR	+2	+5
1					80	17	12	11	13	10			5	8															12	6
2					20	28	21	3	3					13																
3					50	27	16	12	11					9															17	4
4					28	16	4	23						14																
5					35	9	29	4	20	10																				
6					16	33	14	20	13	2				15															7	4
7					16	20	17	13	5	9				13																
8					18	20	6	13	13	9				13															12	17
9					9	30		29	25	16				15																
10					20	24	14	26	17	8				8																
11					39	40	12	23	16	15				6																
12					18	29	38	13	8	7				8															10	15
13					9	26	13		16	16				13																

Prolactin
MW II

rat #	Group	-3WK	-2WK	0WK	1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
14				19	10			7		12	12	✱																		
15				20	8			19		17	12		13										2			17			14	12
16				31	3			4			10		19										23			22				
17					6			26		41	14		18										7			1			13	16
18				20	6																		19			✱2				
19					23			4		12	✱		✱		15		6						10			14				
20				40	23			4		7					9		16						9			24			11	10
21					14			24							18		15													
22				27	22			16		4					18		5						9			18			19	13
23					21			17		3					17		20						✱							
24				19	16			23		3					25		9						1			4			7	9
25				26	17			17		11					10		22						2			5			8	14
26				24	11			16		14					13		16						9			8				

Prolactin
MW III

rat #	Group	-3WK	-2WK	0WK	TIME 1WK	2WK	3WK	4WK	5WK	6WK	7WK	8WK	9WK	10WK	11WK	12WK	13WK	14WK	15WK	16WK	17WK	18WK	19WK	20WK	21WK	22WK	23WK	24WK	+2	+5
27					11	35		16		13					13			13					15							
28					10	13		15		13					3			9					15						16	21
29					20	11		2							18			9					8						17	10
30					10	24		25		3					16			17					12							
31					52	14		5		20					20			15												
32					45	14		17		6					3			11					6							
33					21			25		31					23			19					12						12	10
34					8	8		19							4			14					21							
35					4	16				23					10			18					13							
36					6	14		3		13					13			7					6							
37					7	12		20							14															
38					6	9		4		21					11								6							
39					12	10		14		21					2								14						13	14

Prolactin
MW IV

Rat #	Group	-3HR	-2HR	-1HR	TIME 1HR	2HR	3HR	4HR	5HR	6HR	7HR	8HR	9HR	10HR	11HR	12HR	13HR	14HR	15HR	16HR	17HR	18HR	19HR	20HR	21HR	22HR	23HR	24HR	+2	+3
40					8	15	3	11					4					15					2						10	12
41					22	12	21						10					8					1							
42					9	18	1	16					13					12					4							
43					4	28	8	16					14										8						14	14
44					8	7	5	16					11					21					1							
45					5	3	17	21										13					5							
46					19	3	15	13					18					6					2						7	14
47					10	12	25	16					23					13					20							
48					5	2		13					6					6					2						16	18
49					14	3	17	14					4					14					16							
50					13	3	15	10										8					14						12	14
51					19	8	24	16					12					16												
52					3	12	26	15					17					12					17						11	9

Prolactin
MW V

Rat #	Group	-3HR	-2HR	-1HR	TIME 1HR	2HR	3HR	4HR	5HR	6HR	7HR	8HR	9HR	10HR	11HR	12HR	13HR	14HR	15HR	16HR	17HR	18HR	19HR	20HR	21HR	22HR	23HR	24HR	+2	+3
53					8	4	23	2					16					15					17						12	16
54					4	3	18	8					17										12						9	12
55					26	12	7	13					12					23											29	17	14	18
56					12	12	2	8										16					17						18	12	22	16
57					8	11	8	21					21					7											18	11	24	12
58					20	10	13	19					18					16					12						3	12	20	8
59					13	9	11	9										27					12						20	2	2	3
60					7	15	4	9					20										11						17	3		23
61					24	16	29	18					14					5					22						17	15	24	27
62						23	2	14					13					16					6						11	18		
63						7	7	13										14					7						7	26		
64						19	18	11					16					13					15						19	14		
65																															

APPENDIX B

STATISTICAL METHODOLOGY

APPENDIX B

STATISTICAL METHODOLOGY

The balanced design of this experiment (requiring that 25 animals from each group be sampled once every 3 weeks for stress hormones) should have produced data easily tested by balanced, 2-way analysis of variance (ANOVA) statistics with 12 levels of factor A (time) and 2 levels of factor B (RF radiation). However, data collection did not proceed according to protocol in that, in numerous cases, samples were collected at odd intervals (invalidating the orthogonality of the design) and the number of samples taken per week varied more or less than 25 (unbalancing the design). These two factors combined to lower the power of ANOVA statistics (power is defined as the ability to reject the null hypothesis given the null hypothesis should be rejected) trying to test the model

$$y_{ijk} = \mu + \tau_i + \beta_j + \tau\beta_{ij} + \varepsilon_{ijk}, \qquad \text{(B-1)}$$

where y_{ijk} = hormone concentration (response),
μ = the normal hormone resting concentration,
τ_i = the change in hormone resting concentration induced by RFR,
β_j = the change in hormone resting concentration induced by time,
$\tau\beta_{ij}$ = the change in hormone resting concentration induced by the interaction between RFR and time, and
ε_{ijk} = noise within the system (sampling and assaying errors)

for the following hypotheses:

H_0: $\tau_0 = \tau_1 = 0$,
H_1: τ_0 or $\tau_1 \neq 0$ (RFR-induced effects), (B-2)

H_0: $\beta_1 = \beta_2 = \ldots = \beta_{12} = 0$,
H_1: at least one $\beta_j \neq 0$ (time-induced effects), (B-3)

H_0: $\tau\beta_{ij} = 0$, and
H_1: at least one $\tau\beta_{ij} \neq 0$ (interaction between RFR and time). (B-4)

However, examination of the collected data suggested an alternative approach in that the data resembled what might have been collected in an unplanned experiment monitoring over time the operation (in this case, characterized by resting animal hormone concentrations) of an established RF radiation facility. Data of this type are often successfully treated by employing linear regression techniques to develop, build, and test a linear (or intrinsically linear) model whose parameters can be used to predict the system response at various treatment levels. Therefore, we decided to proceed with a regression approach to data analysis.

The first step in the regression approach to data analysis was to define an initial model to fit the data, and to test the properties of this model.

Visual inspection of the scatter diagrams of Figures 3 and 4 showed an essentially linear plasma prolactin response versus time. Therefore, there was a nonzero β_0 in the final model, and tests were conducted for a RFR-induced effect on this intercept with the term $\alpha_0 z$. Also, there was sufficient curvature in the plot (particularly at exposure onset and termination) to justify the inclusion of linear terms (β_1 and $\alpha_1 z$) and quadratic terms (β_{11} and $\alpha_{11} z$).

The initial model therefore became:

$$y = \beta_0 + \beta_1 x + \beta_{11} x^2 + \alpha_0 z + \alpha_1 zx + \alpha_{11} zx^2 \qquad \text{(B-5)}$$

where y = the plasma prolactin concentration,

x = the time (in weeks), and

z = a categorical variable with value of 0 for animals in the sham-exposure group and 1 for animals in exposure group.

At this point, raw data from the prolactin spreadsheet were put on computer file (see Appendix A). A Statistical Analysis System (SAS) program (see Appendix C) was then written to read the raw data file, format the data for analysis, and perform a variety of statistical tests on the model.

The first test identified terms in the general model which contributed the least to forming a statistically significant regression. Two stepwise regression procedures were employed: forward regression and maximum R^2 regression. Forward regression procedures entered variables into the model in such a way as to produce the greatest increase in R^2 (R^2 being a measure of the

percentage of variation in the data set which is explained by the statistical model) while ensuring that the variable entered was statistically significant at a significance of 0.15. The forward stepwise regression produced the model (see Appendix D for the SAS forward and maximum R^2 analysis output):

$$y = \beta_0 + \beta_1 x + \beta_{11} x^2, \qquad \text{(B-6)}$$

where all variables were as previously defined.

The second stepwise procedure employed was maximum R^2 regression (MAXR). Maximum R^2 regression functioned essentially the same way as the forward procedure, the distinction being that MAXR entered a variable into the model so long as the introduction of that variable increased the R^2 ratio (even if the variable was found to be otherwise statistically insignificant). Thus, MAXR first found the best possible 2-parameter model, then the best possible 3-parameter model, up to the best all-factor model.

The combined output of these 2 programs gave a good indication (when viewed with estimates of the coefficients in the all-parameter model) of which terms in the original model could be removed without compromising the final model's predictive power. Both forward and maximum R^2 regression determined that, at the 0.15 significance level, neither α_0, α_1, or α_{11} were important to the original model. (α_0 was significant at $\alpha = 0.3818$, α_1 was significant at $\alpha = 0.8850$, and α_{11} was significant at $\alpha = 0.7264$.) Thus, since the terms modeling the RFR interaction effect were insignificant, the conclusion was drawn that RFR exposure did not produce a detectable effect on plasma prolactin concentrations.

Note that the estimated values for β_0, β_1, and β_{11} were all found to be significant at a level greater than $\alpha = 0.001$ (Appendix E, page 44). This indicated that the plasma prolactin concentrations in both exposure and sham-exposure groups varied over the duration of the experiment. This curvature from the straight line case ($y = \beta_0$, which would indicate that all hormone concentrations remained constant over time) took into account the slightly higher values of plasma prolactin at the experiment onset and conclusion (17 to 19 ng/mL) as compared to plasma prolactin concentrations in the middle of the study (approximately 11 ng/mL). These predictions are rough estimations from the model since the confidence intervals (provided under a separate cover) on plasma prolactin concentration were the same width as normal hormone ranges (10 to 15 ng/mL).

To complete the analysis (with regards to the question of RFR-induced bioeffects) required the subsequent determination of the maximum perturbation in resting prolactin levels that the experimental protocol was capable of detecting. However, in order for results from the linear regression to be considered significant, it was first important to verify that the assumptions made in forming the linear model were not violated during the model-building procedure. These assumptions included no lack-of-fit in the model, and that the residuals from the fitted model followed a normal, independent distribution (termed NID $(0, \sigma^2)$). First, a lack-of-fit test was performed on the data by obtaining (in the revised model) sum-of-squares regression error and sum-of-squares pure error. Since there were repeated measurements taken at each week for both the exposed and sham-exposed animals, it was therefore possible to break the model sum-of-squares error into lack-of-fit and pure error terms. First, the model sum-of-squares error was obtained by running a regression on the revised model and reading the term from the resulting ANOVA table. To obtain a sum-of-squares pure error term, the SAS General Linear Models (GLM) procedure was applied to the data (33 levels of time treatment, 2 levels of RFR treatment). The sum-of-squares error term yielded by the GLM represented a sum-of-squares pure error (due to sampling variation) in the regression. Sum-of-squares lack-of-fit was then the regression sum-of-squares error minus the sum-of-squares pure error. Calculations to compute the critical value F_0 from these sum-of-squares terms are detailed in Appendix E.

The computed test statistic F_0 exceeded the critical value, thereby indicating significant lack-of-fit. Normally, this result would be faintly disturbing since it would require refitting the model using transformed rather than raw data values. The transformation of the dependent variable y was definitely undesirable, since the residual plots indicated that the residuals of y (using the revised model) conformed to the NID $(0,\sigma^2)$ requirement. Additionally, transformation of the predictor variables x and x^2 to yield a model displaying no lack-of-fit, although theoretically possible, would be a long and time-consuming process.

Fortunately, the experimental design helped compensate for the model lack-of-fit deficiency. First of all, the lack-of-fit was comparatively small. Under optimal conditions (lack-of-fit statistically insignificant), both the mean square error and the mean square pure error estimate the population variance. If there is a lack-of-fit, the mean square pure error estimates the

variance plus a bias term. From the ANOVA (regression and GLM) tables, the tabulated values for MS_E and MS_{pe} were 54.58 and 52.59 respectively. Thus, although the lack-of-fit was statistically significant, it was also practically insignificant. In other words, the development of an alternative model displaying no lack-of-fit would yield essentially (within 1 or 2 %) the same results as the present model displaying lack-of-fit. Rather than identify an alternate model (which would not be that much better a predictive tool than the model currently being used), we decided to proceed with the stepwise model and modify the significance of the tests to compensate for model lack-of-fit. Thus, all α's listed are somewhat higher than they should be, and the confidence intervals established are somewhat wider than indicated in the appendix tables.

The final step in determining model accuracy involved examining the residual and partial residual plots to verify the least-squares regression assumption that the model errors were NID $(0,\sigma^2)$. Confirming this assumption confirmed the basis of the F tests used to determine the statistical significance of the parameters, and confirmed the statistics which produced the tables listing confidence intervals of the prolactin concentrations. A number of residual plots suggested themselves immediately: residuals versus time, residuals versus predicted value of prolactin concentration, and residuals versus animal case number; studentized residuals versus the 3 plots just mentioned, and partial residual plots corrected for the parameters β_0, β_1, and β_{11}.

Examination of the original residual plots essentially confirmed the NID $(0, \sigma^2)$ hypothesis. However, there was one outlier in the data set (case number 101, week 0, prolactin concentration 80 ng/mL) whose studentized residual was 8.16 (Cook's distance of 0.097). This value was most likely due to an error in assaying or reporting the results, and was sufficiently anomalous to be discarded from the data set. The residual plots were then regenerated and rechecked for their distributional properties. The new plots (Appendix F) indicated no further problems.

Diagnostics to check for model multicollinearity and correlation between the terms were then employed. Examination of the listed condition numbers and matrix eigenvalues detected no troublesome values. This indicated that the model did not display a significant degree of multicollinearity. Similarly, examination of the correlation matrix showed that correlation between the estimated values of β were all within tolerable limits. The highest degree of

correlation was between the x and the x^2 term, which often occurs when using a polynomial model in linear regression.

For future reference, and for the sake of completeness, tables listing animal case number, observations (if taken) at each week, predicted value of prolactin concentration, standardized error of prediction, 95% confidence intervals on the mean value of the prolactin concentration, and residuals were prepared as were tables containing animal case number, regular and studentized residual values, a graphical display of student residual values, and influence statistics (such as Cook's D). These tables were used to detect both outliers and influential data points in the prolactin data set.

Since the null hypothesis in the study was not rejected in the analysis, it was necessary to determine the smallest difference between the exposed and sham-exposed means that the protocol could reliably detect. A conservative estimate of this sensitivity was obtained by finding this difference δ in a simpler experimental setting. Since the experimental hypothesis being tested in this alternative model was more general than the hypotheses given in the original model, the difference obtained in the calculations would be somewhat larger than the difference that the ANOVA design was capable of detecting.

To begin, it was assumed that the experimental hypothesis was merely one testing the equality of the means between the exposed and sham-exposed groups

$$\begin{aligned} H_0&: \mu_{\text{sham-exposed}} = \mu_{\text{exposed}} \\ H_1&: \mu_{\text{sham-exposed}} \neq \mu_{\text{exposed}} \end{aligned} \tag{B-7}$$

This type of hypothesis could be tested using a 2-sided t-test. The equation to determine the type II error in this test was then

$$\begin{aligned} d &= \frac{\mu_{\text{sham-exposed}} - \mu_{\text{exposed}}}{\sigma} \\ &= \delta/\sigma \end{aligned} \tag{B-8}$$

This equation assumed equality in the variances of the exposed and sham-exposed populations. In general, this assumption was acceptable since there was no evidence that RFR affected the variance of prolactin parameters differently in the exposed and sham-exposed groups. In equation (B-8), the square root of the MS_E estimated the population standard deviation.

The number of replications per group, n, was computed by the following equation:

$$n = \frac{\sigma_1^2 + \sigma_2^2}{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}} \qquad \text{(B-9)}$$

and the computation yielded n = 63.

Then, the tabulated value of d was read from the 2-sided t-test operating curve for $\alpha = 0.05$, $\beta = 0.10$, and n=63. Returning to the original equation:

$$0.45 = \delta/7.387 \qquad \text{(B-10)}$$

$$\delta = 3.3242$$

Therefore, the protocol was able to detect a $\pm$ 3.32 ng/mL change in resting prolactin concentration approximately 90% of the time.

At the conclusion of the statistical analysis, it was evident that, if there were any RFR-induced effects on plasma prolactin concentration, these effects were within a range of $\pm$ 3.32 ng/mL from the normal resting value. Since this range was within the normal range of plasma prolactin concentration variation (10 to 15 ng/mL), from a practical standpoint, there was no indication of RFR-induced stress affecting animal resting plasma prolactin concentrations.

We gratefully acknowledge the assistance of Dr. Russell G. Heikes of Georgia Tech's Department of Industrial and Systems Engineering in developing the statistical methodology of this appendix.

APPENDIX C

PROLACTIN SAS FORMATTING PROGRAM

```
1          SAS(R) LOG  CMS SAS 5.16      VM/CMS CMS USER QSECLSB

NOTE: COPYRIGHT (C) 1984,1986 SAS INSTITUTE INC., CARY, N.C.  27511, U.S.A.
NOTE: CMS SAS RELEASE 5.16 AT GEORGIA INSTITUTE OF TECHNOLOGY (03559001).

NOTE: CPUID   VERSION = FF  SERIAL = 012242  MODEL = 4381 .

NOTE: SAS OPTIONS SPECIFIED ARE:
      LEAVE=0

  1 DATA TESTP;
  2 CMS FILEDEF X DISK PROLAC DAT A;
  3 CMS FILEDEF 20 DISK PROLAC0 LISTING A1 (BLKSIZE 141 RECFM VBA LRECL 133;
  4 CMS FILEDEF 21 DISK PROLAC1 LISTING A1 (BLKSIZE 141 RECFM VBA LRECL 133;
  5 CMS FILEDEF 22 DISK PROLAC2 LISTING A1 (BLKSIZE 141 RECFM VBA LRECL 133;
  6 CMS FILEDEF 23 DISK PROLAC3 LISTING A1 (BLKSIZE 141 RECFM VBA LRECL 133;
  7 CMS FILEDEF 24 DISK PROLAC4 LISTING A1 (BLKSIZE 141 RECFM VBA LRECL 133;
  8 CMS FILEDEF 25 DISK PROLAC5 LISTING A1 (BLKSIZE 141 RECFM VBA LRECL 133;
  9 CMS FILEDEF 26 DISK PROLAC6 LISTING A1 (BLKSIZE 141 RECFM VBA LRECL 133;
 10 CMS FILEDEF 27 DISK PROLAC7 LISTING A1 (BLKSIZE 141 RECFM VBA LRECL 133;
 11 ARRAY WEEK {33} WKN3 WKN2 MISSN1 WK0-WK24 MISS25 WKP2 MISS27 MISS28 WKP5;
 12 KEEP X XSQR Y Z XZ XSQRZ CASE;
 13 INFILE X;
 14 INPUT CASE 1-3
 15       WKN3 5-6
 16       WKN2 8-9
 17       WK0 11-12
 18       WK1 14-15
 19       WK2 17-18
 20       WK3 20-21
 21       WK4 23-24
 22       WK5 26-27
 23       WK6 29-30
 24       WK7 32-33
 25       WK8 35-36
 26       WK9 38-39
 27       WK10 41-42
 28       WK11 44-45
 29       WK12 47-48
 30       WK13 50-51
 31       WK14 53-54
 32       WK15 56-57
 33       WK16 59-60
 34       WK17 62-63
 35       WK18 65-66
 36       WK19 68-69
 37       WK20 71-72
 38       WK21 74-75
 39       WK22 77-78
 40       WK23 80-81
 41       WK24 83-84
 42       WKP2 86-87
 43       WKP5 89-90
 44 ;
 45 MISSN1=.;
 46 MISS25=.;
 47 MISS27=.;
 48 MISS28=.;
 49 IF CASE < 100 THEN Z = 0;
 50 IF CASE >= 100 THEN Z = 1;
```

```
 51 IF Z = 1 THEN CASE = CASE - 100;
 52 DO I = 1 TO 33;
 53 X = I-4; XSQR = X*X ; XZ = X*Z; XSQRZ = X*X*Z; Y = WEEK {I};OUTPUT;
 54 END;


NOTE: INFILE X IS FILE PROLAC DAT A1
NOTE: 126 LINES WERE READ FROM INFILE X.
NOTE: DATA SET WORK.TESTP HAS 4158 OBSERVATIONS AND 7 VARIABLES.
NOTE: THE DATA STATEMENT USED 0.66 SECONDS AND 200K.

 55 PROC CONTENTS;
NOTE: THE PROCEDURE CONTENTS USED 0.18 SECONDS AND 456K AND PRINTED PAGES 1 TO 2.

 56 PROC PRINTTO NEW UNIT=20;

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 328K.

 57 PROC SORT OUT=SCTR;
 58      BY Z X Y;

NOTE: DATA SET WORK.SCTR HAS 4158 OBSERVATIONS AND 7 VARIABLES.
NOTE: THE PROCEDURE SORT USED 0.93 SECONDS AND 2952K.

 59 PROC SUMMARY;
 60      BY Z X;
 61      VAR Y;
 62      OUTPUT OUT=OVLMN MEAN=MEAN;
NOTE: THE DATA SET WORK.OVLMN HAS 66 OBSERVATIONS AND 5 VARIABLES.
NOTE: THE PROCEDURE SUMMARY USED 0.68 SECONDS AND 456K.

 63 DATA SPROLAC;
 64      SET SCTR OVLMN;
 65      BY Z;

NOTE: DATA SET WORK.SPROLAC HAS 4224 OBSERVATIONS AND 10 VARIABLES.
NOTE: THE DATA STATEMENT USED 0.69 SECONDS AND 328K.

 66 PROC PLOT NOLEGEND DATA=SPROLAC;
 67      BY Z;
 68      PLOT MEAN*X='X' Y*X='.' / VAXIS=0 TO 55 BY 5 OVERLAY;
 69      TITLE 'PROLACTIN SCATTER DIAGRAM';
NOTE: THE PROCEDURE PLOT USED 1.34 SECONDS AND 456K AND PRINTED PAGES 3 TO 4.

 70 PROC PRINTTO NEW UNIT=21;

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 328K.

 71 PROC PLOT NOLEGEND DATA=SPROLAC;
 72      PLOT MEAN*X='X' / VAXIS=0 TO 55 BY 5;
 73      TITLE 'Mean Plasma Prolactin Concentrations Versus Time';
NOTE: THE PROCEDURE PLOT USED 1.04 SECONDS AND 456K AND PRINTED PAGE 5.

 74 PROC PRINTTO NEW UNIT=22;
 75      TITLE 'PROLACTIN ANALYSIS';

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 328K.
```

```
3          SAS(R) LOG  CMS SAS 5.16      VM/CMS CMS USER QSECLSB

 76 PROC DATASETS;
 77
LIST OF MEMBERS BEFORE UPDATE OF DIRECTORY.
NAME     MEMTYPE             OBS  TRACKS PROT
OVLMN    /DATA                66       1
SCTR     /DATA              4158       1
SPROLAC  /DATA              4224       1
TESTP    /DATA              4158       1
 77       DELETE SCTR;
 78       DELETE OVLMN;
LIST OF MEMBERS AFTER UPDATE OF DIRECTORY.
NAME     MEMTYPE             OBS  TRACKS PROT
SPROLAC  /DATA              4224       1
TESTP    /DATA              4158       1
NOTE: THE PROCEDURE DATASETS USED 0.11 SECONDS AND 456K.

 79 PROC STEPWISE;
 80       MODEL Y = X XSQR Z XZ XSQRZ
 81             / STEPWISE MAXR;
NOTE: THE PROCEDURE STEPWISE USED 0.69 SECONDS AND 456K AND PRINTED PAGES 6 TO 8.

 82 PROC PRINTTO NEW UNIT=23;

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 328K.

 83 PROC REG;
 84       MODEL Y = X XSQR / PARTIAL;
 85       ID CASE;
NOTE: ACOV AND SPEC OPTION ONLY VALID WITH RAWDATA
NOTE: THE PROCEDURE REG USED 1.88 SECONDS AND 648K AND PRINTED PAGES 9 TO 12.

 86 PROC PRINTTO NEW UNIT=24;

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 328K.

 87 PROC GLM;
 88       CLASS X Z;
 89       MODEL Y = X Z X*Z;
NOTE: THE PROCEDURE GLM USED 4.13 SECONDS AND 1032K AND PRINTED PAGES 13 TO 14.

 90 PROC PRINTTO NEW UNIT=25;

NOTE: THE PROCEDURE PRINTTO USED 0.03 SECONDS AND 328K.

 91 PROC REG;
 92 *-------------------------------------------------------------*
 93 *                                                             *
 94 *     to obtain tables listing the variance inflation factors,*
 95 *     influence statistics, and tolerances, the following SAS *
 96 *     statements were used in this partition:                 *
 97 *                                                             *
 98 *      PROC REG;                                              *
 99 *           MODEL Y = X XSQR / TOL VIF INFLUENCE;             *
100 *           ID CASE;                                          *
101 *      OUTPUT OUT=RPROLAC P=PREDICT R=RESID STUDENT=STUDENT;  *
102 *                                                             *
103 *-------------------------------------------------------------*;
104       MODEL Y = X XSQR / I SS1 SS2 STB COVB CORRB SEQB COLLIN
```

```
4          SAS(R) LOG  CMS SAS 5.16      VM/CMS CMS USER QSECLSB

105                        . COLLINOINT ACOV P R CLM;
106        ID CASE;
107        OUTPUT OUT=RPROLAC P=PREDICT R=RESID STUDENT=STUDENT;
NOTE: THE DATA SET WORK.RPROLAC HAS 4224 OBSERVATIONS AND 13 VARIABLES.
NOTE: THE PROCEDURE REG USED 8.88 SECONDS AND 648K AND PRINTED PAGES 15 TO 104.

108 PROC PRINTTO NEW UNIT=26;

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 328K.

109 PROC PLOT DATA=RPROLAC;
110        PLOT RESID*X='*';
111        PLOT RESID*PREDICT='*';
112        PLOT STUDENT*X='*';
113        PLOT STUDENT*PREDICT='*';
114        TITLE 'PROLACTIN ANALYSIS';
NOTE: THE PROCEDURE PLOT USED 1.76 SECONDS AND 456K AND PRINTED PAGES 105 TO 108.

115 PROC PRINTTO NEW UNIT=27;

NOTE: THE PROCEDURE PRINTTO USED 0.02 SECONDS AND 328K.

116 PROC PLOT DATA=RPROLAC;
117        BY Z;
118        PLOT RESID*CASE='*' / HAXIS=1 TO 63 BY 2;
119        PLOT STUDENT*CASE='*' / HAXIS=1 TO 63 BY 2;
120        TITLE 'PROLACTIN ANALYSIS';
NOTE: THE PROCEDURE PLOT USED 1.44 SECONDS AND 456K AND PRINTED PAGES 109 TO 112.

NOTE: SAS INSTITUTE INC.
      SAS CIRCLE
      PO BOX 8000
      CARY, N.C. 27511-8000
```

APPENDIX D

STEPWISE REGRESSION AND MAXIMUM R^2 REGRESSION PROCEDURES

STEPWISE REGRESSION PROCEDURE FOR DEPENDENT VARIABLE Y

WARNING: 3272 OBSERVATIONS DELETED DUE TO MISSING VALUES.

NOTE: SLENTRY AND SLSTAY HAVE BEEN SET TO .15 FOR THE STEPWISE TECHNIQUE.

STEP 1 VARIABLE X ENTERED R SQUARE = 0.03908007 C(P) = 36.41210587

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	1	2189.05893585	2189.05893585	38.64	0.0001
ERROR	950	53825.65955155	56.65858900		
TOTAL	951	56014.71848739			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	15.81519624				
X	0.20183162	0.03247083	2189.05893585	38.64	0.0001

BOUNDS ON CONDITION NUMBER: 1. 1

STEP 2 VARIABLE XSQR ENTERED R SQUARE = 0.07535017 C(P) = 1.25529105

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	2	4220.71829140	2110.35914570	38.67	0.0001
ERROR	949	51794.00019600	54.57745015		
TOTAL	951	56014.71848739			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	17.05565731				
X	-0.74687256	0.09484699	3384.22401522	62.01	0.0001
XSQR	0.02597365	0.00425710	2031.65935555	37.23	0.0001

BOUNDS ON CONDITION NUMBER: 8.857537, 35.43015

NO OTHER VARIABLES MET THE 0.1500 SIGNIFICANCE LEVEL FOR ENTRY INTO THE MODEL.

SUMMARY OF STEPWISE REGRESSION PROCEDURE FOR DEPENDENT VARIABLE Y

STEP	VARIABLE ENTERED	VARIABLE REMOVED	NUMBER IN	PARTIAL R**2	MODEL R**2	C(P)	F	PROB>F
1	X		1	0.0391	0.0391	36.4121	38.6360	0.0001
2	XSQR		2	0.0363	0.0754	1.2553	37.2253	0.0001

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MAXIMUM R SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE Y

WARNING: 3272 OBSERVATIONS DELETED DUE TO MISSING VALUES.

STEP 1 VARIABLE X ENTERED R SQUARE = 0.03908007 C(P) = 36.41210587

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	1	2189.05893585	2189.05893585	38.64	0.0001
ERROR	950	53825.65955155	56.65858900		
TOTAL	951	56014.71848739			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	15.81519624				
X	-0.20183162	0.03247083	2189.05893585	38.64	0.0001

BOUNDS ON CONDITION NUMBER: 1, 1

THE ABOVE MODEL IS THE BEST 1 VARIABLE MODEL FOUND.

STEP 2 VARIABLE XSQR ENTERED R SQUARE = 0.07535017 C(P) = 1.25529105

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	2	4220.71829140	2110.35914570	38.67	0.0001
ERROR	949	51794.00019600	54.57745015		
TOTAL	951	56014.71848739			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	17.05565731				
X	-0.74687256	0.09484699	3384.22401522	62.01	0.0001
XSQR	0.02597365	0.00425710	2031.65935555	37.23	0.0001

BOUNDS ON CONDITION NUMBER: 8.857537, 35.43015

THE ABOVE MODEL IS THE BEST 2 VARIABLE MODEL FOUND.

STEP 3 VARIABLE XSQRZ ENTERED R SQUARE = 0.07605837 C(P) = 2.52977744

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	3	4260.38790545	1420.12930182	26.01	0.0001
ERROR	948	51754.33058195	54.59317572		
TOTAL	951	56014.71848739			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	17.08636975				
X	-0.77407363	0.10008391	3265.68547553	59.82	0.0001
XSQR	0.02908957	0.00561156	1467.05353842	26.87	0.0001
XSQRZ	-0.00244038	0.00286285	39.66961405	0.73	0.3942

BOUNDS ON CONDITION NUMBER: 15.38603, 86.87415

MAXIMUM R-SQUARE IMPROVEMENT FOR DEPENDENT VARIABLE Y

THE ABOVE MODEL IS THE BEST 3 VARIABLE MODEL FOUND.

STEP 4 VARIABLE XZ ENTERED R SQUARE = 0.07650540 C(P) = 4.07181096

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	4	4285.42858499	1071.35714625	19.61	0.0001
ERROR	947	51729.28990241	54.62438216		
TOTAL	951	56014.71848739			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	17.06421002				
X	-0.83496062	0.13457174	2102.86216142	38.50	0.0001
XSQR	0.03288023	0.00792796	939.57766719	17.20	0.0001
XZ	0.10359845	0.15301129	25.04067954	0.46	0.4985
XSQRZ	-0.00819214	0.00896482	45.61397198	0.84	0.3610

BOUNDS ON CONDITION NUMBER: 36.38056, 428.518

THE ABOVE MODEL IS THE BEST 4 VARIABLE MODEL FOUND.

STEP 5 VARIABLE Z ENTERED R SQUARE = 0.07657550 C(P) = 6.00000000

	DF	SUM OF SQUARES	MEAN SQUARE	F	PROB>F
REGRESSION	5	4289.35506258	857.87101252	15.69	0.0001
ERROR	946	51725.36342482	54.67797402		
TOTAL	951	56014.71848739			

	B VALUE	STD ERROR	TYPE II SS	F	PROB>F
INTERCEPT	16.98394713				
X	-0.82316380	0.14165188	1846.46157551	33.77	0.0001
XSQR	0.03248926	0.00806491	887.34507653	16.23	0.0001
Z	0.23006938	0.85854579	3.92647759	0.07	0.7888
XZ	0.06529851	0.20943368	5.31527650	0.10	0.7553
XSQRZ	-0.00691488	0.01015700	25.34258699	0.46	0.4962

BOUNDS ON CONDITION NUMBER: 46.65425, 714.6894

THE ABOVE MODEL IS THE BEST 5 VARIABLE MODEL FOUND.

APPENDIX E

LACK-OF-FIT TEST CALCULATIONS

DEP VARIABLE = Y

ANALYSIS OF VARIANCE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PROB>F
MODEL	2	4220.71829	2110.35915	38.667	0.0001
ERROR	949	51794.00020	54.57745015		
C TOTAL	951	56014.71849			

ROOT MSE	7.387655	R SQUARE	0.0754
DEP MEAN	14.18697	ADJ R SQ	0.0734
C.V.	52.07351		

PARAMETER ESTIMATES

VARIABLE	DF	PARAMETER ESTIMATE	STANDARD ERROR	T FOR H0: PARAMETER=0	PROB > |T|
INTERCEP	1	17.05565731	0.40590938	42.018	0.0001
X	1	0.74687256	0.09484699	7.874	0.0001
X**2	1	0.02597365	0.00425703	6.101	0.0001

the error term contains both the sum of squares pure error and the sum of squares lack-of-fit.

PROLACTIN ANALYSIS 16:03 WEDNESDAY, JANUARY 7, 1987

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: Y

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	
MODEL	38	7995.94618315	210.41963640	4.00	0.0001	0.142747	51.
ERROR	913	48018.77230425	52.59449321		ROOT MSE		Y
CORRECTED TOTAL	951	56014.71848739			7.25220609		14.1869

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE III SS	F VALUE	PR
X	24	7195.15718450	5.70	0.0001	24	7089.40318751	5.62	0.
Z	1	27.47190052	0.52	0.4700	1	1.30398626	0.02	0.
X*Z	13	773.31709813	1.13	0.3282	13	773.31709813	1.13	0.

this term is solely a measure of sum-of-squared pure error

Partitioning SS_E into SS_{PE} and SS_{lof}:

$SS_E = 51794.00 \quad df = 949$

$SS_{PE} = 48018.77 \quad df = 913$

$SS_{lof} = 3775.23 \quad df = 36$

$MS_{lof} = 104.8675$

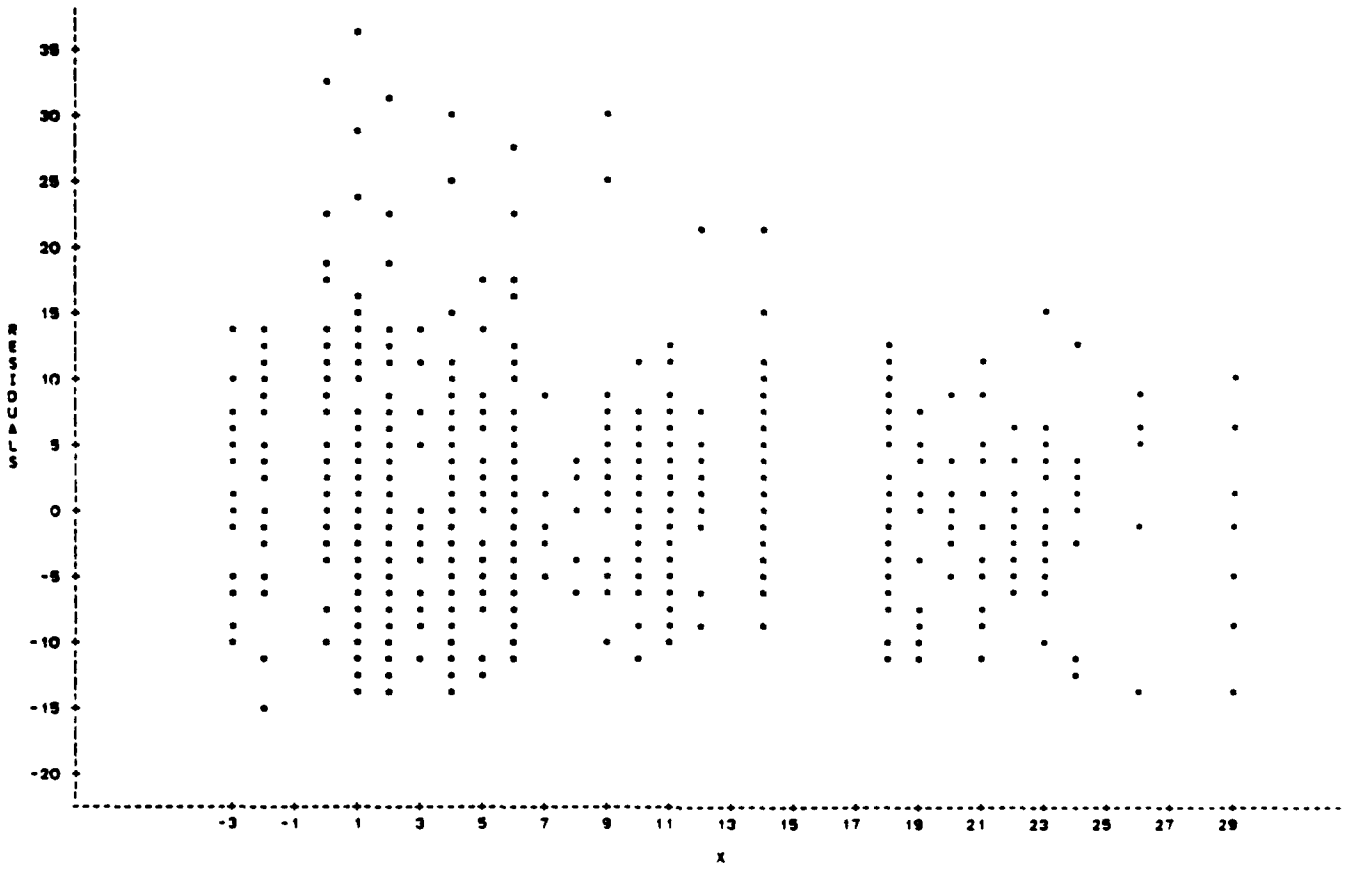
$MS_{PE} = 52.5945$

$\therefore F_0 = \frac{MS_{lof}}{MS_{PE}} = 1.9939$

$F_{0.05,36,913} \approx 1.42$

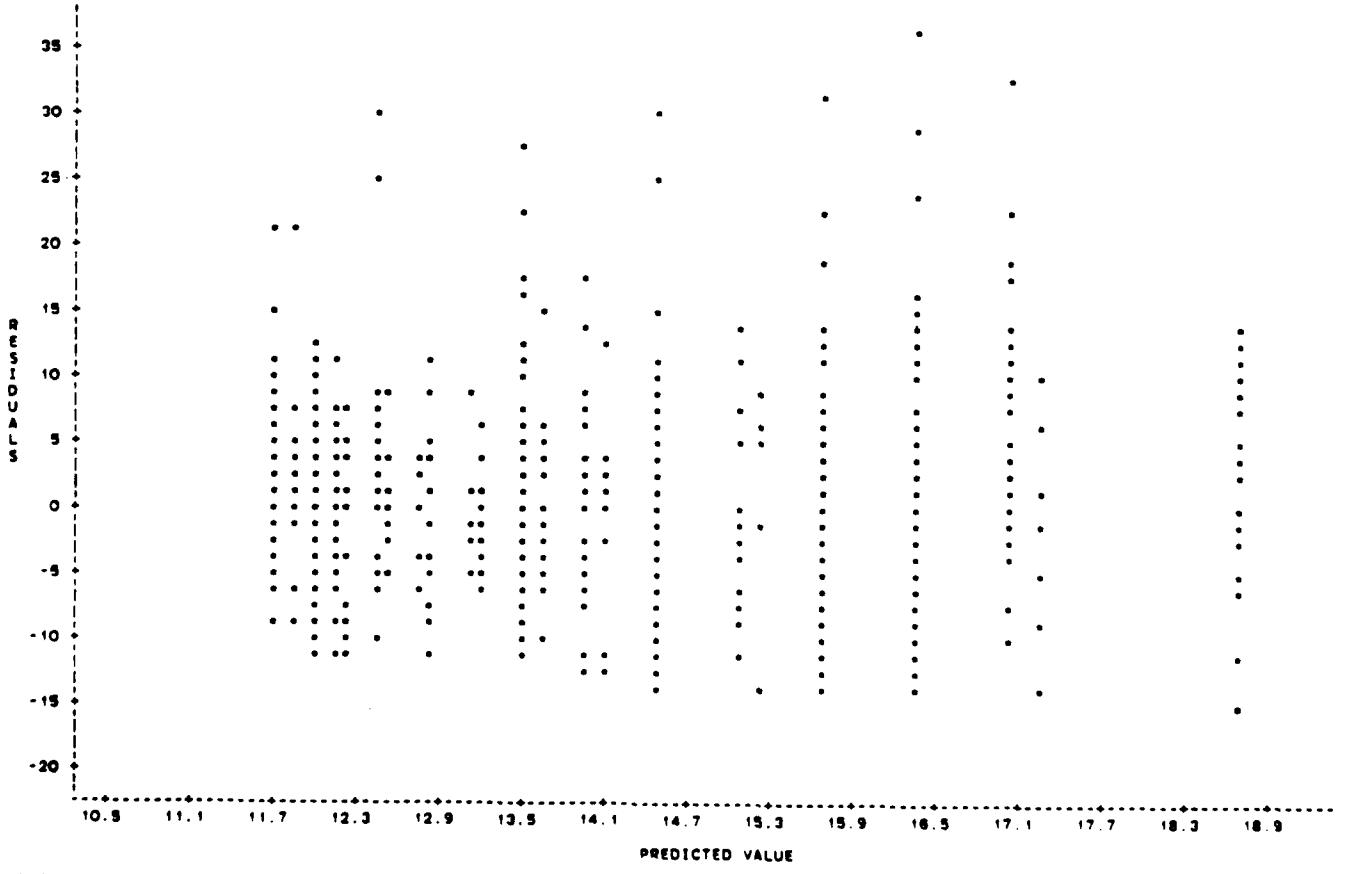
APPENDIX F

RESIDUAL PLOTS



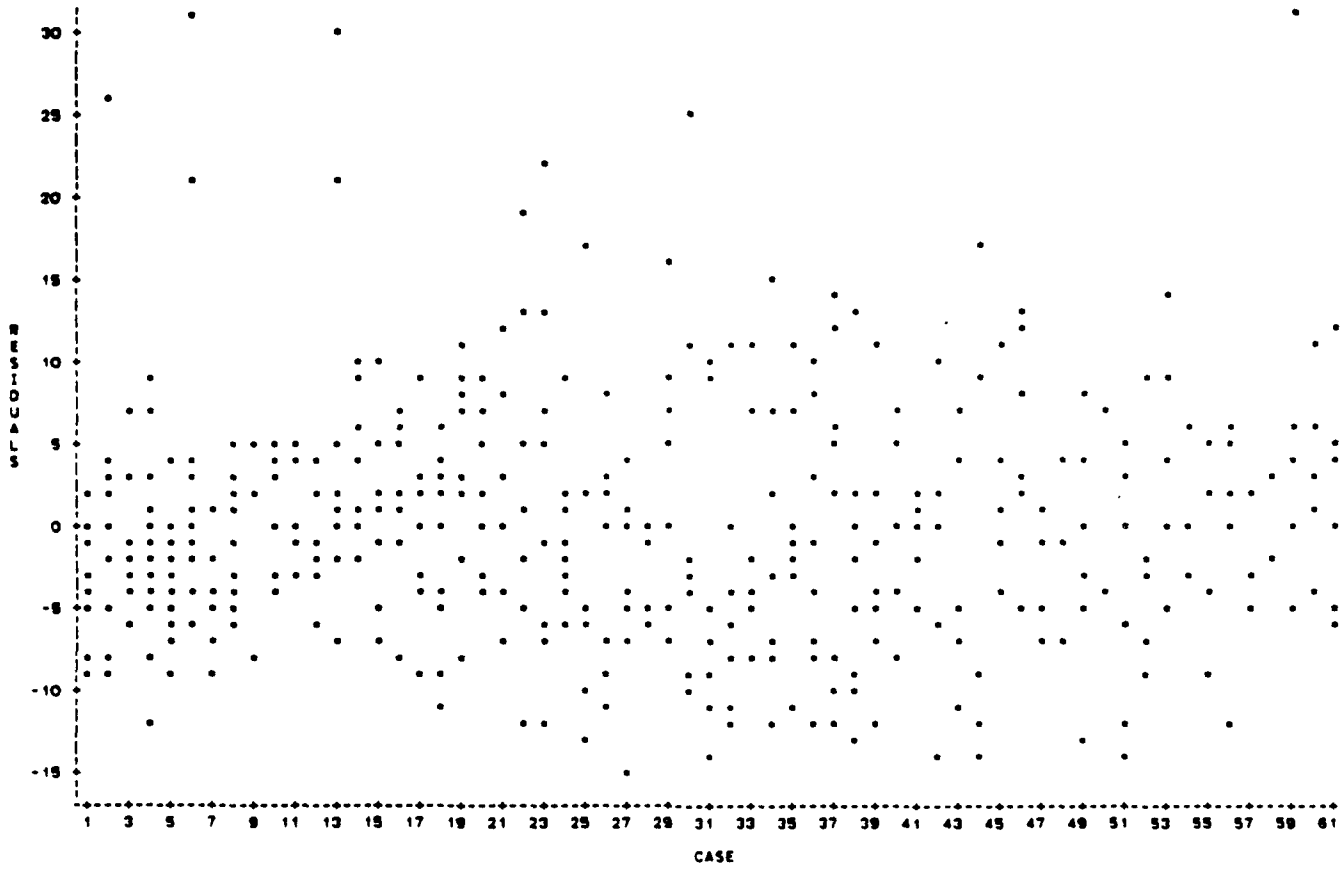
NOTE: 3272 OBS HAD MISSING VALUES 604 OBS HIDDEN

Residuals versus time.



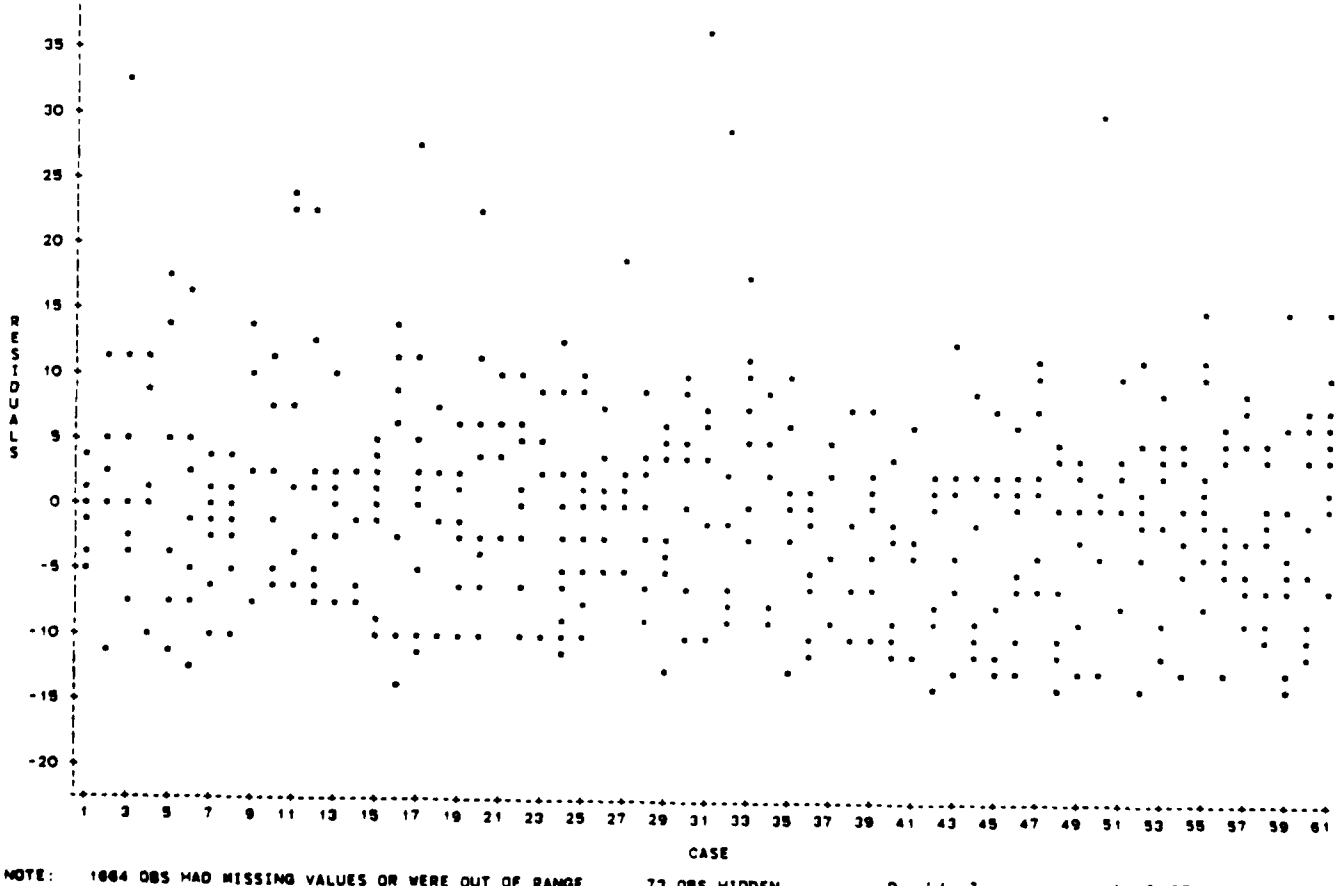
NOTE: 3272 OBS HAD MISSING VALUES 620 OBS HIDDEN

Residuals versus predicted value of plasma prolactin concentration.



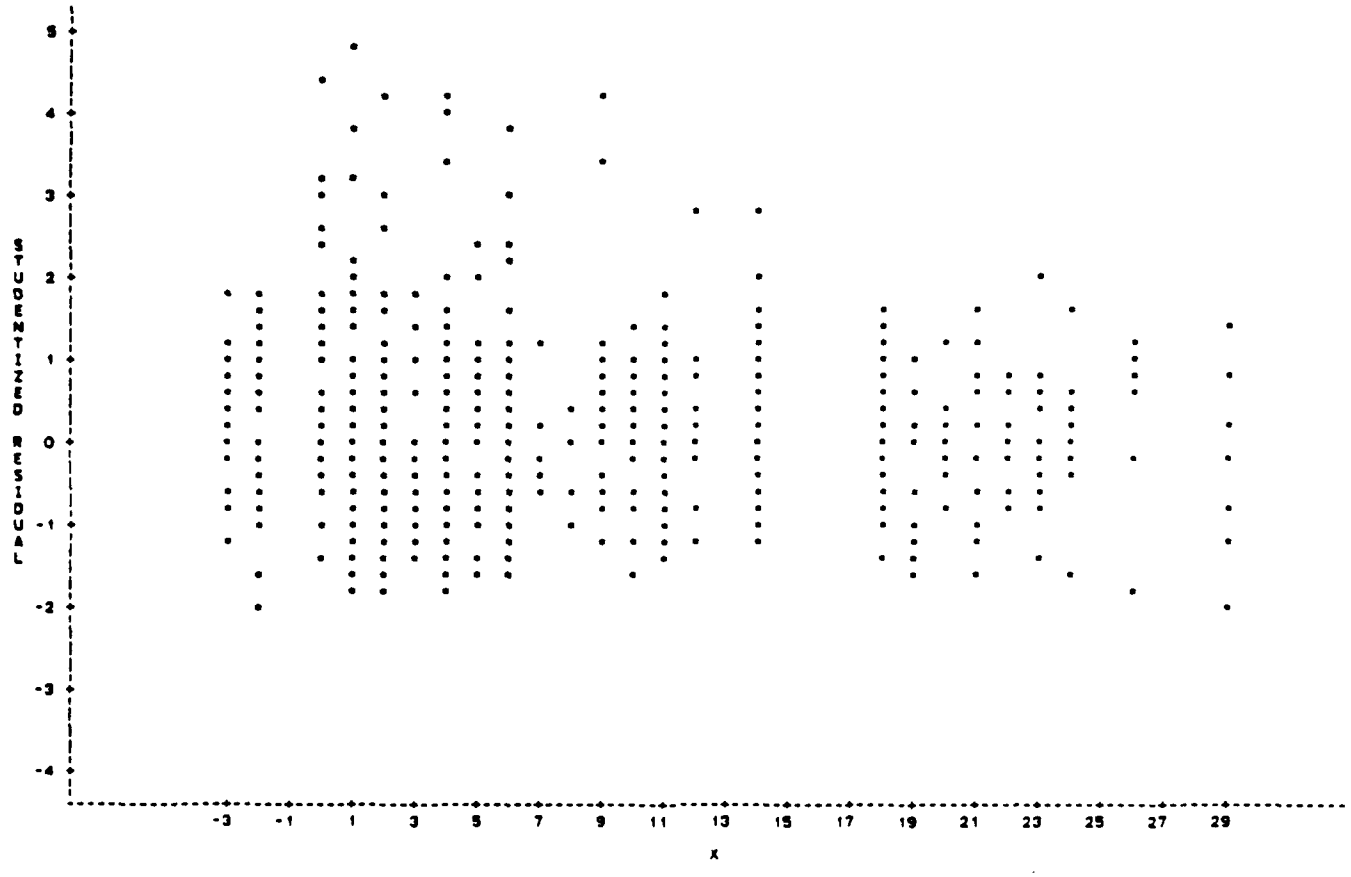
NOTE: 1616 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 74 OBS HIDDEN

Residuals versus animal ID number (sham-exposure group).



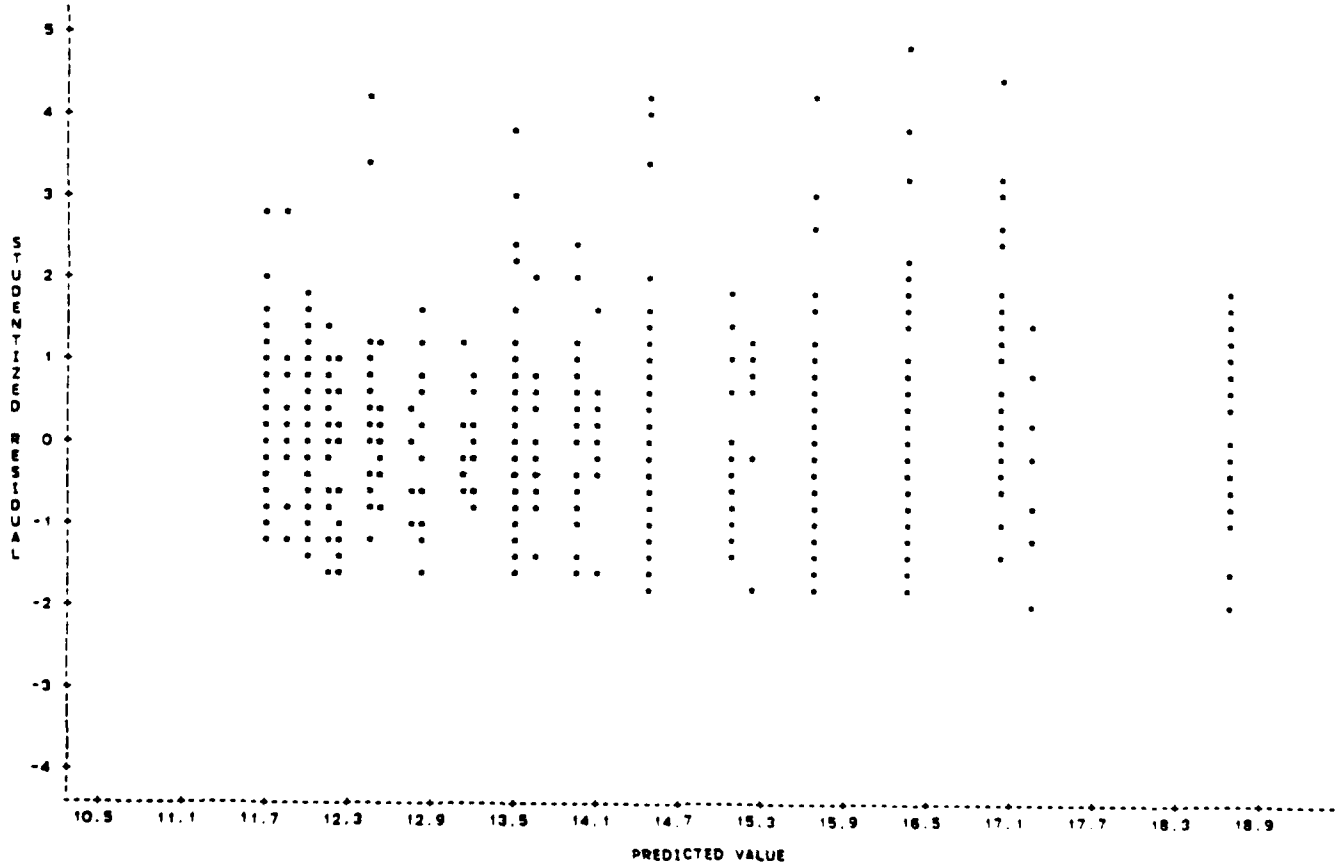
NOTE: 1664 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 73 OBS HIDDEN

Residuals versus animal ID number (exposure group).



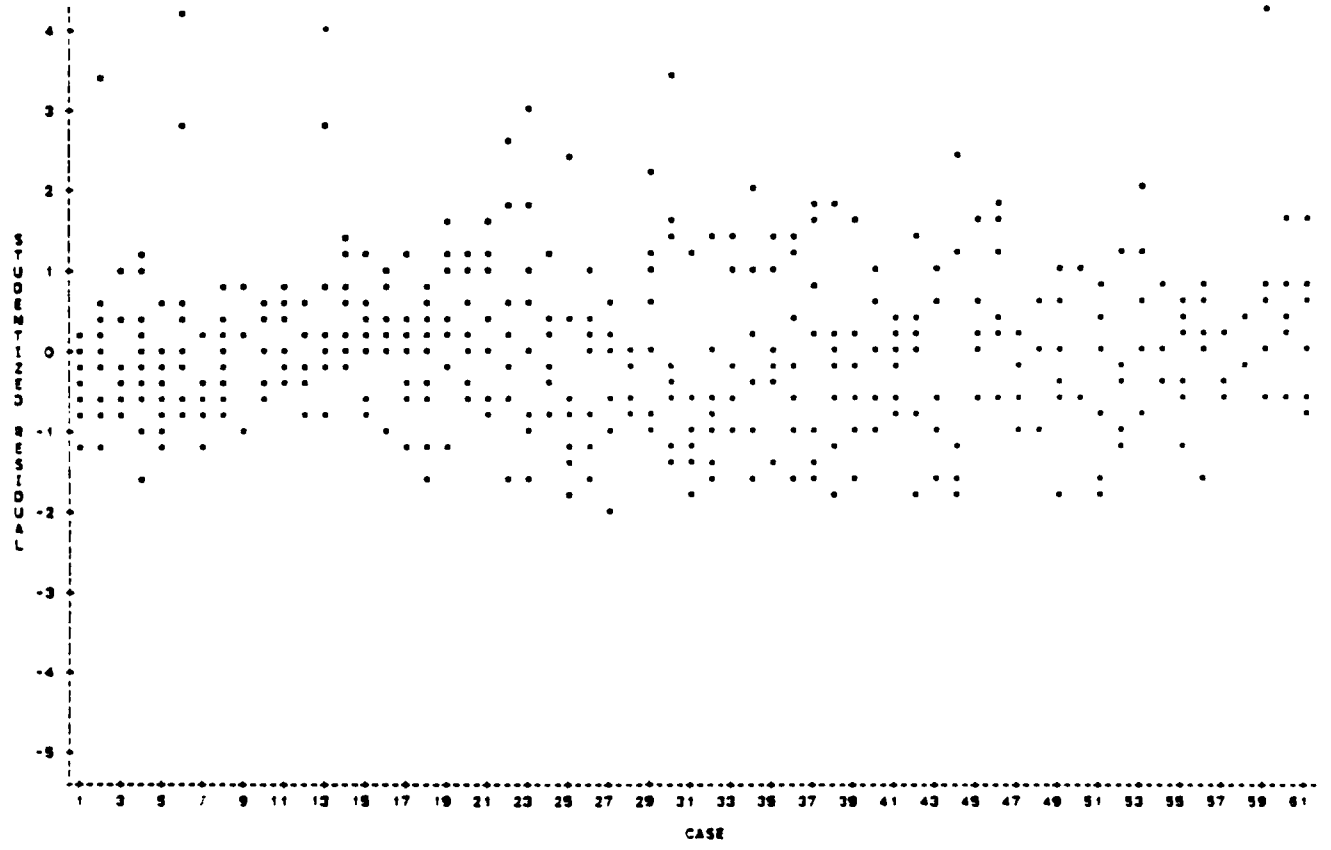
NOTE: 3272 OBS HAD MISSING VALUES 637 OBS HIDDEN

Studentized residuals versus time.



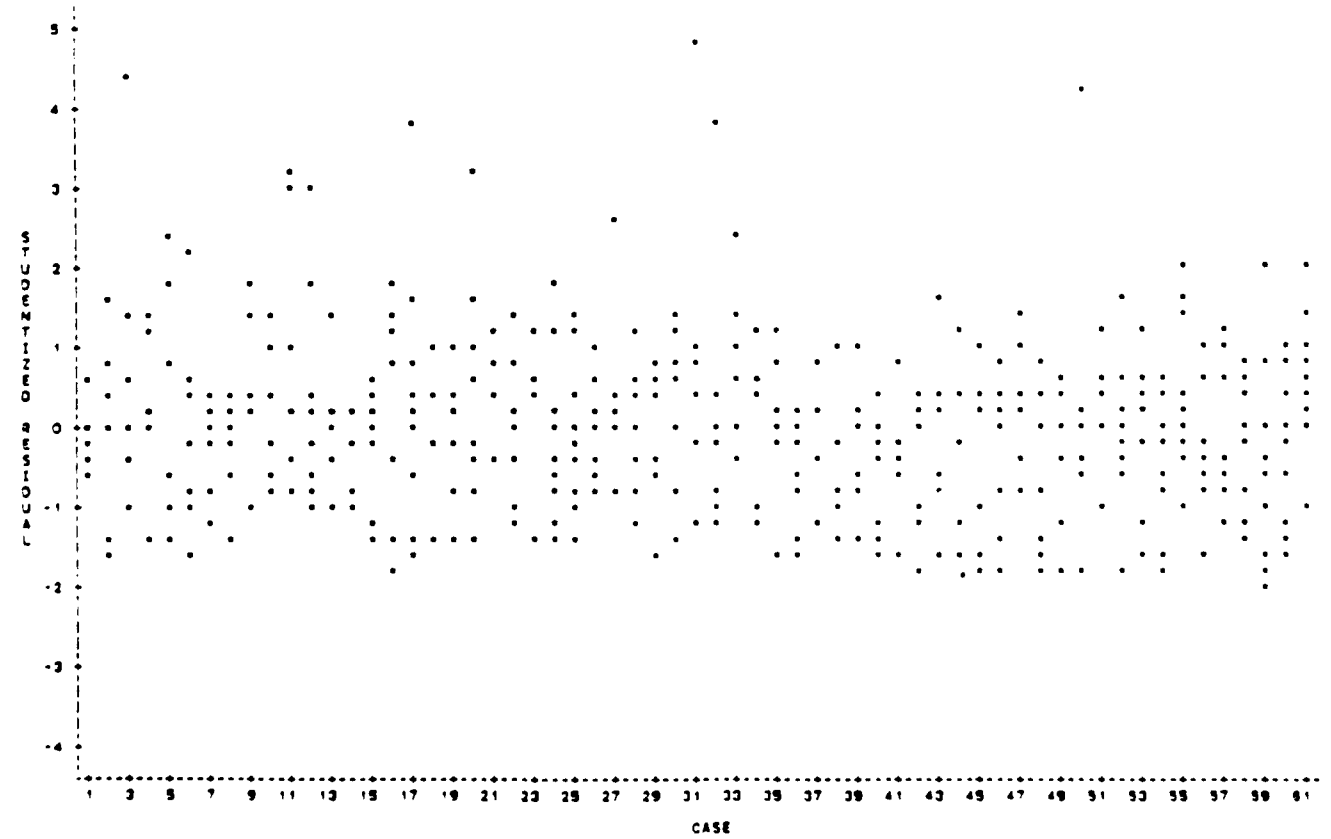
NOTE: 3272 OBS HAD MISSING VALUES 651 OBS HIDDEN

Studentized residuals versus predicted value of plasma prolactin concentration.



NOTE: 1616 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 92 OBS HIDDEN

Studentized residuals versus animal ID number (sham-exposure group).



NOTE: 1664 OBS HAD MISSING VALUES OR WERE OUT OF RANGE 78 OBS HIDDEN

Studentized residuals versus animal ID number (exposure group).

END

9-87

DTIC