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ABSTRACT

There is no widely accepted protocol for the assessment of the toxic effects of irritant gases on humans. Most procedures employed for this purpose utilize endpoints that assess time-to-incapacitation and lethality. The Army Medical Research and Development Command has recognized the need to evaluate potential toxic hazards resulting from human exposures to irritant gases (Hydrogen chloride, ammonia, and formaldehyde) associated with weapons systems exhaust emissions.

We have, within the scope of the Phase I Program exposed Long Evans rats to three levels of hydrogen chloride gas in flowing air. Hydrogen chloride, a respiratory irritant present in the exhausts of solid propellant weapon systems using ammonium perchlorate as the oxidizer, was selected for use in evaluating the feasibility of protocols employing quantitative neurophysiologic monitoring procedures as a means of assessment of the potential toxicological hazard to humans. This specific respiratory irritant was also selected because the of extensive prior experience by the project staff in the toxicological characteristics of hydrogen chloride. We have demonstrated, within the scope of the Phase I Program, our ability to detect early changes in nervous system activity using such quantitative neurophysiological monitoring techniques as (electroencephalographic monitoring, [EEG]; brainstem evoked response, [BAER]; somatosensory evoked potentials, [SEP]; and visual evoked potentials, [VEP]) in rodents exposed to preselected levels of hydrogen chloride gas in flowing air. These changes were observed prior to seeing significant changes in animal homeostasis, as determined by monitoring important blood parameters, in time periods much earlier than would be required to observe significant behavioral changes.

The utilization of the unique animal exposure chamber which has been interfaced to the Quantified Signal Imaging, Inc. Model 9000 Quantitative Neurophysiological Monitoring System (QSI-9000 System) has permitted us to monitor the development of statistically significant changes in neurophysiologic activity as early as during the first epoch following the start of the exposure episode. Having demonstrated our ability to identify early decrements of performance in nervous system activity, we will, within the scope of the Proposed Phase II Program, if funded, utilize the quantitative neurophysiological techniques to identify the foci of abnormal brain activity resulting from the exposure to combinations of toxicants used to model weapon systems' exhausts. We will also follow the development of late occurring sequelae and study pathologic lesions grossly and where indicated on the ultrastructural level.

We will, after confirmation of our results in higher mammalian species, develop a cost-effective protocol for the first-tier screening assessment of complex mixtures of toxicants present in weapon systems' exhausts. We will employ a variety of techniques, including but not limited to thermodynamic equilibrium calculations, to predict the presence and most probable concentrations of the toxicants likely to be encountered by soldiers during training exercises, simulated battle conditions, as well as under combat conditions. By determining the foci resulting from chronic exposure to volatile toxicants we should be able to determine the mechanisms involved in early intoxication and, hopefully, be able to develop protocols of the assessment of such hazards to permit detection prior to reaching a point of irreversible physiological damage. Knowledge resulting from such studies will provide the baseline information for the development of early warning detector systems capable of providing an early alarm of pending toxicological hazard to military personnel

ANTICIPATED BENEFITS/POTENTIAL COMMERCIAL APPLICATIONS

The assessment of human response to exposure to complex mixtures of environmental pollutants is extremely complex. New screening procedures are required to provide a greater degree of confidence in public safety pertaining to the development and commercialization of of materials, products, and systems early in their development. The studies conducted within this **Phase I Program** has demonstrated our ability to detect and quantify the early changes in neurophysiological activity within very short periods of time during actual laboratory animal exposure episodes. The developments anticipated during the **Phase II Program** are expected to meet the goals and objectives of the U.S. Army Medical Research and Development Command. We have developed a unique animal exposure chamber which has permitted direct interfacing with a state-of-the-art quantitative neurophysiological system. We will during the **Phase II Program** develop the computer programs and hardware to permit complete monitoring and control of the exposure environment (complex mixtures of gases, aerosols, and particulates). We are seeking support to undertake a research program which will permit the use of a quantitative neurobehavioral assessment of animal performance during the development of the intoxication syndrome in the animals subjects. The results obtained during this **Phase One Program** has provided us with the capability to provide a means of screening pesticides, growth control agents, fire retardants, plasticizers, and new chemical compounds before such materials are introduced into our environment. We have proposed a novel concept for the first-tier assessment of potential toxic agents employing protocols utilizing quantitative neurophysiological protocols and ancillary employing brain electrical activity mapping procedures (to be studies during the **Future Phase II Program** to provide information pertaining to the foci for early decrement of human performance as a consequence of exposure to toxic agents. We have established the feasibility of utilizing BAER and EEG protocols to identify the loss of physiological functions which indicate early intoxication during exposures to hydrogen chloride. This capability together with our previous experience in studying the interactions of toxic thermal decomposition products on animal and human performance should provide considerable knowledge required by the Department of Transportation to increase the safety of materials used in the construction and furnishing of aircraft, trains, automobiles, buses, subway cars, and recreational vehicles. The Veterans Administration has maintained an active interest in the fire safety of materials used in its hospitals and health-care facilities. The Environmental Protection Agency and the Occupational Health and Safety Administration have major interests in the role of environmental pollutants in affecting the health of the nation's citizens.

Interest has been expressed by several industrial organizations in the development of a cooperative research effort using the results anticipated during the **Future Phase II Program** to form the basis of a joint-venture organization to commercialize the hardware, software, and "know how" developed in future programs.

KEY WORDS

Toxicology, Decrement, Neurophysiological, Performance, Incapacitation, Lethality, Screening, Protocol, First-tier

UTILIZATION OF NEUROPHYSIOLOGICAL PROTOCOLS TO CHARACTERIZE SOLDIER RESPONSE TO IRRITANT GASES

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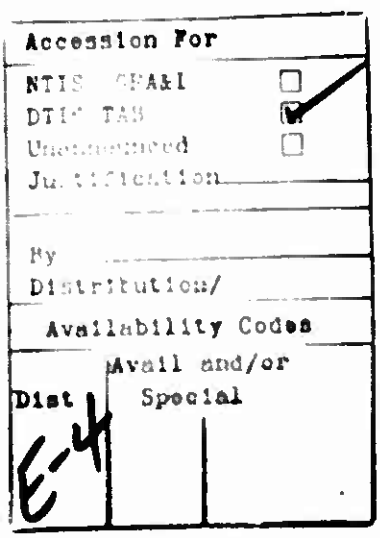

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UTILIZATION OF NEUROPHYSIOLOGICAL PROTOCOLS TO CHARACTERIZE SOLDIER RESPONSE TO IRRITANT GASES

1. INTRODUCTION

This **Final Report** is submitted to the Department of the Army, U.S. Army Medical Research and Development Command, U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, Frederick, Maryland, in compliance with requirements for Contract DAMD17-89-C-9136, 1989 SBIR Award entitled **Utilization of Neurophysiological Protocols to Characterize Soldier Response to Irritant Gases.**

1.1 Significance of the Problem

The Army Medical Research and Development Command has recognized the need to evaluate potential toxic hazards resulting from human exposures to irritant gases (Hydrogen chloride [HCl]; ammonia [NH_3]; and formaldehyde [CH_2CO]) associated with weapons systems' exhaust emissions. Of principal importance is the need to develop a protocol capable of providing a quantitative assessment of the decrement of performance which may occur as the result of chronic intermittent exposures to one or more of the noxious gases released into the environment as weapons systems' exhausts during routine training exercises as well as under simulated or actual combat conditions.

It is well known in the toxicological research community that behavioral deficits which result as a consequence of exposure to irritant gases are often not observed until late in the exposure episode by which time permanent central or peripheral nervous system impairment may have occurred. The apparent insensitivity of behavioral indices due to toxic effects of combustion products on natural and synthetic materials which contain many of the irritants found in weapon systems exhausts has been shown by numerous researchers. Einhorn et al[1,2,3], Kimmerle et al[4,5], Pryor et al[6], Alarie et al[7,8], and Smith et al[9] have employed various protocols such as the hindlimb conditioned avoidance response, the rotorod, the pole jump, the bar press, and the ability to perform in a maze as indicators of a developing intoxication syndrome. The use of many of these protocols has led to the ability to predict the **"dose"** required to obtain a **Lethal Dose - 50 (LD_{50})** and in some situations the prediction of **Incapacitation Dose - 50 (EC_{50})**. Almost all of these procedures which have been shown to provide the capability to indicate the incapacitation EC_{50} endpoint have been able to indicate such endpoint only during the development of the anoxic or hypoxic state of intoxication. The presence of respiratory irritants such as hydrogen chloride, aldehydes, ammonia, or organic acids complicates the intoxication syndrome and usually results in the development of severe pulmonary edema and insult to the respiratory system. Laboratory animals exposed to concentrations of irritants usually well below that required to produce an LD_{50} response are incapacitated very early during the exposure episode. Many of the animal subjects exposed to such irritants have been observed to die within several days following exposure.

At the present time there is no widely accepted methodology or protocol for the assessment of human toxicity induced by exposure to irritant gases. Most procedures used by the toxicological research community usually employ endpoints that assess **time-to-incapacitation** and **lethality**. These procedures, including those employed by the fire research community, such as the National Bureau of Standards (NBS) protocol, are based on sample decomposition techniques which often do not resemble conditions to which military personnel will be exposed. The results which are obtained using such

protocols are difficult to interpret and often there is insufficient data obtained using such procedures to permit an assessment of the potential toxic hazard to humans.

Incapacitation, as defined in our earlier studies, is the state where the animal (or human) cannot carry out normal functions. This endpoint is much more severe and usually occurs much later in an exposure episode than the military is interested in with regards to personnel being able to carry out assigned functions without a sufficient decrement of performance which would result in the loss of the ability to conduct assigned tasks in the manner expected. The experiments which were conducted within the scope of the **Phase I Program** were be designed to identify the first observable decrement in animals as a consequence of their exposure to irritant gases.

2. PHASE I TECHNICAL GOALS AND OBJECTIVES

2.1. Specific Goals and Objectives - Phase I Program

2.1.1 Feasibility of Development of a Combined Quantitative Neurophysiologic, Bioassay, and Behavioral Protocol for the Assessment of the Decrement of Performance Produced by Respiratory Irritants

2.1.1.1 Selection of a Quantitative Neurophysiologic Monitoring System

2.1.1.2 Development of an Interfaced Animal Exposure Chamber/Quantitative Neurophysiologic Monitoring System

2.1.1.3 Characterization and Control of the Animal Exposure Chamber Environment

2.1.1.4 Development of a Protocol for Hydrogen Chloride Exposure Studies

2.1.1.5 Determination of the Intoxication Syndrome in Long Evans Rats Exposed to Concentrations of Hydrogen Chloride in Air Ranging from 75 ppm to 300 ppm

3 EXECUTIVE SUMMARY OF RESULTS OBTAINED IN THE PHASE I PROGRAM

3.1 Feasibility of Development of a Combined Quantitative Neurophysiologic, Bioassay, and Behavioral Protocol for the Assessment of the Decrement of Performance Produced by Respiratory Irritants

The principal goal within the scope of the **Phase I Program** was to determine the feasibility of development of a combined neurophysiological, bioassay, and behavioral protocol for the assessment of a decrement of performance produced by respiratory irritants present in the exhaust emissions from guns and rockets.

We elected, after discussions with the Contracting Officer's Representative, to confine the animal exposure experiments to be conducted during the **Phase I Program** to HCl because of its presence in the exhausts of weapon systems utilizing ammonium perchlorate as an oxidizer as well as our extensive previous experience with this toxicant. Because of time constraints and financial limitations during the **Phase I Program** we limited our animal subjects to Long Evans rats. There are three major drawbacks

in using the rodent as a model for the study inhalation toxicology: (1) the Long Evans rat is a "nose breather", and thus, its respiratory physiology does not closely resemble that of humans, (2) the rat has a very small volume of blood, thus, care must be exercised not to withdraw quantities of blood which would affect the physiological response to the toxicant studied, and (3) there are severe limitations in being able to utilize brain electrical activity mapping procedures. It is necessary to employ approximately 20 electrodes to conduct full-scale brain mapping procedures, using a rat model we were limited to the use of 4 to 6 electrodes.

We determined, within the scope of the **Phase I Program** that the brainstem auditory evoked response (BAER), and electroencephalographic activity (EEG) were the most appropriate quantitative neurophysiological monitoring techniques of those evaluated for use in assessing the early decrements assessing the early decrements of performance developed as a consequence to exposure to respiratory irritants.

A bioassay protocol was utilized to determine whether the exposed animal subjects exhibited a significant deviation from homeostasis as a consequence of exposure to HCl concentrations ranging from 75 ppm to 300 ppm in flowing air. As will be presented later in this report, measurements of blood pH, pO_2, and pCO_2, obtained in both control and exposed animals prior to the exposure episode, from 5-8 minutes into the exposure episode, and just following the venting of the animal exposure chamber showed no statistically significant differences. The animal's weight gain (or weight loss) were checked on a daily basis commencing 7 days prior to the exposure episode and continuing for a minimum of 14 days postexposure showed no statistically significant differences between the control and exposed groups. Random gross physiological examination of the control and exposed animal groups, likewise, showed no observable differences in the animal's exploratory behavior, nuzzle response, lid-corneal and ear reflexes, pain response, the righting reflex, or posture. General observations conducted randomly during the **Phase I Program** showed no significant differences in the lack of spontaneous activity such as preening or movement. There was no evidence of respiratory distress observed in control or exposed animals at periods greater than 24 hours postexposure. Because of severe time constraints and consistent with the lack of significant differences in homeostasis or the gross physiological examinations of the control and exposed animal groups, we eliminated the use of the modified "T" maze behavioral tests within the scope of the **Phase I Program**.

3.2 Selection of a Quantitative Neurophysiologic Monitoring System

A detailed study was made by senior project staff members of the major quantitative neurophysiologic monitoring systems which are commercially available. Visits were made to the Nicolet Instruments Company in Madison, Wisconsin, the University of Wisconsin Medical School, and to the Quantified Signal Imaging, Inc. in Toronto, Canada. In addition to these visits, telephone conversations were made to selected references provided by the manufacturer's of the neurophysiologic system to discuss the ease of use, the developmental status, and interactions between the user and manufacturer of the equipment following purchase. A visit was also made to Dr. Professor Frank, Duffy, M.D., Harvard Medical School and The Children's Hospital Medical Center, Boston, Massachusetts, a world recognized leader in topographic mapping of brain electrical activity. Following these meetings and discussions, a decision was made to purchase the QSI-9000 Quantitative Neurophysiologic Monitoring System. Special adaptations were specified to permit our initial rodent studies.

3.3 Development of an Interfaced Animal Exposure Chamber/Quantitative Neurophysiologic Monitoring System

To accomplish the **Phase I Program Goals** it was necessary to design, construct, and test a state-of-the-art animal exposure chamber and to interface this exposure chamber with a quantitative neurophysiologic monitoring system which was selected early in the project. The animal exposure chamber, which will be described fully later in this report, has been used successfully throughout the **Phase I Program** in a series of actual animal exposures after being fully interfaced with the QSI-9000 Quantitative Neurophysiological Monitoring System. Additional modifications were incorporated into the animal exposure chamber to provide a fixed assembly for the audio speakers used to provide the stimulus for the brainstem auditory evoked response. Similar modifications were made to provide a constant positioning of the strobe lights used to provide the stimulus for the animal's visual evoked response.

We have demonstrated during the **Phase I Program** that this newly designed chamber permits us to determine, usually within the first monitoring epoch, when the animal subjects reach the first observable decrement of neurophysiologic activity.

Because of the uniqueness in the design of the animal exposure chamber and its utility when interfaced with a quantitative neurophysiologic monitoring system, the Northeast Research Institute, Inc. will apply for U.S. and foreign patents covering the animals exposure chamber.

3.4 Characterization and Control of the Animal Exposure Chamber Environment

The redesign of the animal exposure chamber greatly facilitated our ability to regulate the concentration of toxicant which was introduced into the chamber during the exposure episode. The use of a two-section partitioned animal exposure chamber has permitted us to obtain baseline neurophysiologic monitoring while the animals were exposed in the "nose only" mode in a flowing air environment. During the period of time that the animal subjects were being exposed to the flowing air environment, the environment within the sealed partitioned section of the exposure chamber was brought to the preselected level of HCl in air.

A series of experiments were conducted in which certified gas cylinders $\pm$ 1.0 % of the stated value of HCl in air were diluted to provide a chamber environment consistent with preselected concentrations of HCl in air using the Environics 100 Computerized Multi-Gas Calibrator.

The approximate concentrations of gases metered into the exposure chamber during the **Phase I Program** were verified using a Miran 1A Gas Analyzer (Foxboro Wilkes Corporation). This single beam, variable filter spectrometer is capable of scanning the infrared spectral range from 2.5 microns to 14.5 microns in conjunction with a 5 liter capacity cell whose pathlength is variable from 5.0 to 20.25 meters. The Miran Spectrometer is equipped with a pump for drawing air samples from the animal exposure chamber at a rate of 2.0 liters/minute.

The concentrations of gases within the flow-through chamber were balanced to maintain the constant preselected level. The Miran Spectrometer was calibrated throughout the **Phase I Program** using certified gas cylinders $\pm$ 1.0 % of the stated value that will be diluted to selected concentrations by the Environics Series 100 Computerized Multi-Gas Calibrator. A standard curve was chart recorded over each of the expected working ranges of the chamber's environment. The atmosphere within the animal

exposure chamber was continuously monitored from the animal exposure chamber during all exposure episodes and compared to the standard curves for accuracy.

Since a single gas was used within the scope of the **Phase I Program** and because the redesigned two-sectioned animal exposure chamber provided very rapid mixing of the environments within the two sections of the animal exposure chamber, when the dividing door was opened, we were able to control precisely the preselected levels of HCl in flowing air. Master curves were developed using the Miran Infrared Spectrometer, with appropriate GC confirmation. Although not required during the **Phase I Program** HCl exposure studies, NERI's analytical laboratories will be used during the **Phase II Program** when more complicated mixtures of weapons systems' exhausts will be modeled and used in animal exposure studies.

3.5 Development of a Protocol for Hydrogen Chloride Exposure Studies

A computerized review of the literature (Defense Technical Information Center, Chemical Abstracts, Medline, Toxline, NTIS, Biosis, Embase, NIOSH, Federal Research In Progress, Enviroline, and Hazardline) followed by discussions with key personnel of the U.S. Army Biomedical Research and Development Laboratory has indicated that sufficient information is not available to provide the quantitative chemical characterization of typical weapon systems exhaust mixtures and the duration of time that the concentrations of toxicants present in such mixtures remain in the soldier's environment. We will include an Optional Task within the scope of the **Proposed Phase II Program** to characterize such weapons systems' exhaust mixtures. The NERI laboratory has facilities and extensive experience in the characterization of very complex and dynamically changing mixtures of combustion products of natural and synthetic materials which should enable us to identify and quantify most environments to which soldiers may be exposed during the firing of guns and rockets.

Within the scope of the **Phase I Program** we exposed instrumented Long Evans rats to flowing air, using the same flow rates as the animals would be subjected to during the actual exposure episodes. Because of the rapid changes observed using the unique animal exposure chamber designed for the present study we were able to quantify changes from baseline in the animal's neurophysiological activity often within the first epoch following exposure to varying concentrations of HCl in flowing air. The duration of the exposure episode was reduced from the proposed 30-minute exposure to an exposure duration of 15 minutes at the request of the Army Medical Research and Development Laboratory personnel. Specific details of the protocols used within the **Phase I Program** will be presented later in this report

As based on our extensive previous studies of the toxicology of combustion products, we believe that it is very feasible to conduct a limited series of field analytical studies employing the appropriate analytical methodologies necessary to more precisely characterize the complex mixture of low-boiling volatiles, aerosols, and particulates present in weapons systems' exhausts. Once a realistic assessment of the concentration and time of duration has been developed for the major toxic species present in weapons systems' exhausts, the use of thermodynamic equilibrium calculations will provide the information necessary for developing a toxicological model of the soldier's response to such exhausts. This model will permit the development of envelopes of response based upon the additive, antagonistic, or synergistic interactions of toxicant species in dynamically changing exhaust plumes. This model will utilize the types of quantitative neurophysiologic monitoring developed within the **Phase I Program** together with the improved capabilities which we hope to develop in the **Proposed Phase II Program.**

3.6 Determination of the Intoxication Syndrome in Long Evans Rats Exposed to Concentrations of Hydrogen Chloride in Air Ranging from 75 ppm to 300 ppm

We have, during the Phase I Program, observed using quantitative neurologic monitoring protocols that there are statistically significant changes in the latencies of the BAER and EEG activities. These changes are such that they would indicate levels of changes of sufficient magnitude as to indicate the late development of pathologic lesions. The bioassay protocol (measurement of key blood parameters) did not indicate a significant deviation from normative data. These observations were significantly less than we have observed in previous studies when Long Evans rats were exposed to concentrations of HCl ranging from 1200 ppm to 3000 ppm in flowing air, in HCl concentrations ranging from 1200 ppm to 3000 ppm in the presence of 5 volume percent CO_2, or in exposure studies involving HCl concentrations ranging from 1200 ppm to 3000 ppm HCl in the presence of 5 volume percent CO_2 and CO concentrations ranging from 1000 ppm to 3500 ppm.(10)

Note: The results obtained during this Phase I Program should be considered as trends as the lack of sufficient time and finances did not permit the number of replications necessary to totally verify all of the results obtained.

4. METHODS

4.1 Task 1. - Animal Exposure Chamber

The animal exposure chamber, illustrated in Figures 1 and 2, has been designed and assembled. Four animal ports are located on the side of the animal exposure chamber. The Long Evans rats were placed into the exposure chamber using the "nose-only exposure mode." Those animals used to measure neurophysiological activity were fully instrumented when placed into the exposure chamber. The leads attached to the scalp of the instrumented animals were then connected to the QSI-9000 Quantitative Neurophysiologic Monitoring System. The spring activated door, illustrated in Figure 1 (bottom), is held in the closed position to insure that the animals are exposed only to flowing air during the period of time when baseline neurophysiological activity is being monitored. When sufficient baseline data has been obtained, the spring activated door is released permitting a rapid mixing of the preselected mixture of hydrogen chloride gas in air to flow throughout the entire animal exposure chamber. This newly designed chamber has permitted us to determine within a very short period of time when the animal subjects reached the first observable decrement of neurologic activity.

It should be noted that this prototype chamber permits the simultaneous exposure of four animal subjects, rather than the five animals per exposure as listed in our proposal. This modification was adopted after discussions with personnel of the Nicolet Biomedical Division as well as the neurophysiologists representing QSI Corporation. The reduction in the number of animals used per exposure episode was governed by the number of channels available for monitoring the preselected neurophysiological functions during a single experiment.

Under practical laboratory conditions, we have limited our animal exposures to one instrumented animal per exposure episode. Up to three additional animals were used during the exposure episode to provide additional information utilizing the blood monitoring bioassay protocol.

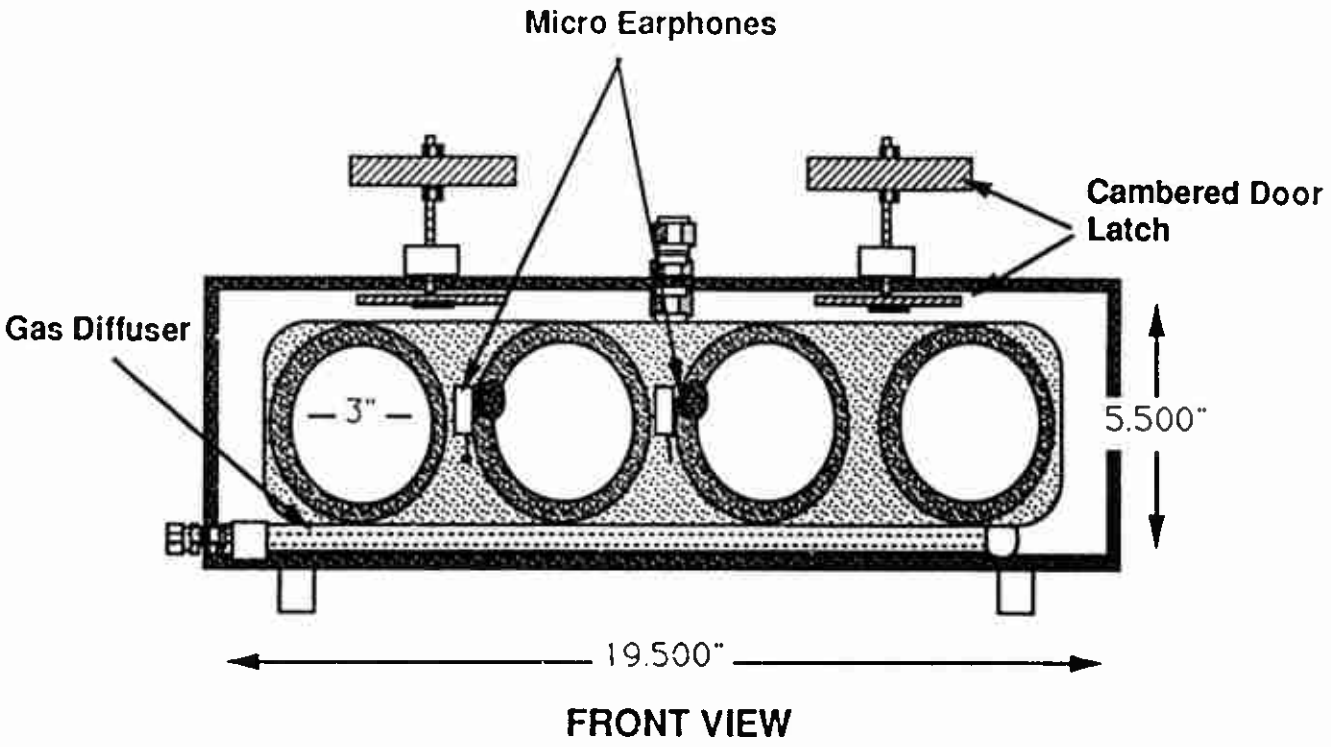


Figure 1. Head-Only Animal Exposure Chamber Showing Front and Side Views

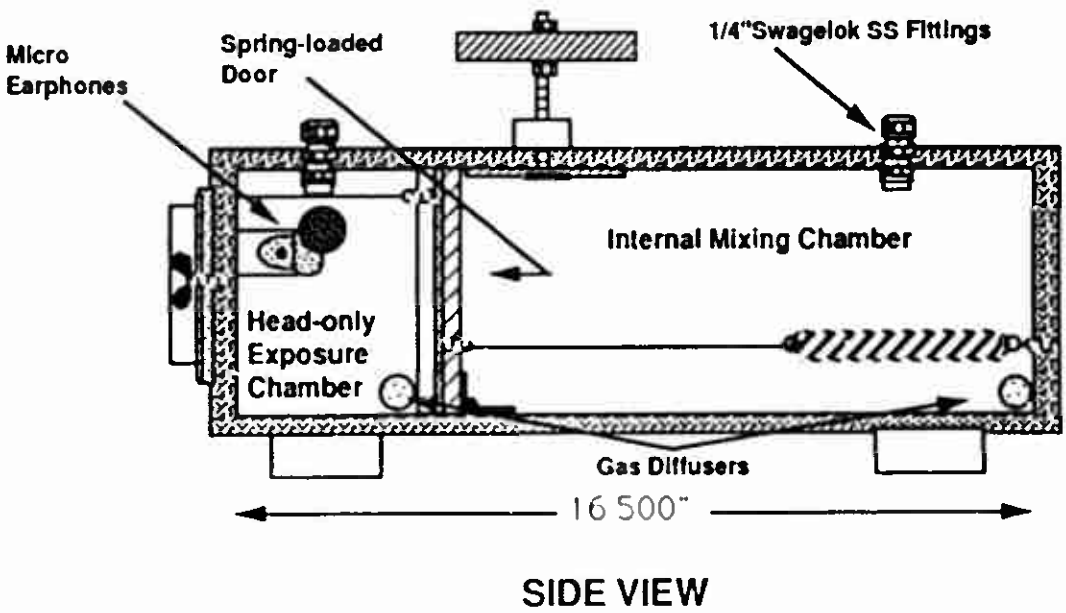


Figure 2. Head-Only Animal Exposure Chamber Showing View From Top

4.2 Task 2 - Characterization and Control of the Animal Exposure Chamber's Environment

The principal objective of Task 2 was to develop and characterize a computerized system capable of delivering and maintaining a preselected set of concentrations of the respiratory irritant HCl selected for study. The redesign of the animal exposure chamber greatly facilitated our ability to regulate the concentration of toxicant which was introduced into the chamber during the exposure episode. The use of a two-section partitioned animal exposure chamber has permitted us to conduct baseline neurophysiologic monitoring while the animals were exposed in the "nose only" mode in a flowing air environment. During the period of time that the animal subjects were being exposed to the flowing air environment, the environment within the sealed partitioned section of the exposure chamber was brought to the preselected level of HCl in air.

A series of experiments were conducted in which certified gas cylinders $\pm$ 1.0 % of the stated value of HCl in air were diluted to provide a chamber environment consistent with preselected concentrations of HCl in air using the Environics 100 Computerized Multi-Gas Calibrator. The Environics Series 100 System used in our most recent animal exposure studies consists of a single chassis supporting mass flow meters, a mixing chamber, a reaction chamber, and an electronics module. All wetted surfaces are stainless steel or Teflon and all seals are made of Viton.

The mass flow meters are controlled by a microcomputer with 32 bit computational precision. The systems software allows the user to conduct precision multipoint calibration checks of the sample environment. Each mass flow meter is factory calibrated with 20 points of data stored in the systems microcomputer memory. These baseline data points are averaged by a computer generated least squares line. This statistical technique improves by a factor of 10 the accuracy of the system's mass flow meters and, thus, precisely delivers the concentration and flow of gas required for the preselected exposure episode. Table 1 contains a listing of the Environics 100 specifications.

TABLE 1

SPECIFICATIONS FOR THE ENVIRONICS SERIES 100
GAS STANDARDS GENERATOR

Accuracy of Flow	0.15 % of Full Scale
Linearity	0.15 % of Full Scale
Repeatability	0.10 % of Full Scale
Dilution Ratio	Up to 1:30,000
Operating Temperature	-40 to 120° F.

The computerized unit provides the user with display prompts that permit automatic and highly accurate mixtures to be introduced into the animal chamber during the exposure episode. The approximate concentrations of gases metered into the exposure chamber during the Phase I Program were verified using a Miran 1A Gas Analyzer (Foxboro Wilkes Corporation). This single beam, variable filter spectrometer is capable of scanning the infrared spectral range from 2.5 microns to 14.5 microns in conjunction with a 5 liter capacity cell whose pathlength is variable from 5.0 to 20.25 meters. The Miran Spectrometer is equipped with a pump for drawing air samples from the animal exposure chamber at a rate of 2.0 liters/minute.

The concentrations of gases within the flow-through chamber were balanced to maintain the constant preselected level. The Miran Spectrometer was calibrated throughout the **Phase I Program** using certified gas cylinders ± 1.0 % of the stated value) that werediluted to preselected concentrations by the Environics Series 100 Computerized Multi-Gas Calibrator. A standard curve was chart recorded over each of the expected working ranges of the chamber's environment. The atmosphere within the animal exposure chamber was continuously monitored from the animal exposure chamber during all exposure episodes and compared to the standard curves for accuracy. Figure 3 is an infrared scan obtained using the Miran IA Gas Analyzer. Examination of Figure 3 illustrates, even with the slow acting Miran infrared Spectrophotometer, show how closely we were able to maintain the exposure chamber's environment. The approximate times for sampling (Observations or Treatments) are indicated along with the HCl concentrations (ppm) within the chamber. This is typical of the animal exposure episodes used within the scope of the **Phase I Program.**

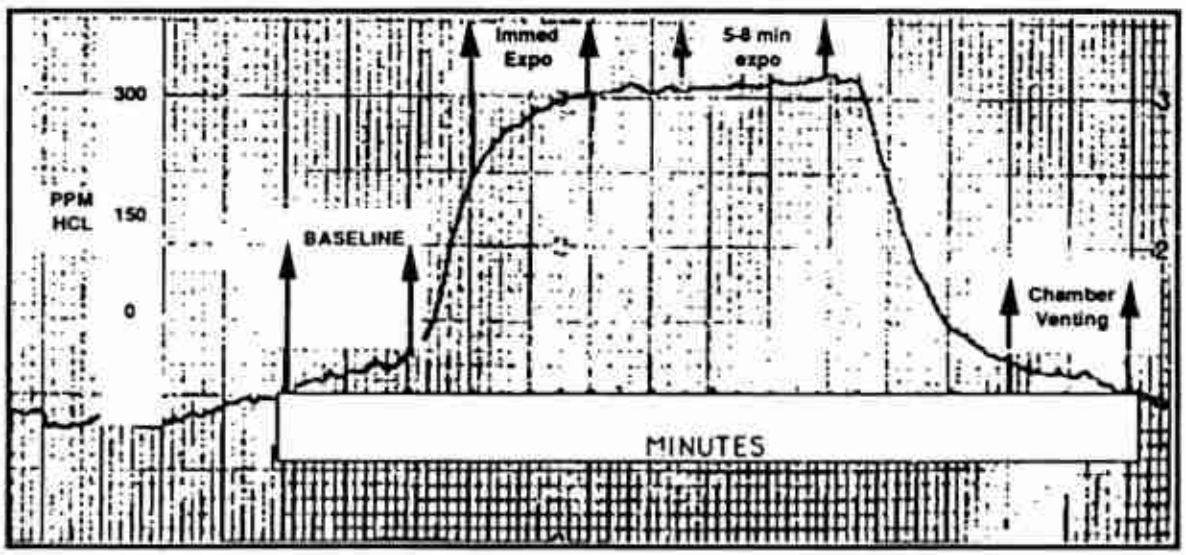


Figure 3. Continuous Infrared Spectroscopy Monitoring Profile of HCl in Air Captured at the Nose Level Within the Animal Exposure Chamber

4.3 Task 3 - Quantitative Neurophysiologic Monitoring

4.3.1 Task 3.1 - Selection of a Neurophysiologic Monitoring System

4.3.1.1 Task 3.1.1 - Evaluation of Nicolet Neurophysiologic Monitoring Systems

I.N. Einhorn and M.L. Grunnet visited the Biomedical Division of the Nicolet Corporation to review in detail the Nicolet Pathfinder System. During this visit the various techniques and computer programs used to develop quantitative EEG, EMG, and Evoked Potentials were demonstrated. Specifications were jointly developed for the instrument recommended by Nicolet Corporation.

A visit was made to the Department of Neurology, University of Wisconsin College of Medicine. The use of quantitative neurophysiologic procedures to monitor auditory evoked responses were observed and discussions held pertaining to detection and elimination of artifacts.

I. Einhorn and G. Moore met with D. Chillemi, District Sales Manager, Nicolet Instrument Corporation to discuss the characteristics of the Nicolet Pathfinder System, the Nicolet Brain Laboratory System, the Viking Electromyograph System, and the Nicolet BEAM System. Each of these systems was reviewed in detail with emphasis placed upon the use the project staff would require during the **Phase I Program.**

4.3.1.2 Task 3.1.2 - Evaluation of Quantified Signal Imaging, Inc. (QSI) Systems

I. Einhorn and G. Moore met in Toronto, Canada with the technical personnel involved in the development and applications of QSI's neurophysiological monitoring systems. Considerable time was spent in discussions pertaining to the present and future status of these novel systems. We also had the opportunity to personally operate the computer system.

During the visit to the QSI facility, it became apparent that the present QSI-9000 System was significantly more advanced than other systems investigated, thus, the decision was made for the Northeast Research Institute, Inc. to obtain the QSI-9000 System.

The QSI-9000's electrode headbox, which can be positioned near the subject, contains 20 channels of optically isolated amplifiers. When the animal subjects are prepared, a single keyboard command will begin the EEG recording for the preselected analog channels, and the electrode impedance can be checked at any time during the experiment. The operator can select the following 200 or 400 samples per second; amplifier gain; display gain; high and low filter settings, as low as 0.5 Hz and as high as 200 Hz.

In order to eliminate artifacted EEG from being included in the frequency analysis, the operator can choose which EEG epochs are to be selected for Fast Fourier Transform (FFT) calculation . Power and spectral amplitude are calculated for absolute and relative power and the results of the FFT can be recalled in the following four ways:

1. Table of mean values for both absolute and relative calculations;

2. Trended histograms of the frequency bands using successive vertical bars to represent each epoch of EEG;

3. Averaged frequency spectrum histograms of a specific EEG segment;

4. Compressed Spectral Array (CSA) of two user-selectable channels shown side-by-side which allows the user to analyze the trended spectral components of the EEG.

After analyzing the EEG using the FFT, the user can generate topographic maps of special amplitude or power for each of the four conventional EEG frequency bands, or maps of individual points of frequency from the histogram of the frequency spectrum. These can be stored for future comparisons on the same screen using maps created from different EEG's. The voltage distribution across the scalp can also be topographically mapped which can indicate the location and degree of spread of certain EEG

events, such as spikes. These mapping procedures can generate individual maps, sequential maps, continuous maps, and averaged user-selected ranges.

The EEG data can be further processed to provide the interpreter with several comprehensive displays utilizing measures of absolute and relative power, coherence, amplitude asymmetry, and phase. The displays can take the form of topographical distributions or tables of values of the raw data, the normative data, and z-score data. The data collected during the **Phase 1 Program** was also analyzed using external Fisher PLSD, Scheffe F-Test, and Dunnett T-Test statistical programs.

The normative data base which was generated during the **Phase I and Phase II Programs** permitted the statistical comparisons of animals using the t-test techniques. The systems software programs will allow the user to group together specific sets or subsets of data which will facilitate customized research-oriented studies.

System modifications and selection of appropriate electrodes and operational modes during the **Phase II Program** will permit the users to obtain EMG data similar to that described for EEG data.

The QSI-9000 System permits the user to acquire and monitor 2,4,8,or 20 channels of evoked potentials. The principle modalities include BAER, SEP, VEP, MLAR, and P300. All of the stimulation parameters are user-definable and protocols can be stored for various subject types, which can be immediately recalled. The effect of the variable artifacts reject level can be monitored by displaying individual sweeps. With a 20-channel recording the QSI-9000 System is capable of generating a topographic map of the voltage distribution of the evoked potential. When preparing topographic maps the operator can choose individual points, automatically generate maps of 8 successive points, or cartoon through the evoked potential, in effect, showing a slow-motion replay of the amplitude distribution over the entire scalp. After the averaging, the evoked potentials can be digitally filtered to remove undesirable frequency components. The EP can be superimposed, added, subtracted and averaged across separate trials. A summary report including waveform, peak latency, and amplitude tables, animal demographics, and interpreter's conclusions can easily be prepared using the QSI-9000 report generating routines.

4.3.2 Task 3.2 Interface the Quantitative Neurophysiologic Monitoring System to the Animal Exposure Chamber

We have, within the scope of the **Phase I Program**, interfaced the animal exposure chamber with the QSI-9000 Quantitative Neurophysiologic Monitoring System. The auditory stimulus and strobelight stimulus devices have been mounted within the animal exposure chamber. When the computerized signal is given to start the collection of the BAER and VEP responses these stimulus devices are turned on and regulated according to preset specifications for the duration of the exposure episode. The SEP stimulus (an electric shock) is also controlled in a similar manner, however, the stimulus is given directly to the animal subjects via electrodes attached to a hindlimb. The recording leads from the animals are interconnected with the QSI-9000 System, thus, facilitating measurement of the appropriate stimulus via the Sun 386 Computer System. Following the completion of the exposure episode, the data may be recalled from the hard disk, reviewed, analyzed statistically, and hard copies of the output obtained, and if desired stored on an optical disk for future use.

We will during the **Proposed Phase II Program** work with QSI personnel to develop software that will permit communication between the computerized gas standards generator system (CGS) and the QSI-

9000 Neurophysiologic Monitoring System. This communication through the RS232 port will permit the operator to command the CGS System to produce controlled test atmospheres on cue and to initiate monitoring of preselected neurophysiologic parameters by custom designed defaults and macro programs.

4.3.3 Task 3.3 - Development of Surgical Techniques

A variety of surgical techniques were developed within the scope of the **Phase I Program**. We have successfully developed procedures for implantation of BAER, EEG, VEP, and SEP electrodes. The cranial electrodes (three-electrode system) requires placement of two electrodes centered to the coronal suture on the parietal plates at 4 mm distance from each other. The third electrode, which serves as an isoelectric ground, is placed in the nasal arch. The five-electrode system includes placement of two additional electrodes 4 mm anterior to the electrodes centered to the coronal suture on the parietal plates. The electrodes are 5 mm x 1/2 mm stainless steel philster-head screws that are placed into previously drilled locations and then further secured in place with a fast-curing dental cement.

Only mixed success was achieved in the development of the femoral artery cannulation system. The surgical team under the direction of Professors G. Howe, University of Massachusetts, School of Veterinary and Animal Sciences and Professor J. Ryder, Department of Neurosurgery, Mt. Sinai College of Medicine. Further development of the cannulation technique will be required early in the **Phase II Program**.

4.4 Hydrogen Chloride/Air Exposure Studies

4.4.1 Background Information

A computerized review of the literature (Defense Technical Information Center, Chemical Abstracts, Medline, Toxline, NTIS, Biosis, Embase, NIOSH, Federal Research In Progress, Enviroline, and Hazardline) followed by discussions with key personnel of the U.S. Army Biomedical Research and Development Laboratory has indicated that sufficient information is not available to provide the quantitative chemical characterization of typical weapon systems exhaust mixtures and the duration of time that the concentrations of toxicants present in such mixtures are remain in the soldier's environment. We will include an Optional Task within the scope of the **Proposed Phase II Program** to characterize such weapons systems' exhaust mixtures. The NERI laboratory has facilities and extensive experience in the characterization of very complex and dynamically changing mixtures of combustion products of natural and synthetic materials which should enable us to identify and quantify most environments to which soldiers may be exposed during the firing of guns and rockets.

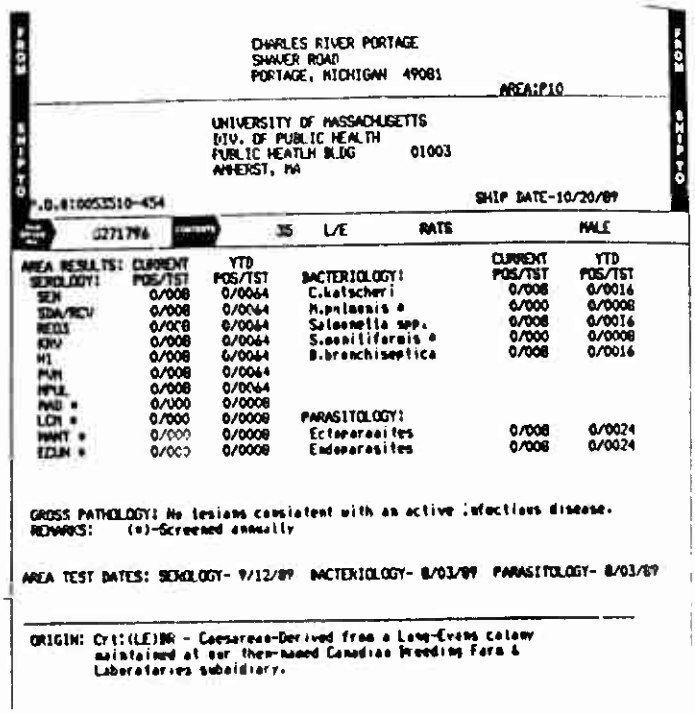
After discussions with personnel of the U.S. Army Biomedical Research and Development Laboratory, we designed the HCl exposure studies conducted within the scope of the **Phase I Program** to measure the effects on Long Evans rats during the exposure to 75 ppm, 150 ppm, and 300 ppm HCl in flowing air. Within the scope of the **Phase I Program** we exposed instrumented Long Evans rats to flowing air (0 ppm HCl), utilizing the same flow rates as the animals would be subjected to during the actual exposure episodes. Because of the rapid changes observed using the unique animal exposure chamber designed for the present study we were able to quantify changes from baseline in the animal's neurophysiological activity often within the first epoch following exposure to varying concentrations of HCl in flowing air. The duration of the exposure episode was reduced from the proposed 30-minute

exposure to an exposure duration of 15 minutes at the request of the Army Medical Research and Development Laboratory personnel.

4.4.2. Task 4.1.a. - Effects of HCl on the Brainstem Auditory Evoked Responses (BAER) in Long Evans Rats

4.4.2.1 Animal Preparation

Viral free male Male Long Evans rats in the weight range 225-250 were received from the Charles River Laboratories, Inc, Wilmington, Massachusetts. Figure 4 is a copy of a typical VAFIPLUS Report certification received with each animal shipment. All animals were held in the University of Massachusetts vivarium for a minimum of 7 to 14 days prior to surgical implantation of electrodes. All animals were subjected to a holding period of a minimum of 72 hours following surgery to minimize artifacts during the exposure episodes.



CHARLES RIVER PORTAGE
SHAVER ROAD
PORTAGE, MICHIGAN 49081

AREA:P10

UNIVERSITY OF MASSACHUSETTS
DIV. OF PUBLIC HEALTH
PUBLIC HEATLH BLDG 01003
AMHERST, MA

P.O.#10055510-454

SHIP DATE-10/20/89

2271796 35 L/E RATE MALE

AREA RESULTS: SEROLOGY:	CURRENT POS/TST	YTD POS/TST
SEN	0/008	0/0064
SDA/RCV	0/008	0/0064
REO3	0/008	0/0064
KRV	0/008	0/0064
H1	0/008	0/0064
PVM	0/008	0/0064
MPUL	0/008	0/0064
PNU *	0/000	0/0008
LCM *	0/000	0/0008
MART *	0/000	0/0008
EDIM *	0/000	0/0008

BACTERIOLOGY:	CURRENT POS/TST	YTD POS/TST
C.kutscheri	0/008	0/0016
M.pulmonis *	0/000	0/0008
Salmonella spp.	0/008	0/0016
S.moniliformis *	0/000	0/0008
B.bronchiseptica	0/008	0/0016
PARASITOLOGY:		
Ectoparasites	0/008	0/0024
Endoparasites	0/008	0/0024

GROSS PATHOLOGY: No lesions consistent with an active infectious disease.
REMARKS: (o)-Screened annually

AREA TEST DATES: SEROLOGY- 9/12/89 BACTERIOLOGY- 8/03/89 PARASITOLOGY- 8/03/89

ORIGIN: Crl:(LE)BR - Caesarean-Derived from a Long-Evans colony maintained at our then-named Canadian breeding farm & Laboratories subsidiary.

Figure 4. A Typical VAFIPLUS (Viral Antibody Free) Report from Charles River Breeding Laboratories

4.4.2.2 BAER Preparation

The brainstem auditory evoked responses (BAER's) were recorded prior to, during, and postexposure to animal exposure to 75 ppm, 100 ppm, or 300 ppm HCl in flowing air. Control animals were exposed under identical conditions used for exposure of animals to HCl in flowing air, except that no HCl was added to the air stream. The BAER responses were obtained using the placement of electrodes as described previously in Section 4.3.3. A needle electrode implanted behind the rat's left ear served as an isoelectric ground. An additional ground electrode was placed in the nasal arch. A series of 11.1 clicks per second (condensation 121 dB) were delivered through a microearphone placed within 10 mm of the animal's left ear while the animal was held in the "nose only" position in the animal exposure chamber.

Just prior to placemcnt of the Long Evans rats into thc animal exposure chamber the rats were anesthetized with 30 mg/kg of a ketamine (Ketalar; Parke Davis, Morris Plains, New Jersey) Rompun (Cutter Laboratories, Shawnee, Kansas) mixture intraperitoneally.

The placement of the animal within the exposure chamber and the constant positioning of the microearphone assured reproducibility of experimental conditions. Each epoch consisted on 800 sweeps. The following conditions were fixed for this series of experiments:

Amplifier gain	160 K
Filter Low	150 Hz
Filter High	3000 Hz
Stimulus Rate	11.1/Sec

4.4.2.3 BAER Experimental Results

Figure 5 illustrates the normal baseline BAER results obtained in Long Evans rat # LE005 during exposure in a flowing air environment. Figure 5 (right) illustrates the excellent reproducibility obtained during the recording of three BAER epochs of 800 sweeps each using a 121 db click delivered to the animal's left ear. Figure 5 (left) is the BAER obtained as the average of the three individual epochs illustrated in Figure 5 (right).

Figure 6 contains the statistical information obtained during the recording of the baseline BAER's shown in Figure 5. This statistical data has been obtained corresponding the the five peaks illustrated in Figure 6 (Average of 3 epochs) and Table II below.

TABLE II

BAER BASELINE PEAK LATENCIES AND AMPLITUDES
(Animal LE005-Flowing Air)

Peak	Latencies of Peaks	Peak Amplitudes
1	1.06	0.38
2	1.39	1.12
3	1.68	1.10
4	2.40	0.91
5	2.93	-1.01

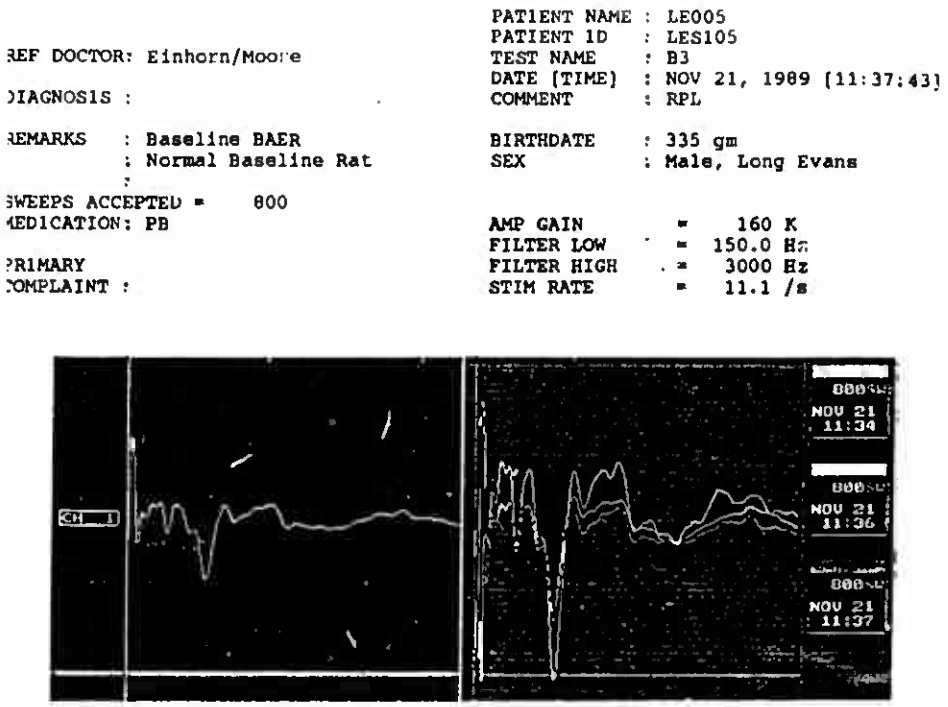


Figure 5. Typical BAER Response from Male Long Evans Rats Showing Repeated Epochs (Right) and Averaged Results (Left)

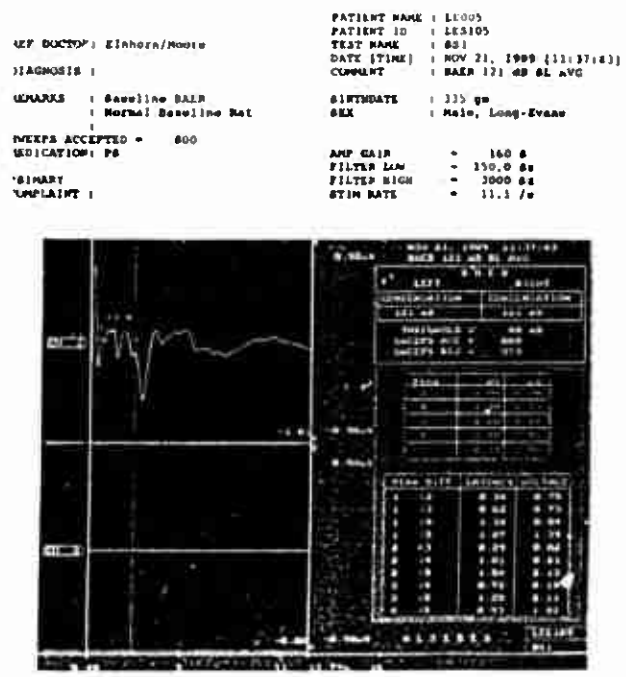


Figure 6. Typical BAER Response Observed in Long Evans Rats Showing Quantified Peaks and Latencies Including Peak Differences or Interpeak Latencies (IPL)

4.4.2.3.1 Process for the Analysis of BAER Experimental Data

The experimental design for the BAER experiments consisted of four experiments in which Long Evans rats were exposed to environments containing 0 ppm HCl (controls) in flowing air; 75 ppm HCl in flowing air; 150 ppm HCl in flowing air; and 300 ppm HCl in flowing air. Each experiment was run in triplicate, usually on different days to minimize experimental artifacts. Prior to the introduction of the preselected volume of HCl into the exposure chamber, three consecutive epochs of BAER data were obtained. As soon as the third epoch of BAER data was obtained the chamber door was dropped and mixing occurred. Distribution of the HCl throughout the animal exposure chamber occurred within 15 to 20 seconds following the opening of the door dividing the two chamber sections as was confirmed by continuous infrared analysis. The first series of BAER epochs were started 20 seconds following the introduction of HCl into the chamber's environment. As soon as the third BAER exposure epoch of this set was completed, the audio stimulus was turned off. A third set of three BAER exposure epochs were taken starting 5 minutes into the exposure episode. After the completion of the third BAER epoch in this series (approximately 8 minutes into the exposure episode), the audio signal was again tumed off. A final set of three BAER epochs was obtained from each animal subject commencing approximately 30 seconds following chamber venting (at approximately 15 minutes + 15 to 20 seconds after the start of the exposure episode.

Figures 7a-7l is a complete data set which is representative of the BAER studies conducted within the scope of the **Phase I Program**. Figures 7a-7c are representative of the three consecutive epochs of BAER baseline data obtained in a Long Evans rat exposed to an environment of flowing air. Figures 7d-7f are representative of the three consecutive epochs of BAER data obtained in the same Long Evans rat immediately after exposure to 150 mm HCl in flowing air. Figures 7g-7i are representative of the three consecutive epochs of BAER data obtained in the same Long Evans rat between 5 to 8 minutes into the exposure episode in which 150 ppm HCl is present in an environment of flowing air. Figures 7j-7l are representative of the three consecutive epochs of BAER data obtained in the same Long Evans rat immediately following chamber venting and replacement of the contaminated chamber environment with flowing uncontaminated air.

Table III is a representative sample of interpeak latency data as entered into a 3-factor analysis of variance statistical program. The three factors represent: (1) the animal subjects (2) the interpeak latencies (1:3; 1:5:, 3:5), and (3)the treatments (baseline, immediate exposure, 5-minute exposure, and chamber venting) resulting in a total of 36 category combinations. Note: **The data entered in Table III must be in the form of integers, it is necessary to divide this number by 100 to obtain the actual value of the latency in msec.**

Table IV is an ANOVA table for a 3-factor analysis of variance on BAER IPL for Long Evans rats exposed to 75 ppm HCl. The analysis of variance shows that there was a significance at $p = 0.0001$ between observations (treatments), more specifically there were very significant differences in interpeak latencies between baseline, immediate exposure, 5-minute exposure, and chamber venting (15-minute exposure) when averaged over all three-peak latencies (1:3; 1:5; and 3:5). (See Table V) Examination of Table V shows the IPL values for each of the measured interpeak latencies (1:3; 1:5; and 3:5) over the various observations (treatments).

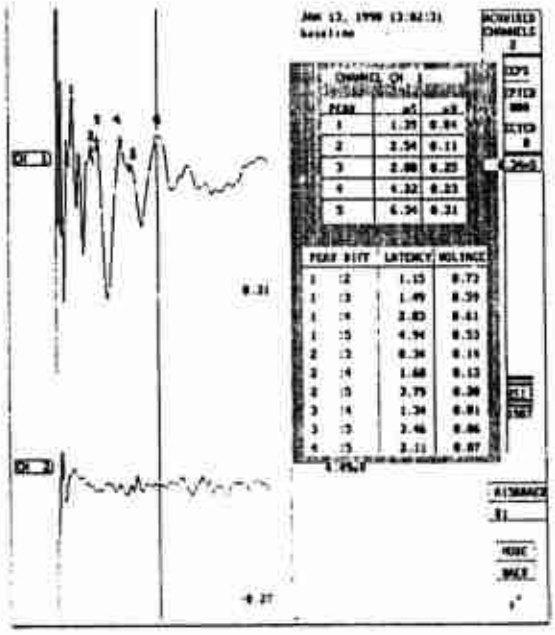


Figure 7a. First Baseline (0 ppm HCl)

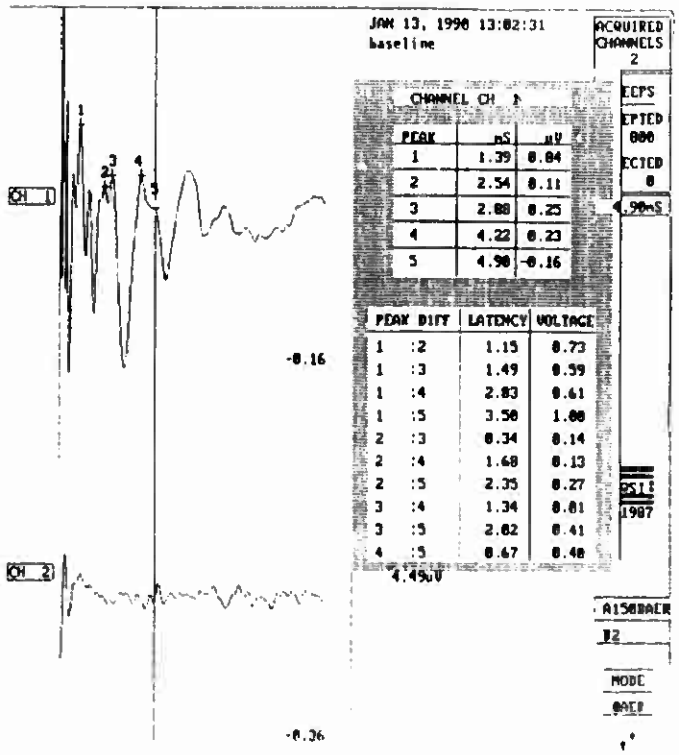


Figure 7b. Second Baseline (0 ppm HCl)

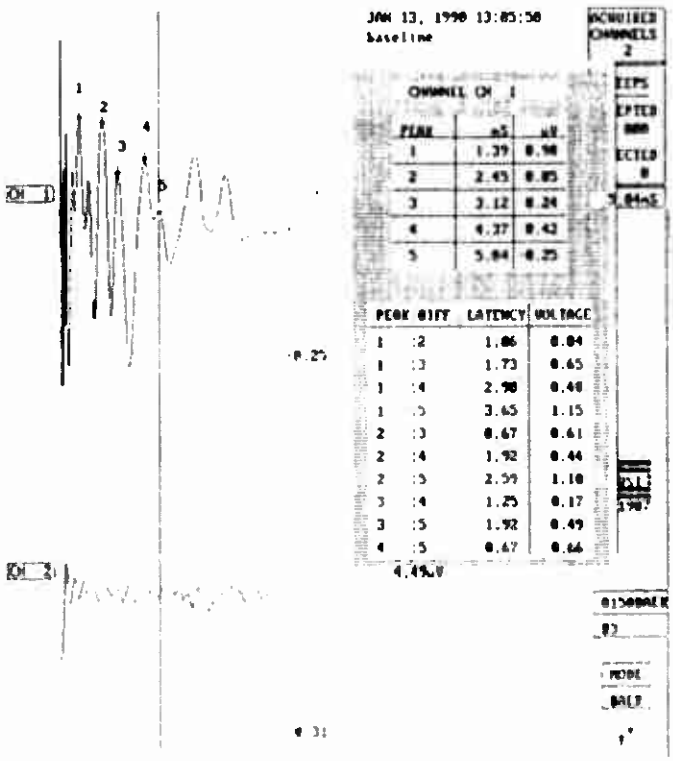


Figure 7c. First Baseline (0 ppm HCl)

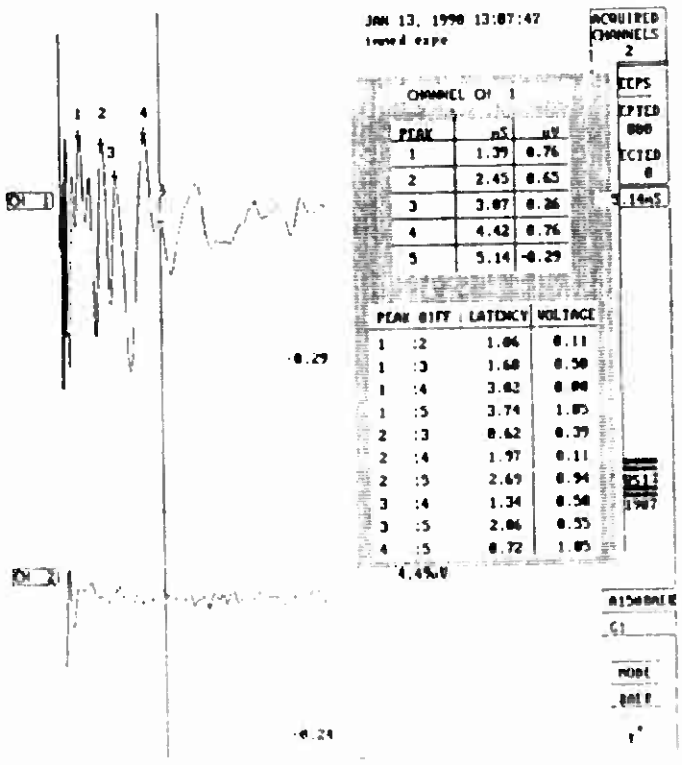
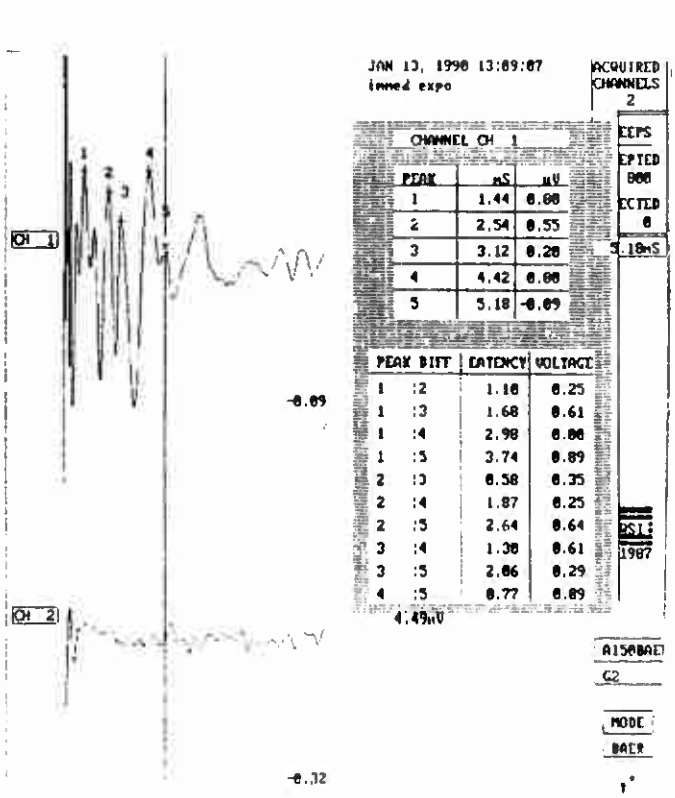
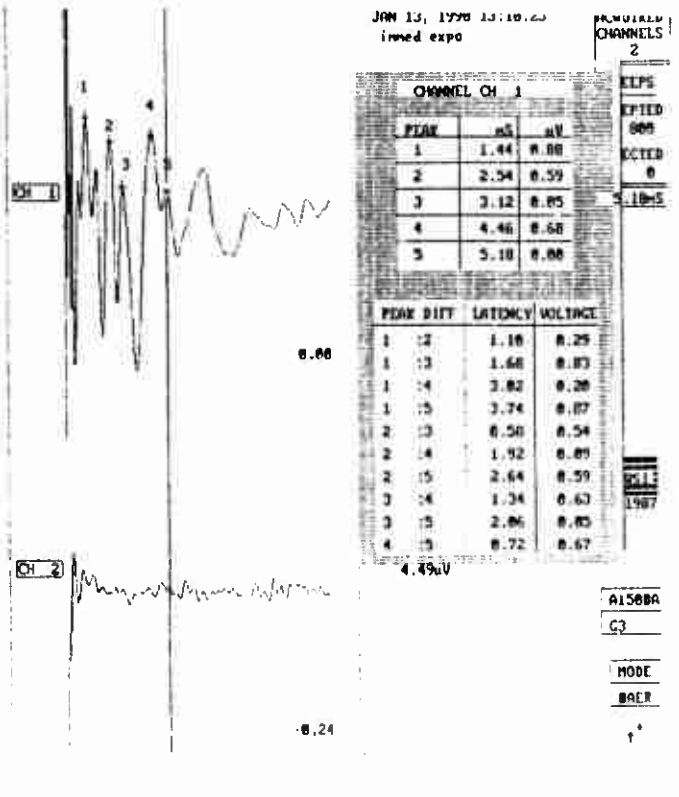


Figure 7d. First Exposure(150 ppm HCl)

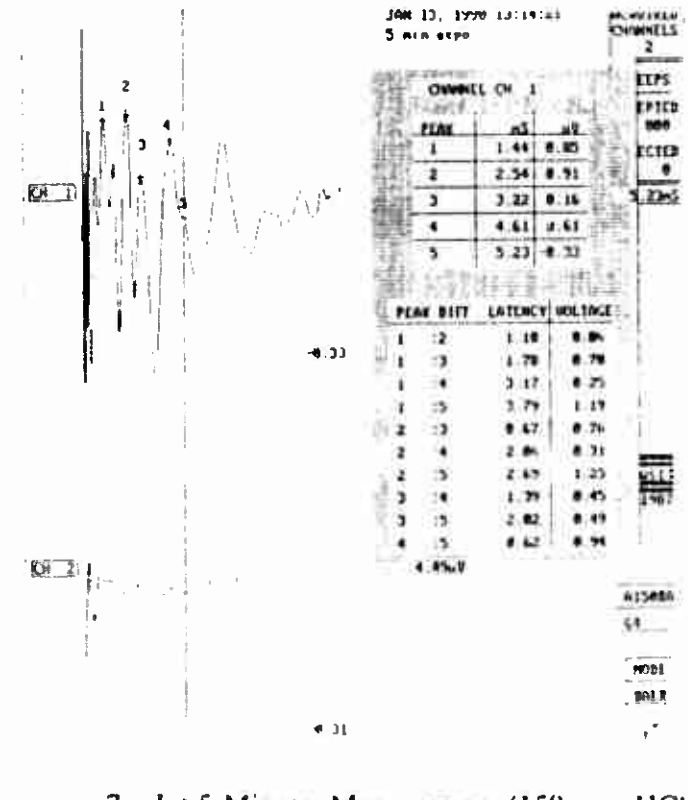
Figures 7A-7l: Complete Series of BAER Measurements Representing a Single Animal Exposure Sequence from Baseline Through Chamber Venting



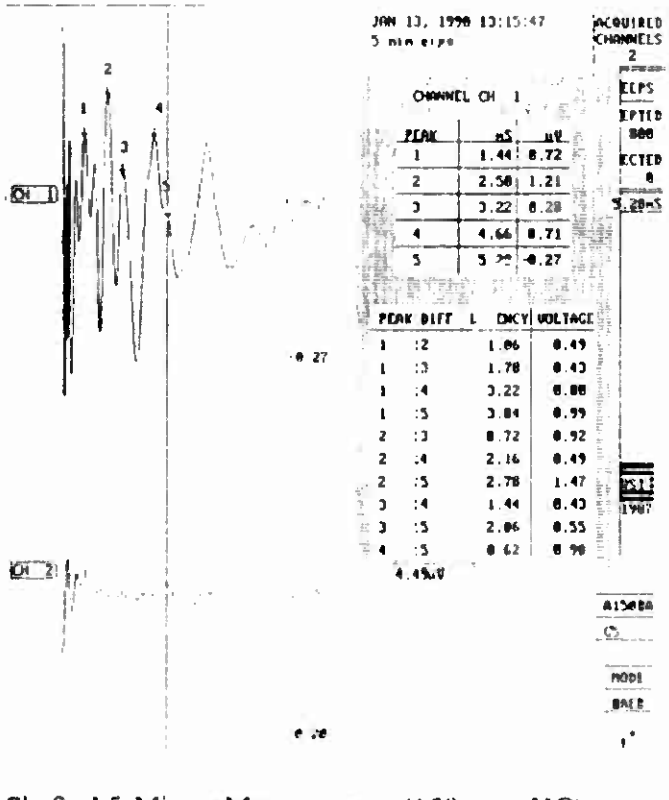
7e. 2nd Measurement (150 ppm HCl)



7f. 3rd Measurement (150 ppm HCl)

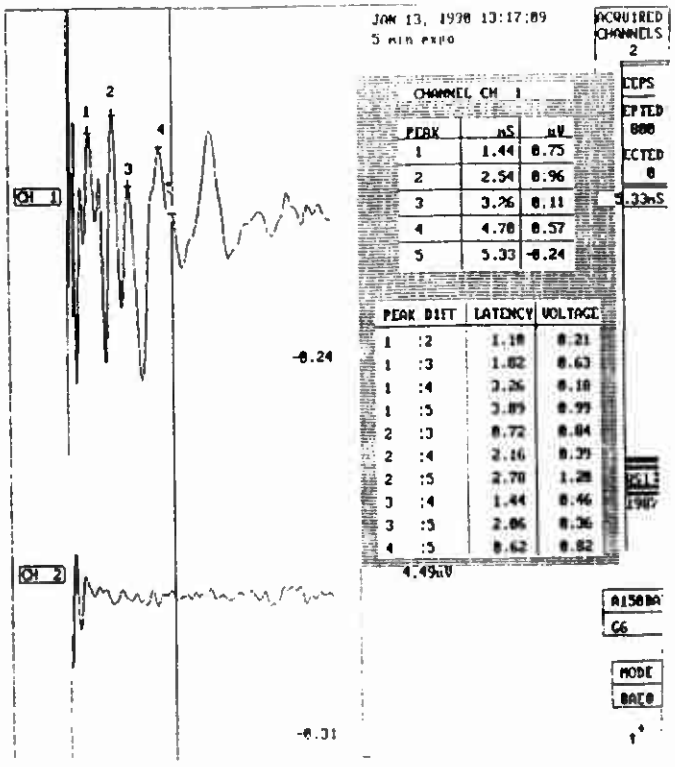


7g. 1st 5-Minute Measurement (150 ppm HCl)

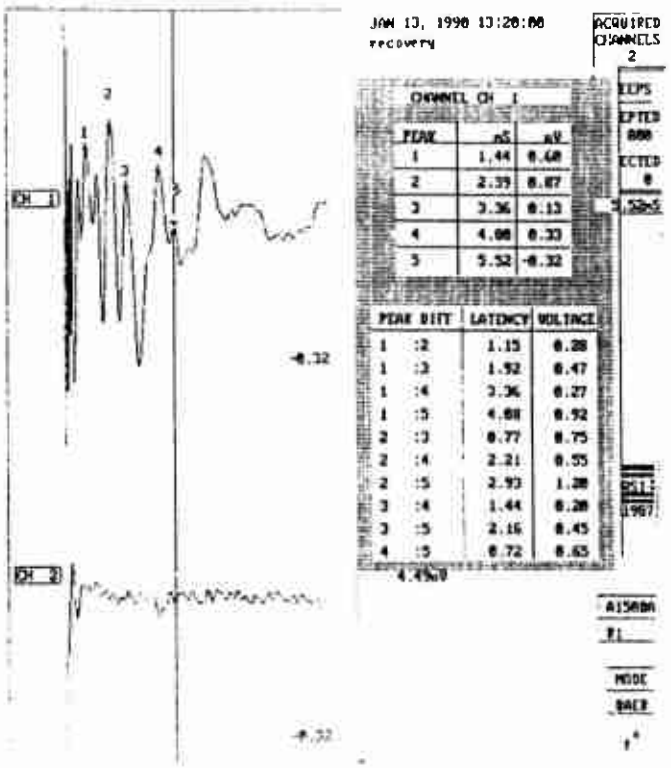


7h. 2nd 5-MinuteMeasurement (150 ppm HCl

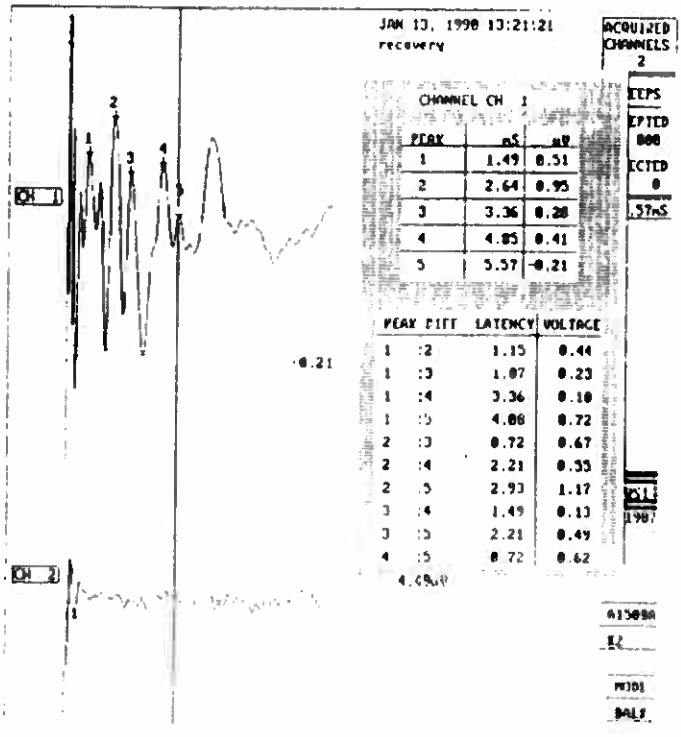
Figures 7A-7I: Complete Series of BAER Measnrements Representing a Single Animal Exposure Sequence from Baseline Through Chamber Venting



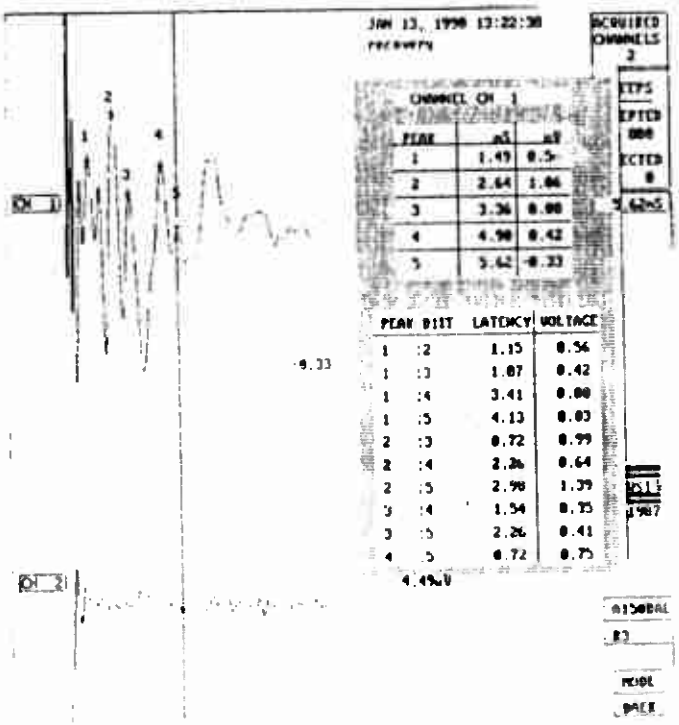
7i. 3rd 5-Minute Measurement
(150 ppm HCl)



7j. 1stMeasurement Chamber Vented
(150 ppm HCl)



7k. 2nd Measurement Chamber Vented
(150 ppm HCl)



7l. 2nd Measurement Chamber Vented
(150 ppm HCl)

Figures 7A-7l: Complete Series of BAER Measurements Representing a Single Animal Exposure Sequence from Baseline Through Chamber Venting

TABLE III

A REPRESENTATIVE SAMPLE OF INTERPEAK LATENCIES (IPL) ; DATA AS ENTERED INTO A 3-FACTOR ANALYSIS OF VARIANCE STATISTICAL PROGRAM

(Data from Three Test Animals Exposed to 75 ppm HCl in Flowing Air)

	LONG-EVANS RAT	LATENCY IPL	OBSERVATION	LATENCY (mS)
81	C	THREE:FIVE	FIVE MIN EXP...	197
82	A	ONE:THREE	CHAMBER VENT	182
83	B	ONE:FIVE	CHAMBER VENT	384
84	A	THREE:FIVE	CHAMBER VENT	202
85	A	ONE:THREE	CHAMBER VENT	178
86	A	ONE:FIVE	CHAMBER VENT	309
87	A	THREE:FIVE	CHAMBER VENT	211
88	A	ONE:THREE	CHAMBER VENT	182
89	A	ONE:FIVE	CHAMBER VENT	379
90	A	THREE:FIVE	CHAMBER VENT	197
91	B	ONE:THREE	CHAMBER VENT	182
92	B	ONE:FIVE	CHAMBER VENT	413
93	B	THREE:FIVE	CHAMBER VENT	230
94	B	ONE:THREE	CHAMBER VENT	178
95	B	ONE:FIVE	CHAMBER VENT	309
96	B	THREE:FIVE	CHAMBER VENT	211
97	B	ONE:THREE	CHAMBER VENT	173
98	B	ONE:FIVE	CHAMBER VENT	394
99	B	THREE:FIVE	CHAMBER VENT	221
100	C	ONE:THREE	CHAMBER VENT	182
101	C	ONE:FIVE	CHAMBER VENT	379
102	C	THREE:FIVE	CHAMBER VENT	197
103	C	ONE:THREE	CHAMBER VENT	192
104	C	ONE:FIVE	CHAMBER VENT	379
105	C	THREE:FIVE	CHAMBER VENT	197
106	C	ONE:THREE	CHAMBER VENT	182
107	C	ONE:FIVE	CHAMBER VENT	379
108	C	THREE:FIVE	CHAMBER VENT	197
109	•	•	•	•
56	B	ONE:FIVE	FIVE MIN EXP...	370
57	A	THREE:FIVE	FIVE MIN EXP...	197
58	A	ONE:THREE	FIVE MIN EXP...	173
59	B	ONE:FIVE	FIVE MIN EXP...	370
60	B	THREE:FIVE	FIVE MIN EXP...	197
M1	A	ONE:THREE	FIVE MIN EXP...	178
62	B	ONE:FIVE	FIVE MIN EXP...	370
M3	A	THREE:FIVE	FIVE MIN EXP...	192
M4	B	ONE:THREE	FIVE MIN EXP...	173
65	B	ONE:FIVE	FIVE MIN EXP...	398
66	B	THREE:FIVE	FIVE MIN EXP...	226
67	B	ONE:THREE	FIVE MIN EXP...	173
MM	B	ONE:FIVE	FIVE MIN EXP...	394
69	B	THREE:FIVE	FIVE MIN EXP...	221
70	B	ONE:THREE	FIVE MIN EXP...	173
71	B	ONE:FIVE	FIVE MIN EXP...	389
72	B	THREE:FIVE	FIVE MIN EXP...	216
73	C	ONE:THREE	FIVE MIN EXP...	189
74	C	ONE:FIVE	FIVE MIN EXP...	380
75	C	THREE:FIVE	FIVE MIN EXP...	192
76	C	ONE:THREE	FIVE MIN EXP...	173
77	C	ONE:FIVE	FIVE MIN EXP...	370
78	C	THREE:FIVE	FIVE MIN EXP...	197
79	C	ONE:THREE	FIVE MIN EXP...	173
80	C	ONE:FIVE	FIVE MIN EXP...	320

TABLE IV

ANOVA TABLE FOR A 3-FACTOR ANALYSIS OF VARIANCE ON BAER (IPL) FOR LONG EVANS RATS EXPOSED TO 75 PPM HCl IN FLOWING AIR

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
LONG-EVANS RAT (A)	2	4930.574	2415.297	81.823	.0001
LATENCY IPL (B)	2	927042.907	413521.454	14009.892	.0001
AB	4	2746.426	667.106	23.277	.0001
OBSERVATION (C)	3	10130.943	3376.949	114.401	.0001
AC	6	669.796	111.633	3.782	.0025
BC	9	1451.241	241.973	8.194	.0001
ABC	12	275.67	22.989	.779	.97
Error	72	2125.333	29.519		

There were no missing cells found. 1 case deleted with missing values.

TABLE V

THE LATENCY vs OBSERVATION (TREATMENT) BC INTERACTION TABLE FROM THE ANOVA ANALYSIS SHOWING THE LATENCIES FOR EACH (IPL) AND FOR EACH TREATMENT

	OBSERVATION	BASELINE	IMMED EX	FIVE MIN ...	CHAMBER...	Totals:
LATENCY IPL	ONE-THREE	9 184.111	9 195.222	9 173	9 190.111	36 170.611
	ONE-FIVE	9 350.333	9 358.778	9 379.778	9 387.222	36 399.278
	THREE-FIVE	9 199.111	9 183.556	9 203.999	9 207	36 197.939
Totals		27 233.518	27 238.185	27 251.222	27 258.111	108 245.509

The next phase in the statistical analysis of the data was to determine which if any of the IPL were significantly altered by the animal's exposure to HCl. An analysis of variance was conducted for BAER IPL (1:3) for control animals and all HCl exposed animals. A typical analysis is shown in Table VI. Table VI contains the statistical information developed as a one factor ANOVA-repeated measures for BAER IPL (1:3) in control and HCl exposed animals. There was a significant difference between controls and all HCl exposed groups with respect to the (1:3) IPL at P = 0.045. Figure 8 illustrates the IPL latencies (1:3) for control and HCl exposed Long Evans rats. (Note: The IPL values shown in Figure 8 should be divided by 100 to obtain the actual values, typically these values range from 1.55 to 1.85 msec). The final step in the statistical analysis required us to determine the significance between the controls and the effects observed as a consequence to animal exposure to the different levels of HCl. This was accomplished by determining the percent change from baseline of the IPLs for each of the observations (treatments). A typical example is seen in Figure 9 showing a comparison of BAER IPL (1:3) in Long Evans rats following exposure to the preselected levels of HCl in flowing air. Examination of Figure 9 shows that there is a significant difference between BAER IPL (1:3) and the controls with the controls showing, as would be expected, insignificant change from baseline. A t-test analysis was performed comparing the BAER IPL (1:3) and controls against each of the three different HCl exposure levels. Included in Figure 9 is a series of statistical analyses including the ANOVA and results of the t-test. The IPL (1:3) of the HCl exposed Long Evans rats were significantly different from those observed in the control group at P = 0.05 or less.

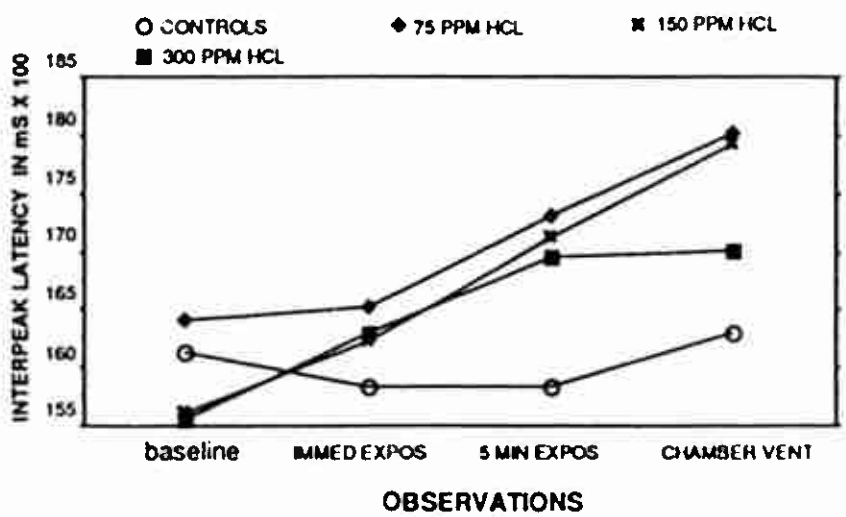


Figure 8. Interpeak Latencies (1:3) for Controls and HCl Exposed Long Evans Rats Graphed as a Function of Treatment

TABLE VI

ONE FACTOR ANOVA-REPEATED MEASURES FOR BAER IPL (1:3) COMPARING CONTROLS TO LONG EVANS RATS EXPOSED TO HCl

One Factor ANOVA Repeated Measures for X_1 ... X_4

Source	df	Sum of Squares	Mean Square	F-test	P value
Between subjects	3	455.188	151.729	4.488	.0246
Within subjects	12	404.78	33.729		
treatments	2	232.188	77.389	4.037	.045
residual	8	172.592	19.174		
Total	15	859.938			

Reliability Estimates for: All treatments: .778 Single Treatment: .467

1

One Factor ANOVA-Repeated Measures for X_1 ... X_4

Group	Count	Mean	Std. Dev.	Std. Error
CONTROL	4	180	2.449	1.225
75 PPM HCL	4	170.5	7.506	3.753
150 PPM HCL	4	187	10.1	5.05
300 PPM HCL	4	154.75	6.702	3.351

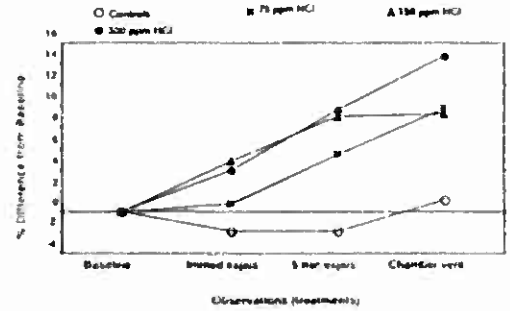
2

One Factor ANOVA-Repeated Measures for X_1 ... X_4

Comparison	Mean Diff.	Fisher PLSD	Scheffe F-test	Dunnett t
CONTROL vs. 75 PPM HCL	10.5	7.005*	3.833	3.381
CONTROL vs. 150 PPM HCL	-7	7.005	1.704	2.261
CONTROL vs. 300 PPM HCL	4.75	7.005	.784	1.534
75 PPM HCL vs. 150 PPM...	3.5	7.005	.426	1.13
75 PPM HCL vs. 300 PPM	5.75	7.005	1.15	1.857

* Significant at 95%

3



STATISTICAL ANALYSIS

Variables Compared	Statistic Used	Result
Difference between treatments Baseline vs Inhaled expos vs 5 min Expos vs Chamber vent for HCl exposures	3 factor ANOVA	p=0.0001
Difference between treatments for Controls considered	3 factor ANOVA	NS at 95%
Control vs 75 ppm expos	T test	p=.0534
Control vs 150 ppm expos	T test	p=.0343
Control vs 300 ppm expos	T test	p=.0479

Figure 9. Percent Change from Baseline of BAER IPL (1:3) in Long Evans Rats Exposed to Various Levels of HCl Gas in Flowing Air Compared with Controls

4.4.2.3.2 Statistica' Results BAER Studies of the Effects of HCl on Long Evans Rats

Using the same procedures as discussed in Section 4.4.2.3.1, the following is a summary of BAER results obtained during the **Phase I Program.**

Tables VII - IX are ANOVA tables for a 3-factor analysis of variance on BAER IPL for Long Evans rats exposed to 0, 150, and 300 ppm HCl. As shown in Table VII, the analysis of variance shows no significant differences in observations (treatments) for BAER IPL control animals (0.0 ppm HCl). This indicates, as expected, that the IPL latencies do not vary with respect to experimental conditions with the exclusion of HCl.

TABLE VIIA

THREE-FACTOR ANOVA OF BAER IPL (1:3) FOR CONTROL RATS IN FLOWING AIR
(ANOVA Table for a 3-Factor Analysis of Varience on Y_1 : LATENCY (mS)

Anova table for a 3-factor Analysis of Variance on Y_1: LATENCY

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
LONG-EVANS RAT (A)	2	2068.366	1034.184	.	.
LATENCY (IPL) (B)	2	222765.056	111382.528	.	.
AB	4	1134.444	283.611	.	.
OBSERVATION (C)	3	1422.306	474.102	.	.
AC	6	536.278	89.38	.	.
BC	6	987.611	161.266	.	.
ABC	12	396.556	33.296	.	.
Error	0	0	.		

There were no missing cells found.

TABLE VIIB

TIIREE-FACTOR ANOVA OF BAER IPL (1:3) FOR CONTROL RATS IN FLOWING AIR
(The AC Incidence Table on Y_1 : LATENCY (mS)

The AC Incidence table on Y_1: LATENCY

OBSERVATION		BASELINE	IMMED EX...	FIVE MIN ...	CHAMBER...	Totals:
LONG-EVANS RA	A	3 214.333	3 236.667	3 237	3 233.333	12 230.333
	B	3 204.667	3 206	3 214.333	3 220.667	12 211.417
	C	3 214.333	3 227.333	3 230.333	3 220.667	12 223.167
Totals		9 211.111	9 224	6 227.222	6 224.889	36 221.806

TABLE VIIIA

THREE-FACTOR ANOVA OF BAER IPL (1:3) FOR RATS EXPOSED TO 150 PPM HCl IN FLOWING AIR

(ANOVA Table for a 3-Factor Analysis of Varience on Y_1 : LATENCY (mS)

Anove table for a 3-factor Analysis of Varience on Y_1: LATENCY (mS)

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
LONG-EVANS RAT (A)	1	17766.125	17766.125	932.333	.0001
LATENCY IPL (B)	2	516052.333	258026.167	13540.732	.0001
AB	2	3796	1898	99.603	.0001
OBSERVATION (C)	3	9812.819	3270.94	171.653	.0001
AC	3	597.153	199.051	10.446	.0001
BC	6	1438.222	239.704	12.579	.0001
ABC	6	660.556	110.093	5.777	.0001
Error	48	914.667	19.056		

TABLE VIIIB

THREE-FACTOR ANOVA OF BAER IPL (1:3) FOR RATS EXPOSED TO 150 PPM HCl IN FLOWING AIR

(The AC Incidence Table on Y_1 : LATENCY (mS)

The AC Incidence table on Y_1: LATENCY (mS)

OBSERVATION:		BASELINE	IMMED EX...	FIVE MIN ...	CHAMBER...	Totals:
LONG-EV...	A	9 236.889	9 249.333	9 256	9 273.111	36 253.833
	B	9 204.889	9 221.889	9 230.333	9 232.556	36 222.417
Totals:		18 220.889	18 235.611	18 243.167	18 252.833	72 238.125

TABLE IXA

THREE-FACTOR ANOVA OF BAER IPL (1:3) FOR RATS EXPOSED TO 300 PPM HCl IN FLOWING AIR

(ANOVA Table for a 3-Factor Analysis of Varience on Y_1 : LATENCY (mS)

Anova table for a 3-factor Analysis of Variance on Y_1: LATENCY (mS)

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
LONG-EVANS RAT (A)	2	8813.483	4406.731	95.02	.0001
LATENCY IPL (B)	2	752298.88	376149.44	8110.722	.0001
AB	4	1147.105	286.776	6.184	.0003
OBSERVATION (C)	3	11729.58	3909.86	84.308	.0001
AC	6	884.253	147.375	3.178	.0082
BC	6	1809.235	301.539	6.502	.0001
ABC	12	891.817	57.851	1.243	.2728
Error	69	3200	46.377		

There were no missing cells found. 1 case deleted with missing values.

1

TABLE IXB

THREE-FACTOR ANOVA OF BAER IPL (1:3) FOR RATS EXPOSED TO 300 PPM HCl IN FLOWING AIR

(The AC Incidcnce Table on Y_1 : LATENCY (mS)

The AC Incidance table on Y_1: LATENCY (mS)

OBSERVATION:		BASELINE	IMMED EX...	FIVE MIN ...	CHAMBER...	Totals:
LONG-EVANS RAT	A	9 216.667	9 225	9 232.444	9 250.556	36 231.167
	B	9 235.867	9 246.556	9 260.333	8 265.333	33 250.758
	C	9 218.667	9 233.444	9 236.869	9 243.111	36 233.028
Totals:		27 223.667	27 235	27 243.222	24 251.458	105 237.962

3

Tables VIII and IX indicate a very strong significant difference p = 0.0001 between observations (treatments) for Long Evans rats exposed to 150 and 300 ppm HCl in flowing air.

The next phase in the statistieal analysis of the data was to determine which if any of the IPL were significantly altered by the animal's exposure to HCl. We have previously shown that there is a statistically significant difference in the BAER IPL (1:3) for control animals and all HCl exposure levels.

Normally the next phase in the analysis of BAER IPLs would include the statistical analysis of the BAER IPL peaks (1:5) and (3:5). The changes which occur in the latency of peak 5 is very important as this relates to changes associated with the nervous system centers controlling vital functions. We have observed major changes in the latencies and amplitudes associated with peak V. In some cases peak V is absent, in other cases it is difficult to assign the latency associated with peak V due to the poor definition of this peak. In preliminary trials our attempts to evaluate the statistical significance associated with IPLs (1:5; and 3:5) has led to a large coefficient of variation because of the inability to exactly locate peak V. Within the scope of the **Phase I Program** sufficient time and resources were not available to determine the mechanism related to the intoxication syndrome associated with HCl which might produce the observed changes. Attention will be directed to determining the causes of changes in peak V during the **Phase II Program.**

4.4.3 Task 4.1.b - Effects of HCl on the Electroencephalographic Activity (EEG) in Long Evans Rats)

4.4.3.1 Background Information

Electroencephalographic analysis techniques have been divided into two basic categories that emphasize different aspects of the EEG signal. The frequency-domain category contains techniques such as spectral analysis, which isolates the amplitude or power of the signal associated with various frequencies.[11] This technique has been used extensively in both clinical and basic research and has been useful in identifying organismic and task related differences in the EEG.[12-14] Spectral analysis, however, separates the EEG from its time-related dimension so that information pertaining to the amount of time a given frequency is present in the signal is lost. Time-domain techniques, such as amplitude and period analysis, preserve this time-related information. Period analysis identifies the proportion of time that an EEG signal falls within various signal ranges.([15]. whereas amplitude analysis typically provides the integrated amplitude of the signal for a specified interval.[13]

Giannitripiani[13] has suggested the use of factor analytic studies of EEG spectra and task performance in order to establish a set of tasks that have similar neurophysiological characteristics.

Initial studies designed to determine the results of animal exposure to environmental toxicants were useful in showing that there were significant changes in the latencies and amplitudes of EEG activity.[10]

The present studies direct emphasis toward determining which quantitative neurophysiologic monitoring protocols offer the greatest potential to characterize the intoxication syndrome produced as a consequence of exposure to a single toxicant or mixtures of toxicants.

4.4.3.2. EEG Preparation

The final electrode placement which was used throughout the **Phase I Program** involved the use of four screw electrodes, two in the anterior region of the skull and two in the posterior region of the skull with the reference EEG electrode implanted on the nose. This placement of electrodes has greatly reduced the number of EEG epochs which were rejected, and has also reduced the earlier problem with signal clipping.

We commenced monitoring of EEG activity one minute after the animals have been placed in the animal exposure chamber. We collected a two-minute epoch of EEG data prior to the introduction of the HCl gas as baseline data. With the instantaneous introduction of the HCl gas and additional 2-minute epoch of EEG data was collected to determine the effects of intermediate exposure on the quantitative EEG (qEEG). After an additional 5 minutes into the exposure an additional 2-minute EEG epoch was collected. An additional 2-minute EEG epoch was collected following the venting of the exposure chamber with HCl free filtered air (15 minutes into the exposure episode).

4.4.3.3 Process for Analysis of EEG Results

The quantitative analysis of the EEG data was obtained on control and exposed animals using the most appropriate of the following three quantitative procedures.

1. Table of mean values for both absolute and relative calculations;
2. Trended histograms of the frequency bands using successive vertical bars to represent each epoch of EEG;
3. Averaged frequency spectrum histograms of a specific EEG segment;

4.4.3.4 Statistical Analysis of Quantitative EEG Studies of the Effect of HCl on Long Evans Rats

The qEEG data which we have used to show the statistical processes employed in the analysis of data obtained within the scope of the **Phase I Program** represent data developed during the exposure of control animals and animals exposed to 300 ppm HCl in flowing air. Figure 10 includes trended histograms, a table of mean values for both absolute and relative amplitudes for the four major EEG frequencies (Delta, Beta, Alpha. and Theta) for data collected using 4 electrodes.

The typical process used within the scope of the Phase I Program included analyzing each major frequency for both absolute and relative percent amplitudes at each exposure level by one factor ANOVA repeated measures. The results for the 300 ppm HCl exposures are presented in Tables X - XV. Tables X through XIII show significant differences between observations (treatments) for the relative percent of the major frequencies: Delta ($p = 0.0133$); Theta ($p = 0.0523$); Alpha ($p = 0.0202$); and Beta ($p = 0.0001$).

Examination of the data contained in these tables show comparisons of baseline vs immediate exposure; 5-minute exposure; and at chamber venting (approximately 15 minutes into the exposure episode). Those values which are significant at the 95 % level for the Fischer PLSD and Scheffe F-test are indicated by an asterix.

The Delta and Beta frequencies (Relative %) exhibited the highest level of significance with respect to

significant changes at the 300 ppm HCl exposure levels. The ANOVA showed no significant differences between observations (trcatments) for the Delta and Beta (relative %) frequencies at 150 ppm and 75 ppm HCl exposure levels. (See Tables XVI - XIX)

We also statistically analyzed the effects of HCl exposure on absolute total amplitudes in Long Evans rats. The results of this analysis are presented in Figure 15 which shows the percent change and absolute total amplitudes of control and HCl exposed Long Evans rats at the three exposure levels. A paired t-Test was performed comparing each exposure group with the control group. This analysis revealed that the 300 ppm and 150 ppm exposures produced significantly reduced absolute total amplitudes compared with controls. The 75 ppm HCl exposures produced a significant increase in the absolute total amplitudes with respect to controls. In addition to examining the four major EEG frequencies for amplitude changes, we also investigated the percent change in absolute total amplitudes of control and HCl exposed Long Evans rats.

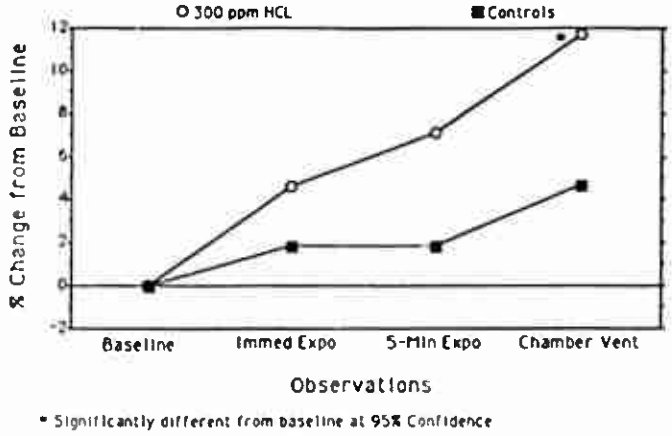


Figure 11. Percent Change in EEG Delta Frequency (Relative Percent) Observed in Control and HCl Exposed Long Evans Rats

significant changes at the 300 ppm HCl exposure levels. The ANOVA showed no significant differences between observations (treatments) for the Delta and Beta (relative %) frequencies at 150 ppm and 75 ppm HCl exposure levels. (See Tables XVI - XIX)

We also statistically analyzed the effects of HCl exposure on absolute total amplitudes in Long Evans rats. The results of this analysis are presented in Figure 15 which shows the percent change and absolute total amplitudes of control and HCl exposed Long Evans rats at the three exposure levels. A paired t-Test was performed comparing each exposure group with the control group. This analysis revealed that the 300 ppm and 150 ppm exposures produced significantly reduced absolute total amplitudes compared with controls. The 75 ppm HCl exposures produced a significant increase in the absolute total amplitudes with respect to controls. In addition to examining the four major EEG frequencies for amplitude changes, we also investigated the percent change in absolute total amplitudes of control and HCl exposed Long Evans rats.

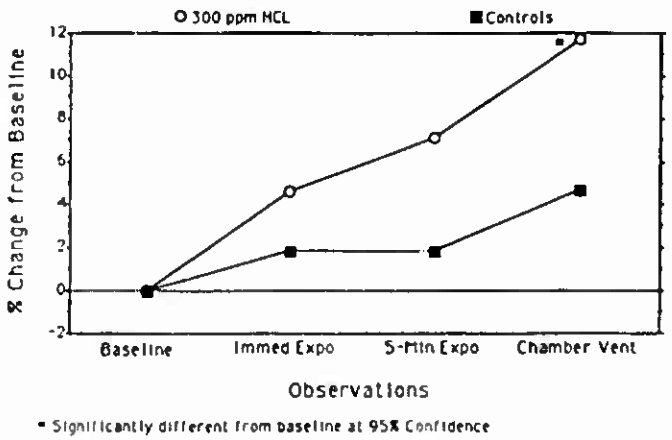


Figure 11. Percent Change in EEG Delta Frequency (Relative Percent) Observed in Control and HCl Exposed Long Evans Rats

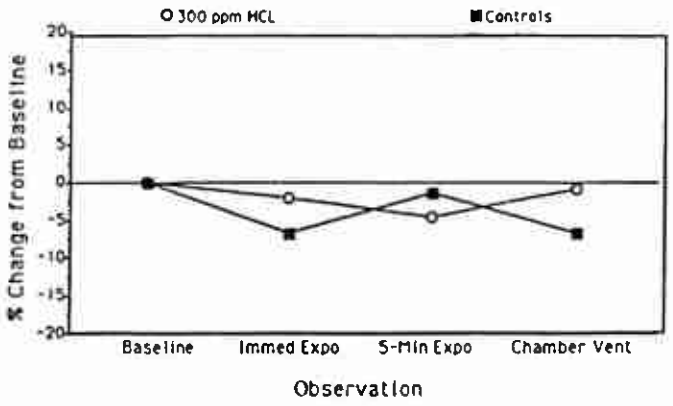


Figure 12. Percent Change in EEG Theta Frequency (Relative Percent) Observed in Control and HCl Exposed Long Evans Rats

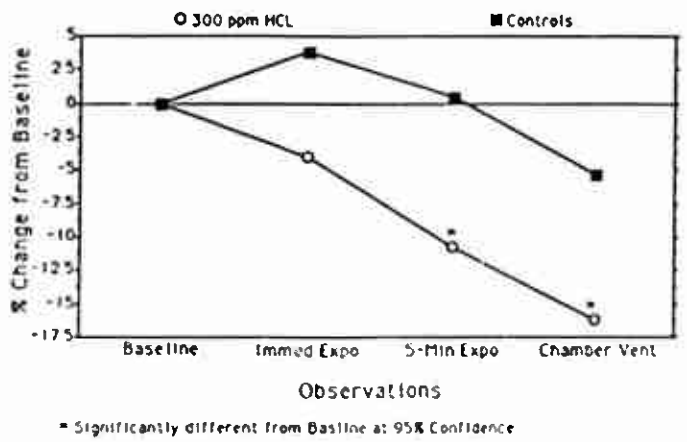


Figure 13. Percent Change in EEG Alpha Frequency (Relative Percent) Observed in Control and HCl Exposed Long Evans Rats

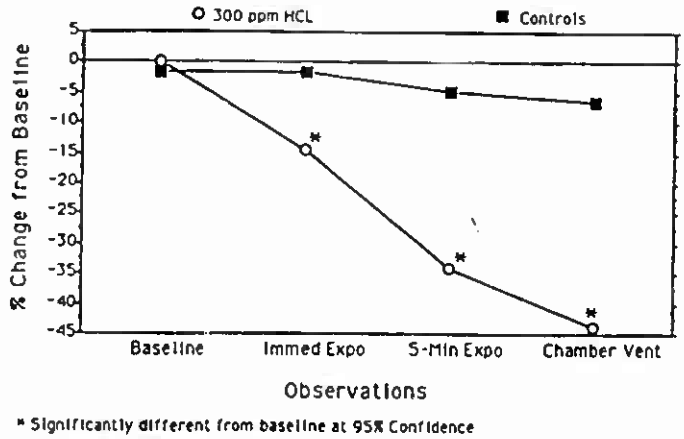


Figure 14. Percent Change in EEG Beta Frequency (Relative Percent) Observed in Control and HCl Exposed Long Evans Rats

TABLE X

ONE FACTOR ANOVA-REPEATED MEASURES OF DELTA (RELATIVE PERCENT) EEG FREQUENCY FOR LONG EVANS RATS EXPOSED TO 300 PPM HCl IN FLOWING AIR

One Factor ANOVA-Repeated Measures for X_1 ... X_4

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Between subjects	7	924.375	132.054	5.497	.0007
Within subjects	24	576.5	24.021		
treatments	3	226.625	75.542	4.534	.0133
residual	21	349.875	16.661		
Total	31	1500.875			

Reliability Estimates for: All treatments: .616 Single Treatment: .529

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
BASELINE	8	58.75	4.773	1.688
IMMED EXPO	8	80	6.094	2.155
5 MIN EXPO	8	82.875	8.843	2.349
RECOVERY	8	85.625	8.831	3.122

One Factor ANOVA-Repeated Measures for X_1 ... X_4

Comparison:	Mean Diff.:	Fisher PLSD	Scheffe F-test:	Dunnett t:
BASELINE vs IMMED EXPO	-1.25	4.245	.125	.612
BASELINE vs 5 MIN EXPO	-4.125	4.245	1.382	2.021
BASELINE vs RECOVERY	-6.875	4.245*	3.783*	3.369
IMMED EXPO vs 5 MIN EX...	-2.875	4.245	.661	1.409
IMMED EXPO vs RECOVERY	-5.625	4.245*	2.532	2.756

* Significant at 95%

TABLE XI

ONE FACTOR ANOVA-REPEATED MEASURES OF THETA (RELATIVE PERCENT) EEG FREQUENCY FOR LONG EVANS RATS EXPOSED TO 300 PPM HCl IN FLOWING AIR

One Factor ANOVA-Repeated Measures for X_1 ... X_4

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Between subjects	7	151.719	21.674	7.622	.0001
Within subjects	24	68.25	2.844		
treatments	3	20.594	6.865	3.025	.0523
residual	21	47.656	2.269		
Total	31	219.969			

Reliability Estimates for: All treatments: .869 Single Treatment: .623

1

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
BASELINE	8	18.375	2.825	.999
IMMED EXPO	8	18	2.777	.962
5 MIN EXPO	6	17.5	2.268	.802
RECOVERY	8	16.25	2.765	.977

2

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
BASELINE vs. IMMED EXPO	.375	1.567	.083	.496
BASELINE vs. 5 MIN EXPO	.875	1.567	.45	1.162
BASELINE vs. RECOVERY	2.125	1.567*	2.653	2.821
IMMED EXPO vs. 5 MIN EX...	.5	1.567	.147	.664
IMMED EXPO vs. RECOVERY	1.75	1.567*	1.799	2.323

* Significant at 95%

3

TABLE XII

ONE FACTOR ANOVA-REPEATED MEASURES OF ALPHA (RELATIVE PERCENT) EEG FREQUENCY FOR LONG EVANS RATS EXPOSED TO 300 PPM HCl IN FLOWING AIR

One Factor ANOVA-Repeated Measures for X_1 ... X_4

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Between subjects	7	856.375	122.339	25.421	.0001
Within subjects	24	115.5	4.812		
treatments	3	42.375	14.125	4.056	.0202
residual	21	73.125	3.482		
Total	31	971.875			

Reliability Estimates for: All treatments: .961 Single Treatment: .859

1

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
BASELINE	8	16.5	4.071	1.439
IMMED EXPO	8	17.75	6.065	2.144
5 MIN EXPO	6	16.5	6	2.121
RECOVERY	8	15.5	6.59	2.33

Comparison	Mean Diff.:	Fisher PLSD	Scheffe F-test:	Dunnett t:
BASELINE vs. IMMED EXPO	75	1.941	.215	.804
BASELINE vs. 5 MIN EXPO	2	1.941*	1.532	2.144
BASELINE vs. RECOVERY	3	1.941*	3.446*	3.215
IMMED EXPO vs. 5 MIN EX...	1.25	1.941	.598	1.34
IMMED EXPO vs. RECOVERY	2.25	1.941*	1.938	2.412

* Significant at 95%

TABLE XIII

ONE FACTOR ANOVA-REPEATED MEASURES OF BETA (RELATIVE PERCENT) EEG FREQUENCY FOR LONG EVANS RATS EXPOSED TO 300 PPM HCI IN FLOWING AIR

One Factor ANOVA-Repeated Meeeuree for X_1 ... X_4

Source:	df:	Sum of Squares:	Mean Square:	F-teat:	P value:
Between subjects	7	7.375	1.054	.887	.5315
Within subjects	24	28.5	1.188		
treatments	3	24.375	8.125	41.364	.0001
residual	21	4.125	.196		
Total	31	35.875			

Reliability Estimates for- All treatments: ·.127 Single Treatment: ·.029

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
BASELINE	8	5.125	.835	.295
IMMED EXPO	8	4.375	.518	.183
5 MIN EXPO	8	3.375	.516	.183
RECOVERY	8	2.875	.641	.227

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
BASELINE vs. IMMED EXPO	.75	.461*	3.818*	3.364
BASELINE vs. 5 MIN EXPO	1.75	.461*	20.766*	7.897
BASELINE vs. RECOVERY	2.25	.461*	34.364*	10.153
IMMED EXPO vs. 5 MIN EX...	1	.461*	6.788*	4.513
IMMED EXPO vs. RECOVERY	1.5	.461*	15.273*	6.769

* Significant at 95%

TABLE XIV

ONE FACTOR ANOVA-REPEATED MEASURES OF DELTA (ABSOLUTE POWER) EEG FREQUENCY FOR LONG EVANS RATS EXPOSED TO 300 PPM HCI IN FLOWING AIR

One Factor ANOVA-Repeated Messures for X_1 ... X_4

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Between subjects	7	388913480.719	55559068.674	44.979	.0001
Within subjects	24	29645195.25	1235216.469		
treatments	3	20478730.594	6825576.865	15.634	.0001
residual	21	9166464.656	436593.555		
Total	31	418558675.969			

Reliability Estimates for- All treatments: .976 Single Treatment: .917

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
BASELINE	8	12210.5	3643.042	1286.01
IMMEDIATE EXPO...	8	11645.25	3670.186	1297.807
5 -8 MINUTE EXP...	8	12786.375	4009.705	1417.645
RECOVERY	8	13612	3748.21	1325.192

Comparison	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
BASELINE vs. IMMEDIATE...	565.25	687.128	.978	1.711
BASELINE vs. 5 -8 MINUT...	-565.875	687.128	.944	1.683
BASELINE vs. RECOVERY	-1401.5	687.128*	7.833*	4.648
IMMEDIATE ... vs. 5 -8 MI...	-1121.125	687.128*	3.839*	3.393
IMMEDIATE ... vs. RECOVE...	-2166.75	687.128*	14.338*	6.558

* Significant at 95%

TABLE XV

ONE FACTOR ANOVA-REPEATED MEASURES OF BETA (ABSOLUTE POWER) EEG FREQUENCY FOR LONG EVANS RATS EXPOSED TO 300 PPM HCl IN FLOWING AIR

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Between subjects	7	2874789.969	410684.281	10.142	.0001
Within subjects	24	971879.75	40494.99		
treatments	3	852005.844	284001.948	49.753	.0001
residual	21	119873.906	5708.281		
Total	31	3846669.719			

Reliability Estimates for: All treatments: .901 Single Treatment: .696

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
BASELINE	8	1048.5	405.925	143.516
IMMED EXPO	8	837.25	310.375	109.734
5 MIN EXPO	8	675.75	298.395	105.499
RECOVERY	8	632.875	278.676	98.527

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
BASELINE vs. IMMED EXPO	211.25	78.569*	10.424*	5.592
BASELINE vs. 5 MIN EXPO	372.75	78.569*	32.454*	9.867
BASELINE vs. RECOVERY	415.625	78.569*	40.349*	11.002
IMMED EXPO vs. 5 MIN EX...	161.5	78.569*	6.092*	4.275
IMMED EXPO vs. RECOVERY	204.375	78.569*	9.756*	5.41

* Significant at 95%

TABLE XVI

ONE FACTOR ANOVA-REPEATED MEASURES FOR THE DELTA (RELATIVE PERCENT) EEG OBSERVED IN LONG EVANS RATS EXPOSED TO 150 PPM HCl IN FLOWING AIR

One Factor ANOVA-Repeated Measures for X_1 ... X_4

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Between subjects	11	445.229	40.475	7.973	.0001
Within subjects	36	182.75	5.078		
treatments	3	9.729	3.243	.619	.8079
residual	33	173.021	5.243		
Total	47	627.979			

Reliability Estimates for: All treatments: .875 Single Treatment: .635

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Baseline	12	43.333	3.229	.932
Immed Expo	12	43.167	2.791	.806
5-Min Expo	12	42.75	3.934	1.136
Chamber Vent	12	42.167	4.745	1.37

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Baseline vs. Immed Expo	.167	1.902	.011	.178
Baseline vs. 5 Min Expo	.583	1.902	.13	.624
Baseline vs. Chamber Vent	1.167	1.902	.519	1.248
Immed Expo vs. 5 Min Expo	.417	1.902	.066	.446
Immed Expo vs. Chamber ...	1	1.902	.381	1.07

TABLE XVII

ONE FACTOR ANOVA-REPEATED MEASURES FOR THE BETA (RELATIVE PERCENT) EEG OBSERVED IN LONG EVANS RATS EXPOSED TO 150 PPM HCI IN FLOWING AIR

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Between subjects	11	64.729	5.864	1.5	.1744
Within subjects	36	141.25	3.924		
treatments	3	28.729	9.576	2.609	.0547
residual	33	112.521	3.41		
Total	47	205.979			

Reliability Estimates for: All treatments: .333 Single Treatment: .111

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Baseline	12	13.417	1.621	.468
Immed Expo	12	13.5	1.382	.399
5-Min Expo	12	11.833	1.115	.322
Chamber Vent	12	13.833	3.215	.928

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Baseline vs. Immed Expo	-.083	1.534	.004	.111
Baseline vs. 5-Min Expo	1.583	1.534*	1.47	2.1
Baseline vs. Chamber Vent	-.417	1.534	.102	.553
Immed Expo vs. 5-Min Expo	1.667	1.534*	1.629	2.211
Immed Expo vs. Chamber ...	-.333	1.534	.065	.442

* Significant at 95%

TABLE XVIII

ONE FACTOR ANOVA-REPEATED MEASURES FOR THE DELTA (RELATIVE PERCENT) EEG OBSERVED IN LONG EVANS RATS EXPOSED TO 0 PPM HCI IN FLOWING AIR

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Between subjects	11	1570.5	142.773	38.582	.0001
Within subjects	36	140.5	3.903		
treatments	3	28.667	6.556	2.62	.054
residual	33	111.833	3.366		
Total	47	1711			

Reliability Estimates for: All treatments: .973 Single Treatment: .899

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Baseline	12	46.25	5.426	1.567
Immed Expo	12	47.083	7.66	2.296
5-Min Expo	12	47.25	5.172	1.493
Recov	12	48.417	5.775	1.667

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Baseline vs. Immed Expo	-.833	1.529	.41	1.109
Baseline vs. 5-Min Expo	-1	1.529	.59	1.331
Baseline vs. Recov	-2.167	1.529*	2.77	2.883
Immed Expo vs. 5 Min Expo	-.167	1.529	.016	.222
Immed Expo vs. Recov	-1.333	1.529	1.049	1.774

* Significant at 95%

TABLE XIX

ONE FACTOR ANOVA-REPEATED MEASURES FOR THE BETA (RELATIVE PERCENT) EEG OBSERVED IN LONG EVANS RATS EXPOSED TO 0 PPM HCl IN FLOWING AIR

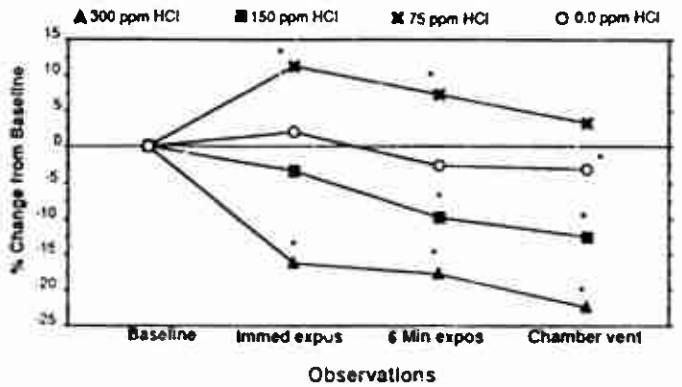
One Factor ANOVA-Repeated Measures for X_1 ... X_4

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Between subjects	11	176.667	16.061	21.414	.0001
Within subjects	39	27	.75		
treatments	3	3.333	1.111	1.649	.2202
residual	33	23.967	.717		
Total	47	203.667			

Reliability Estimates for- All treatments: .953 Single Treatment: .836

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
Baseline	12	10.617	2.353	.679
Immed Expo	12	10.76	2.596	.75
5-Min Expo	12	10.417	1.676	.464
Recovery	12	10.26	1.795	.509

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
Baseline vs. Immed Expo	.167	.703	.077	.462
Baseline vs. 6-Min Expo	.5	.703	.697	1.446
Baseline vs. Recovery	.667	.703	1.239	1.929
Immed Expo vs. 5-Min Expo	.333	.703	.31	.994
Immed Expo vs. Recovery	.5	.703	.667	1.446



* = Significantly different from baseline at the 95% level or greater

Paired t-Test Comparing Each Exposure Group with the Control Group

	DF:	Mean X-Y:	Paired t value	Prob. (1-tail)
300 ppm HCl vs Control	3	-13.31	-2.942	.0302
150 ppm HCl vs Control	3	-5.64	-2.731	.036
75 ppm HCl vs Control	3	6.36	2.803	.0338

Figure 15. Percent Change in Absolute Total Amplitudes Observed in Control and Long Evans Rats Exposed to HCl in Flowing Air

4.4.4 Task 4.1.c - Effects of HCl on the Somatosensory Evoked Potentials (SEP) in Long Evans Rats

4.4.4.1 Background

Afferent electrical activity evoked by stimulation of the face, limb, foot, etc. can be recorded from the postcentral cortex according to a homunculus-like distribution. An analogous placement of skull electrodes is suited to record the maxima of the early cortical complexes of somatosensory evoked potentials (SEP). As reported by Emmert and Flugel[16] the use of a multielectrode array and an electrical mapping system enables us to visualize the area of, for example, the negativity at 20 ms following nerve stimulation. The size of such an area can be used as a measure of the primary somatosensory representation field. Thus, topographic brain mapping might provide a tool to record possible changes of cortical representation resulting from animal or human exposure to volatile toxicants.

4.4.4.2 Somatosensory Preparation

We have employed a 3.0 mA square wave stimulus at a stimulation rate of 8.1 stimuli/second to the biceps femoris. The SEP responses were obtained using a vertex epidural screw electrode and a needle electrode behind the left ear. A series of 3.5 mA square wave electrical stimuli (8.1/sec) were delivered through surface electrodes placed in the biceps femoris while the animal was held in the "nose only" position in the animal exposure chamber. Each epoch consisted on 500 sweeps. The following conditions were fixed for this series of experiments:

Amplifier gain	40 K
Filter Low	50 Hz
Filter High	2800 Hz
Stimulus Rate	11.1/Sec

Figure 16 (right) illustrates the excellent reproducibility obtained during the recording of three baseline (animals exposed to flowing air) SEP epochs of 500 sweeps each using a 3.0 mA square wave at a stimulation rate of 8.1 stimuli/second delivered to the animal's biceps femoris muscle. Figure 16 (left) is the SEP obtained as the average of the three individual SEP epochs illustrated in Figure 16 (right).

Figure 17 contains the statistical information obtained from another SEP recording. The statistical data presented in this figure has been obtained corresponding to the the three major SEP peaks.

The latencies associated with SEP peak number 1 hold the predominant interest of clinical neurophysiologists. The latencies for peak 1 were examined for Long Evans rats exposed to 300 ppm HCl and 0 ppm HCl (controls), the SEP data obtained during the exposure episode were plotted for control and 300 ppm HCl exposed animals. (See Figure 18) Examination of Figure 18 reveals that there is no significant differences between the controls and exposed animals. Consequently we did not conduct exposures at 150 ppm and 75 ppm HCl based on the assumption that these lower levels of the toxicant would not show differing results.

4.4.5 Visual Evoked Potentials

4.4.5.1 Background

Visual stimuli are processed at the cortical level in several different sensory projection areas, most of

Visual stimuli are processed at the cortical level in several different sensory projection areas, most of which display an orderly retinotopic organization.(17) Skrandies(18) has reported that various areas investigated with single-cell recordings appear to be involved in different aspects of visual information processing, and already primary areas of the visual cortex may show functional differences as reflected by the local distribution and densities of physiologically different cell types like simple, complex, and hypercomplex cells.

Human visual evoked brain activity recorded from the intact scalp is influenced by physical stimulus parameters like luminance, contrast, or pattern size as well as by the location of the stimulus on the retina.

When responses elicited by stimuli presented to the upper or lower retinal half were compared by Skrandies[18] differences between stimulus conditions in terms of component latency and component location becomes obvious. Such factors must be analyzed in a future **Phase II Program** together with the additional effects that acid gases such as HCl may have on the visual processes.

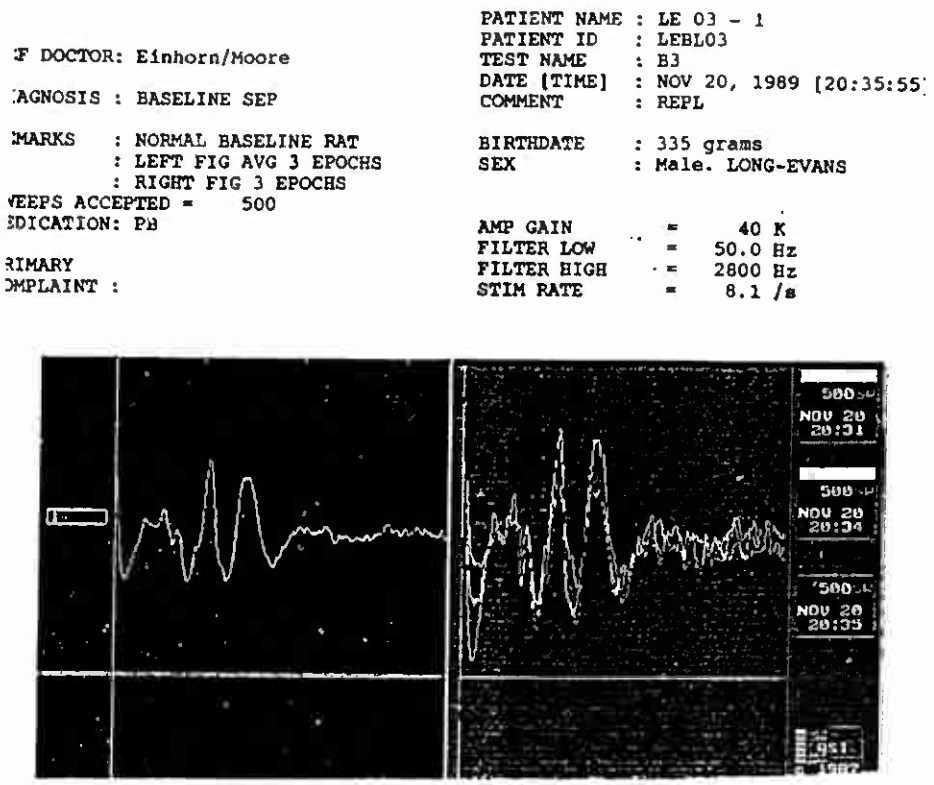


Figure 16. Typical Computer Output of Somatosensory Evoked Potential (SEP) Observed in Long Evans Rats

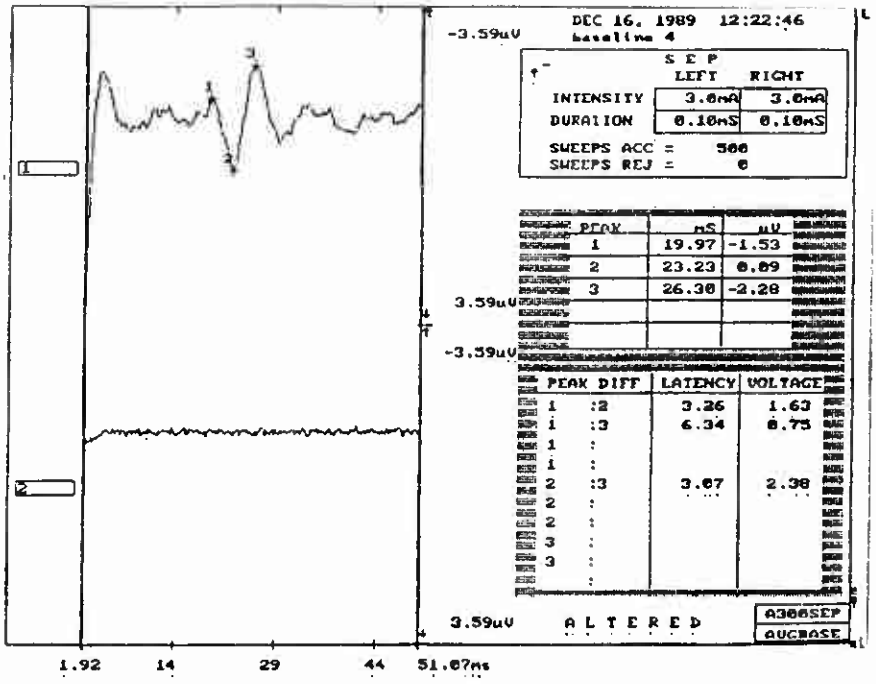


Figure 17. Typical Computer Output of Somatosensory Evoked Potential (SEP) Showing Quantified Peak Latencies and Amplitudes Observed in Long Evans Rats

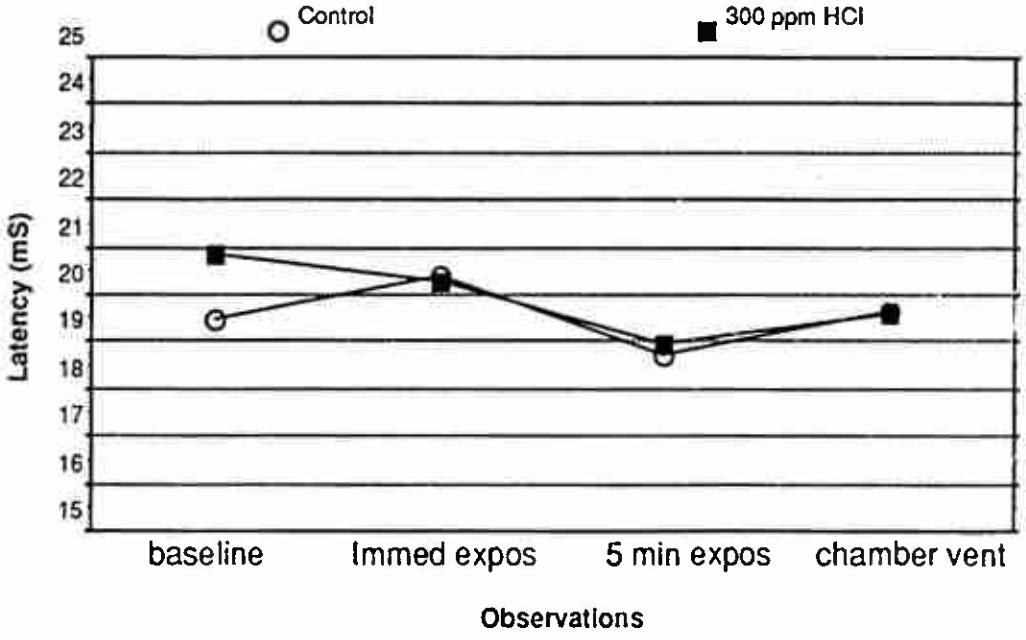


Figure 18. Latencies (mS) for SEP Observed in Long Evans Rats Exposed to 300 ppm HCl and Controls (0 ppm HCl) in Flowing Air

4.4.5.2 Visual Evoked Potential (VEP) Preparation

We have employed the QSI-9000's strobe system to deliver 3.7 stimuli/sec with a 1.96 ms delay time. The VEP responses were obtained using a vertex epidural screw electrode and a needle electrode behind the left ear. The flash stimuli were delivered while the animal was held in the "nose only" position in the animal exposure chamber. Each epoch consisted on 200 sweeps. The following conditions were fixed for this series of experiments:

Amplifier gain	40 K
Filter Low	50 Hz
Filter High	200 Hz
Stimulus Rate	3.7/Sec

```
REF DOCTOR: Einhorn/Moore

DIAGNOSIS :

REMARKS    : RCl 300 ppm
           : LEFT AVG 3 EPOCHS
           : RIGHT 3 EPOCHS
SWEEPS ACCEPTED =    200
MEDICATION: PB

PRIMARY
COMPLAINT :
```

```
PATIENT NAME : LONG-EVANS
PATIENT ID   : BE
TEST NAME    : 3
DATE [TIME]  : NOV 30, 1989 [12:55:46]
COMMENT      : HCI 300 ppm

BIRTHDATE    : Aug 31, 1989
SEX          : Male

AMP GAIN       =    40 K
FILTER LOW     =   0.5 Hz
FILTER HIGH    =   200 Hz
STIM RATE      =   3.7 /s
```

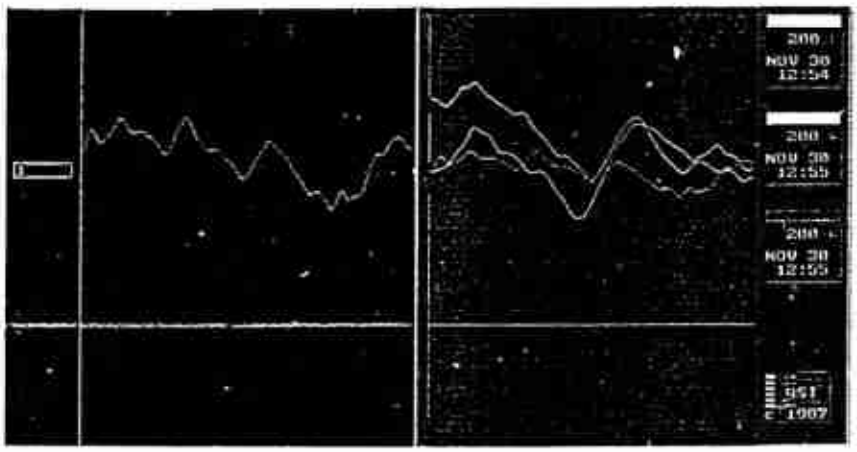


Figure 19. Typical Visual Evoked Potential (VEP) Observed in Long Evans Rats

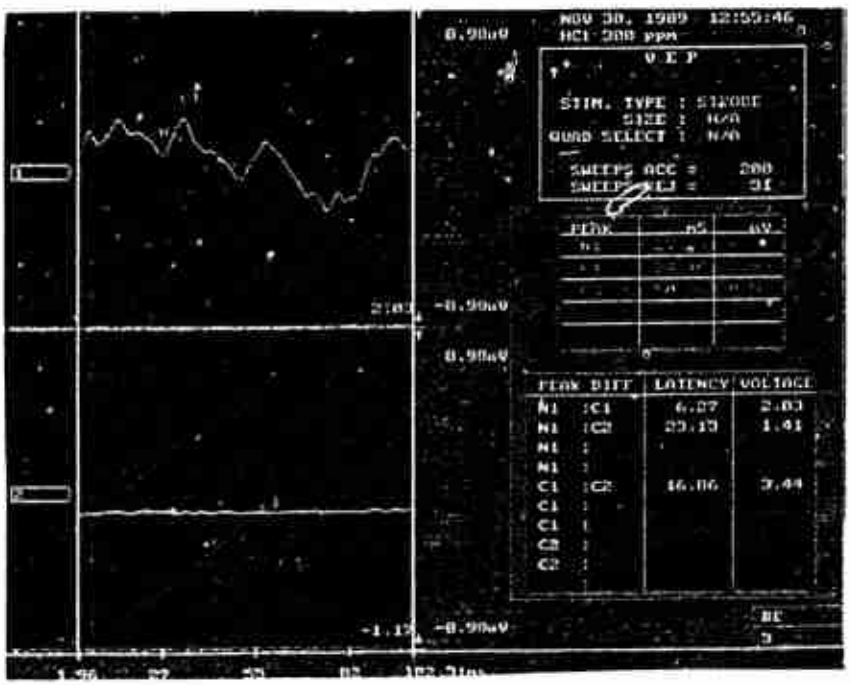


Figure 20. Typical Visual Evoked Potential (VEP) Observed in Long Evans Rats Showing Quantified Peak Latencies and Amplitudes

Figure 19 (right) illustrates the results obtained during the recording of three baseline (animals exposed to flowing air) VEP epochs of 200 sweeps each using the strobe flash at a stimulation rate of 3.7 stimuli/ second. Figure 19 (left) is the VEP obtained as the average of the three individual VEP epochs illustrated in Figure 19 (right).

Figure 20 contains the statistical information obtained during the recording of the baseline VEP's shown in Figure 19 (right). This statistical data presented in Table XX has been obtained corresponding to the the three VEP peaks illustrated in Figure 19 (left).

TABLE XX

VEP BASELINE PEAK LATENCIES AND AMPLITUDES
(Animal BB-Flowing Air)

Peak	Latencies of Peaks	Peak Amplitudes
N1	25.48	4.22
C1	29.01	5.00
C2	47.43	0.00

Figure 21 (right) illustrates the reproducibility obtained during the recording of three HCl exposure (animals exposed to 300 ppm HCl in flowing air) VEP epochs of 200 sweeps each using the strobe flash at a stimulation rate of 3.7 stimuli/second. Figure 21 (left) is the VEP obtained as the average of the three individual SEP epochs illustrated in Figure 21 (right).

Figure 22 contains the statistical information obtained during the recording of three HCl exposure (animals exposed to 300 ppm HCl in flowing air) VEP epochs of 200 sweeps each using the strobe flash at a stimulation rate of 3.7 stimuli/second. The statistical data presented in Table XXI has been obtained corresponding to the the three VEP peaks illustrated in Figure 21 (left).

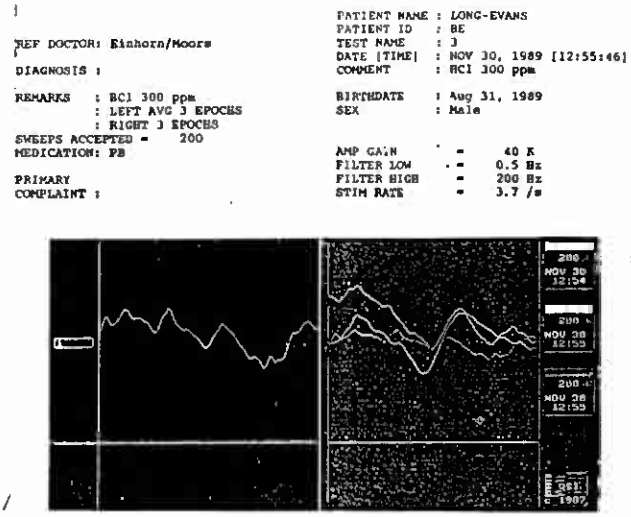


Figure 21. Typical Visual Evoked Potential (VEP) Observed in Long Evans Rats Showing Overlapping of Three VEP Determinations from the Same Long Evans Rat

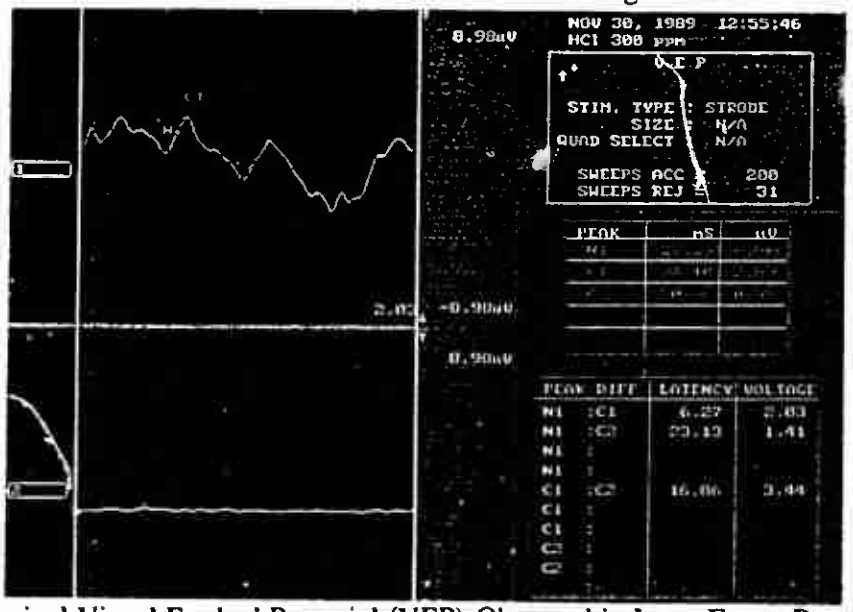


Figure 22. Typical Visual Evoked Potential (VEP) Observed in Long Evans Rats Showing Quantified Amplitudes and Latencies Based on Three Determinations in the Same Animal

TABLE XXI

VEP PEAK LATENCIES AND AMPLITUDES
(Animal BE - 300 ppm HCl in Flowing Air)

Peak	Latencies of Peaks	Peak Amplitudes
N1	27.83	0.86
C1	34.10	2.89
C2	50.96	-0.55

A review of the statistical data contained in Tables XX and XXI indicates that there were marked increases in the VEP latencies in animals exposed to 300 ppm HCl in flowing air as compared to the latencies obtained in the control animals. There also appears to be a significant lowering of VEP peak amplitudes generally decreasing with peak number during the development of the HCl induced intoxication syndrome. Early analysis of data obtained during work conducted following the close of this reporting period seems to indicate that the VEP values tend to reach levels which remain almost constant once the animal reaches an apparent incapacitation level.

Additional VEP studies conducted within the scope of the **Phase I Program** produced marked variations between animals within a given group as well as between the control and exposed animals. There exists the possibility that the interaction of HCl on the cornea may account in part for some of the observed variability. This variability precluded further work within the limited scope of the **Phase I Program.** Further studies will be required to substantiate these trends prior to utilizing the VEP protocol for the assessment of HCl induced toxicity.

4.5 Analysis of pH and Blood Gases

The pH and the blood gases pO_2 and pCO_2 were determined using the Ciba-Corning Model 178 pH/ Blood Gas System. A complete analysis requires only 85 microliters of blood. Baseline blood chemistry measurements will be obtained from preselected animals prior to the start of the exposure episode.

Handling of the instrumented animals during withdrawal of blood specimens effected the measurements of EEG and EMG activity during our previous studies. Our experimental protocol was modified from the initial design which called for extraction of blood samples from all animals during the exposure episode. We will use the modified procedure during the **Proposed Phase I Program;** blood will be withdrawn only from the uninstrumented animals during the actual exposure episode, where feasible blood specimens were taken from the instrumented animals at the end of the exposure episode. Justification for this approach is based on the fact that blood values measured from instrumented and uninstrumented at the end of the exposure episodes in our previous studies showed little differences.

A three-factor ANOVA was performed for pH and blood gas parameters of Long Evans rats before, during, and after exposure to 0 ppm (controls), 75 ppm, 150 ppm, and 300 ppm HCl in flowing air. A typical set of ANOVA results are shown in Table XXII indicating no level of significance for differences in observations (treatments). Figures 23-25 illustrate the results of blood pH, pO_2, and pCO_2, respectively. Examination of these figures indicate that the concentration of HCl and exposure time had little effect of the blood parameters. This finding was in keeping with previous studies of HCl intoxication by the project team. There is evidence that the pO2 results may have been affected as a result of the animal's breath holding in an attempt to avoid the noxious effect of HCl at 300 ppm HCl.

TABLE XXII

ANOVA OF pH AND BLOOD GAS PARAMETERS OBSERVED IN LONG EVANS RATS BEFORE, DURING, AND AFTER EXPOSURE TO 75 PPM HCl IN FLOWING AIR

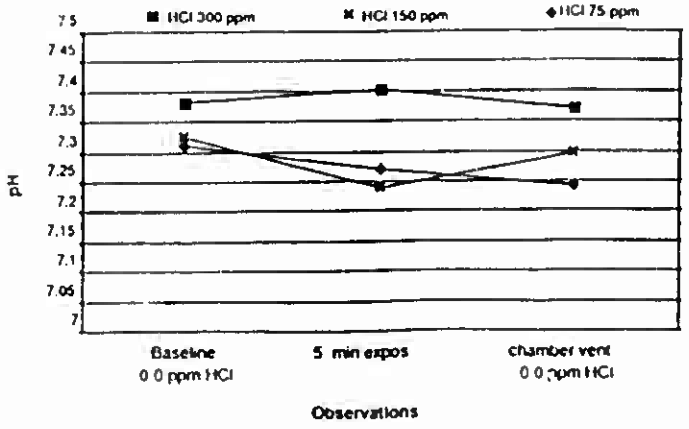
Anova table for a 3-factor Analysis of Variance on Y_1: Value

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Column 1 (A)	2	232.074	116.037	.	.
OBSERVATION (B)	2	364.963	182.481	.	.
AB	4	836.815	209.204	.	.
BLOOD PARAMETER (C)	2	274194464.963	137097232.481	.	.
AC	4	26660.148	6665.037	.	.
BC	4	17973.926	4493.481	.	.
ABC	8	5705.63	713.204	.	.
Error	0	0	.		

There were no missing cells found.

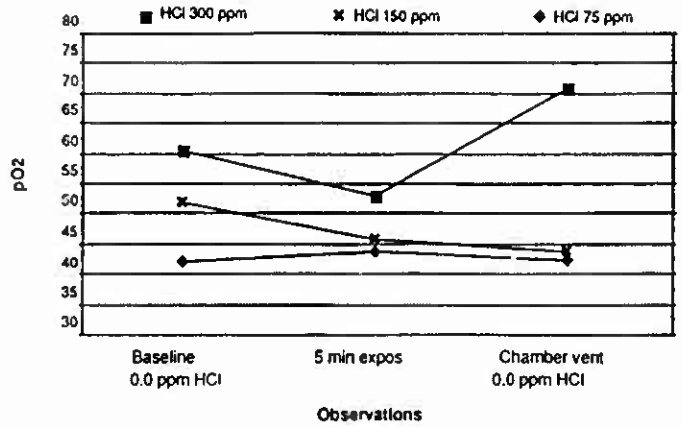
The BC Incidance table on Y_1: Value

	BLOOD PARA...	pH	pCO2	pO2	Totals:
OBSERVATION	BASELINE	3 7319	3 573.333	3 420.667	9 2771
	FIVE MIN E...	3 7275.333	3 625.333	3 439.333	9 2780
	CHAMBER...	3 7248.667	3 654.667	3 424	9 2775.778
Totals:		9 7281	9 617.778	9 428	27 2775.593



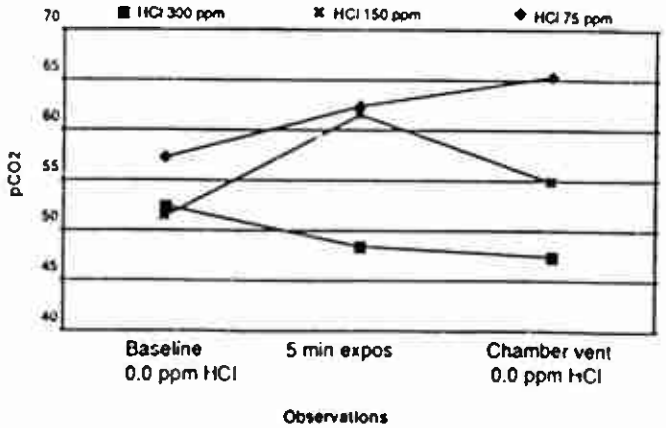
A 3-factor ANOVA shows no significant difference between observations (treatments) at any HCl exposure level

Figure 23. Blood pH Observed in Long Evans Rats as a Function of HCl Exposure in Flowing Air



ANOVA 3-factor analysis shows no significant difference between observation (treatments) at any exposure level.

Figure 24. Blood pO_2 Observed in Long Evans Rats as a Function of HCl Exposure in Flowing Air



A 3-factor ANOVA shows no significant difference between observation (treatments) at any exposure level.

Figure 25. Blood pCO_2 Observed in Long Evans Rats as a Function of Exposure to HCl in Flowing Air

4.6 Analysis of Ancillary Effects of the Exposure to HCl on Long Evans Rats

4.6.1 Weight Gain (or Loss)

The Long Evans rats used studied within the scope of the **Phase I Programwere** examined periodically prior to, and during the 14-day postexposure period. The average daily weight gain for the Long Evans rats used in this study ranged between 3.65 and 4.2 grams. Figure 26 illustrates the average weight gain between the control groups and the animal groups exposed to 75 ppm; 150 ppm; and 300 ppm HCl. The weight gain results are contrary to those observed in our previous studies when animals were exposed to sufficiently higher levels of HCl (600 ppm to 3000 ppm).

A one factor ANOVA using repeated measures showed no statistical difference between treatment groups with respect to weight with a $p = 0.906$. (See Table XXIII)

4.6.2 Miscellaneous Observations

Random gross physiological examination of the control and exposed animal groups, likewise, showed no observable differences in the animal's exploratory behavior, nuzzle response, lid-corneal and ear reflexes, pain response, the righting reflex, or posture. General observations conducted randomly during the Phase I Program showed no significant differences in the lack of spontaneous activity such as preening or movement. There was no evidence of respiratory distress observed in control or exposed animals at periods greater than 24 hours postexposure. Because of severe time constraints and consistent with the lack of significant differences in homeostasis or the gross physiological examinations of the control and exposed animal groups, we eliminated the use of the modified "T"-maze behavioral tests within the scope of the **Phase I Program.**

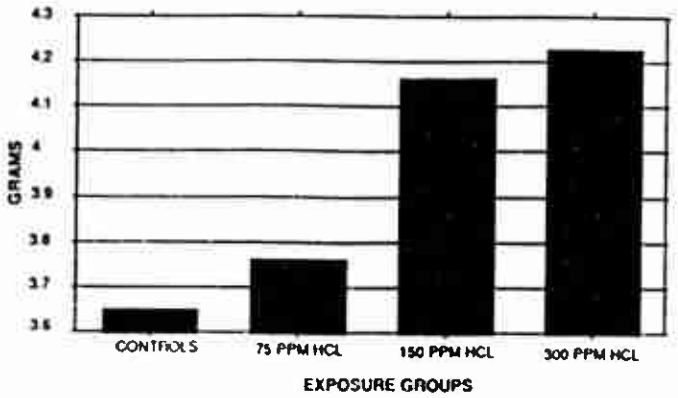


Figure 26. Average Daily Weight Gain Observed in Long Evans Rats During the 14-Day Postexposure Period Following Exposure to HCl in Flowing Air

TABLE XXIII

ONE FACTOR ANOVA-REPEATED MEASURES OF AVERAGE DAILY WEIGHT GAIN FOR HCl EXPOSED LONG EVANS RATS

One Factor ANOVA-Repeated Measures for X_1 ... X_4

Source:	df:	Sum of Squares:	Mean Square:	F-test:	P value:
Between subjects	4	4.644	1.161	.896	.4903
Within subjects	15	19.429	1.295		
treatments	3	.849	.283	.183	.906
residual	12	18.58	1.548		
Total	19	24.074			

Reliability Estimates for- All treatments: -.116 Single Treatment: -.027

1

Group:	Count:	Mean:	Std. Dev.:	Std. Error:
CONTROLS	5	3.74	.799	.357
75 PPM HCL	5	3.76	1.128	.505
150 PPM HCL	5	4.066	1.527	.683
300 PPM HCL	5	4.226	1.251	.559

2

Comparison:	Mean Diff.:	Fisher PLSD:	Scheffe F-test:	Dunnett t:
CONTROLS vs. 75 PPM HCL	-.02	1.715	2.153E-4	.025
CONTROLS vs. 150 PPM ...	-.326	1.715	.057	.414
CONTROLS vs. 300 PPM ...	-.486	1.715	.127	.618
75 PPM HCL vs. 150 PPM...	-.306	1.715	.05	.389
75 PPM HCL vs. 300 PPM...	-.466	1.715	.117	.592

3

4.7 Interpretation of the Phase I Program Results

The interpretation of these findings must be postponed until further data are collected and analyzed. We have during the **Phase I Program** demonstrated our ability to develop a quantitative neurophysiologic monitoring protocol which is capable of identifying the early decrements in neurophysiological activity in response to a toxicant. At present there is insufficient information to determine the mechanism for the changes observed nor to fully evaluate those specific effects in terms of neurophysiological function. However, we can postulate several hypothesis based on the limited data review.

First, the BAER appears to be the most sensitive EP measure in this protocol. We have, despite the small number of animals used in our experiments, observed statistically significant changes in the BAER response when the normal control group was compared to the combined exposure groups. Secondly, it appears that the greatest changes were recorded after the five minute exposure period and the the immediate period following chamber venting. This may be attributed in part to the animal's use of breath holding to delay or mitigate the initial toxic exposure. In this case the animal's later response, after holding its breath, continues to increase in the degree of change from baseline values. Similar responses were reported previously by Einhorn and Grunnet [3] when Long Evans rats were exposed to the thermal decomposition products of polyvinyl chloride polymers. It should be noted that the major thermal decomposition products was HCl. In these previous studies the plots of carboxyhemoglobin (COHb) saturation vs oxyhemoglobin (O_2Hb) saturation was a straight line at during the exposure of Long Evans

rats to levels of carbon monoxide ranging from 500 ppm to 15,000 ppm. When HCl was mixed with carbon monoxide during other exposure experiments the Long Evans rats exhibited a major deviation in the plot of COHb vs O_2Hb. Immediately following the introduction of HCl mixed with CO into the animal exposure chamber, the COHb vs O_2Hb curve was parallel to the x-axis. The period of time that the COHb vs O_2Hb curve remained parallel to the x-axis was directly proportional to the concentration of HCl in the HCl/CO mixture within the chamber's environment. At a period of time ranging from one to three minutes, the animals were forced to breath more deeply and more rapidly facilitated by the drive for the system to obtain more oxygen. During the next several minutes the COHb vs O_2Hb rapidly returned to normal. This response was observed over a wide range of HCl concentrations until the level of HCl (approximately 1200 ppm) produced massive edema within the animal subjects.

We plan to monitor respiratory rate and blood pressure during animal exposures to be conducted within the scope of the **Phase II Program.** Finally, regardless of the exposure condition, we have demonstrated that the BAER may be sensitive enough to effectively measure the changes of neurophysiologic functioning during and following exposure of animals to potentially dangerous neurotoxins.

With respect to the qEEG results, we observed statistically significant changes in the activity of delta, alpha, and beta in exposed animals as compared to the control. These changes, the increases in delta and decreases in alpha and beta activity, reflect the deviations in the brain's electrical activity which apparently are caused by the exposure to HCl gas.

The changes observed are classical signs of functional pathology in the EEG caused by stroke, tumor, or vascular disease of the brain. Zappulla and Ryder[19] described changes in qEEG in a group of patients with structural lesions of the brain. They reported statistically significant decreases in alpha and increases in delta activity ipsilaterally to the side of the lesion. Often the area of enhanced or decreased activity extended further than the boundaries of the lesions as seen on radiologic studies. The pattern of changes reported in this pilot **Phase I Program** study are similar to those classical signs of abnormal EEG. Despite the relatively short and limited exposure of the animals to HCl in flowing air specific trends did appear which based on clinical experience may be construed as abnormal findings. However, any conclusions regarding the results reported here must first be replicated in a larger number of experiments and supported by additional evidence.

This project offered a valuable avenue in the early assessment of neurologic damage caused by animal exposure to HCl, normally considered as a respiratory irritant and not a neurotoxin. The qEEG data presents information regarding a variety of very subtle indicators of brain activity which could be used to determine when hazardous agents first have their impact on humans as well as providing the means to monitor such changes over time to provide the researcher with a quantitative method for following neurologic functioning.

5 DISCUSSION

5.1 Overview of Electrophysiologic Assessment of Neurologic Functioning)

Evoked potentials and other neurophysiological procedures offer the opportunity for better neuro-evaluative techniques. Such techniques reflect rather directly the functioning of neural tissue sampled by the electrodes. Some of the measurements obtained using these neurophysiological techniques are extremely reliable both within and between the animal subjects or human subjects, and in some cases,

they are similar even among several species. The neurophysiological procedures which employed within the scope of the **Phase I Program** permitted the objective quantification and extensive evaluations of the nervous system using advanced recording methods.

It should be noted that although the evoked potential methods have been highly developed in clinical neurology and the responses are routinely recorded from animals, the procedures and electrophysiologic endpoints have not been widely applied in the areas of toxicology and neurotoxicology. We have, within the scope of Task 3, attempted to develop and validate in rodents, and will in the future develop within the scope of the **Phase II Program** utilize in pigs and primates methods that should prove to be applicable to military personnel exposed to weapon system exhausts. We will utilize epidurally recorded potentials elicited by stimuli that are routinely used in the clinic.[20-24]

There are two compelling reasons why evoked response methods like those used in neurological studies of humans need to be developed in animals: (1) it is not clear from the clinical literature how evoked potentials, such as we demonstrated in our **Phase I Program** HCl exposure studies, change in response to variations in the concentration-time product (Ct) and (2) the neurotoxicity of irritants, such as those found in weapon systems exhausts, can be ethically studied only in animals.

Field potentials generated in the brain or from the PNS can be detected on the scalp. When elicited by specific sensory, motor, or cognitive events they constitute evoked potentials (EPs) or event-related potentials (ERPs). We have, within the scope of Task 3, identified which of the several types of electrophysiological responses will provide a reliable characterization of nervous system activity in response to the previously mentioned categories of responses commonly found in humans exposed to volatile irritants. We have attempted to develop an understanding of causal mechanisms pertaining to the inhalation of the most common irritants found in weapon systems exhausts through the detailed mapping of foci for toxic effects, especially those associated with early decrements of performance, such as those we have identified in our recent animal exposure studies associated with the early response to high levels of CO, CO and CO_2, or CO and HCl, prior to observance of major changes in blood parameters associated w h homeostasis.

The development of the digital averager and the discovery of potentials related to complex psychological processes - the P300 wave, the contingent negative variation, and the readiness potential have led to a greatly expanded study of evoked potentials

Figure 27 relates EEG and event-related potentials (ERPs) in a paired stimulus (W and S) paradigm illustrating transient and slow potentials associated with sensory and cognitive processes. Hillyard and Galambos[25] have used the schematic representation illustrated in Figure 27 to demonstrate this paradigm and response. The subject shown in Figure 27 was stimulated by a flash of light which functions in the paired stimulus paradigm to warn the subject that a click will occur 1 second after the flash, and further that the subject must engage in some meaningful covert or overt act within some minimum time after occurrence of the click in order to be considered adequately functional. After processing the flash, which is illustrated by the occurrence of long-latency components labeled N_1 and P_2 the EEG showed a slow negative shift of the EEG baseline (the contingent negative variation - CNV) reflecting the subject's developing state of anticipation and preparatory activity related to culminating this act. Successful performance of the task permitted the subject's brain to continue processing information, as shown by the complex sequence of poststimulus potentials encompassing early components generated in the auditory brainstem nuclei (I-V) and very late components reflecting attentional (N_1) and mnemonic (P_3) processes.

Potentials evoked by somatosensory stimulation have been reported to be extremely useful because they can be recorded at several levels of the neuroaxis, providing information about conduction in various parts of the peripheral and central pathways as indicated hy Figure 28.[26] This type of measurement was used within the scope of Task 4.1 c of the **Phase I Program**, however, additional studies will be required before the SEP protocols will be usefull in the assessment of the toxic response to respiratory irritants. The somatosensory evoked potentials are useful in confirming early decrement of performance such as we have reported previously on the reduction of nerve conduction velocity as measured in the ventral caudal nerve of the Long Evans rat as an indicator of CO intoxication.[27-29] The recording of somatosensory response from different regions of the neuroaxis should permit us to identify localization of deficits to peripheral nerves, sections of the spinal cord, brainstem, and cortex.

We have previously reported on changes in the nerve conduction velocity of Long Evans rats as a consequence of changes observed during ultrastructural examinations at the node of Ranvier.[2,30] Such changes were long lasting, but developed only when the animals had been exposed to concentrations of CO sufficient to induce anoxic shock in test animals. If we are able to utilize the recording of evoked potentials to indicate very early changes, as suggested by our feasibility studies conducted within the **Phase I Program**, we should then be able to develop a protocol for the quantitative measurement of decrements of performance in the intoxication syndrome.

Xintaras et al[31] utilized the evoked potential technique to investigate the consequences of exposure to CO on the visual system. In a series of unique experiments, they recorded the averaged evoked potentials from both the superior colliculus and the visual cortex of rats. In these early studies the superior colliculus evoked potential was a better indicator of CO toxicity than was the visual evoked potential. Changes in amplitudes and latencies were reported at concentrations of 50 ppm CO after exposure for 1 hour. The COHb saturation levels were less than 4 %. The major shortcomings of this early work was the informal nature of the experimental design and the largely qualitative nature of the results.
Dyer[32] attempted to determine answers to the following questions in his studies conducted on Long Evans rats: (1) What is the best way to measure waveform, peak-to-peak or baseline-to-peak?; (2) How stable are different peaks with respect to each other?; (3) How stable are different peaks over time?; (4) How sensitive are the different peaks to variations in stimulus intensity and stimulus frequency?; (How great is the between-animal variability of the recorded response?

In his study Dyer[32] implanted bipolar leads straddling the stratum griseum superficiale of the superior colliculus. Figure 29 is a typical waveform recorded from the superior colliculus of the rat. The arrow indicates the flash stimulus. Negative deflections are upward.

Dyer[32] also reported that the amplitudes of the superior colliculus evoked potential increased with increasing concentrations of CO up to 500 ppm. At 1000 ppm CO (55 % COHb) Dyer[32] reported a decline in amplitudes. These results coincide with the results of the EEG and EMG measurements we made during our recent animal exposure studies.

Dyer[32] further reported that in his studies of the visual evoked response, the first major deflection (P_1-N_1) increased in amplitude with exposure to CO. Unlike Dyer's[32] measured evoked potential from the superior colliculus, the (P_1-N_1) amplitudes in the cortex were significantly greater than the controls at 6 % COHb.

Unlike the results obtained with the superior colliculus, the (P_1-N_1) amplitudes obtained in measurements

of the visual evoked response at 55 % COHb were significantly less than amplitudes at 38 % COHb. It should be noted that the reported results by Dyer are in agreement with the data obtained using the EEG and EMG monitoring techniques within the scope of our recent HCl studies.

During the Future Phase II Program we plan to use the QSI multisignal stimulator in our behavioral audiology studies with the the QSI-9000 System in an attempt to refine our measurements of the early decrement of performance during the development of the intoxication syndrome associated with HCl. This multisignal stimulator provides a wide variety of click, tonal, and noise signals for behavioral audiology as well as an auditory evoked potential tests. These signals include filtered and unfiltered clicks, single and half-cycle sinusoids, and gated tones with a variety of envelopes. The sophisticated QSI-9000 System will permit evaluation of the amplitude and periodicity during the critical 100 - 300 seconds following the startle stimulus.

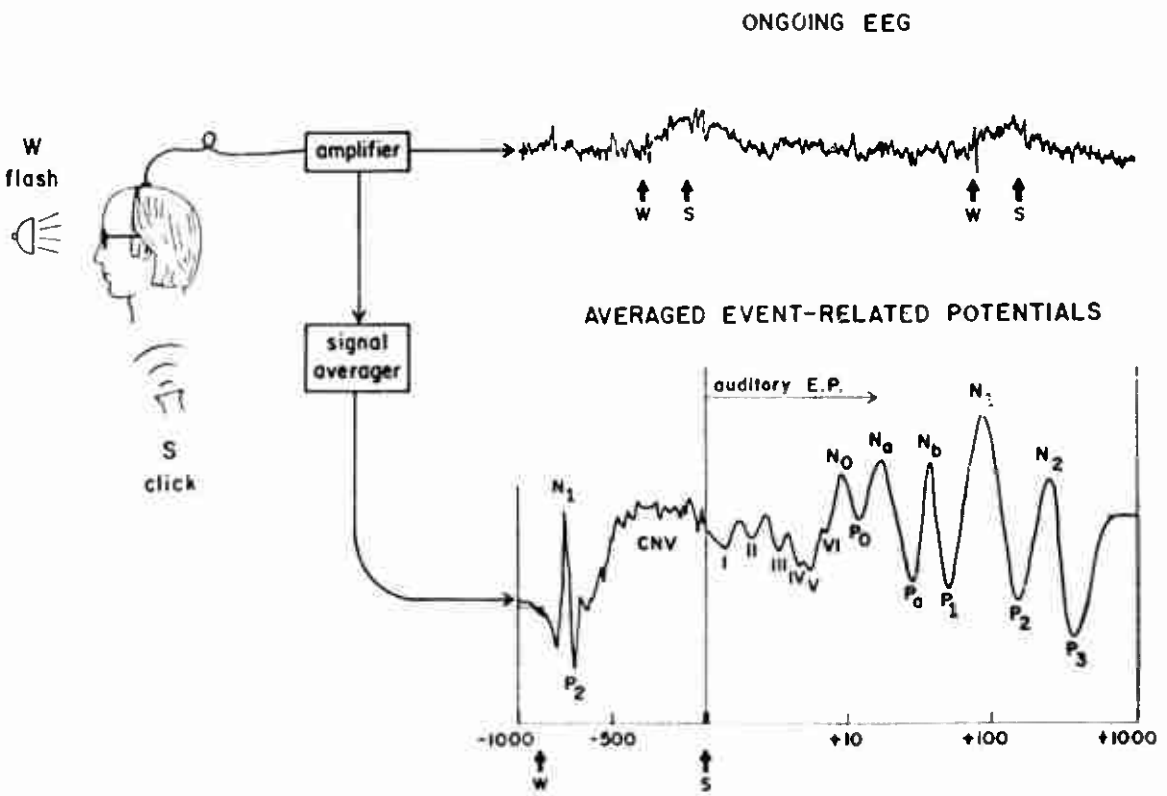


Figure 27. Schematic EEG and Event-related potentials (ERPs) in a Paired Stimulus (W and S) Paradigm Showing Transient and Slow Potentials Associated with Sensory and Cognitive Processes. In the Ongoing EEG a Slow Potential - the Contigent Negative Variation (CNV) - is evident, but the Other Components are Obviously Only in the Averaged EPR. The Earliest Compontents Elicited by a Click(s) Represent Activity in the Brainstem Auditory Nuclei, and the Late Waves Represent Thalamic and Cortical Processes. [25]

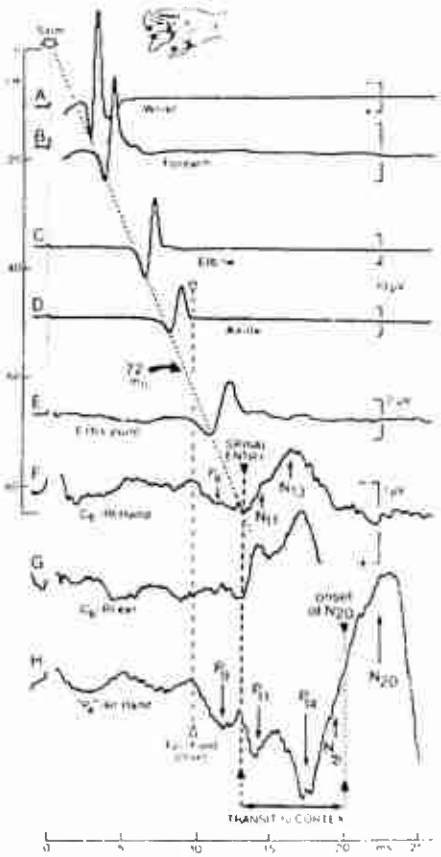


Figure 28. Sensory Nerve Potentials Recorded from the Median Nerve at the Wrist and otherroximal Locations. The Onset of the Cortical N_{22} recorded from the Contralateral Parieatal Scalp INdicates a Spinocortical Transit Time of 6.0 msec[26]

Figure 29. A Typical Flash Evoked Potential from the Visual Cortex of the Male Hooded Rat. Arrow Indicates F. Negative Deflections are Upward[32]

5.2 Utility of Brainstem Auditory Evoked Potentials in Diagnosis of Neurologic Disorders

Evoked potentials are obtained by stimulating a specific sensory modality and recording a short epoch of the brain's electrical activity that is "time locked " to the stimulation. Over time, with several hundred replications the computer can average and summate the activity of the brain while all other activity (presumably random) cancels itself out. What is obtained is an evoked response, a series of waves that represent the the neurologic activity caused by the discrete stimulation of the nervous system. These waveforms can then be examined with respect to their latency, amplitude, and morphology. Because of the excellent replicability of evoked responses they serve as an ideal, sensitive method of evaluating changes in the functioning of the nervous system. Of all of the various kinds of evoked potentials described from man and laboratory animals, the brainstem auditory evoked potentials lend themselves most readily to comparison among widely different species, both mammalian and nonmammalian, because of their robustness, relative independence of electrode placement, state of arousal, or anesthesia.[33] Comparison among diverse vertebrates is needed because neither anatomy no behavior predicts whether sharks, fish. frogs, turtles, porpoises, rats, or humans will have similar evoked responses; comparison might yield clues to the mechanisms of the response or of audition. Presumahly these far-field potentials depend on at least two special features of the relevant neurons: a high degree of synchrony of discharge and a favorable open-field geometry. The BAER also requires a series of brainstem nuclei that preserve synchrony in an adequate subset of the neurons, while they relay responses through successive levels.

Bullock[33] has stated that electrodes and their placement raise questions pertaining to equivalent recording geometry in animals of different size. Approximations to vertex and mastoid placement are not essential since considerable differences in locus make little difference in the recorded wave forms. Corwin et al[34] have suggested that to be in the most consistent relation to the generators, which are not precisely known but probably within the brainstem, that the placement of one electrode just behind the cerebellum, thc other in front of the cerebrum, both in the midline.

The brainsten auditory evoked response is shown schematically in Figure 30.[35] The BAER, as illustrated in Figure 30, consists of five major components additively derived from potentials occurring in the nuclei and leemnisci of this system. Figure 31 illustrates the BAER measured in various species. The most significant finding in the study of BAER's in all mammals to date is that the morphology of the BAER, while divergent in detail, (See Figure 31), is notably uniform in number and latency of the first four or five waves. The relative amplitudes vary widely but peaks I to V, and usually VI, can be identified by latency. Even when comparing species as diverse in size and auditory specialization as rat and dolphin, the latencies of waves I, II, III, AND IV are within 0.1 msec, in spite of a large difference in the conduction distance. The human is curious in having a slower sequence, the time between waves I and IV is 1.1 msec longer.

Maurer [36] has described the auditory pathway as follows: The peripheral portion of the auditory pathway originates from the branches of the bipolar ganglionic cells of the ganglion spirale. The nervous processes contain myelinated axons and nonmyelinated fibers that connect to the hair cells through synapses. After the afferent peripheral processes form a bundle in the ganglion spirale, the acoustic nerve passes through the internal auditory canal and the cerebellopontine angle to the posterior brain stem where the central impulse transmission begins. Anatomically we recognize nerve cell aggregations and nervous pathways. In the medulla we find the dorsal and ventral cochlear nuclei, in the pons, the superior olive, and in the pontomesencephalic area the lateral lemniscal nuclei and the inferior colliculi.

The cortical projection field, the gyrus of Heschl in the temporal lobe, is reached via the medial geniculate body and the acoustic radiation. The nuclei are interconnected with a complex system of pathways, which results in the organs of Corti being connected not only with all nuclear areas, but also with both cortical projection fields as illustrated in Figure 32.

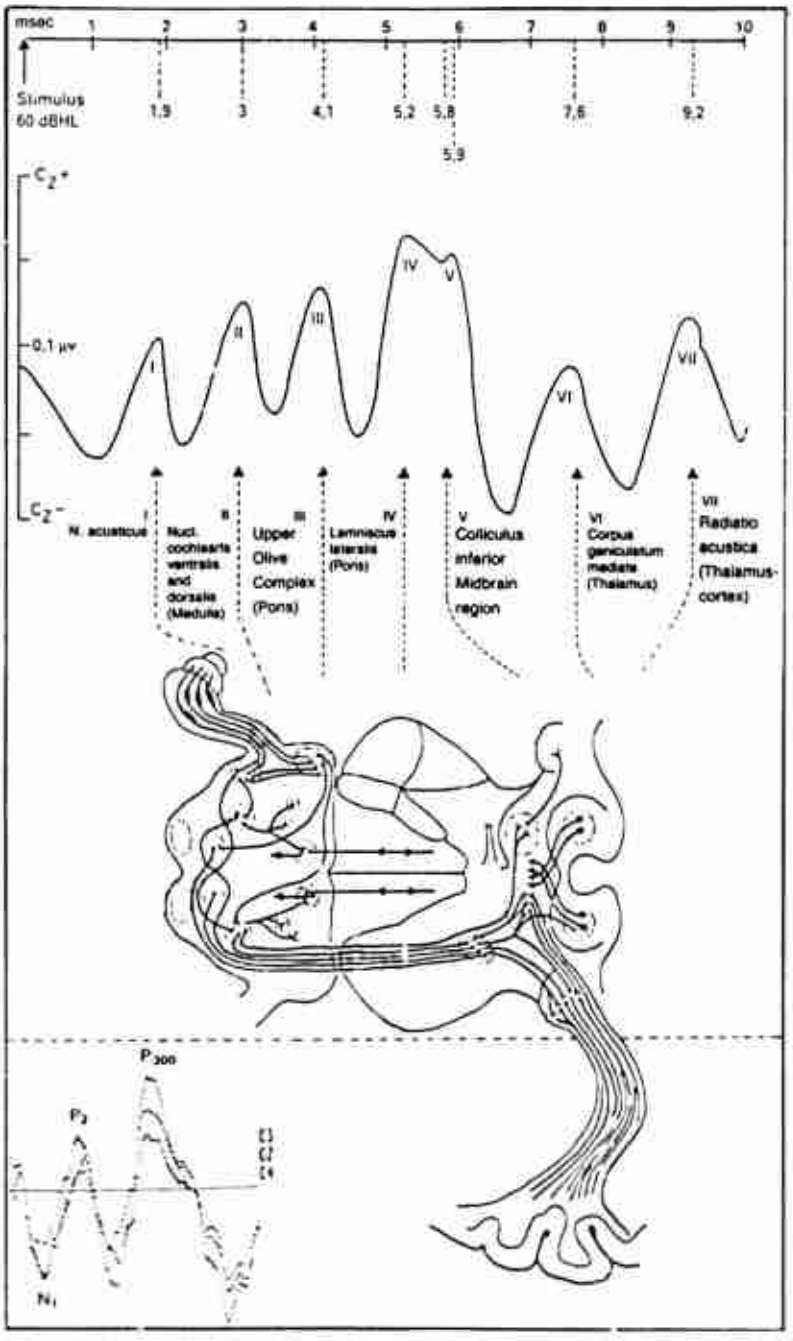


Figure 30. Simplified Schema of Generator Sites of Brainstem Auditory Evoked Response Components, Giving Rise to the Scalp-recorded Composite Potential Abbreviations: AN - auditory nerves; CN - cochlear nuclei; SO - superior olives; LL - lateral lemnisci; IC - inferior colliculi; MG - medial geniculates [35]

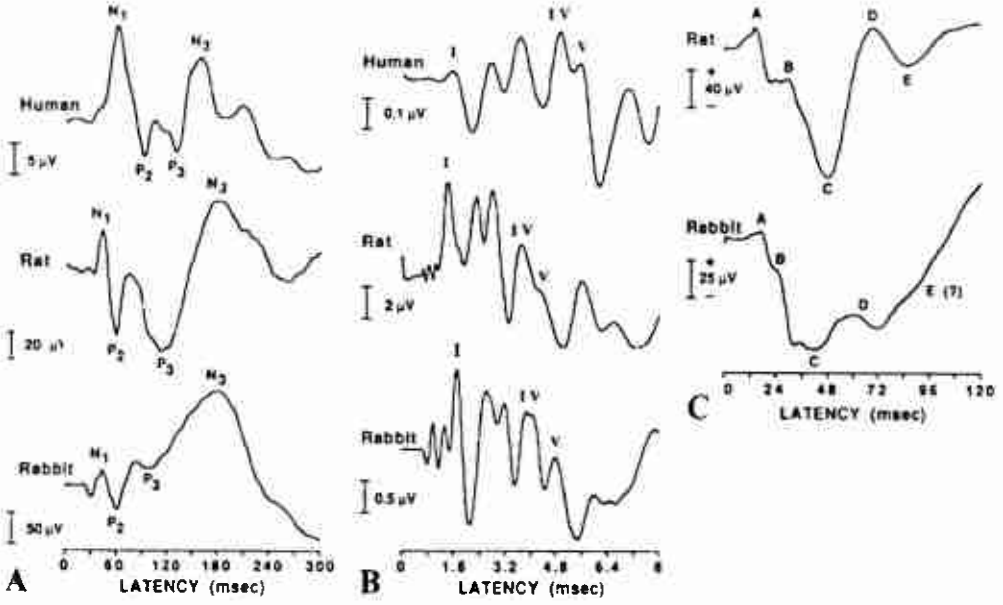


Figure 31. (A) Composite Flash Evoked Potentials (VEP) Recorded from the Rat (n=8), rabbit (n=8), and a Human . (B) Composite Brainstem Auditory Evoked Responses (BAER) recorded from the rat (n=11), and rabbit (n=8), and a Human. Note: the Fusion of Peaks of II and III in each BAER, the Fusion of Peaks IV and V in rRat and Human BAERs, and the Deep Valley After Peak V in All Three Species. (C) Composite Somatosensory Evoked Potentials (SEP) Recorded from the Rat (n=12) and rabbit (n=8). The Early Components of the SEP (Peaks A and B) are Similar, Whereas the Later Components (D and E) are More Variable and There is Some Uncertainty of Peak Correspondence Between the Two Species.[37]

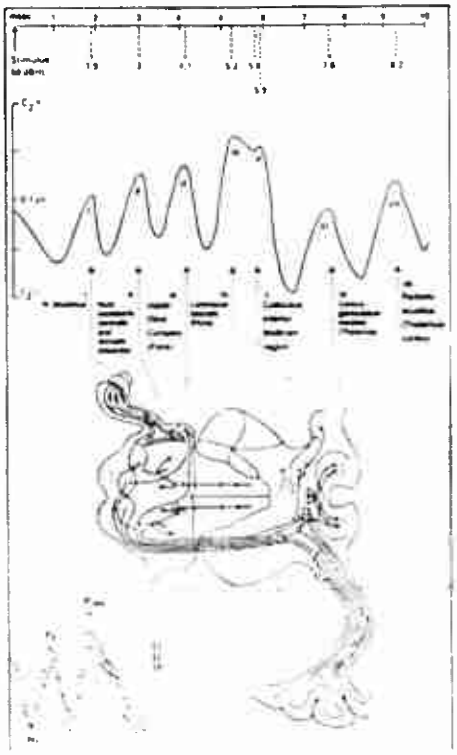


Figure 32. Waves I - VII of the BAER and Their Assignment to Structures Such as the Acoustic Nerve and Brainstem [38]

Only waves I-V of the BAER can be assigned clinically by the auditory evoked potentials to any definite nuclear region in the central nervous system (CNS). Although the site of origin of waves I-V is still a matter of dispute, wave I can be assigned with considerable confidence to the distal end of the acoustic nerve. It originates as the result of a volley of action potentials.[39-45] Wave II has been considered by most authors to be a central wave.[41,46,47] Moeller et al[48] and Hashimoto et al[49] believe that wave II originates at the proximal end of the acoustic nerve, or just beyond the entry of the acoustic nerve into the brainstem. There is also evidence for a central (medullary) component, since deep brainstem lesions do affect wave II.[50] It should be noted that if wave II originated exclusively in the acoustic nerve, it would remain intact in a much higher percentage of these cases. Electroclinical correlations suggest an inferior and superior pontine origin for waves III and IV and a midbrain origin for wave V.[49,51] The laterality of waves I and II is well known. Both potential arise on the side of the stimulus; i.e., they are ipsilateral. A primarily ipsilateral origin can be assumed for waves III-V as well.[52,53] Evidence for this can be obtained from large acoustic neuromas, which not only exert pressure on the acoustic nerve, but are in contact with the brainstem as well.[50] In these cases waves I-IV were of normal intensity and amplitude, provided that hearing on that side was normal. If waves III-IV originated on the contralateral side, pressure from the tumor would affect these waves.

Starr and Achor[54] reported that the brainstem auditory evoked response (BAER) is reliable diagnostic measure of acoustic nerve and brainstem integrity. Because of the stability of the latency of the five initial peaks at constant stimulus levels, an abnormality can be predicted primarily on changes in individual peak or interpeak latency (IPL). Among the advantages of the BAER technique is the fact that the BAER is relatively unaffected by the subject's mental status, which is an advantage when recording from uncooperative subjects. Campanella et al[55] have reported that BAER's tend to indicate progression of disease and severity of the illness as they have been reported to do in Friedreich's ataxia.

The factors that affect normative BAER data are presently being defined. Houston and McClelland[57] have reported on gender contributions to intersubject variability of the auditory brainstem potentials.

5.2.1 Use of BAER Protocol To Assess The Effects of Exposure to HCl in Flowing Air on Long Evans Rats

As discussed earlier (See pages 14-27) the BAER Protocol was extremely sensitive in determining the early decrements in neurophysiological activity in Long Evans rats exposed to levels of HCl ranging from 75 ppm to 300 ppm in flowing air.

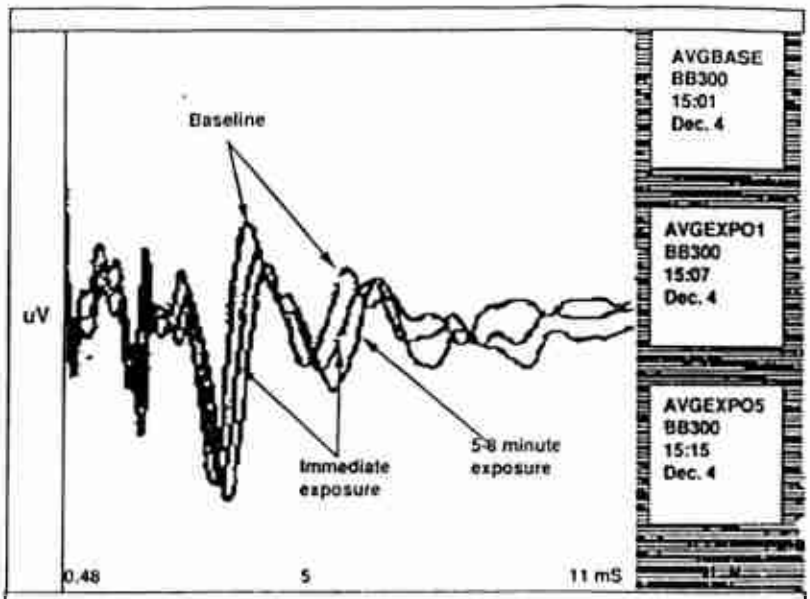
A review of the information presented in Figure 33, which is typical of the results observed in the BAER studies, illustrates the reproducibility of the BAER curves obtained during 3 consecutive epochs prior to the introduction of HCl into the exposure chamber, the reproducibility of the BAER curves obtained in 3 consecutive epochs commencing approximately 30 seconds into the exposure episode in which the animal subject was exposed to an environment containing 300 ppm HCl in flowing air, and the reproducibility of the BAER curves obtained in 3 consecutive epochs commencing 5 minutes into the exposure episode. A similar set of curves (not shown) also confirmed the reproducibility of 3 consecutive BAER curves taken commencing 15 seconds following the venting of the exposure chamber following the termination of the exposure episode.

This data was analyzed statistically by ANOVA which showed differences between treatments at $p = 0.0001$ with an F-test of 63.391. When individual test groups were examined for standard error the

values ranged from 4.2 for the baseline and immediate exposure BAERs to 7.3 for the 5-8 minute exposures (#2). A standard error of 6.0 was obtained during the early recovery period.

The comparison of the various statistical treatments showed statistical significance for all groups indicating indicating that there was an immediate increase in latency from a baseline level of 2.82 msec to 2.96 msec following exposure to 3.00 ppm HCl. The latency increased to an average of 3.02 msec (measured 5-8 minutes into the exposure period. This was followed by an additional increase in the BAER latency to 3.12 msec immediately following the venting of the exposure chamber at the termination of the 15-minute exposure period. This is in agreement with earlier studies conducted by Einhorn and is attributed to the onset of deeper breathing in the exposed animals as the HCl concentration within the chamber is decreased.[10] The increase has been attributed to the deeper inhalation of the irritant in the dead space in the respiratory system. These results were in complete agreement with the Student's t-Test applied to each treatment vs baseline resulting in p values of 0.0001-0.0002. Consequently the reliability of the test system can be considered excellent.

The BAER Protocol proved to be the sensitive technique for detecting the early decrements in neurophysiological activity used during the **Phase I Program**. This technique will be further refined during the early stages of the **Phase II Program** prior to its use in assessing the consequences of animal exposure to model mixtures of toxicants which are present in weapons systems' exhausts.



PAIRED T-TEST X : BASELINE

	DF	MEAN X-Y	PAIRED T-VALUE	PROBABILITY(2 TAIL)
IMMEDIATE	6	-.144	-66.9	.0001
5-8 MINUTE	6	-.207	-9.6	.0002
RECOVERY	6	-.303	-14.6	.0001

Figure 33. Comparison of Brainstem Auditory Evoked Response (BAER) in Long EvansRats, Prior to and During, and Postexposure to 300 ppm HCl in Flowing Air

5.3 Utility of EEG in Diagnosis of Neurologic Disorders

The EEG is a continuous varying voltage measured from the scalp, surface of the brain, or points within the brain. The peak-to-peak voltage of the EEG wave rarely exceeds 100 microvolts on the skull and can approach several hundred microvolts in deep structures. The EEG voltage is a function of the structure of the measuring electrode and its location. The frequency (rate of fluctuation) of the EEG varies with behavioral activities and brain intactness. Figure 34 illustrates a few examples of EEG segments.[58]

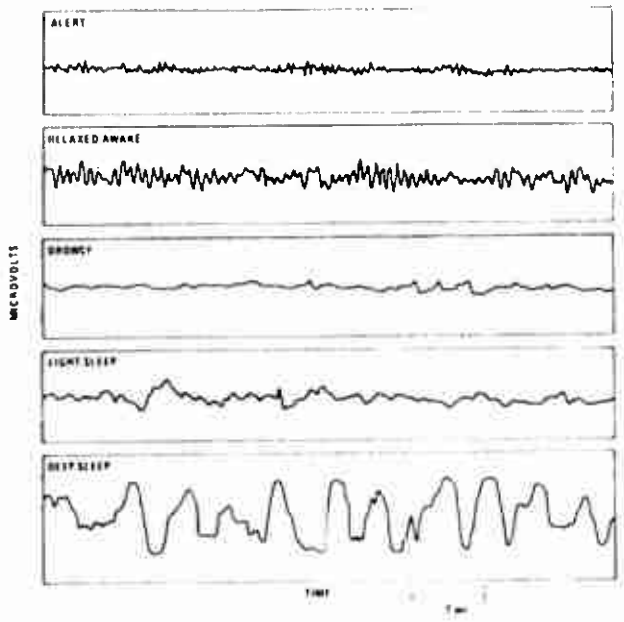


Figure 34. Examples of EEG during various states of alertness[58]

5.3.1 Time Domain Quantification

5.3.1.1 Voltage-Amplitude

During the analysis of the EEG wave one is primarily concerned with the variations of voltage over time and not with the absolute voltage relative to zero. The peak-to-peak amplitude of the wave is the range of voltages in the statistical sense. The amplitude of a segment of the EEG wave may be expressed as the root mean square (RMS) amplitude, which is the square root of the averaged squared voltage deviation around the mean which can be computed by taking voltage samples over time. The RMS amplitude is recognizable as the statistical descriptor of dispersion, the standard deviation (SD) of the voltages which are monitored. In the different waves shown in Figure 34 the RMS amplitude describes the "average amplitude" (root mean variation or SD). Monitored EEG signals are usually found to follow Gaussian distributions.

5.3.1.2 Frequency-Period

The rate at which EEG voltage fluctuates is another of the major time domain descriptors of waves. This rate is the "frequency" and is measured in cycles of the wave per second expressed as Hertz (Hz). The fluctuation of rate may be expressed as the period (P) of time required to complete one cycle or the wavelength. $P = 1/f$ in which f is the frequency of the wave.

In stochastic waves such as the EEG no fixed frequency wave occurs. It may be shown that waves of many periods occur either alternately or simultaneously by being superimposed on each other. Burch[59] provided a review and classifications used in the analysis of EEG waves. A simple descriptor of the length of each wave is the time between baseline crosses (periods of half waves). By estimating the distribution of these periods it is possible to describe the frequency content of a segment of EEG. The baseline crossing technique only describes the "major" periods and not the superimposed faster activity which appears in the composite wave. By computing the first derivative of the wave, one can make an estimate of the characteristics of the faster activity. The first derivative is roughly analogous to the original EEG wave with the slow waves removed.

It should be noted that the baseline cross methods have several advantages as descriptors. Benignus[58] has stated that the outcome of the baseline cross method is analogous to the probability density distribution of voltages. Thus, along with amplitude distribution analysis there exist two distributional descriptors of a wave, one for amplitude and the second for the time dimension. Due to the inability to provide a physical interpretation of baseline cross analysis only a limited use is made of this technique.

5.3.2 Frequency Domain Analysis

Fourier synthesis and Fourier analysis are symmetrical procedures such that (1) any waveshape may be produced by scaled sine and cosine waves of appropriate frequencies and (2) any empirical wave may be decomposed into sine and cosine waves of appropriate amplitudes and frequency.[60-62]

As reported by Benignus[58] Fourier analysis consists of estimating the proportion of the amplitude of the EEG which can be accounted for by each of a series of sine and cosine waves at different frequencies. This is achieved by multiple regression analysis using sines and cosines as predictors and EEG waves as the dependent variable. The result is a set of regression coefficients, one pair for each frequency, resulting in a spectrum.

5.3.2.1 Power Spectra

One power estimate can be computed for each of a limited spectrum of frequencies. Thus, all of the variance in an EEG wave can be accounted for by the sum of the powers at each frequency. Figure 35 shows a typical power spectrum of a waking relaxed human subject.[58] Using power spectrum analysis one can develop an estimate of the frequency content of a population of EEG waves. Unlike time domain analysis, which require two plots to describe amplitude and frequency, power spectra provides this information in a single plot.

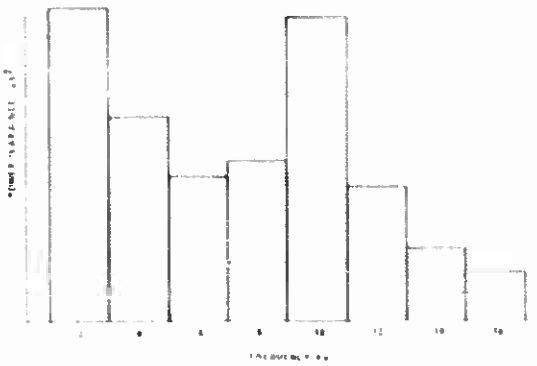


Figure 35. Power spectrum of the EEG of a quantified waking human subject[58]

5.3.2.2 Reduction of Spectral Cumplexity

After an EEG has been quantified in the frequency domain there remains a great deal of complexity and a large mass of data still to be interpreted. Additional procedures such as factor analysis,[63-65] pattern recognition algorithms,[66] various spatial displays,[67-68] and Fuzzy groups analysis,[69-70] have been used for further interpretation of the EEG spectra.

5.3.3 Use of EEG in Neurotoxicological Studies

There is considerable information in the literature which may be used to shed light on the neurotoxicological effects of chemical compounds, either present as individual species or complex mixture of species. Depoortere et al[71] and Glatt et al[72] have reported on the effects of drugs upon the EEG. In many instances a suspected neurotoxin may be studied in a paradigm in which a drug may be used as a positive control. Data are available to indicate that known stimulants and sedatives frequently affect cortical EEG in a way which would be predictable from activation-EEG relationships in the cat or rat.[71,72] researchers have used EEG as part of a battery of tests to classify drug effects in cats[73] and man.[74-75]

Dolce and Decker[76] have used multivariate statistical methods in the analysis of spectral values of the EEG. More than 50 drugs were studied on cat, rat, and man[76] using EEG spectra in a program of "quantitative pharmaco-electorencephalography." The Dolce and Decker study has shown that not all drugs fall on a simple activation scale and further that some drugs have individual effects which are difficult to classify. Stermin et al[77] reported similar complications in their study of the effects of anticonvulsant drugs in rhesus monkeys.

Many of the studies reported in literature pertaining to the effects of drugs on the EEG do not report the dose used. When only one dose level is used, as is typical in many of the reported studies, it is difficult to generalize to other levels. Bronzino et al[78] in their study on the effect of morphine on the rat reported that high and low dosages produced different EEG effects corresponding to different behavioral effects. A review of several studies reported in the literature shows that while sufficient information may not have been available to describe the mechanisms of effects on the EEG there were reasonably consistent indicators across species.

Hudson et al[73] developed a model for studying depth of anesthesia and acute tolerance to thiopental. In this model they used the following EEG characteristics to evaluate the depth of anesthesia:

1. Stage 1 anesthesia is characterized by low voltage fast irregular patterns,
2. Stage 2 is characterized by high amplitude irregular rhythm,
3. Stage 3 is characterized by short bursts or irregular luw frequency and high frequency mixed potentials separated by "silent" periods of about 1-2 seconds duration,
4. Stage 4 is similar to Stage 3 except that the "silent" periods rang ing from 4-6 seconds in duration.

Stage 1 corresponds to loss of consciousness, Stages 2 and 3 to surgical levels, and Stage 4 to a comatose state.

5.3.4 Pathology

One of the more common uses of EEG in clinical applications is the diagnosis and location of epileptic foci.[79-80] Success in the detection and spectral description of seizure patterns in man has been quite good.[81-84] The fact that seizure patterns are generally the same across species is attested to by the use of nonhuman models to study the seizure process. Seizure research has been conducted using EEG spectra in the cat[81], Gerbil[85], and dog.[86]

5.4 Utility of Somatosensory Evoked Potentials in Diagnosis of Neurologic Disorder

The somatosensory evoked potentials (SEP) is one of the most complex of the evoked potential protocols used clinically to determine neurologic disorders. The response, which may be be recorded from the scalp or cortex, contains both local and far-field components.[87] The SEP is obtained following stimulation of any sensory nerve in the peripheral nervous system. The time course and topography of the SEP will vary as a function of the locus of stimulation.[88-89]. There may be considerable variations in the SEPs observed in different subjects (humans vs animals) since the distance between the standard stimulus locations and the recording location will vary considerably with the size of the subject. The SEP technique is a well-used response in clinical studies.[90-91]

As we have observed during our **Phase I Program** studies, there is a greater degree of variability between animal subjects than was observed in either the BAER of EEG studies. We believe that in future studies it will be necessary to determine the appropriate level of stimulus for each subject, since the desired intensity is usually a function of the extent to which the underlying peripheral nerve has been activated. Dyer[27] has reported that the stimulus which bears a constant relationship to the threshold intensity for producing the maximal compound action potential from the nerve should elicit consistent activation of the system across subjects, but individual differences, such as differences in skin resistance and the distance of the nerve from the surface of the skin, may be a major source of variability in SEP measurements.

Figure 36 shows schematic SEPs derived from four human studies following stimulation of the median nerve as well as two SEPs obtained in the stimulation of the tail nerve in rats.[27] Giblin[92] has reported that the interpretation of SEP is less direct than in the case of BAER in that there may be at least two "typical" configurations in normal subjects. Hume and Cant[93] have observed in their studies of humans with various lesions of the cortex, thalamus, brainstem, and spinal cord that the N_{20} wave recorded following stimulation of the median nerve at the wrist represents the first sign of cortical activity. They further reported that alterations in peak latencies and amplitudes may reflect anything from changes in conduction velocity or fiber populations of the peripheral nerve involved to increased synaptic delay or loss from selected neurons in the spinal cord, brainstem, or thalamus. It should be noted that while changes in the latencies and amplitudes may not be diagnostic in all cases such changes often serve to identify foci of pathologic change. Obeso et al[94], for example, have reported that cortical lesions restricted to the primary sensorimotor area tend to produce increases in latency and decreases in amplitude of peaks N_{20}, N_{40}, and N_{70}, while cortical lesions outside of this areas tended to produce increased amplitude and increased duration of these peaks. Allison et al[87], Crespi et al[95], and Arezzo et al[96] have demonstrated techniques which they used to further define the foci of pathologic change after observing alterations in the N_{20} response recorded at the scalp.

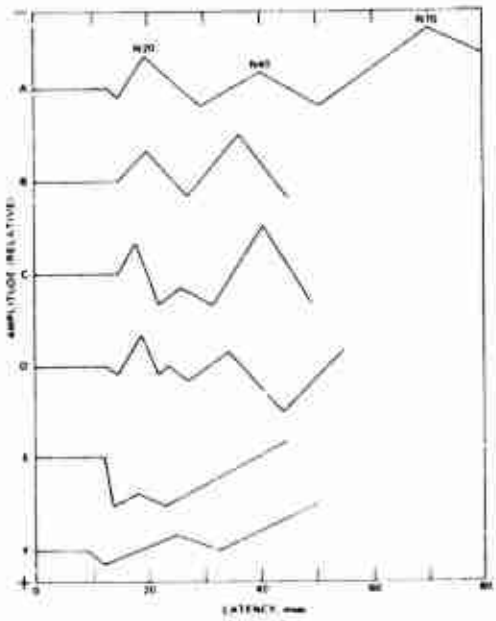


Figure 36. Schematic Somatosensory Evoked Potentials Obtained from Published Latency Data. Negativity is up. (A-D) Human SEPs Recorded from the Scalp Following Stimulation of the Contralateral Median Nerve. (A) data Based on 40 Normal Subjects (93); B "V" Configuration, Representative of About 72 % on Normal Subjects (90); (C) "W" Configuration Representative of 28 % of Normal Subjects(90); (D) Data based on 21 Normal Subjects((91); (E) Schematic of SEP Recorded from Rat Skull Following Stimulation of the Tail Nerve, with Rat Anesthetized and Tail Warmed to 37 0C (97); (F) Same as E except that Rat Unanesthetized and Tail Not Warmed[97]

Since many neurotoxic substances produce dysfunction in the peripheral nervous system, it is not surprising that SEPs recorded from various locations along the somatosensory pathway have detected toxicological effects. Boyes and Cooper[98] have reported that exposure to acrylamide produced alterations in far-field SEP recorded in rats. Arezzo et al[96] confirmed these findings in monkeys by showing the somatosensory responses recorded from the cervical spinal cord detected acrylamide toxicity earlier than do responses recorded from the peripheral nerve.

Rebert et al[97] have included the SEP as part of an electrophysiologic battery for assessment of neurotoxicity. They have investigated the effects of carbon disulfide, hexane, vincristine, maytansine, and cis-platinum upon the SEP recorded from the skull following electrical stimulation of the ventral caudal tail nerve in the rat. Significant SEP alterations were found in all cases, but only in the case of cis-platinum did the SEP technique appear to detect toxicity at lower dosages than other techniques included in the battery.

Howell et al[99,100] have shown, as illustrated in Figure 37, that SEPs detect dysfunction induced by trimethyltin (TMT) that resulted in increased latencies. These results were particularly significant in that they contributed to increasing evidence in the literature that in addition to being a limbic system neurotoxicant, TMT also affects sensory systems.

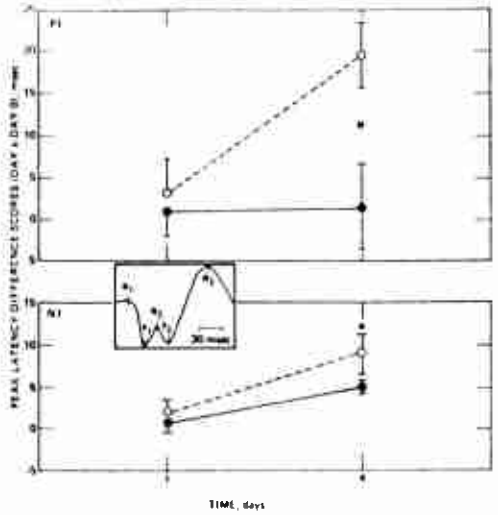


Figure 37. Effects in Rats of a Single Exposure to 7mg/kg of Trimethyltin on the SER. The Data Indicate a Progressive Increase in Latency of Peaks P_1 and N_1 with Time Since Dosing[98]

Within the scope of the **Phase II Program** we propose to further the development of SEP protocols by producing a standardized recording paradigm useful for rats as well as further studying the morphology of a "prototype" SEP waveform in Long Evans rats.

5.5 Utility of Visual Evoked Potentials (VEP) in Diagnosis of Neurologic Disorders

The visual system is unique in that it is embryologically a pure CNS system, while other sensory systems contain peripheral nervous system components.

The VEPs have been used by clinicians of routine evaluation of neurotoxicity. We have had problems utilizing VEPs during the **Phase I Program**. The problems encountered may have been due to the effects of HCl on the cornea, as we have observed in previous studies.[3] Dyer[27] has reported that the major problem in ensuring reproducibility of the stimulus from trial to trial and from subject to subject is one of dark adaptation. Dyer has stated that if testing occurs in an environment which is darker than the ambient environment, then dark adaptation must be allowed to occur before testing can begin.

Dyer and Swartzwelder[101] have developed a normative VEP database for albino and hooded rats. These researchers have used their database to conduct some comparisons of latency and amplitude values across experiments. Dyer and Annan[102] reported that more consistent results could be obtained in measured VEPs, in situations where the compound being studied would alter pupil diameter, if prior to testing the animal's pupils were dilated with atropine.

An example of the utility of the VEP as a protocol for screening neurotoxic effects follows. Morphological studies had previously concluded that TMT-induced damage is restricted largely to the limbic system.[103] Dyer et al[104] treated rats with trimethyltin and recorded VEPs before and 1, 2, 8, and 16 days following dosing. Statistical analysis revealed that the effects observed were not time dependent within the range of test time. Figure 38 illustrates the alterations observed in peak latency collapsed across different posttreatment times. The alterations which were observed in the earliest peak of the response were suggested to represent subcortical changes. To test the hypothesis that the alterations

were retinal in origin, a second experiment was conducted during which electrodes were placed in the optic nerve as well as over the visual cortex. Dyer et al[104] showed, as was expected, that the changes in the magnitude and direction of change observed in the cortex were also present in optic tract recordings. Subsequent neuropathological examination by Chang and Dyer[105] demonstrated retinal lesions in the animal subjects, thereby confirming the physiologically predicted results.

We will within the **Phase II Program** attempt to improve upon the VEP protocol used during the **Phase I Program** to enable us to use VEP as part of a battery of procedures recommended for the assessment of the effects of weapons systems' exhausts on humans. Where appropriate we will use established neuropathological techniques to assess the pathological lesions which may result as a consequence of human exposure to weapons systems's exhausts.

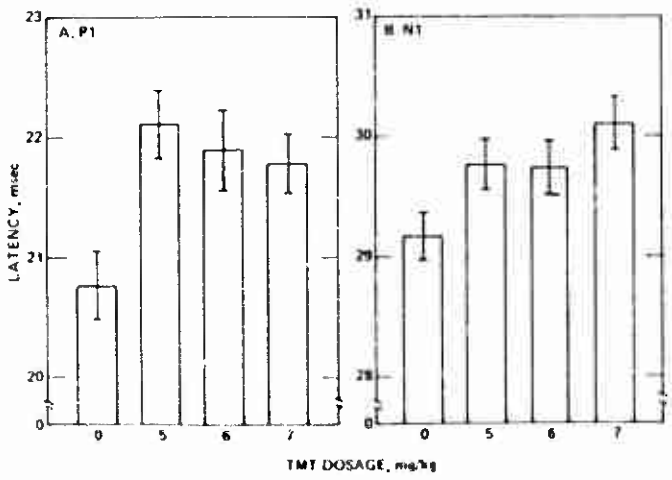


Figure 38. Effects in Rats of a Single Injection of TMT on the VEP. The Data Indicate Increases of P_1 and N_1 Latencies, Thereby Suggesting Altered Inputs to the Cortex [27]

6 SUMMARY AND CONCLUSIONS PHASE I PROGRAM

6.1 Caveat

The Small Business Innovation Research Programs (SBIR) were established by the Small Business Innovation Development Act of 1982 (Public Law 97-219 and Public Law 99-443). The SBIR Program supports creative advanced research in important scientific and engineering areas and is designed to encourage the conversion of research and development into technological innovation and commercial applications for its potential economic benefits to the Nation.

The funded SBIR projects explore advanced ideas that can lead to important new technology, breakthroughs, innovative new products, or to the next generation of a product or process. The SBIR Program emphasizes cutting-edge, high-risk, potentially high payoff research in hundreds of areas identified by the sponsoring government agencies as important to technical progress in their fields. The

SBIR Program also funds the gap from a research-based idea to a prototype that many industrial and venture capital companies find difficult to support.

6.2 SUMMARY AND CONCLUSIONS - PHASE I PROGRAM

There is no widely accepted protocol for the assessment of the toxic effects of irritant gases on humans. Most procedures employed for this purpose utilize endpoints that assess time-to-incapacitation and lethality. The Army Medical Research and Development Command has recognized the need to evaluate potential toxic hazards resulting from human exposures to irritant gases (hydrogen chloride, ammonia, formaldehyde, and carbon monoxide) associated with weapons systems' exhaust emissions.

We have, within the scope of the **Phase I Program** exposed Long Evans rats to three levels of hydrogen chloride gas in flowing air. Hydrogen chloride, a respiratory irritant present in the exhausts of solid propellant weapon systems using ammonium perchlorate as the oxidizer, was selected for use in evaluating the feasibility of protocols employing quantitative neurophysiologic monitoring techniques (electroencephalographic monitoring, [EEG]; brainstem auditory evoked response, BAER]; somatosensory evoked potentials, [SEP]; and visual evoked potentials, [VEP] in rodents exposed to preselected levels of hydrogen chloride gas in flowing air. This specific respiratory irritant was also selected because the of extensive prior experience by the project staff in the toxicological characteristics of hydrogen chloride. We have demonstrated, within the scope of the **Phase I Program**, our ability to detect early and significant changes in nervous system activity during the animal exposure episodes. These changes were observed prior to seeing significant changes in animal homeostasis, as determined by monitoring important blood parameters, in time periods much earlier than would be required to observe significant behavioral changes.

The utilization of the unique animal exposure chamber which has been interfaced to the Quantified Signal Imaging, Inc. Model 9000 Quantitative Neurophysiological Monitoring System (QSI 9000 System) has permitted us to monitor the development of statistically significant changes in neurophysiologic activity as early as during the first epoch following the start of the exposure episode. Having demonstrated our ability to identify early decrements of performance in nervous system activity, we will, within the scope of the **Proposed Phase II Program**, if funded, utilize the quantitative neurophysiological techniques to identify the foci of abnormal brain and nervous system activity resulting from the exposure to combinations of toxicants used to model weapons systems' exhausts. We will also follow the development of late occurring sequelae and study pathologic lesions grossly and where indicated on the ultrastructural level.

We will, after confirmation of our results in higher mammalian species, develop a cost-effective protocol for the first-tier screening assessment of complex mixtures of toxicants present in weapons systems' exhausts. We will employ a variety of techniques, including but not limited to thermodynamic equilibrium calculations, to predict the presence a most probable concentrations of the toxicants likely to be encountered by soldiers during training exercises, simulated battle conditions, as well as under combat conditions. By determining the foci resulting from chronic exposure to volatile toxicants we should be able to determine the mechanisms involved in early intoxication and, hopefully, be able to develop protocols of the assessment of such hazards to permit detection prior to reaching a point of irreversible physiological damage. Knowledge resulting from such studies will provide the baseline information for the development of early-warning detector systems capable of providing an early alarm of pending toxicological hazard to military personnel.

6.3 Potentials for Phase II Program Development

Having demonstrated our ability to identify early decrements of performance in nervous system activity in Long Evans rats exposed to 0 ppm, 75 ppm, 150 ppm, and 300 ppm HCl in flowing air, we will, within the scope of the **Proposed Phase II Program**, if funded, utilize advanced quantitative neurophysiologic techniques, such as qEEG, BAER, VEP, SEP, and topographic mapping protocols to identify foci of abnormal brain activity resulting from exposure to simulated weapons systems' exhausts.

We will then, after confirmation of our results in higher mammalian species, develop a cost-effective protocol for the first-tier screening assessment of weapons systems' exhausts of interest to the U.S. Army Biomedical Research and Development Laboratory.

Due to the limitations of time and financial support imposed within the concept of proving feasibility within the **Phase I SBIR Program**, as well as the inability to predict the potential experimental results which might be obtained within a six-month program, we have been limited as to the number of experiments as well as the number of animal subjects which could be employed during such initial studies.

We have utilized a variety of statistical procedures to analyze our experimental results. The statistical analyses conducted, although greatly limited by the small number of animals as well as the limited number of replicates possible within the scope of the **Phase I Program** clearly have substantiated the trends observed in our experiments. The results obtained agree for the most part with the prior studies conducted by the Principal Investigator and his colleagues as well as with many of the studies reported in the literature by other researchers within the toxicological research community. We have, within the scope of this **Phase I Program**, been able to observe and measure changes in neurophysiologic activity and animal homeostasis that clearly indicates the feasibility of detecting and quantitating early decrements of performance, especially during the development of animal intoxication resulting as a consequence of exposure to four levels of HCl in flowing air. We have been able to detect these changes induced by exposure to HCl, one of the suspected important thermal decomposition products present in the exhausts of solid propellants using ammonium perchlorate as an oxidizing agent long before there were significant changes measured in animal homeostasis as determine by convention use of blood bioassay procedures.

Once we have been able to confirm the results obtained within the scope of the **Phase I Program** we should be able in future studies to provide considerable insight into the human effects resulting from short-time exposures toxic chemical species. These protocols, when fully developed should provide considerable insight not only to the development of the first observable decrements in human performance as a consequence of exposure to these toxic species, but should also provide a means of determining untoward human response to environmental toxicants present in the environments within our "build environment" as well as to determine human effects from environmental pollutants present in soil gas and groundwater. By determining the foci resulting from chronic exposure to volatile toxicants, we should be able to determine the mechanisms involved in early intoxication and, hopefully, be able to develop protocols for assessment of such human hazards to permit detection prior to reaching a point of irreversible physiological damage. Knowledge resulting from such studies will provide the baseline of information for development of early warning detector systems capable of providing an early alarm of pending toxicological hazard to humans.

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