CHEMICAL MANUFACTURERS ASSOCIATION PROPOSAL

"Vinyl Chloride Combined Inhalation Two-Generation Reproduction and Developmental Toxicity Study in CD Rats"

Huntingdon
Life Sciences

7 December 1995

Hasmakh C. Shah, Ph.D.
Chemical Manufacturers Association
2501 M Street, NW
Washington, D.C. 20037

Dear Dr. Shah:

Enclosed are one bound and one unbound copy of our cost and technical proposal for the Vinyl Chloride Combined Inhalation Two-Generation Reproduction and Developmental Toxicity Study in CD Rats program.

Our price to conduct this program is $555,000. We could initiate this study in February 1996 and will provide a draft audited final report within 6 months of study termination.

We appreciate your interest in Huntingdon Life Sciences and look forward to the opportunity of working with you and your colleagues at the Chemical Manufacturers Association. Please feel free to contact either Ray Schroeder (Project Manager-Reproduction and Developmental Toxicology, x2910) or Paul Newton (Director-Inhalation Toxicology, x2760) if you have any questions or wish to discuss any of the information provided.

Sincerely,

Ira W. Daly, Ph.D., D.A.B.T.
Vice President, Directorate of
Agrochemical and Industrial Toxicology

IWD/pds

Attachment

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CHEMICAL MANUFACTURERS ASSOCIATION

TEST MATERIAL REQUIREMENTS

Test material	Vinyl Chloride
Molecular Weight	62.5
Chamber	ppm
I	10
III	100
IV	1100
V	-
Chamber size (liters)	6000
Number of exposure days	225
Hours/day	6
Nominal/Analytical	1.05
Percent extra	50
Total test material required (kg)	482.19

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Introduction

On 15 November 1995, Chemical Manufacturers Association requested a technical and cost proposal for a Vinyl Chloride Combined Inhalation Two-Generation Reproduction and Developmental Toxicity Study in CD Rats. Herein this document presents the capabilities of Huntingdon Life Sciences to conduct the requested program.

Huntingdon Life Sciences has a wealth of experience including conducting numerous Inhalation Reproductive-Developmental Inhalation toxicity studies.

In the capabilities section of this document, the Inhalation Toxicology segment discusses various aspects of how state-of-the-art inhalation toxicity testing is conducted. Huntingdon Life Sciences' experience in conducting over 570 development toxicity studies is presented in the Reproductive and Developmental segment of the capabilities section.

Two very important areas in this research are analytical support capabilities and the performance of the testing laboratory in meeting regulatory requirements. These areas are also discussed in the capabilities section.

In the sample protocol section of this document, is a detailed draft protocol. Curricula vitae for the key staff involved in the conduct of the program are included in the next section.

This testing would be conducted at our laboratory in East Millstone, NJ., where we have issued over 1000 on-time final reports since 1992. This record of timeliness combined with our decades of experience and scientific expertise, positions us well to meet Chemical Manufacturers Association's expectations for performance and quality.

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Inhalation Toxicology

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Potential exposure to toxic materials is greater via inhalation than any other route of exposure. The potential for exposure is greatest through the lung, as more air is inhaled each day than water or food is ingested. The surface area of the human lung far exceeds the surface area of the skin and gastrointestinal tract. Some exposures may be intentional, such as with inhaled drugs. However, most exposures are unintentional via environmental pollutants in the industrial setting or in the ambient air. Inhalation toxicity studies determine the health effects of these materials through exposure to animals, which then allows for a human risk assessment. Inhalation studies involve all the standard types of toxicity studies including acute, subchronic, chronic, oncogenicity, reproductive, teratology and neurotoxicity.

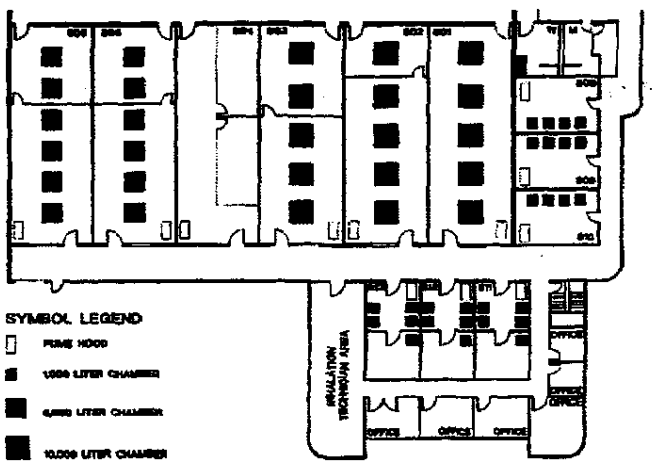


Fig 1
Inhalation Facility at Huntingdon Life Sciences Toxicology Services, North America

I. NOSE-ONLY VERSUS WHOLE-BODY EXPOSURES

Inhalation exposures have been predominantly conducted using either whole-body or nose-only exposures. Whole-body exposures are usually conducted for chemicals or agrochemicals where there is minimal concern about dermal absorption of gases or vapors. Whole-body inhalation exposures to aerosols, either liquid or solid, lead to the potential problem of deposition on the fur and dermal absorption. Oral absorption of the test material from the gastrointestinal tract may also occur as a result of the animal preening itself. Regulatory agencies have recognized these concerns, thus, the U.S. EPA recommends that aerosol inhalation studies be conducted via nose-only exposure.

Fig 2

The experimental design determines the size of the chamber to be used. Huntingdon Life Sciences has 54 whole-body inhalation chambers on-line, ranging in size from 100 to 10,000 liters (Figure 1). The whole-body exposure chamber size selected for a study is based on the animal volume being less than 5% of the chamber volume (Figure 2). These chambers are operated with at least 10 air changes per hour, allowing for adequate oxygenation, test material mixing and removal of animal biproducts.

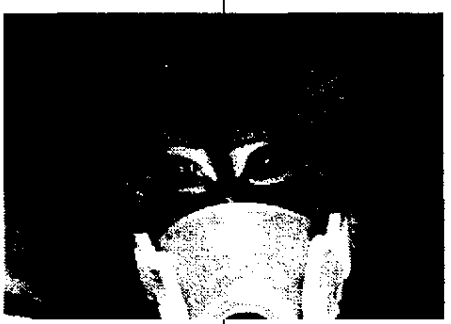
Nose-only exposures are preferred by the pharmaceutical industry to eliminate deposition on the fur and dermal absorption or oral ingestion. Nose-only exposures also require far less drug than whole-body exposures. This is attractive when generation of the new drug is costly and in limited supply.

The flow-past nose-only exposure system flows the test material laden airstream past each individual nose, eliminating rebreathing or cross contamination from other animals (Figure 3). The chambers are modular in design, allowing multiple tiers to be added in order to accommodate the desired number of test animals. Either 8 or 16 animals may be exposed per tier. The chambers typically operate at 1 liter minute/animal to maintain adequate oxygenation and avoid rebreathing. To ensure containment of potentially biologically active test materials and to maintain exposure group separation, the nose-only chambers are set up within the 6,000 or 10,000 liter whole-body chambers.

In larger species, such as dogs and primates, nose-only exposures are conducted using masks (Figure 4). The animals are trained to accept the mask over a 3-week acclimation period. In addition, for metered-dose-inhaler (MDI) studies, dogs can be trained to accept an oropharyngeal tube for dosing. A computerized system allows monitoring of the dog's respiration rate and documents the number of doses as well as the pre- and post-dosing weight of the MDI unit for each animal.

Fig 3

Fig 4



II. EXPOSURE DURATION

The duration of the daily exposure usually differs between chemicals and pharmaceuticals. When chemicals are being tested, the desire is to simulate the work environment with exposures typically 6 hours/day for 5 days/week. Exposures to ambient air levels are typically 23 hours/day. In the case of pharmaceuticals, the exposures are daily for 1 hour/day or, if the drug is formulated in a metered-dose-inhaler (MDI), the exposure for dogs or primates can be single or multiple doses.

The number of exposures varies from study to study depending on the experimental design. Single exposure is typical for acute studies. Animals are exposed daily in subchronic, chronic or oncogenicity studies for up to 91 days, 12 months or 2.5 years, respectively. When conducting developmental studies, the pregnant dams are only exposed during specific portions of their gestational period.

III. TEST ANIMAL SPECIES

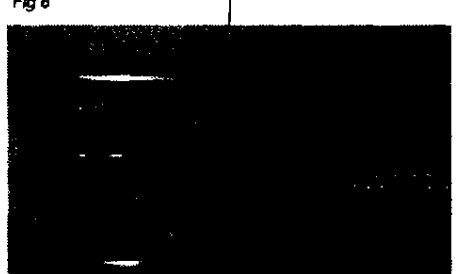
Standard inhalation toxicity studies frequently involve the testing of two species, typically rats (Fischer-344 and CD) and dogs (Beagle). Huntingdon Life Sciences also has experience with other species including mice (CD-1 and B6C3F1), hamsters, guinea pigs and nonhuman primates (cynomolgus and rhesus). Inhalation developmental studies are routinely conducted in rats and rabbits.

Fig 5

IV. EXPOSURE ATMOSPHERE GENERATION

Gases are the easiest test materials to work with. Huntingdon Life Sciences has mass flowmeter-controllers which accurately and precisely dispense the gas into the exposure chamber air supply (Figure 5). Delivery into an exposure prechamber allows for adequate mixing of the test material with the chamber air supply. The gas cylinders are also supplied with cut-off valves. If chamber airflow is interrupted, the valve automatically closes and gas flow from the cylinder is terminated. This prevents over exposure of the test animals or the generation of potentially hazardous or explosive levels of the gas.

Fig 6



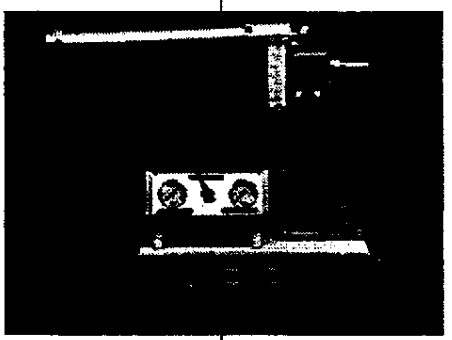
Vapors are generated using bubblers or counter-current evaporation units (Figure 6). In the evaporation unit, the volatile liquid is introduced at the top of the apparatus and spirals down around a heated glass coil, while heated nitrogen flows up through the unit. The counter flow maximizes the evaporation. Evaporation under nitrogen also brings the vapor down below its lower explosive limit without the presence of oxygen. This allows for safe testing of highly flammable materials such as gasoline.

Fig 7

Liquid aerosols are generated using a variety of aerosol generators. When working with chemicals, a spray atomizer or a multi-barrelled nebulizer is used. Huntingdon Life Sciences has had experience in pharmaceutical studies using the DeVilbiss, Solosphere, Pariboy and Hospitak nebulizers (Figure 7). The choice of the nebulizer used is influenced by the specific test material and the Sponsor's intended delivery system. Ultimately, the generator used should give the most consistent and stable output of a respirable aerosol. Materials with viscosities of up to approximately 100 centipoise can be used. More viscous materials may require a vehicle such as water or acetone.

Solid aerosols or dusts are also generated using a variety of dust generators. Huntingdon Life Sciences has the latest version of the Wright Dust Feeder with a larger bore and digital speed control. Other generators include the Spengler, TSI fluidized bed and Jet-O-Miser. All generators must produce an aerosol which is respirable in the test animal.

Fig 8



One distinctive generator is the brush generator, which is suitable for dusts and especially appropriate for fibers (Figure 8). A plug of fiber is slowly advanced while densely-packed, stainless-steel brushes whisk the fibers gently off the plug. Compressed air blows the fibers into the exposure chamber. Generation of the fiber aerosol exposure is accomplished without breaking or grinding the individual fibers.

Another unique generator activates the metered-dose-inhalers (MDIs), and can discharge up to 6 MDIs simultaneously (Figure 9). The MDIs (30 maximum) which are mounted completely on a spinning platter are subsequently inverted between discharges. The exposure concentration is controlled by the frequency of firing and the number of MDIs fired simultaneously. The MDIs fire into an expansion chamber to allow for propellant evaporation and aerosol stabilization prior to entering the exposure chamber. As an alternative, if the components of the formulation are known (drug, propellant and excipients), exposures can be produced by simultaneously generating the components using separate generators. This allows for initial evaluations without wasting time and money by placing the complete drug formulation into the MDI canisters.

Fig 9

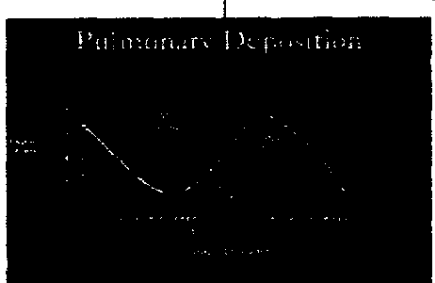
After using these generators to produce the exposure atmospheres, the test material laden air makes a single pass through the exposure chamber. Prior to exhausting from the building, HEPA filters are used to remove all particulates from the air stream.

The gas or vapor laden air exhausted from these chambers flows through an incinerator operating at 1500 degrees Fahrenheit. The residence time in the combustion chamber is 1.9 seconds which gives a designed 99% efficiency in combustion of all vapors and gases within the chamber. This incinerator is required by the State of New Jersey. The amount of carbon monoxide, oxygen and temperature in the incinerator exhaust are monitored by a computerized system to ensure proper operation.

V. PARTICLE SIZE

One crucial factor when conducting aerosol studies is particle size. In the case of small rodents, unless the particle has a mass median aerodynamic diameter less than approximately 4 microns, the test material will not penetrate to the deep lung (Figure 10). Having varied particle sizes in each exposure group is also problematic because the varied sizes lead to differences in the pattern of deposition. This may lead to variances in the observed toxicity. Therefore, a great deal of effort is necessary to yield a small and consistent particle size in all the exposure groups. If the proper size test material cannot be obtained from the Sponsor, on-line micronization of the test material may be possible (Figure 11). The output of the dust generators can also be run through cyclones or inertial impaction devices which allow only the small particles to pass through. Larger particles impact on the walls and are collected at the bottom of the device. In addition, passing the aerosol through a charge neutralizer minimizes the static charge and subsequent loss of a test material to the chamber walls.

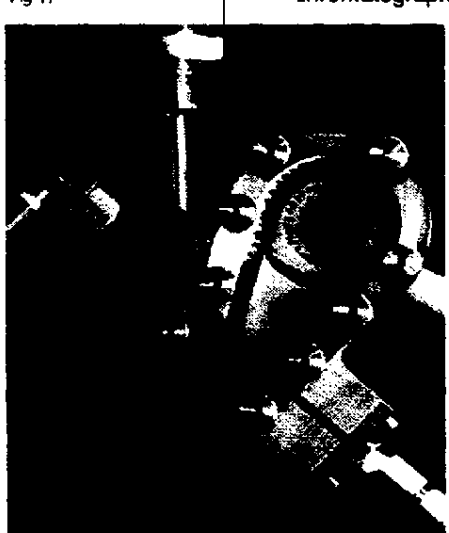
Fig 10



VI. EXPOSURE MONITORING

The actual exposure level is measured in the breathing zone of the animal. Exposure levels of gases and vapors are measured frequently during each exposure using infrared spectrophotometers, on-line gas chromatographs, impingers or absorbent tube samples. The impingers and absorbent tube samples may be analyzed by high-pressure liquid chromatography or gas chromatography. In the case of solid or essentially nonvolatile aerosols, the exposure levels are determined gravimetrically. This is accomplished by measuring the mass gained on a glass fiber filter after drawing a known volume of air from the breathing zone of the animals through the filters. Semi-volatile liquid aerosol exposure levels can be determined gravimetrically by back calculation from the proportion of the non-volatiles in the original sample. Alternatively, both the vapor and solid components may be sampled using impingers or absorbent tubes.

Fig 11

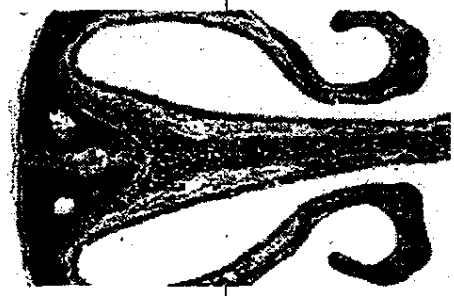


The output of the aerosol generators may also be monitored using real-time-aerosol-monitors (RAMs). These devices give a real time indication of the exposure level and can be used to ensure that the aerosol generator is operating consistently.

Particle size can be determined by a variety of devices including Delron,

Andersen and Intox cascade impactors or a TSI aerodynamic particle sizer. The impactor determines the size distribution of the aerosol by passing it through successive stages which sequentially allows only smaller and smaller particles to pass. These devices all withdraw samples at varying flow rates. Care must be taken not to alter the atmosphere being sampled during the sampling process.

Fig 12



Temperature and relative humidity are measured and controlled to meet all regulatory requirements. Typically this is 20 to 24 degrees Celsius and 40% to 60% relative humidity.

VII. INHALATION TOXICITY STUDIES

All standard toxicity studies from acute up through oncogenicity studies can be conducted via the inhalation route. The standard toxicity studies include the on-line computerized acquisition of body weight, food consumption and detailed physical observation data. A battery of hematology and clinical chemistry parameters are typically evaluated. Macroscopic and microscopic evaluations of numerous tissues are often conducted. The evaluation of 4 sections of the nasopharynx and 3 sensitive areas in the larynx is of particular interest in inhalation studies (Figures 12 and 13).

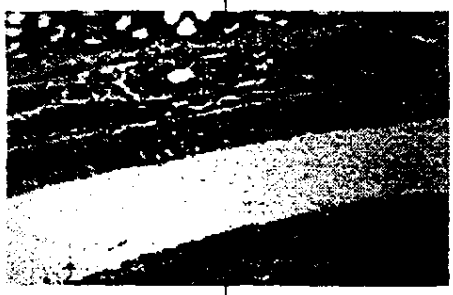


Fig 13

VIII. THE HUNTINGDON LIFE SCIENCES APPROACH

At Huntingdon Life Sciences, the inhalation toxicology laboratory provides the latest directed flow-past nose-only as well as whole-body exposure capability, and two decades of experience in performing all types of toxicity tests for the pharmaceutical, agrochemical and chemical industries. It is our commitment to our Sponsors to provide a scientifically sound, competitively priced study, on time, which will meet or exceed your expectations.

Huntingdon Life Sciences

Neurotoxicology

Huntingdon Life Sciences

In recent years, considerable interest has been given to the evaluation of chemicals and drugs as potential neurotoxic materials. Accordingly, developmental and adult neurotoxicity screens have been developed to evaluate these materials. The neurotoxicity screening battery for adults discussed here evaluates: a functional observational battery of assessments (FOB), motor activity and neuropathology. The functional observational battery consists of non-invasive procedures designed to detect gross behavioral deficits in animals. The motor activity test uses a computerized system to measure the level of spontaneous locomotor activity of individual animals. Neuropathology techniques are designed to provide data to detect and characterize morphologic changes in the central and peripheral nervous systems.

The adult neurotoxicity battery is designed to be used separately, or in conjunction with, general toxicity studies. Any changes should be evaluated in the context of both the concordance between functional and neuropathological effects and with respect to any other toxicological effects seen. The battery is not meant to provide a complete evaluation of neurotoxicity. Further functional and morphological evaluations may be necessary.

I. EXPERIMENTAL DESIGN

The experimental designs follow traditional toxicity studies with multiple groups including varying test material dose levels as well as negative and vehicle controls. At least 10 male and 10 female rats are used in each group with the neurobehavioral evaluations (FOB and motor activity) being conducted in all animals. The neuropathology evaluations are conducted in at least 5 animals per sex per group. The high dose level is set at the highest estimated non-lethal dose and the lower doses in fractions thereof.

In acute studies, neurobehavioral evaluations are performed before initiation of dosing, at the time of estimated peak effects within 8 hours of dosing, and at 7 and 14 days post dosing at a minimum. The time of peak effect may be estimated during a range-find study where animals are regularly observed post dosing. For subchronic and chronic studies, the neurobehavioral evaluations are performed before initiation of dosing, during the 4th, 8th, 13th weeks of exposure, and every 3 months thereafter (if applicable).

II. FUNCTIONAL OBSERVATIONAL BATTERY

For the FOB, animals are observed by trained technicians who are unaware of the animal's treatment using standardized procedures to minimize observer variability. The FOB includes a thorough description of the animal's appearance, behavior and functional integrity. Observations are assessed in each animal's home cage, while the animal is moving freely in an open field, and while undergoing manipulative tests. Test conditions must be consistent, as behavior can be affected by noise level, temperature, humidity, odor, time of day of measurements or other changes in the test animal's environment.

The undisturbed animal is observed for posture, vocalization and palpebral closure in its home cage. Each animal is then removed from its cage, noting ease of removal and response to handling. The animal is observed for chromodacryorrhea, lacrimation, fur appearance and salivation. The animal is then placed in an open area and observed for gait, locomotion, arousal, piloerection, exophthalmia, fecal boluses and urination. Responses to approach, tail pinch, auditory startle, pupil reflex and any abnormal movements are also recorded. Finally, grip strength, landing foot splay and air righting are then evaluated prior to returning the animal to its cage.

III. MOTOR ACTIVITY

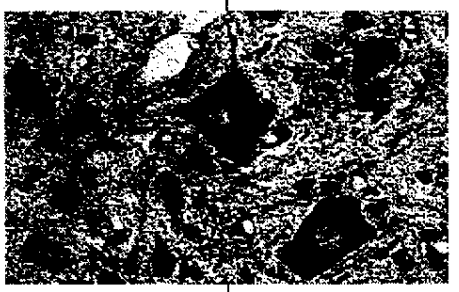
Motor activity is measured by using a computerized apparatus to measure an animal's movement in a novel environment. When a rat is placed into a clear plastic cage, the animal moves about exploring the cage. Movement is detected by the animals breaking photobeams going across the bottom of the cage. Initially the activity is high and decreases over time. An hour is sufficient for control animals activity to asymptotically approach a steady level. Up to 20 animals can be tested individually at a time. Therefore for a large study, the test is counter-balanced across all groups with similar numbers of animals per sex per group being tested each day.

Activity measurements, scored by the number of photobeam breaks within 5-minute intervals for an hour, are captured by a fully validated computer program. As with the FOB, care must be taken to standardize the testing environment to avoid effects due to changes in environmental conditions.

IV. NEUROPATHOLOGY

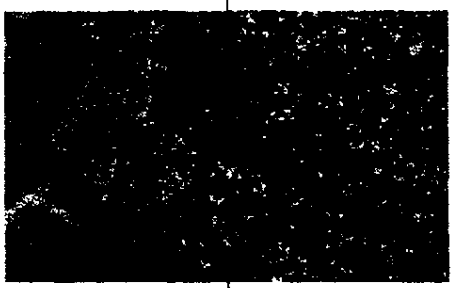
To provide for adequate sampling and optimal preservation of cellular integrity, all tissues are prepared for histological analysis by in-situ perfusion and paraffin and/or plastic embedding procedures. At least 5 animals per sex per group are anesthetized with sodium pentobarbital and then trans-cardially perfused with phosphate buffered saline, followed by 1% glutaraldehyde/4% paraformaldehyde in the same buffer. Paraffin embedding is acceptable for the central nervous system; however, plastic embedding is required for the peripheral nervous system.

Fig 1



Stains used for microscopic evaluations include Hematoxylin and Eosin for cell bodies, Luxol Fast Blue for myelin (Figure 1), Sevier-Munger for axons (Figure 2), and Toluidine Blue for peripheral nerves (Figure 3). Routine sections taken for microscopic evaluation include six coronal sections of the brain, cross and longitudinal sections through the cervical, thoracic and lumbar regions of the spinal cord, and plastic sections of the sciatic, tibial and sural nerves.

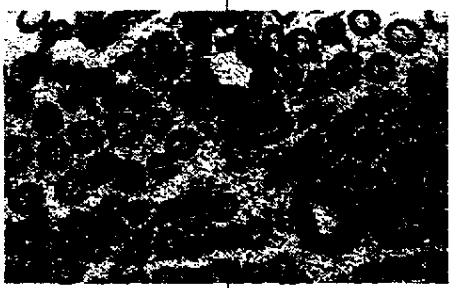
Fig 2



V. GLIAL FIBRILLARY ACIDIC PROTEIN

Chemical-induced injury of the nervous system is associated with astrocytic hypertrophy at the site of damage. Assays of glial fibrillary acidic protein(GFAP), the major intermediate filament protein of astrocytes, can be used to document this response. Moreover, increases in GFAP can be seen at doses less than those doses necessary to produce changes seen microscopically. Animals designated for this evaluation are decapitated and six regions of the brain are dissected, weighed, homogenized in hot, 1% sodium dodecyl sulphate and then frozen. GFAP levels can then be assayed using either radioimmunoassay or ELISA techniques. This biochemical test is intended to be used in conjunction with the neurotoxicity screening battery described above including FOB, motor activity and neuropathology.

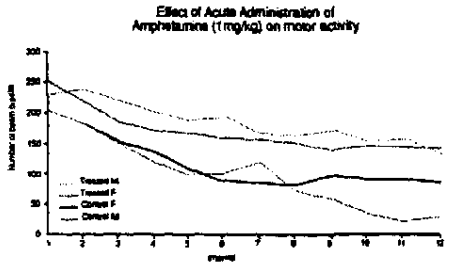
Fig 3



VI. POSITIVE CONTROL DATA

Positive control data provide evidence of the sensitivity of the methods used to detect neurotoxic endpoints. Positive control studies demonstrating neurotoxicity in adult animals have been conducted at Huntingdon Life Sciences for d-amphetamine sulfate, triethyltin bromide, acrylamide, and 2,5-hexanedione. Study results have been previously presented (*Toxicologist* 14:1379, 1994) and are summarized below.

Fig 4

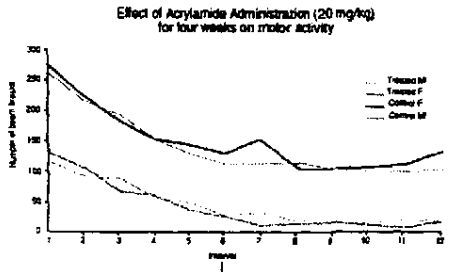


A single dose of 1 mg/kg of d-amphetamine administered intraperitoneally increased motor activity for both male and female rats on the day of dosage (Figure 4).

Four 1.5 mg/kg doses of triethyltin bromide administered by oral intubation produced vacuolation of myelinated areas in the brains of all animals and in the spinal cord of some animals. Lesions were noted in the cerebrum, cerebellum and pons.

Doses of 20 mg/kg of acrylamide administered by oral intubation for 4 weeks produced decreased motor activity (Figure 5), as well as loss of limb co-ordination, mobility and normal gait. Many animals demonstrated ataxia, hindlimb splay, impaired locomotion and were unable to demonstrate a normal air righting response. Decreases in grip strength were also noted. Microscopic changes in peripheral nerves included myelin bubbles, loss of myelinated fibers and neuronal degeneration.

Fig 5



Doses of 750 mg/kg of 2,5-hexanedione administered by oral intubation for 2 weeks decreased motor activity due to the paralytic effect of the compound. Observations noted in the FOB included altered gait, impaired locomotion, lack of air righting response and decreased grip strength. Peri-neuronal vacuolation was noted in the brains. Myelin bubbles, loss of myelinated fibers and neurodegeneration were noted in the peripheral nerves.

VII. THE HUNTINGDON LIFE SCIENCES APPROACH

At Huntingdon Life Sciences, the neurotoxicology laboratory provides validated testing methods for motor activity, FOB and neuropathology; computerized data acquisition; highly qualified senior staff with experience in neuroanatomy, neurochemistry, neurohistological techniques coupled with an overall understanding of potential correlations among behavioral, biochemical and morphologic alterations. It is our commitment to our Sponsors to provide a scientifically sound, competitively priced study, on time, which will meet or exceed your expectations.

Huntingdon Life Sciences

Electron Microscopy

Huntingdon Life Sciences

"There is probably no disease or pathological condition where electron microscopy has not added new details and dimensions to existing knowledge. The innumerable published papers and books on the ultrastructure of tissues altered by disease or experimental procedures bear eloquent testimony to the many major contributions made by the technique." (F. Ghadially, 1988)

The high resolving power of the transmission electron microscope allows detailed investigation of cellular and sub-cellular morphology. Qualitative/quantitative changes in rough and smooth endoplasmic reticulum, alterations in the nature and numbers of secondary lysosomes or peroxisomes, and the identification of abnormal cytoplasmic inclusions are just a few examples of the power of this tool in the field of toxicology. It allows detailed examination of tissue structures which is beyond the limits of the light microscope.

I. EXPERIMENTAL DESIGN

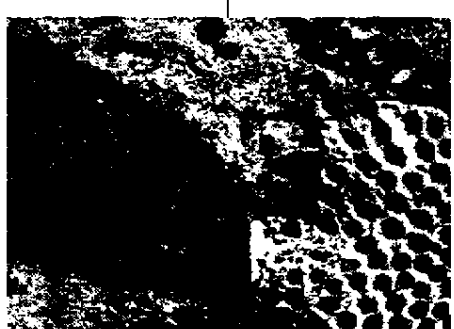
This is derived from the design of the overall study. The required tissue samples are taken from a recommended minimum of 3 male and 3 female animals, of each treatment group, and these are individually preserved in aldehyde fixatives developed especially for electron microscopy. Identification of the toxicologically susceptible tissues is a prime consideration in any electron microscopic investigation, as it allows for correct and prompt dissection and fixation of affected regions, criteria which are essential to the success of the investigation. Where dissection of a particular organ or tissue may compromise its availability for standard light microscope examination, satellite animals can be incorporated into the study design to facilitate electron microscopic examination. This can be the case in inhalation studies where examination of epithelia from the upper respiratory tract is required. Sampling of nasal or laryngeal tissues might also require this study design.

II. FIXATION AND EMBEDDING PROCEDURES

The most fundamental requirement of good electron microscopy is the correct dissection and initial fixation of the required target organs/tissues. Our approach includes the presence, at necropsy, of trained staff to carry out this crucial step. Our standard procedure involves immersion fixation in an appropriate aldehyde fixative. However, whole-body or *in vivo* organ perfusion can be used to ensure good preservation of delicate ultrastructure. This is generally recommended when investigating tissues such as those of the central nervous system. In the case of respiratory tract investigations, fixation can be performed in this manner or by direct infusion of the fixative into the airways. All resulting samples are secondarily fixed in 1% osmium tetroxide, dehydrated in acetone, and embedded in an epoxy resin medium.

III. ELECTRON MICROSCOPY

Investigations are initially carried out only on those samples taken from the top dosage group and the control group. The samples from intermediate groups are held in reserve pending the results of other investigations. Ultra-thin sections are cut from each sample and these are photographed using a Philips 300 Transmission electron microscope. The resulting micrographs are analyzed to establish any differences in ultrastructure between samples from treated animals and those from controls.



Perfusion fixed Rat Bronchus

Examinations have been made of many tissues for a variety of purposes. Following are some examples:

Identification of myelinoid bodies in possible cases of phospholipidosis.

Examinations of the epithelial layers of the respiratory tract.

Examination of corneal layers in the eye has helped to clarify changes identified at light micro.

Electron microscopy of lysosomes has enabled pathologists to clarify whether changes seen in the liver may have been as a result of adaptive change or a direct toxic effect. In this instance, electron microscopy can provide an invaluable aid to support regulatory submissions by helping to explain the source and nature of effects. Peroxisome proliferation has been linked with development of liver tumors in rodents. Electron microscopy is a useful way in which this effect can be assessed.

IV. REPORTING

A selection of the micrographs is chosen to represent the findings of the ultrastructure analysis. These are annotated to describe the cellular structures present in each micrograph and, together with the results and conclusion of the investigation, are bound in a report suitable for submission to the regulatory authorities.

V. OPTIONS

The flexible nature of electron microscopy investigations allows the possibility of a number of study designs.

All samples can be held as processed blocks, without investigation, while awaiting the outcome of other study procedures.

The use of resin embedding media additionally offers the advantages of high resolution light microscopy. Sections can be cut significantly thinner than standard wax embedded histology samples, and this feature can prove extremely useful to the pathologist and regulators.

Fully quantified reporting of ultrastructural changes can be carried out when micrographs are used in conjunction with available image analysis facilities.

VI. THE HUNTINGDON LIFE SCIENCES APPROACH

At Huntingdon Life Sciences, electron microscopic investigations are regularly used both to investigate specific characteristics of test materials and also to clarify and expand on light microscopic interpretation. The investigations are conducted by a skilled and experienced team of technologists and pathologists. It is our committment to our Sponsors to provide a scientifically sound, competetively priced study, on time, which will meet or exceed your expectations.

After the last day of treatment, males are caged with one or more untreated, virgin females and co-housed continuously for one week. The females are evaluated daily to determine the day of mating. After one week, the male is provided with two new untreated females and co-housed with these animals for the week. This mating routine is repeated over an 8–10 week period with a new set of untreated females being caged with the male each week. To determine dominant lethality the mated females are sacrificed mid-gestation and the uterine implants scored as viable or resorbed fetuses. The latter can also be further classified as early or late fetal deaths. The number of corpora lutea of pregnancy is also counted on each ovary.

Dominant lethality is evaluated on the basis of both the ratio of dead to total implants and pre-implantation loss which is the ratio of implants to corpora lutea. The sequential mating scheme used for these studies is useful in identifying the stage in the spermatogenic cycle that was most susceptible to the mutagenic effects of the treatment. Evidence of dominant lethality seen in females mated over the first three weeks indicates effects on the post-meiotic stages of spermatogenesis, while effects seen at week 4 or later indicate that sperm in the pre-meiotic stages were most susceptible. Effects seen late in the mating period (i.e., Weeks 8–10 post-treatment) would indicate that stem cell stages were affected. These studies are generally performed with both a negative control and a positive control group.

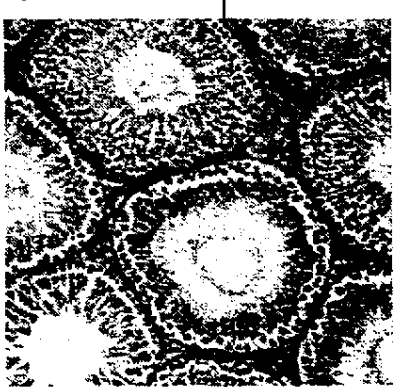
V. THE HUNTINGDON LIFE SCIENCES APPROACH

At Huntingdon Life Sciences, we have conducted over 570 developmental studies and over 310 reproduction studies. Therefore, the developmental and reproductive laboratory has abundant experience in the performance of a comprehensive range of studies for submission to regulatory authorities worldwide, aimed at the detection of adverse effects upon all aspects of reproduction and development. It is our commitment to our Sponsors to provide a scientifically sound, competitively priced study, on time, which will meet or exceed your expectations.

Females are sacrificed mid-gestation and uterine implants evaluated for number and viability status. Males continue on treatment until sacrificed at completion of the mating period. Along with receiving a gross postmortem evaluation, the reproductive tissues (testes, epididymides, prostate and seminal vesicles) are weighed and preserved. Spermatogenesis is assessed from histopathological evaluation of the testes and a sperm count and motility assessment performed (Figures 6a and 6b).

Fig 6a

Fig 6b



The second phase of study in these harmonized guidelines evaluates for potential adverse effects of treatment on pre- and post-natal development. These studies are designed to evaluate at least three dose levels and include a concurrent control group. Group sizes are large enough to provide at least 2 litters for evaluation. Treatment of the pregnant female initiates at the point in gestation that the embryo implants in the uterus (approximately Day 6 of gestation in rats and mice) and continues throughout lactation to weaning of the litter (Day 21 of lactation). During the study the maternal animals are evaluated for evidence of toxicity (clinical signs of toxicity, body weight data and food consumption, gross postmortem findings). An extensive evaluation of the pups is also conducted during this phase of study. Pups are evaluated for physical development and maturity (vaginal patency and preputial separation), sensory functions and reflexes, motor activity, learning and memory, and reproductive performance/fertility. The third phase of study in these harmonized guidelines is an assessment of developmental toxicity with pregnant females being treated over the period from implantation of the embryo in the uterus to closure of the palatal shelves (e.g., period of organogenesis).

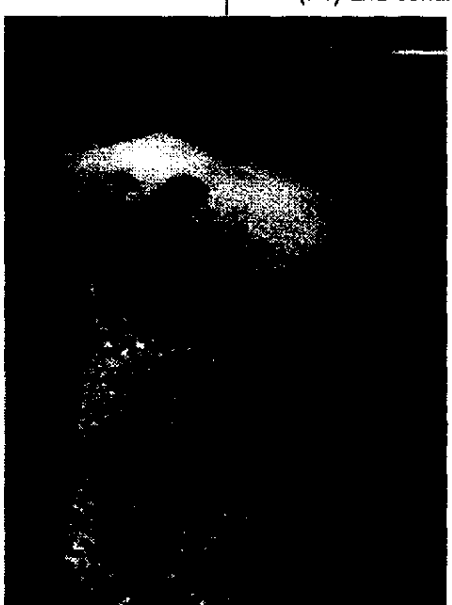
IV. DOMINANT-LETHAL MUTAGENICITY

One of the specialized studies conducted within the reproduction and developmental toxicology group is the *in vivo* dominant-lethal mutagenicity assay. Some agents demonstrate mutagenic effects by producing breaks in the chromosomes that result in gains, losses or rearrangement of pieces of chromosomes (clastogenic changes) and/or additions or deletions of entire chromosomes (aneuploidy). These types of chromosomal changes when they occur in the gametes (sperm, ova) can be passed along into the conceptus at fertilization and expressed as non-viable zygotes (i.e., dead embryo). The test involves treatment of male rats or mice and subsequently mating these animals with untreated, virgin females over a period of 8–10 weeks. Usually animals are treated for 1–5 days but longer treatment periods have also been used in this assay.

For mating, males are co-housed nightly with females (generally in a 1:1 ratio) from the same treatment group until evidence of mating is seen. Once mated, the females are housed individually for the remainder of gestation.

Treatment of the mated females continues over the 22-day gestation period and 21-day lactation period (Figure 5). Weanlings are selected to become the next parental generation (F1) and continue to be treated at the same dose level as their parents over an 11–14 week pre-mating period (both sexes), and throughout the ensuing mating, gestation and lactation periods. The study ends with the sacrifice of the F1 parental animals and the F2 pups at weaning. These types of studies elucidate potential effects of treatment on gonadal function, estrus cycling, mating behavior, conception, parturition, neonatal morbidity, mortality, weaning and the growth and development of the offspring.

Fig 5



Huntingdon Life Sciences has considerable experience with the performance of rat reproduction studies. Mating procedures, vaginal smearing and estrus cycle determination, parturition and neonatal observations, scoring of pup developmental landmarks to include vaginal patency and preputial separation, pup gross postmortem examinations, and other evaluation procedures unique to reproduction studies, are performed by a trained technical staff with years of experience.

An extensive historical control data base is maintained for many parameters to include mating, pregnancy and fertility indices, gestation length and parturition data, neonatal growth and survival to weaning. These data bases are updated regularly and used for interpretation of study results.

III. PRE- AND POST-NATAL TOXICITY

In the recently issued international harmonization guidelines to assess reproductive and developmental toxicity of medicinal products, a three-phase study approach is presented to evaluate adverse effects of treatment on reproduction and developmental toxicity through one complete life cycle.

The first phase evaluates the effects of treatment before mating (males and females treated for several weeks prior to mating) with treatment continuing through mating to the point in gestation when the embryo implants in the uterus.

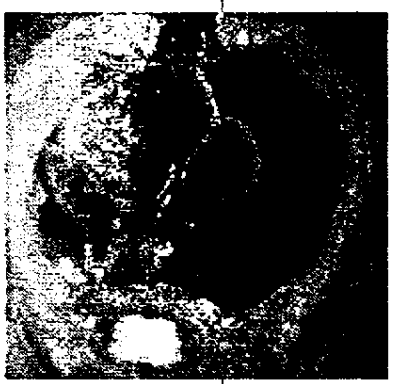
Fetal external, visceral and skeletal evaluations are performed by a trained technical staff. External examinations are performed shortly after the fetuses are removed from the uterus.

Fig 3

Visceral evaluations are performed by a microdissection procedure. Viscera in the thoracic, abdominal and pelvic cavities are evaluated in situ under a dissecting microscope (Figure 3). Another visceral evaluation procedure in which we have considerable experience is the free-hand, razor blade sectioning technique on Bouin's fixed fetuses (Figures 4a and 4b). Skeletal evaluations are performed on Alizarin-stained fetal specimens. The latter are evaluated under appropriate magnification for malformations and evidence of delayed ossification.

Historical control data bases are maintained for rats, mice and rabbits for corpora lutea/uterine implantation data, fetal weight and sex distribution data and all fetal evaluations. These data bases are updated regularly and compilations of these control data are routinely included in the final reports to assist in the interpretation of study results.

Fig 4a



II. REPRODUCTION STUDIES

There are a number of study types to evaluate the potential adverse effects of treatment on reproductive performance or fertility. These studies are usually performed with standard laboratory rodent species, either the rat or mouse. Considerable information is available on the reproductive cycle of these animals. They reproduce well in the laboratory, have high mating and fecundity indices, short gestation periods, large litters, short lactation period and a high success rate in rearing their offspring to weaning. A common study design to evaluate reproductive toxicity is the two-generation study.

This study design involves continuous treatment through two consecutive parental generations. Usually there are three treatment levels evaluated along with a concurrent control group. Each group is comprised of 25–30 animals per sex. Young animals (P1 generation) are treated for a period of time prior to mating. For males, this usually involves a period of at least 10 weeks so that animals are treated through one complete spermatogenic cycle (8–11 weeks in rats). In females, this should be a period of at least two weeks which provides treatment over two estrus cycles prior to mating. Treatment continues during the ensuing mating period.

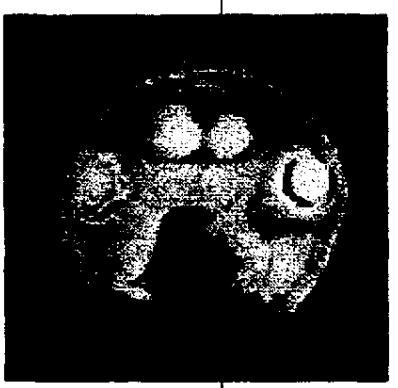


Fig 4b

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Reproductive and Developmental Toxicology

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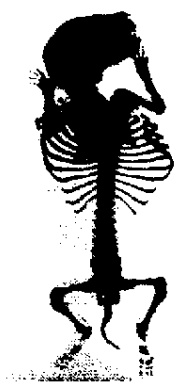
Research in the areas of reproductive and developmental toxicity has been active since the 1966 publication of the first FDA reproduction and teratology study guidelines for the evaluation of drugs. The nature of the materials studied has broadened over the years with the issuance of new and revised guidelines from regulatory agencies around the world. These agencies recognized the need for extending reproductive and developmental toxicity testing to a host of other materials to which man is exposed including pesticides, industrial chemicals, food additives and environmental pollutants.

Fig 1



Developmental and reproduction studies conducted today include: developmental toxicity studies in mice, rats and rabbits; reproduction and fertility studies, pre- and post-natal studies in rats; rat multi-generation studies; and dominant-lethal mutagenicity studies in rats. Protocols are developed and studies designed to meet requirements of various regulatory agencies, including those of the United States, the European Union, Japan and Canada. There has also been a movement over the last few years to adopt internationally harmonized protocols. All studies must be performed in full accordance with international Good Laboratory Practice regulations.

I. DEVELOPMENTAL TOXICITY

Developmental toxicity studies are conducted to detect potential adverse effects of treatment on the pregnant female and the developing embryo/fetus (Figure 1). These studies are traditionally conducted with standard laboratory small animal species such as the rat, mouse and rabbit. The reproductive performance of these species in the laboratory is good, and these species have well defined fetal developmental profiles. They also have high fertility indices, relatively short gestation periods and produce litters containing a large number of offspring. Treatment in developmental toxicity studies initiate in the pregnant animal after the embryo has implanted in the uterus (approximately Gestation Day 6) and continues over the period of major fetal organogenesis to closure of the palatal shelves (Gestation Day 15 in rats and mice and Day 18 in rabbits).

Fig 2

During the treatment period, animals are weighed periodically and food consumption recorded. Animals are also observed for mortality and clinical signs of toxicity. To maximize the opportunity for identifying malformations or other developmental irregularities in the offspring, females are sacrificed 1–2 days prior to expected delivery and the uterus evaluated in situ for the number of live, dead and resorbed fetuses. Upon removal from the uterus, fetuses are weighed, sexed, and evaluated for external, visceral and/or skeletal malformations or other irregularities (Figure 2). Other parameters evaluated at maternal sacrifice include a gross postmortem examination, a count of corpora lutea on the ovaries and evaluation of the placentae.

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Analytical Chemistry

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Analytical chemistry capabilities include development of analytical methods, when required, and validation of all analytical methods used to support toxicology studies. Separation and detection of analytes are accomplished using state-of-the-art instrumentation consisting mainly of gas and liquid chromatographs with a choice of appropriate detectors. Standard Operating Procedures and Good Laboratory Practice procedures are established for dose confirmation, stability determination, homogeneity and bioanalytical investigations.

Each year, analytical support is provided for many active ingredients in toxicology testing programs which results in a broad exposure of personnel to many different classes of chemical entities.

I. DOSE CONFIRMATION

Preparation of all dosage forms includes ensuring uniformity in the formulation whether administered as an injectable or in the diet. Replicate samples are collected from the formulation (sample matrix) from the top, middle and bottom layers, for example, to provide representative data to meet specified requirements. Typical results for a dietary mixture are shown in Table I.

TABLE I.

Typical Analysis of a Test Material in a Prepared Rat Chow at One Dose Level.

Sample	Replicate #	Target Dose Level - ppm	Found ppm	% of Target Level
Top	1	44.4	41.4	93.2
	2	Avg. % Target	39.4	88.7
	3	93.0	43.1	97.1
Middle	1		42.6	95.9
	2	Avg. % Target	41.0	92.3
	3	95.6	43.8	98.6
Bottom	1		37.2	83.8
	2	Avg. % Target	42.0	94.6
	3	90.2	40.9	92.1

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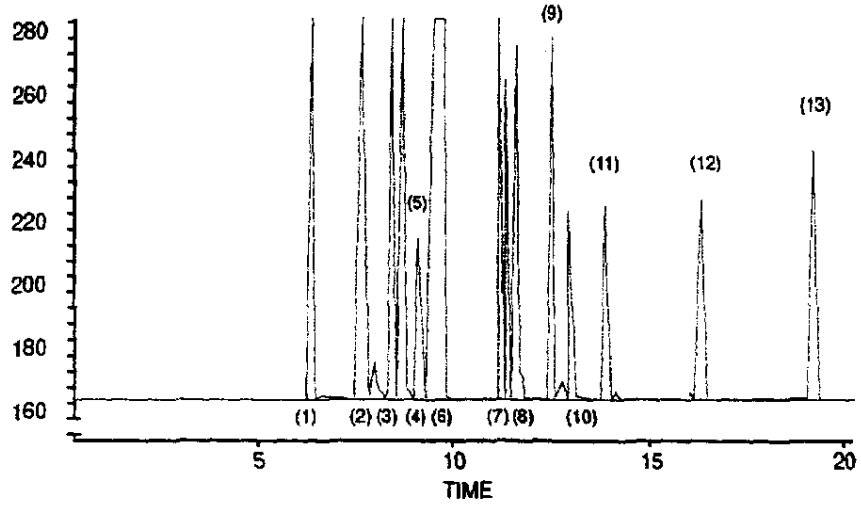
In addition, analytical chemistry support is provided for inhalation studies. Chambers are monitored for concentrations of a single or multiple components of a test material. Samples are analyzed on-line or may be collected in appropriate adsorbents with subsequent analysis usually by gas (GC) or liquid (HPLC) chromatography.

Results from a typical analysis of a multicomponent test substance are shown in Figure 1 which represents a hydrocarbon mixture sample collected on charcoal tubes and subsequent gas chromatographic analysis. Data are collected to ensure the presence of each component in the required concentrations and a uniform distribution in the inhalation chamber.

Fig 1. Analysis of a Hydrocarbon Mixture Containing 13 Components by Gas Chromatography.



II. STABILITY OF TEST MATERIALS

Stability of dosage forms is investigated at ambient temperatures or below to demonstrate storage conditions required prior to administering the dose. For chronic studies, stability of the test material in a dietary admixture, for example, may be investigated over a duration of several weeks. Results for a test substance previously blended into a rodent chow are shown in Table II. Results indicate the test material is not stable in the diet over an 8-day storage interval at ambient (room) temperature.

TABLE II.
Stability of an Organophosphorous Compound in Rat Diet at Ambient Temperature

Days / Replicate	Concentration (ppm) Level Added	Level Found	% Found
0 - 1	10.0	9.5	95
- 2	Avg. % Added	8.9	89
- 3	94	9.9	99
1 - 1	Avg. % Added	9.5	95
- 2	93	9.2	92
5 - 1	Avg. % Added	8.5	85
- 2	84	8.3	83
8 - 1		6.9	69
- 2	Avg. % Added	7.2	72
- 3	72	7.8	78
- 4		6.9	69

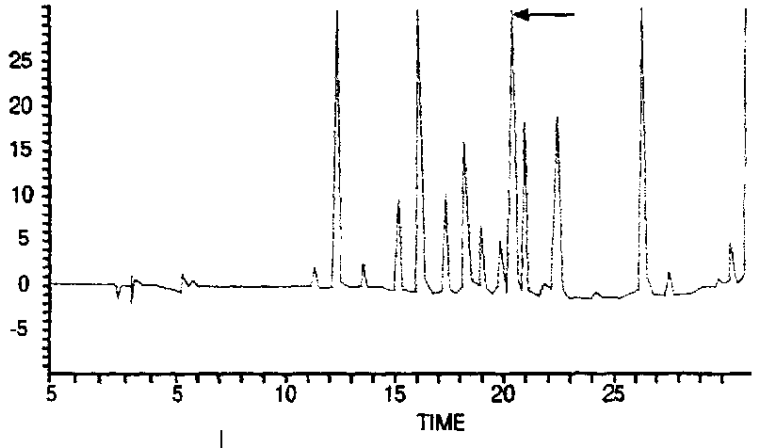
III. BIOANALYTICAL SUPPORT

Analysis of plasma from several test animal species is routinely conducted after validation of an analytical method. Analytical methodology can be developed, if not already available. Quantification of the test material, whether parent or its metabolites, is determined using internal or external standards. Intra- and inter-assay variance is established as well as accuracy and precision. Analysis may be conducted by gas chromatography with electron capture, flame ionization detection or selected ion monitoring by mass spectrometry or by liquid chromatography with ultra-violet or fluorescence detection. Analytical results for a typical drug substance in a plasma test sample are illustrated in Figure 2. Toxicokinetic parameters can then be calculated from the plasma data, as required.

IV. THE HUNTINGDON LIFE SCIENCES APPROACH

At Huntingdon Life Sciences, the analytical laboratory uses the latest state-of-the-art techniques to provide the most accurate, precise and timely quantitation of test material levels in numerous matrices. If analytical methodology is not available, we have the expertise to develop appropriate methods. It is our commitment to our Sponsors to provide a scientifically sound, competitively priced study, on time, which will meet or exceed your expectations.

Fig 2. Levels of a Drug Substance in Plasma from Monkeys.



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Regulatory Compliance

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Over the years growing emphasis has been placed on the need for a clinical research organization to have a good regulatory history with both foreign and domestic agencies. In response to this, Huntingdon Life Sciences has developed the expertise to run studies in full compliance with U.S., European and Japanese regulations. Policy in the regulatory arena is to meet or exceed the expectations of the governing agency, as well as those of our clients. Huntingdon Life Sciences is proud of its history with the regulatory agencies and makes available a package of its most recent inspections upon request. This package includes the inspection reports as well as any associated correspondence. No study, or portion of a study, that we have conducted has ever been rejected by a regulatory agency.

Regulatory agencies are frequent visitors to our facility to inspect data from studies which have been submitted by our clients. One of the most recent inspections conducted by the FDA in November 1994 at our East Millstone, New Jersey facility was a directed inspection (i.e. study specific). The inspection was conducted over several days covering two studies and no FDA-483 was issued. At our Eye, Suffolk facility in the United Kingdom, frequent inspections by the governing agency, the Department of Health (DoH) are conducted. The Eye, Suffolk site is a fully certified laboratory by the DoH. Huntingdon Life Sciences has also embarked on an effort to become a ISO 9000 certified laboratory.

THE HUNTINGDON LIFE SCIENCES APPROACH

With the integration of our US and UK preclinical testing capabilities, clients can be assured that with our combined expertise we will be able to provide them a compliant study conducted with the highest quality standards around the world. It is our commitment to our Sponsors to provide a scientifically sound, competitively priced study, on time, which will meet or exceed your expectations.

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VINYL CHLORIDE COMBINED INHALATION TWO-GENERATION REPRODUCTION AND DEVELOPMENTAL TOXICITY STUDY IN CD RATS

STUDY NO.: *

ISSUE NO.: 1

SPONSOR
STUDY NO: *

SPONSOR: CHEMICAL MANUFACTURERS ASSOCIATION

SPONSOR
REPRESENTATIVE: Hasmukh C. Shah, Ph.D

DATE: 5 December 1995

Huntingdon Life Sciences Inc. PO Box 2360, Mettlers Road, East Millstone, NJ 08875-2360 USA.
Tel: +1 908 873 2550 Fax: +1 908 873 3992

1 INTRODUCTION:

1.1 STUDY NO.: *

1.2 ISSUE NO.: 1

1.3 STUDY TITLE: Vinyl Chloride Combined Inhalation Two-Generation Reproduction and Developmental Toxicity Study in CD Rats.

1.4 TEST MATERIAL: Vinyl Chloride

1.5 SPONSOR:
Chemical Manufacturers Association
2501 M Street, NW
Washington, DC 20037

1.6 SPONSOR REPRESENTATIVE:
Hasmukh C. Shah, Ph.D.
Work - 202-887-1192
Fax - 202-887-4756

1.7 TESTING FACILITY:
Huntingdon Life Sciences
Mettlers Road
P.O. Box 2360
East Millstone, NJ 08875-2360

1.8 PURPOSE:

The objectives of the combined inhalation two-generation reproduction and development toxicity study outlined in this protocol are to evaluate the effects of the test material on parental toxicity, reproductive capability, in utero development, and neonatal growth and survival in rats.

2 REGULATORY REFERENCES:

2.1 TEST GUIDELINE:

This study is designed to meet or exceed the guideline requirements of the EPA (Environmental Protection Agency: TSCA Test Guidelines (EPA, 1985); the Organization for Economic Co-operation and Development (OECD, Guidelines for testing of chemicals, Section 4: Health Effects, (OECD, 1981); and the European Economic Community (EEC), Methods for the Determination of Toxicity (EEC, 1988).

2.2 GOOD LABORATORY PRACTICES:

This study will be conducted in compliance with the FDA (Food and Drug Administration) Good Laboratory Practice (GLP) Regulations for nonclinical studies (FDA, 1988), the EPA TSCA Good Laboratory Practice Standards (EPA,1990), the OECD Good Laboratory Practice Procedures (OECD,1982) and the standard operating procedures of the Testing Facility.

2.3 FACILITIES MANAGEMENT/ANIMAL HUSBANDRY:

Currently acceptable practices of good animal husbandry will be followed, e.g., Guide for the Care and Use of Laboratory Animals; DHHS Publication No. (NIH) 86-23, Revised 1985. Huntingdon Life Sciences (East Millstone, NJ) is fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

2.4 ANIMAL WELFARE ACT COMPLIANCE:

This study will comply with all appropriate parts of the Animal Welfare Act regulations: 9 CFR Parts 1 and 2 Final Rules, Federal Register, Volume 54, No. 168, August 31, 1989, pp. 36112-36163 effective October 30, 1989 and 9 CFR Part 3 Animal Welfare Standards; Final Rule, Federal Register, Volume 56, No. 32, February 15, 1991, pp. 6426-6505 effective March 18, 1991. The Sponsor should make particular note of the following:

1. The Sponsor's signature on this protocol documents for the study described, there are no generally accepted non-animal alternatives and the study does not unnecessarily duplicate previous experiments.

2. All procedures used in this study have been designed to avoid discomfort, distress and pain to the animals. All methods are described in this study protocol or in written laboratory standard operating procedures.

3. Any aspects of this study which cause more than momentary or slight pain or distress to the animals will be performed with appropriate sedatives, analgesics or anesthetics unless the withholding of these agents is justified for scientific reasons, in writing by the Sponsor and the Study Director, in which case the procedure will continue for the minimum time necessary.

4. Animals that experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanatized as deemed appropriate by the Testing Facility's veterinary staff and the Study Director. The Sponsor will be advised by the Study Director of all circumstances which could lead to this action in as timely a manner as possible.

2.4 ANIMAL WELFARE ACT COMPLIANCE:

5. Methods of euthanasia used during this study are in conformance with the above referenced regulations.

3 QUALITY ASSURANCE MONITORING:

The Huntingdon Life Sciences Quality Assurance Unit (East Millstone, NJ) will monitor the facilities, equipment, personnel, methods, practices, records and controls used in this study to assure that they are in conformance with this protocol, company standard operating procedures, and the appropriate Good Laboratory Practice regulations.

4 ALTERATION OF DESIGN:

Alterations of this protocol may be made as the study progresses. No changes in the protocol will be made without the consent of the Sponsor. In the event that the Sponsor authorizes a protocol change verbally, such changes will be honored by the Testing Facility and will be followed by a written verification. All protocol modifications will be signed by the Study Director and a Sponsor representative. Any modifications potentially affecting animal welfare will also be signed by two members of the Institutional Animal Care and Use Committee prior to the modification's implementation.

5 STUDY PERSONNEL:

Study Director: *

Director of Inhalation Toxicology: *

Study Pathologist: *

Additional personnel will be documented in the project file and presented in the final report.

6 PROPOSED STUDY DATES:

Reproduction Study:

Initiation date:	Date Study Director Signs protocol - See Section 18.2.
Receipt of test animals:	*(to be addressed by amendment)
Initiation of exposures (P_1): (Experimental start date)	*
Termination of exposures (P_1):	*
Selection (P_2 from the F_1):	*
Termination of exposures (P_2):	*
Necropsy	
P_1:	*
P_2:	*

Developmental Toxicity Study:

Receipt of test animals:	*
Initiation of mating:	*
Initiation of exposures (first Day 6 gestation):	*
Termination of exposures (last Day 19 gestation):	*
Experimental termination: (date of last data collection)	*
Submission of draft final report:	*
Study completion date:	Date final report is signed by Study Director.

7 EXPERIMENTAL DESIGN:

7.1 Two-generation Reproduction Study:

Group	Exposure Level (ppm)	Number of Animals							
		Mated Adults				Microscopic Pathology Adult Generations[b]			
		P_1		P_2		P_1		P_2	
		M	F	M	F	M	F	M	F
I (control)	0[a]	30	30	30	30	30	30	30	30
II	10	30	30	30	30	A.R.	A.R.	A.R.	A.R.
III	100	30	30	30	30	A.R.	A.R.	A.R.	A.R.
IV	1100	30	30	30	30	30	30	30	30

[a] Control animals will be chamber-housed and sham-treated with clean room air for a comparable period of time as the test animals.
[b] Histologic examinations will be performed for tissues listed in Appendix A.

Key: A.R. = As Required: 1) tissues that demonstrate treatment-related histologic changes in Group IV (additional cost); 2) gross lesions from animals found dead or euthanatized in a moribund condition during the study (additional cost); and 3) gross lesions terminal animals (additional cost); M = Male; F = Female.

7.2 Developmental Toxicity Study:

The developmental toxicity study will be performed with a unique population of animals during the exposure period for the P_1 parental animals. This will be during the mating and gestation periods for the F_1 pregnancies when animals are being exposed 7 days/week. Animals in the developmental toxicity study will be exposed in the chamber along with the animals in the two-generation reproduction study.

Group	Exposure Levels (ppm)	Treatment Schedule	Number of Animals				
			Mated	Sacrificed[a]	Proportion of Gestation Day 20 Fetuses/Litter Malformation/Variation Evaluations		
				Gestation Day 20	External	Soft Tissue	Skeletal
I (chamber - housed, sham air control)	0	Gestation Days 6-19	25	A.S.	All	½	½
II	10	Gestation Days 6-19	25	A.S.	All	½	½
III	100	Gestation Days 6-19	25	A.S.	All	½	½
IV	1100	Gestation Days 6-19	25	A.S.	All	½	½

[a] Gross postmortem examination, liver and kidneys will be weighed and preserved along with gross lesions. Microscopic examinations of these tissues will not be conducted unless deemed necessary to interpret other observations made during the study or requested by the Sponsor (additional cost).

A.S. = All Survivors;

8 TEST MATERIAL:

8.1 TEST MATERIAL: Vinyl Chloride

Description, lot number, storage, expiration date and handling procedures, as well as other pertinent information will be documented in the study data. Properties of the test material are presented in the Appendix B.

8.2 IDENTIFICATION OF TEST MATERIAL:

Unless otherwise noted, the identity, strength, purity, composition, stability, and method of synthesis, fabrication and/or derivation of each batch of the test material will be documented by the Sponsor before its use in the study.

8.3 ANALYSIS OF THE TEST MATERIAL:

The purity of the test material at the start of study and at 6-month intervals will be determined and reported. These analyses will be performed in the analytical laboratory of the Testing Facility.

8.4 ARCHIVAL SAMPLES:

A sample (approximately 10 grams) from each lot of test material used on study will be taken and stored in the Archives of the Testing Facility at East Millstone, NJ.

8.5 UNUSED TEST MATERIAL:

At completion of the study unused test material, waste material and empty test material containers will be returned to the sponsor (information concerning where these materials should be returned will be provided by the Sponsor).

9 TEST ANIMALS: Albino Rats (Outbred) VAF/Plus®

9.1 STRAIN:

Sprague Dawley - derived (CD®)
[Crl: CD® BR]

9.2 SUPPLIER:

Charles River Laboratories
Portage, Michigan

9.3 JUSTIFICATION FOR TEST SYSTEM SELECTION:

The rat is a rodent animal model commonly utilized in reproduction and developmental toxicity studies as recommended in the referenced guidelines. In addition, a historical control data base with this strain of animal and supplier facility is available for comparative evaluation.

9.4 ANIMAL REQUIREMENTS/SPECIFICATIONS:

9.4.1 Number:

9.4.1.1 Two-generation Reproduction Study (P_1):

	Total	Males	Females
Placed on test	240	120	120

9.4.1.2 Developmental Toxicity Study:

Placed on test - 100 mated females (25/group).

9.4.2 Age:

9.4.2.1 Two-generation Reproduction Study (P_1):

Males and females: approximately four weeks at receipt; approximately six weeks (and no more than eight weeks) at initiation of treatment.

9.4.2.2 Developmental Toxicity Study:

Females: eight weeks at receipt and at least 10 weeks at initiation of mating. Females will be nulliparous and non-pregnant.

Males: in-house breeding colony used only for mating.

9.5 ACCLIMATION PERIOD:

Approximately two weeks; all animals will be checked for viability twice daily. Prior to assignment to study all animals will be examined to ascertain suitability for study.

9.6 ANIMAL HUSBANDRY:

9.6.1 Housing:

9.6.1.1 Two-generation Reproduction Study (P_1):

Animals will be housed in suspended, stainless steel cages with wire mesh fronts and floors. For the first week of acclimation, animals will be housed two/sex/cage. Thereafter, animals will be housed individually except as follows:

Mating: one male and one female co-housed nightly.
Lactation: dam with litter.
Postweaning: two littermates/sex until selection for P_2 parental generation.

During exposures, parental animals will be housed individually in suspended stainless steel wire mesh cages. Neonates will be housed 1-2/cage (littermates) until the formal initiation of the premating treatment period.

9.6.1.2 Developmental Toxicity Study:

Animals will be housed in suspended, stainless steel cages with wire mesh fronts and floors except during mating when females will be co-housed overnight with a male. During exposures, animals will be housed in suspended stainless steel wire mesh cages.

9.6.2 Food:

Certified Rodent Diet, No. 5002; (Meal) (PMI Feeds, Inc., St. Louis, MO). Each animal's cage will be fitted to retain a glass feeder cup with a stainless steel lid. Feed will be available *ad libitum* during non-exposure periods except on the evening prior to necropsy when parental animals (P_1 and P_2) will be fasted. During exposures, animals will not have access to feed.

9.6.3 Water:

Facility water supply (Elizabethtown Water Company, Westfield, NJ); without restriction, via an automated water delivery system to individual animal cages.

9.6.4 Bedding Material - Two-generation Reproduction Study:

Hardwood shaving bedding (Lab Aspen Shavings, North Eastern Products Corporation, Warrensburg, NY) will be provided for each mated female on Day 20 of gestation. Fresh bedding will be provided as needed to Day 14 of lactation.

9.6.5 Feed Analysis:

Analytical certification of batches of feed used during the study which are provided by the manufacturer, will be maintained on file at the Testing Facility. There are no known contaminants in the feed which are expected to interfere with the results of this study.

9.6.6 Water Analysis:

Monthly water analyses, provided by the supplier, will be maintained on file at the Testing Facility. Biannual chemical and microbiological analyses of water samples collected from representative rooms in this facility will be conducted to assure that water being provided meets standards specified under the EPA National Primary Drinking Water Regulations (40 CFR Part 141). Results will be maintained on file. There are no known contaminants in the water which are expected to interfere with the results of this study.

9.6.7 Bedding Analyses:

Analyses for each batch of bedding used on study provided by the supplier, will be maintained at the Testing Facility. There are no known contaminants in the bedding which are expected to interfere with the results of this study.

9.6.8 Veterinary Care:

Animals will be monitored by the technical staff for any conditions requiring possible veterinary care. If any such conditions are identified, a staff veterinarian will be notified for an examination and evaluation. Any medical veterinary intervention will be made only with approval of the staff veterinarian and the Study Director. The Sponsor will be consulted whenever possible. However, in emergency situations, decisions will be made as needed and the Sponsor will be advised as soon as possible.

9.6.9 Environmental Conditions:

9.6.9.1 Light/Dark Cycle:

Twelve hour light/dark cycle daily.

9.6.9.2 Temperature:

Monitored and recorded twice daily. Temperature in the animal room will be maintained in the range of approximately 20-24°C to the maximum extent possible.

9.6.9.3 Humidity:

Monitored and recorded once daily. The desired humidity range is 40-70%. Humidity will be maintained in this range to the maximum extent possible.

9.7 SELECTION FOR STUDY:

9.7.1 Two-generation Reproduction Study (P_1) :

More animals than required for the study will be purchased and acclimated. Animals considered suitable for study on the basis of pretest physical examinations, body weight data and any other pretest evaluations will be randomly assigned to control or treated groups in an attempt to equalize mean group body weights. Individual weights of animals placed on test shall not exceed 20% of the mean weight for each sex. Disposition of all animals not used in the study will be maintained in the study file.

9.7.2 Developmental Toxicity Study:

More females than required for the study will be purchased and acclimated. Animals considered suitable for study on the basis of pretest physical examinations will included into the mating phase of the study. Females which mate will be assigned to groups daily in such a way as to most nearly equalize both the Day 0 mean body weights between groups and the distribution of animal into groups.

9.8 ANIMAL IDENTIFICATION:

9.8.1 Two-generation Reproduction Study:

Each animal will be assigned a temporary identification number upon receipt. After selection for study (P_1 generation), each animal will be ear-tagged with a number assigned by the Testing Facility.

9.8.1 Two-generation Reproduction Study:

This number plus the study number will comprise the unique identification for each study animal. Following the procedure (post-weaning) to select the P_2 parental animals from the F_1 offspring, these selected animals will be ear-tagged with a unique number. If the tag is lost, it will be replaced or the animal will be tail tattooed for identification. Each animal's cage will be provided with a card which will be color-coded for dose level identification and will contain the study number and animal number.

9.8.2 Developmental Toxicity Study:

Each female will be assigned a temporary identification number upon receipt. Mated females sorted into study groups will be ear-tagged with a number assigned by the Testing Facility that will be unique from the identification numbers used in the two-generation study. This number plus the study number will comprise the unique identification for each study animal. Females will be eartagged on Day 0 of gestation.

10 MATING, GESTATION AND LACTATION PROCEDURES:

10.1 MATING PROCEDURE:

10.1.1 Two-generation Reproduction Study (P_1, P_2):

After animals have been exposed to the test substance for the appropriate length of time (premating treatment period), one male and one female of equivalent dose levels will be caged together nightly until a sign of mating (microscopic observation of sperm in the vaginal smear and/or a copulation plug in the vagina) is observed or for 14 consecutive days. The day evidence of mating is observed will be defined as Day 0 of gestation. If mating has not occurred after this interval, the animals will be separated without further opportunity for mating. During mating of the P_2 generation cohabitation of male and female littermates will be avoided.

10.1.2 Developmental Toxicity Study:

Females selected for mating will be placed with breeder males nightly in a 1:1 ratio. Vaginal smears will be taken early in the morning following nightly interval of co-housing and females will be considered to have mated if sperm is observed microscopically in the vaginal smear and/or a vaginal plug is observed. The day on

10.1.2 Developmental Toxicity Study:

which evidence of mating is observed will be defined as Day 0 of gestation.

10.2 PARTURITION AND LACTATION - TWO-GENERATION REPRODUCTION STUDY:

On Day 20 of gestation, several days prior to expected parturition, each mated female's cage will be fitted to retain a stainless steel floor pan and bedding material provided. Examination for signs of parturition will be made twice daily (morning and afternoon). Evidence of difficult or prolonged parturition, if observed, will be recorded. The day on which all pups have been delivered will be defined as Day 0 of lactation. For females which were caged with males but exhibited no evidence of mating, preparations for undetermined pregnancies (*i.e., floor pan*, bedding material) will be made when the first animals mated reach their day 20 of gestation.

10.3 SELECTION OF P_2 PARENTAL GENERATION:

At weaning of each F_1 litter, two pups/sex/litter will be chosen at random to become a pool of animals from which the P_2 parental generation will be selected. Pups as chosen will be housed two littermates of the same sex/cage. After weaning of the last F_1 litter, 30 F_1 pups/sex/group will be selected from this pool of animals to become the P_2 parental animals. In this selection procedure, each litter will contribute at least one pup/sex, when possible, so as to maximize the representation of animals from different litters in each group.

After the last litter is weaned, the 30 F_1 pups/sex/group designated for the P_2 parental generation will formally initiate the premating treatment period. Thus, there may be a maximum of two weeks difference in age for the P_2 animals within each treatment group at initiation of the premating growth period. During this postweaning period, the pool of F_1 pups will be chambered and exposed (6 hrs./day) at the treatment level of the dam.

In the selection procedure, grossly malformed or obviously-diseased animals within the litters will be excluded from the pool of animals eligible for selection if, in the judgement of the Study Director, their condition may adversely affect long-term survival. Runts, if otherwise normal, will not be excluded from the selection procedure.

11 TEST MATERIAL ADMINISTRATION:

11.1 ROUTE OF ADMINISTRATION:

Inhalation (whole body).

11.2 JUSTIFICATION FOR ROUTE OF ADMINISTRATION:

The inhalation route is one of the potential routes of human exposure to this test material.

11.3 FREQUENCY OF ADMINISTRATION:

11.3.1 Two-generation Reproduction Study:

P_1 and P_2 generation animals will be exposed 6 hours/day, five days/week for at least 70 days (10-week) prior to mating (designated the premating treatment period). Males will continue to be exposed daily, 6 hrs/day during the 14-day mating period and postmating period until sacrificed. Females will continue to be treated daily, 6 hrs/day during mating and mated females will continue to be treated, 6 hrs/day during the ensuing gestation period through to Day 20. Females will be allowed to deliver (Day 0 of lactation) and initiate lactation and treatment will resume on Day 4 of lactation. Females will continue to be treated (6 hrs/day) thereafter for the remainder of lactation and postweaning period until sacrificed. Unmated females will continue to be treated daily (6 hrs/day) until sacrificed.

11.3.2 Developmental Toxicity Study:

Females will be treated 6 hours/day over the Day 6-19 gestation interval. The exposure interval for these animals will coincide with the exposures for the mating/gestation/lactation periods for the F_1 litters in the two-generation reproduction study.

11.4 GENERATION PROCEDURE:

The test material will be administered as a vapor/aerosol in the breathing air of the animals. The test atmospheres will be generated by an appropriate procedure to be developed at the Testing Facility . The generation method will be described in the final report and maintained with the data package for this study.

The whole-body exposure chambers will have a minimum volume of approximately 6000 liters (6 m^3). Each chamber will be operated at a minimum flow rate of 1200 liters per minute. The final airflow will be set

11.4 GENERATION PROCEDURE:

to provide at least one air change in 5.0 minutes (12 air changes/hour) and a T_{99} equilibrium time of approximately 23 minutes. This chamber size and airflow rate are considered adequate to maintain the oxygen level above 19%.

11.5 CHAMBER MONITORING:

A nominal exposure concentration will be calculated, if possible. The flow of air through the chamber will be monitored using appropriate calibrated equipment. The test material consumed during the exposure will be divided by the total volume of air passing through the chamber (volumetric flow rate times total exposure time) to give the nominal concentration.

During each test material exposure, measurements of airborne concentrations will be made at least hourly using a MIRAN infrared spectrophotometer. Also prior to initiating of animal exposures, additional samples will be taken to determine the distribution of the test material in the exposure chamber.

If more than the normal amount of trialing is required because of test material generation or monitoring problems (two weeks or 150 technician hours), the Sponsor will be consulted prior to additional trialing (additional cost).

The minimum frequency of chamber activity is summarized below:

Activity	Frequency/chamber/day
Analytical Test Material Concentration	6 times (hourly)
Temperature	12 times
Relative Humidity	12 times
Airflow Rate	12 times
Nominal Test Material Concentration (excluding the control chamber)	once
Rotation Pattern of Exposure Cages	once
Loading/Unloading Verification	once

11.6 EXPOSURE CONCENTRATIONS:

For both the two-generation reproduction and developmental toxicity studies the targeted exposure concentrations will be 0, 10, 100 and 1100 ppm of the test material. These exposure levels correspond to oral equivalent doses of approximately 0, 9.2, 92 and 1012 mg/kg/day assuming ventilation rates of 1 L/min/kg, 100 percent absorption and a 6 hr/day exposure. The high concentration of vinyl chloride was selected based on the oral equivalent of the limit dose of 1000 mg/kg body weight/day. The high exposure level of 1100 ppm is also expected to produce effects on the liver and other organ systems (ATSDR, 1993). The middle and low exposure levels were selected to provide a dose response for the observed effects and a no-observed-effect level, respectively.

12 EXPERIMENTAL EVALUATIONS:

12.1 OBSERVATIONS:

12.1.1 Viability Checks - Two-generation reproduction study and developmental toxicity study:

Observations for mortality, general appearance and signs of severe toxic or pharmacologic effects as well as availability of feed and water will be made at least twice daily (morning and afternoon). Animals in extremely poor health or in a possible moribund condition will be identified for further monitoring and possible euthanasia. All animals found dead will be submitted for a gross macroscopic examination. Parental animals found dead after normal working hours will be refrigerated until a necropsy can be performed.

12.1.2 Physical Examinations:

12.1.2.1 Two-Generation Reproduction Study:

Each animal will be removed from its cage and examined at least once pretest (P_1), at the study start (first day of treatment for the P_1) and weekly thereafter during the study period (P_1 and P_2). Examinations will include observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia as well as evaluations of respiration, and palpation for tissue masses. These examinations will be performed postexposure when animals are being transferred to their home cages.

12.1.2.2 Developmental Toxicity Study:

Mated animals will be given a detailed physical examination on Days 0 and 6-20 (daily) of gestation. Examinations will include observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia as well as evaluations of respiration, and palpation for tissue masses. During the test period, these examinations will be performed postexposure when animals are being transferred to their home cages.

12.2 BODY WEIGHTS:

12.2.1 Two-generation Reproduction Study:

Males:

P_1: at the time of sorting into groups, on the day treatment initiates, weekly throughout the study and at termination.
P_2: at the formal initiation of the premating treatment period and weekly throughout the study and at termination.

Females:

P_1: at the time of sorting into groups.
P_1 and P_2: on the day treatment initiates (formal initiation of the premating period for the P_2), weekly during the premating growth periods; gestation - Days 0, 7, 14 and 20; and lactation: days 0, 4, 7, 14, 21 and 25 (P_1 only); and at termination.

12.2.2 Developmental Toxicity Study:

Females will be weighed on Days 0, 6, 9, 12, 15 and 20 of gestation.

12.3 FOOD CONSUMPTION:

12.3.1 Two-generation Reproduction Study:

Males:

P_1: one week pretest.
P_1 and P_2: weekly during the premating treatment period and from termination of mating through to sacrifice (postmating period). Food consumption will not be recorded during the mating periods.

Females:

P_1: one week pretest.
P_1 and P_2: weekly during the premating period; during gestation - Days 0-7, 7-14 and 14-20; during lactation - Days 1, 4, 7, 10 and 14. Food consumption will not be recorded during the mating period or postweaning period prior to termination.

12.3.2 Developmental Toxicity Study:

Food consumption will be recorded for the following intervals during Gestation: Days 0-6, 6-9, 9-12, 12-15 and 15-20.

12.4 ESTROUS CYCLE DETERMINATIONS - TWO-GENERATION REPRODUCTION STUDY:

Estrous cycle length and normality will be evaluated daily by vaginal lavage on the first 15 P_1 and P_2 females per group for the three week period prior to mating and continuing throughout the mating period or until the female is confirmed mated.

12.5 LITTER EVALUATIONS (F_1, F_2) - TWO-GENERATION REPRODUCTION STUDY:

12.5.1 Observations:

Litters will be observed as soon as possible after delivery for the number of live and dead pups and pup abnormalities (Day 0 of lactation). Thereafter, litters will be observed twice daily (morning, afternoon) for the presence of dead pups. These pups and pups euthanized in a moribund condition will be examined to the extent possible for defects and/or cause of death and preserved in neutral, phosphate-buffered 10% formalin. Litter size will be recorded on Days 0, 4, 7, 14, 21 (F_1 and F_2) and 24 (F_1 only) of lactation.

12.5.2 Culling:

On Day 4 of lactation, each litter with more than eight pups will be culled to that number with sex distribution equalized (four/sex) when possible. Pups will be culled using a random number table. Preferential culling of runts will not be performed. Culled pups will be examined grossly for abnormalities and following euthanasia by an overdose of inhaled carbon dioxide (CO_2) will have sex confirmed buy internal inspection of the gonads. Only culled pups with abnormalities will be preserved in 10% neutral buffered formalin.

12.5.3 Physical Examinations:

Each pup will be given a gross physical examination on Days 0, 4, 7, 14, 21 and 24 (F_1 pups only).

12.5.4 Pup Body Weights and Sexing Data:

Individual pup weights and pup sexing data will be recorded on Days 0, 4 (presented for both pre- and postcull intervals), 7, 14, 21 and 24 (F_1 pups only) of lactation.

12.5.5 Physical Maturation Landmarks - Selected P_2 Animals:

All F_1 weanlings selected to become the P_2 parental animals (30 sex/group) will be observed daily for vaginal opening beginning on postnatal day 30 or preputial separation beginning on postnatal day 35. If a treatment-related effect is seen in F_1 sex ratios, age of vaginal opening or preputial separation, then anogenital distance will be measured on postnatal day 4 for all F_2 pups.

13 POSTMORTEM:

13.1 TWO-GENERATION REPRODUCTION STUDY:

13.1.1 GROSS POSTMORTEM EXAMINATION:

13.1.1.1 Parental animals:

Complete macroscopic postmortem examinations will be performed on all adult animals, including animals euthanatized in a moribund condition or found dead. These evaluations will be performed under the direct supervision of a veterinary pathologist. The eyes of the parental animals will be examined *in situ* by gently pressing a moistened glass slide against the cornea and observing the eyes under fluorescent light. During the gross postmortem examination all abnormal observations will be recorded. The necropsy of the

13.1.1.1 Parental animals:

parental animals will include examination of the external surface and all orifices; the external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass. Examination of all parental females which were exposed to males, will include a count of uterine implantation scars, if present.

13.1.1.2 Weanling Pups (F_1 and F_2):

At weaning (Day 24 - F_1 and Day 21 - F_2) one pup/sex/litter/group will be selected at random for a complete gross postmortem examination. These examinations will be performed under the supervision of a veterinary pathologist. To control for variation in body weight and organ weight, all pups will be sacrificed at the same age.

13.1.2 TIME OF NECROPSY:

13.1.2.1 Moribund Animals:

Animals showing signs of severe debility, particularly if death appears imminent, will be euthanatized to prevent loss of tissues through autolysis.

13.1.2.2 Terminal Necropsy Males (P_1, P_2):

P_1 males will be sacrificed after the last F_1 litters have been delivered. This will permit some evaluation of fertility (i.e., number of litters delivered) prior to sacrifice. The P_2 males will be sacrificed approximately one week after completion of the mating period to produce the F_2 pregnancies. The Sponsor will be notified prior to sacrifice of the P_1 and P_2 males.

13.1.2.3 Terminal Necropsy Females (P_1, P_2):

All P_1 and P_2 females, regardless of reproductive status (unmated, mated but not pregnant, or with litter) will be sacrificed as a group after the last litters have weaned for all groups. Parental females (P_1 and P_2) will be necropsied on day 2 of diestrus whenever possible. This will be identified by daily vaginal smearing of the females for the occurrence of at least one estrous cycle after which time females found to be in day one of diestrus will be fasted overnight and necropsied the following day. The presence of diestrus will be confirmed by vaginal smearing the morning of necropsy.

13.1.2.4 Terminal Necropsy Pups (F_1, F_2):

13.1.2.4.1 Dead and Culled Pups:

Pups found dead at birth or during the lactation period will be examined to the extent possible for defects and/or the cause of death, presence or absence of milk in the stomach. Dead pups will not be eviscerated. Viscera will remain intact and the pup will be preserved in 10% neutral buffered formalin. Cannibalized pups will be examined to the extent possible and discarded. Culled pups will be examined for external irregularities, sexed internally to confirm external sexing and if unremarkable these pups will be discarded. Culled pups with external irregularities will be preserved intact in 10% neutral buffered formalin.

13.1.2.4.2 Unselected F_1 Pups:

Pups selected as a pool of animals from which the P_2 parental animals will be selected but which were not identified to continue on study, will be given an external examination and if unremarkable, sacrificed and discarded. Pups with external findings will be given a gross internal examination and only abnormal tissues taken and preserved in 10% neutral buffered formalin. Remaining pups in each litter will be given a gross external examination at weaning and if unremarkable, sacrificed and discarded. Pups with external findings will be given a gross internal examination and only abnormal tissues taken and preserved in 10% neutral buffered formalin.

13.1.2.4.3 Unselected F_2 Pups:

At weaning, unselected F_2 pups in each litter will be given a gross external examination and if unremarkable, sacrificed and discarded. Pups with external findings will be given a gross internal examination and only abnormal tissues taken and preserved in 10% neutral buffered formalin.

13.1.3 METHOD OF EUTHANASIA:

13.1.3.1 Parental Animals:

Exsanguination following anesthesia with inhaled carbon dioxide.

13.1.3.2 Weanlings:

Weanling pups selected for a complete gross postmortem examination will be sacrificed by exsanguination following anesthesia with inhaled carbon dioxide. Remaining pups will be sacrificed with an overdose of inhaled carbon dioxide.

13.1.4 Organ Weights:

13.1.4.1 Parental Animals:

The following organs will be weighed at terminal sacrifice from the first 15 P_1 and P_2 animals in each group:

uterus	ovaries
testes	right epididymis (total and cauda)
seminal vesicles (with coagulating glands and their fluids	prostate
brain	liver
kidneys	lungs
adrenals	spleen
thymus	

13.1.4.1 Parental Animals:

Organ weight data will be presented as absolute values and relative to terminal body weight. Organ weights will not be recorded for animal dying spontaneously or sacrificed moribund.

13.1.4.2 Weanling Pups (F_1 and F_2):

The following organs will be weighed from the first 15 male and female pups per group selected for complete macroscopic examination at weaning:

ovaries	testes
brain	liver
kidneys	adrenals
spleen	thymus

Organ weight data will be presented as absolute values and relative to terminal body weight.

13.1.5 TISSUES PRESERVED:

13.1.5.1 Parental Animals:

Grossly abnormal tissues will be preserved for all parental animals. Tissues listed in Appendix A will be obtained at necropsy and preserved for all P_1 and P_2 parental animals.

13.1.5.2 Weanling Pups:

Grossly abnormal tissues and tissues listed in Appendix A will be preserved for all F_1 and F_2 weanling pups selected for complete gross macroscopic examination.

13.1.6 Preservatives:

All tissues - 10% neutral buffered formalin. Testes and epididymides of the parental animals will be fixed in Bouin's solution for at least 48 hrs prior to permanent storage in formalin. Lungs will be infused with formalin to their approximate normal inspiratory volume. The nasal cavity will be flushed with formalin via the pharyngeal duct to ensure rapid fixation of the tissue.

13.1.7 MICROSCOPIC PATHOLOGY EVALUATIONS:

Slides of tissues listed in Appendix A (under Microscopic Examination) will be prepared and examined microscopically for all P_1 and P_2 animals in the control and high-dose groups. If microscopic findings indicative of an effect of test material administration are seen in high-dose animals, then examinations will be made of these tissues/organs for low- and mid-dose animals (additional cost). Additional examinations will be made only after consultation with the Sponsor and receipt of authorization from the Sponsor. Note: any abnormalities not noted during macroscopic postmortem examinations which are seen during histological processing will be recorded. Gross lesions will be examined at additional cost.

13.1.8 STAINS:

Standard stains used, hematoxylin and eosin. Special stains may be employed on selected tissues to aid in making a diagnosis at the discretion of the Study Pathologist. Special stains may be employed at the request of the Sponsor (additional cost).

13.1.9 SPERM COUNT, MOTILITY AND MORPHOLOGY ASSESSMENTS:

From the first 15 P_1 and P_2 parental males sacrificed at termination in each group, samples of sperm from the vas deferens (left) and distal cauda epididymis (left) will be collected. Motility will be assessed on sperm collected from the vas deferens and a count will be performed on the sperm sample collected from the cauda epididymis. A manual procedure developed in the testing facility will be used to assess motility and perform the count. Additionally, a slide of the sperm sample from the cauda epididymis will be prepared for morphological assessment if deemed necessary (Sponsor decision - additional cost item).

13.2 DEVELOPMENTAL TOXICITY STUDY:

13.2.1 MATERNAL TERM SACRIFICES:

Macroscopic postmortem examinations will be performed on all mated rats, including those dying spontaneously or killed in a moribund condition and on females sacrificed after aborting or premature delivery of a litter. The livers and kidneys will be weighed and preserved in 10% neutral buffered formalin for all females killed on Day 20 of gestation but microscopic examinations of these tissues will not be conducted unless deemed necessary to interpret other observations made during the study or as requested by the sponsor (additional cost item). Gross lesions identified

13.2.1 MATERNAL TERM SACRIFICES:

during the macroscopic evaluations will be saved in 10% formalin. Dams showing signs of abortion or premature delivery (expulsion of concepti) will be killed (overdose of inhaled carbon dioxide) on the day such evidence is observed. Reproductive tracts will be examined and fetuses obtained 19 days or later will be given an external examination, eviscerated and processed for skeletal staining with Alizarin Red S. These fetuses will then be examined for skeletal malformations. Fetuses obtained earlier than Day 19 will be evaluated for external malformations and saved (10% neutral buffered formalin) at the discretion of the Study Director. Examination data for these fetuses (Day 19 of gestation or earlier) will not be analyzed with data for term Day 20 gestation fetuses. Data for these fetuses will be presented in a separate appendix to the final report.

13.2.2 Reproductive System:

The intact uteri (ovaries attached) will be removed from the abdominal cavity and weighed. This uterine weight data will then be used to calculate a corrected Day 20 gestation body weight for each animal. The corrected Day 20 gestation weight will be determined by subtracting the gravid uterine weight from the terminal Day 20 gestation weight. Each uterine horn will then be evaluated for the number and location of the following:

- live fetuses (movement in response to touch);
- dead fetuses (lack of movement in response to touch but with no visible degeneration);
- late resorptions (recognizable dead fetus undergoing degeneration, regardless of size);
- early resorptions (evidence of implantation but no recognizable fetus);
- implantation sites (total of fetuses plus resorptions).

Ovaries: corpora lutea will be counted for each ovary.

Uteri without grossly visible implantations will be stained with ammonium sulfate (Salewski, 1964). If stained foci are present, the female will be considered pregnant for purposes of calculating pregnancy rates. The number of foci will not be used in the calculation of uterine implantation data.

13.2.3 Fetal evaluations:

13.2.3.1 External Evaluations:

All fetuses will be weighed and individually identified. Each fetus will be given a gross external examination for defects to include observation of the palate. The sex of each fetus will be noted by observation of the anogenital distance.

13.2.3.2 Fetal Skeletal Evaluations:

Approximately one-half of the fetuses in each litter (alternating fetuses within the litter) will be processed for Alizarin Red S staining of the skeletal structures. Prior to processing, the intact fetuses will be killed (overdose of inhaled carbon dioxide) and eviscerated (internal sex noted). Following staining, these fetuses will be evaluated for skeletal malformations and ossification variations.

13.2.3.3 Fetal Soft Tissue Evaluations:

The remaining fetuses in each litter will be processed for soft tissue examination using a microdissection technique similar to the procedure of Staples (1974) . The evaluations will be performed on the fresh fetal specimens soon after removal from the uterus. The fetuses designated for soft tissue evaluation will be decapitated (head placed in Bouin's solution for later evaluation). The fetal specimens will then be secured beneath a dissecting microscope and dissected so as to permit evaluation of tissues in the thoracic, abdominal and pelvic cavities. At completion of the examination, the decapitated fetal specimens will be eviscerated and processed for Alizarin Red S staining. These fetuses will not be evaluated skeletally unless authorized by the sponsor (additional cost).

Fetal heads, preserved in Bouin's solution, will be sectioned with a razor blade. The serial, transverse sections generated during this procedure will be evaluated for malformations of the palate, eyes and brain.

Fetal soft tissue and skeletal evaluations (microdissection, stained specimens and head sections) will be performed under a dissecting microscope.

13.2.4 Resorptions:

Late resorptions will be examined externally for malformations. Malformation data for late resorptions will be reported but not included in the analyses of malformation data for live or dead

13.2.4 Resorptions:

fetuses recovered on Day 20 of gestation. Only late resorptions with external malformations will be saved (10% neutral buffered formalin) for future possible examination. Early resorption will be discarded.

14 PRESERVATION OF RECORDS AND SPECIMENS:

All data documenting experimental details and study procedures and observations will be recorded and maintained as raw data.

At the completion of the study, all reports, raw data, preserved specimens and retained samples will be maintained in the Testing Facility's Archives for a period of five years after submission of the signed final report.

The Sponsor will be contacted in order to determine the final disposition of these materials. The Sponsor is responsible for all costs associated with the storage of these materials beyond one year from the issuance of the final report and for any costs associated with the shipment of these materials to the Sponsor or to any other facility designated by the Sponsor.

15 STATISTICAL EVALUATIONS:

The following items will be analyzed statistically in the final report:

15.1 CONTINUOUS DATA:

15.1.1 Two-generation Reproduction Study:

Mean body weights (all recorded intervals - premating, gestation, lactation and postmating);
Mean body weight change;
-entire premating period (males and females) - Weeks 0-10;
-over each weighing interval during the gestation and lactation periods to include Days 0-20 of gestation and Days 0-24 (F_1 litters) or Day 0-21 (F_2) of lactation;
-males during the postmating period (weekly and over the entire period).

Mean food consumption values;
-premating growth period (weekly);
-postmating period (weekly for males);
-gestation (Days 0-7, 7-14, 14-20);
-lactation (all recorded intervals);

15.1.1 Two-generation Reproduction Study:

Organ weight data (absolute and relative to the terminal body weight);
Mean pup weights (all recorded intervals during lactation);
Mean number of pups (live, dead, total) at birth (F_1 and F_2 litters);
Mean gestation length (F_1 and F_2 litters);
Mean pup live birth indices (F_1 and F_2 litters);
Mean pup viability indices (Days 0-4) and weaning indices (Days 4-24 or 21 for the F_1 and F_2 litters, respectively);
Mean age-to-criteria for vaginal opening and preputial separation (P_2 animals);
Mean sperm count and motility data.

15.1.2 Developmental Toxicity Study:

Mean maternal body weights (all recorded intervals during gestation) and weight gain (between all weighing intervals to include Days 6-20 of gestation using both the actual and corrected Day 20 gestation weight);
Mean organ weight data, absolute and relative to the corrected Day 20 gestation weight;
Mean number of corpora lutea, implants, live and dead fetuses, resorptions per pregnant female;
Mean pre- and post-implantation loss indices;
Mean fetal weights (distinguished by sex and as a composite for both sexes);

15.1.3 STATISTICAL ANALYSES CONTINUOUS DATA - MULTIPLE GROUP ANALYSES:

Data will be compared between the sham, chamber-housed control and the treated groups.

Statistical evaluation of equality of means will be made by the appropriate one way analysis of variance technique, followed by a multiple comparison procedure if needed. Bartlett's test will be performed to determine if groups have equal variance. If the variances are equal ($p > 0.01$), parametric procedures will be used; if not ($p < 0.01$), nonparametric procedures will be used. The parametric procedures will be the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means are indicated, Dunnett's test will be used to determine which means are significantly different from the control. If a nonparametric procedure for testing equality of means is needed, the Kruskal-Wallis test will be used, and if differences are indicated, a

15.1.3 STATISTICAL ANALYSES CONTINUOUS DATA - MULTIPLE GROU:P ANALYSES

summed rank test (Dunn) will be used to determine which treatments differ from control.

A statistical test for trend in the dose levels will also be performed. In the parametric case (i.e., equal variance), standard regression techniques with a test for trend and lack of fit will be used. In the non-parametric case, Jonckheere's test for monotonic trend will be used.

The test for equal variance (Bartlett's) will be conducted at the 1% two-sided risk level. All other statistical tests will be conducted at the 5% and 1%, two-sided risk levels.

All ratios (pup survival indices, pre- and postimplantation loss indices) will be transformed via Bartlett's transformation followed by the arc-sine transformation prior to analysis. Data will be presented untransformed.

References for these techniques are Snedecor, G.W., Cochran, W.G., *Statistical Methods*, 6th edition, Iowa State Univ. Press (1967); Hollander and Wolfe, *Nonparametric Statistical Methods*, John Wiley and Sons, New York (1973); Dunnett, C.W., *J. Am. Sta. Assn.* 50: 1096-1121 (1955) and *Biometrics* 20: 482 (1964).

Bartlett's Test	pp. 296-298	Snedecor & Cochran
ANOVA	pp. 277-279	Snedecor & Cochran
Dunnett's Test	pp. 1096-1121	Dunnett
	pp. 482-491	Biometrics
Kruskal-Wallis	pp. 114-116	Hollander & Wolfe
Summed Rank Test (Dunn)	p. 131	Hollander & Wolfe
Arc Sine Transformation	pp. 327-329	Snedecor & Cochran
Bartlett's transformation	p. 329	Snedecor & Cochran
Regression Analysis - Trend	pp. 135-153	Snedecor & Cochran
Lack of fit	pp. 456-459	Snedecor & Cochran
Jonckheere's Statistic	pp. 120-123	Hollander & Wolfe

15.2 INCIDENCE DATA:

15.2.1 Two-generation Reproduction Study:

Mortality rates;
Mating indices (male and female);
Pregnancy rates;
Male fertility indices;
Litter survival indices;

15.2.2 Developmental Toxicity Study:

Maternal Mortality
Pregnancy rates
Incidence of females with resorptions;
Incidence of fetuses with malformations/variations - external, soft tissue and skeletal examinations;
Incidence of litters containing fetuses with malformations/variations -external, soft-tissue and skeletal.

15.2.3 INCIDENCE DATA ANALYSIS:

Data will be compared between the sham, chamber-housed control and the treated groups.

Statistical analysis of incidence data will be performed using contingency tables. First, a standard Chi-square analysis will be performed to determine if the proportion of incidences differed between the groups tested. Next, each treatment group will be compared to the control group using a 2 x 2 Fisher Exact Test; the significance level will be corrected via the Bonferroni inequality to assure an overall test of the stated significance level. Thirdly, Armitage's test for linear trend in the dosage groups will be performed. In keeping with standard statistical practice, if any one cell has an expected value less than 5, the Chi-square and Armitage's tests will not be reported. When this occurs, only the Fisher Exact test (corrected via Bonferroni inequality) will be performed and reported.

All tests will be reported at the 5% and 1% level of significance.

15.2.3 INCIDENCE DATA ANALYSIS:

References for the techniques are Snedecor, G.W., and Cochran, W.G., *Statistical methods*, 6th ed., Iowa State University Press, Ames, Iowa (1971); Bradley, J.V., *Distribution Free Statistical Tests*, Prentice-Hall, Englewood Cliffs, New Jersey (1968); Miller, R.G., Jr., *Simultaneous Statistical Inference*, McGraw-Hill Book Co., New York (1966); Armitage, P., "Tests for Linear Trends in Proportions and Frequencies", *Biometrics*, (Sept. 1955).

Chi-square	pp. 250-253	Snedecor & Cochran
Fisher Exact Test	pp. 195-203	Bradley
Bonferroni Inequality	p. 15	Miller
Armitage's Test	pp. 375-386	Armitage

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FDA (1988). Food and Drug Administration Good Laboratory Practice for Nonclinical Studies. 21 CFR Part 58 (1 April 1988).

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Salewski, E. (1964). Farbemethode zum makroskopischen machweis von implantationsstellen am uterus der ratte. *Archiv. Path. Exp. Pharmakol.*, 247:367

Staples, R. E. (1974). Detection of Visceral Alterations in Mammalian Fetuses. *Teratology*, 9:37 (Abstract).

17 REPORT:

17.1 STATUS REPORT:

For the two-generation reproduction study, status reports will be issued monthly during the premating treatment period and after weaning of the F_1 and F_2 litters. In the developmental toxicity study a status report will be submitted after the last Day 20 gestation maternal sacrifices. These reports will include:

Two-generation Reproduction Study:

Mortality rates
Mean weekly body weight and weight gain data;
Mean weekly food consumption premating period;
Summary of detailed physical examinations;
Mating indices (males and females);
Male fertility indices;
Pregnancy rates;
Gestation length;
Number of pups at birth (live, dead and total) and number of live pups surviving during lactation;
Mean pup weights (lactation);
Individual female litter data (F_1, F_2);
Maternal gestation body weights and weight gains;
Maternal food consumption - gestation/lactation;
Summary of gross postmortem evaluations (adults, weanlings)

Developmental Toxicity Study:

Mortality rates:
Mean body weight and weight gain data during gestation;
Mean food consumption data;
Summary of detailed physical examination data;
Mean corpora lutea and uterine implantation data;

17.1 STATUS REPORT:

Mean number of live and dead fetuses and resorptions per pregnant female;
Mean pre- and post-implantation loss indices;
Incidence of females with resorptions;
Mean fetal weights;
Fetal external examination data;
Individual maternal Day 20 gestation sacrifice data;
Maternal organ weight data;
Summary of maternal gross postmortem examination data.

17.2 FINAL REPORT:

One copy of a draft report will be submitted following termination of the study. After receipt and review of the Sponsor's comments, appropriate changes will be made and two copies of a signed, final report will be issued. (Additional copies will be provided at additional cost). The report will include but not be limited to the following:

17.2.1 General:

Compliance Statement;
Abstract;
Introduction;
Experimental Design;
Materials and Methods;
Discussion of study results;
Conclusion and No Observed Effect Level (NOEL) statement;
Inhalation Exposure Report;
References for experimental methodology;
Senior personnel participating in the study;
Quality Assurance Statement.

17.2.2 Data tabulations for parental generations (P_1, P_2):

Mortality - termination history;
Physical in-life observations (summarized and individual data presented monthly throughout the study);
Mating indices;
Pregnancy rates;
Male fertility indices;
Mean body weight data (all interval);
Mean food consumption data (all intervals);
Mean weight gain data (premating, postmating [males], gestation and lactation intervals);
Statement on estrous cycle data;

17.2.2 Data tabulations for parental generations (P_1, P_2):

Macroscopic postmortem observations (adults, weanlings);
Microscopic pathology examinations;
Organ weight data;
Sperm assessment data.

17.2.3 Data tabulations for litters and offspring (F_1 and F_2):

Mean gestation length;
Mean number of pups (live, dead and total) at birth and live pups at Days 4, 7, 14 and weaning (Day 24 - F_1 and Day 21 - F_2);
Litter survival indices;
Pup live birth index;
Pup viability and weaning indices;
Mean pup weights (all recorded intervals during lactation);
Pup sex ratio at birth, Day 4 (pre- and postcull) and weaning;
Pup gross postmortem observations;
Individual female litter data.

17.2.4 Data tabulation for the developmental toxicity study:

Maternal mortality
Maternal body weight and weight gain data;
Maternal food consumption;
Physical observation data:
Pregnancy rates;
Mean number of corpora lutea and uterine implantations;
Mean number of live and dead fetuses;
Mean pre- and post-implantation loss indices;
Mean number of resorptions;
Incidence of females with resorptions;
Types of findings and incidence of fetuses with external, soft tissue and skeletal malformations/variations;
Incidence of litters containing fetuses with malformations/variations (external, soft tissue and skeletal)
Mean fetal weights;
Fetal sex distribution ratios;

17.2.5 Appendices:

All individual animal data (adults, pups, weanlings and fetuses) including but not limited to the following will be presented in the appendices: body weight and weight gain, food consumption, physical observation data, litter and uterine implantation data, organ weight data, sperm assessment data, estrous cycle data, gross

17.2.5 Appendices:

postmortem findings, microscopic examination data, chamber exposure data.

18 SIGNATURES:

18.1 INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC):

The IACUC Protocol Review Subcommittee has reviewed this protocol and found it to be in compliance with all appropriate regulations.

BY: _____ DATE: _____

TITLE: Institutional Animal Care and Use Committee Member

BY: _____ DATE: _____

TITLE: Institutional Animal Care and Use Committee Member
FOR: Huntingdon Life Sciences
East Millstone Facility

18.2 PROTOCOL REVIEWED AND ACCEPTED:

BY: _____ DATE: _____

TITLE: Study Director
FOR: Huntingdon Life Sciences

BY: _____ DATE: _____

Hasmukh C. Shah, Ph.D.

TITLE: Sponsor Representative
FOR: Chemical Manufacturers Association

APPENDIX A
Tissues Preserved/Examined Microscopically

No.[a]	Tissue	Preserved	Microscopic Examination (Groups)	
			I, IV	II, III
2	adrenals	X		
1	aorta	X		
1	auditory sebaceous glands	X		
1	bone (including joint)	X		
3	brain (cerebrum, brainstem, cerebellum)	X		
1	cecum	X		
2	coagulating glands	X	X	
1	colon	X		
1	duodenum	X		
2	epididymis	X	X	
1	esophagus	X		
2	eyes	X		
1	heart	X		
1	ileum	X		
1	jejunum	X		
2	kidneys	X	X	
2	lacrimal/Hardarian glands	X		
1	larynx	X		
2	liver	X	X	
2	lungs	X	X	
1	mammary glands	X	X	
1	mediastinal lymph nodes	X		
1	mediastinal tissues	X		
1	mesenteric lymph nodes	X		

APPENDIX A
Tissues Preserved/Examined Microscopically

No.[a]	Tissue	Preserved	Microscopic Examination (Groups)	
			I, IV	II, III
1	mesenteric tissues	X		
4	nasal tissues (turbinates)	X	X	
1	oral tissues	X		
1	ovary[b]	X	X	
2	oviducts	X	X	
1	pancreas	X		
2	parathyroid glands	X		
1	peripheral nerve	X		
1	pituitary	X	X	
1	prostate	X	X	
1	rectum	X		
2	salivary glands	X		
2	seminal vesicles	X	X	
1	skeletal muscle	X		
1	skin	X		
3	spinal cord (cervical, thoracic, lumbar)	X		
1	spleen	X		
1	stomach	X		
2	testis	X	X	
1	thymus	X		
2	thyroid gland	X		
1	tongue	X		
1	trachea	X	X	
1	urinary bladder	X		

APPENDIX A
Tissues Preserved/Examined Microscopically

No.[a]	Tissue	Preserved	Microscopic Examination (Groups)	
			I, IV	II, III
2	uterus (body/horns with cervix)	X	X	
1	vagina	X	X	
	gross lesions	X	X	

[a] Number of organs/sections preserved/examined.

[b] Only the right ovary will be routinely processed for microscopic evaluation. The left ovary will be saved for possible oocyte quantification. If deemed necessary by the sponsor (additional cost), oocyte quantification will include evaluation of a minimum of 10 sections randomly selected from one completely sectioned ovary per female of the high-dose and control groups. Ovarian follicles will be placed into one of three categories as described by Plowchalk et al., (1993). The total number of follicles and the number of follicles in each of the three categories will be evaluated. Ovaries from the low- and mid-dose groups may be evaluated if treatment-related changes are observed in the high-dose group.

APPENDIX B

TEST MATERIAL PROPERTIES

Chemical name	Vinyl chloride
Synonyms	Monochloroethylene, VC, VCM, vinyl chloride monomer
Molecular formula	C_2H_3Cl
Molecular weight	62.5
Structures	$CH_2=CHCl$
Appearance	Colorless gas
Vapor pressure	2,530 mm Hg at 20 degrees C
Saturated atmosphere	Gas at room temperature
Vapor density	2.16
Flash point	-77.75 degrees C (open cup)
Boiling point	-13.6 degrees C
Specific gravity	0.9121
Conversion factors	1 mg/mm^3 = 0.39 ppm in air

CURRICULUM VITAE

NAME: Paul E. Newton, Ph.D.
Diplomate, American Board of Toxicology

PRESENT POSITION: Project Manager
(since November 1995) Huntingdon Life Sciences Inc.
East Millstone, NJ 08875-2360

Responsible for all aspects of conducting toxicity studies following GLPs including protocol design, development of test exposure/dose and monitoring systems, evaluation of data, preparation of final reports and client/auditor interface.

EDUCATION:

1979	The Medical College of Wisconsin Ph.D. Respiratory Physiology Dissertation: "Control of Human Ventilation During CO_2 Breathing"
1968-1970	Kansas State University Engineering (Graduate courses in Convective Heat and Mass Transfer, Boundary Layer Theory, Radiation Heat Transfer, Fluid Mechanics, Thermodynamics, Control Theory, Engineering Analysis, Analog Computer and Statistics)
1968	Rose Polytechnic Institute M.S. Biological Engineering
1967	Rose Polytechnic Institute B.S. Mathematics

PROFESSIONAL EXPERIENCE:

1986 - 1995	Director of Inhalation Toxicology Pharmaco LSR Inc., Toxicology Services Worldwide East Millstone, NJ 08875

Responsible for the Department of Inhalation Toxicology, overseeing the activities of the technical staff. As a Study Director, responsible for all aspects of the conduct of inhalation studies. Responsibilities include protocol design, development of test exposure and monitoring systems, evaluation of data, preparation of final reports and client/auditor interface.

Paul E. Newton, Ph.D.

PROFESSIONAL EXPERIENCE-continued:

1985 - 1986 — American Biogenics Corporation
Decatur, Illinois
Director, Inhalation Toxicology

Director of Inhalation Toxicology and responsible for overall operation of inhalation toxicology facility. This included client-sponsored acute, subchronic and chronic studies of vapors and liquid or dust aerosols as well as pyrolysis testing following NBS guidelines.

1979 - 1985 — University of California, Irvine
Toxic Hazards Research Unit, Wright-Patterson AFB, OH
Head, Respiratory Toxicology Department

Responsible for design, implementation and operation of a pulmonary toxicology program including preparation of budgets, protocols and standard operating procedures, management of personnel, equipment specification and computer software development for high speed data acquisition and processing using GLP techniques. Research involved evaluation of pulmonary dynamics, gas exchange, distribution, pulmonary defense mechanisms, and the toxicodynamics and toxicokinetics of various chemical agents on rats, mice and dogs. Compounds studied included: ozone, carbon dioxide, propylene glycol dinitrate, jet fuels JP-8 and shale JP-4, papain, bleomycin, 4-ipomeanol and o-ethyl-o-(2-diisopro-pylamino-ethyl) methylphosphonite.

1971 - 1979 — The Medical College of Wisconsin
Department of Environmental Medicine
Senior Bioengineer

Responsible for specification of instrumentation systems, writing computer programs and the collection, processing and statistical analysis of all pulmonary, cardiovascular, neurological and behavioral test data investigated during human inhalation toxicological experiments. Compounds included: carbon monoxide, methylene chloride, trichloroethylene, 1,1,1-trichloro-ethane, tetrachloroethylene, acetone, toluene, xylene, ethyl alcohol, fluorocarbon-11, fluorocarbon-12, isobutane, propane, propylene glycol dinitrate, methyl chloride, Halon 1211, Halon 2402 and Halon 1301.

PROFESSIONAL ACCREDITATION:

Diplomate, American Board of Toxicology, 1984
Recertified in 1989 and 1993

CERTIFICATES/AWARDS:

Best Poster Presentation, Mid-Atlantic Soc. of Toxicol. (1992)
Distinguished Service Award (1976-79) Milwaukee Fire Department

Paul E. Newton, Ph.D.,

PROFESSIONAL MEMBERSHIPS:

Society of Toxicology, Mid-Atlantic and Midwest Regional Society of Toxicology
American College of Toxicology
American Association of Pharmaceutical Scientists
American Association Aerosol Research
American Thoracic Society
Association of Inhalation Toxicologists
European Society of Toxicology
International Society of Aerosols in Medicine

PUBLICATIONS (Book Chapters):

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Stewart, R.D., Newton, P.E., Hosko, M.J. and Peterson, J.E. 1973. "Effects of Carbon Monoxide on Time Perception", **Arch. Env. Health**, 27: 155-160

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Paul E. Newton, Ph.D.

JOURNAL ARTICLES - continued:

Stewart, R.D., Herrmann, A.A., Baretta, E.D., Sikora, J.J., Newton, P.E. and Soto, R.J. 1977. "Acute and Repetitive Human Exposure to Isobutane", **Scand. J. Work, Environ. & Health** 3: 234-243

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Erk, S.D., Jarboe, C.H., Newton, P.E. and Pfledderer, C. 1982. "Gas Chromatographic Determination of 1, 2-Propanediol Dinitrate in Blood, **Journal of Chromatographic Science**, 240: 117-123

Newton, P.E., Hamilton, L.H. and Forster, H.V. 1983. "Measurement of Ventilation using Digitally Filtered Transthoracic Impedance", **J. Appl. Physiol.: Respirat., Environ. Exercise Physiol.** 54: 1161-1166

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Paul E. Newton, Ph.D.

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Paul E. Newton, Ph.D.

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REPORTS ON PROPRIETARY COMPOUNDS SUBMITTED TO SPONSORS:

Approximately 500 confidential reports on acute, sub-chronic, chronic, and reproduction/teratology inhalation studies in rodents and higher animal species. These reports were issued to the pharmaceutical, agricultural and industrial chemical, food additive and cosmetic industries.

I certify that the above is a true and accurate account of my professional career.

Signed: _____ Date: 1 December 1995

CURRICULUM VITAE

NAME: Henry F. Bolte, D.V.M., Ph.D.

PRESENT POSITION: Senior Staff Pathologist
Huntingdon Life Sciences
East Millstone, NJ 08875

Performs the duties of a pathologist. Conducts macroscopic and microscopic examinations of specimens of animal body tissues, fluids and secretions; diagnoses and dictates reports of findings. Reads and follows protocols in examining slides to determine initial requirements. Records all observations noting any abnormalities which might impact on the results of the study or lead to advanced or expanded explorations.

EDUCATION:

1969	Purdue University Ph.D. Pathology Thesis: "Ultrastructure of Isonicotinic Acid Hydrazide Induced Encephalopathy in the Peking Duck"
1966	Purdue University M.S. Pathology Thesis: "Uveitis, a Sequela to Experimentally Induced Leptospira Pomona Infection in the Shetland Pony"
1963	Iowa State University Dr. Veterinary Medicine

PROFESSIONAL EXPERIENCE:

1/83-11/95	Associate Director of Pathology Pharmaco LSR East Millstone, NJ 08875
5/79-1/83	Staff Pathologist Bio/dynamics, Inc. East Millstone, NJ 08875
1970-1979	Group Leader Lederle Laboratories Department of Experimental Pathology

Henry F. Bolte, D.V.M., Ph.D.

PROFESSIONAL EXPERIENCE - continued:

1968-1970	Administrative Assistant Purdue University Graduate Housing
1967-1970	Special Post-Doctoral Fellow
1964-1967	Graduate Instructor Purdue University Veterinary Pathology

PROFESSIONAL MEMBERSHIPS:

International Academy of Pathology
Society of Toxicologic Pathology
Mid-Atlantic Chapter of the Society of Toxicology
The New York Academy of Science
The American Association for the Advancement of Science
Phi Zeta
Phi Kappa Phi
Gamma Sigma Delta
Sigma Xi

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PRESENTATIONS:

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I CERTIFY THAT THE ABOVE IS A TRUE AND ACCURATE ACCOUNT OF MY PROFESSIONAL HISTORY TO DATE.

SIGNED: _____ DATE: _____

CURRICULUM VITAE

NAME: Raymond E. Schroeder, M.S.

PRESENT POSITION: Project Manager
(since November 1995) Huntingdon Life Sciences Inc.
East Millstone, NJ 08875

Study Director responsibility for the performance of preclinical toxicity and safety assessment studies in small animal species particularly in the areas of developmental toxicity and reproduction/fertility.

EDUCATION:

1967	University of Illinois Champaign/Urbana, Illinois M.S. Zoology
1965	Wheaton College Wheaton, Illinois B.S. Biology
1974	Medical Pharmacology Course Northwestern Medical School Chicago, Illinois

PROFESSIONAL EXPERIENCE:

1990-1995	Sr. Toxicologist/Study Director Pharmaco LSR Inc., Toxicology Services Worldwide East Millstone, NJ 08875
1976-1990	Manager/Study Director Reproduction/Teratology Department Bio/dynamics, Inc. East Millstone, NJ 08875
1975-1976	Manager L.B. Allen, Inc. Schiller Park, Illinois
1967-1975	Sr. Research Assistant (Teratology) Searle Laboratories Department of Pathology/Toxicology Skokie, Illinois

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PROFESSIONAL ACCREDITATION:

Diplomate, American Board of Toxicology

PROFESSIONAL MEMBERSHIPS:

Mid-Atlantic Reproduction Teratology Association (MARTA) .
-Member Steering Committee 1977-1981
-Member Nominating Committee 1984-1985
Mid-Atlantic Society of Toxicology
Society of Toxicology
Society of Toxicology Reproductive Specialty Section
Teratology Society
Metropolitan New York Branch of AALAS (American Association for Laboratory Animal Science)
Midwest Teratology Association (MTA)

PUBLICATIONS:

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"R.E. Schroeder, P.E. Newton, J.B. Sullivan and W.M. Busey. "An Inhalation Developmental Toxicity Study of Phosphine in Rats". The Toxicologist, Vol 12, No. 1, February 1992. (Presented by poster at the 31st Annual Meeting of the Society of Toxicology, February, 1992).

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T.R. Hanley Jr., R.E. Schroeder and W.J. Breslin. "Developmental Studies on a Series of 2,4-D Salts and Esters in the Rat" The Toxicologist, Vol 11, No. 1, February 1991. (Presented by poster at the 30th Annual Meeting of the Society of Toxicology, February, 1991).

Raymond E. Schroeder, M.S.

PRESENTATIONS - continued:

W.J. Breslin, R.E. Schroeder and T.R. Hanley Jr."Developmental Toxicity of Picloram Potassium (L) and Triisopropanolamine (TIPA) Salts in the Rat" **The Toxicologist**, Vol 11, No. 1, February 1991. (Presented by poster at the 30th Annual Meeting of the Society of Toxicology, February, 1991).

C.S. Auletta, R.E. Schroeder, W.J. Krasavage and C. Stack "Toxicology of Diethylene Glycol Butyl Ether: 3. Dermal Subchronic Toxicity/Fertility Study in Rats". **The Toxicologist**, Vol. 11 No. 1, February 1991. (Presented by poster at the 30th Annual Meeting of the Society of Toxicology, February, 1991).

S.J. Murphy, R.E. Schroeder, A.M. Blacker, W.J. Krasavage and J.C. English "Study of Developmental Toxicity of Hydroquinone (HQ) in the Rabbit". **The Toxicologist, Vol. 11, No. 1,** February 1991. (Presented by poster at the 30th Annual Meeting of the Society of Toxicology, February, 1991).

R. Nair, J. Barter, H. Bolte, R. Schroeder and C. Stack. "Evaluation of Reproductive Toxicity of Ortho-Dichlorobenzene (ODCB) in the Rat" (abstract); (presented at the V International Congress of Toxicology, July 1989).

R.S. Nair, F.R. Johannsen, C.S. Auletta and R.E. Schroeder. "Chronic Toxicity and Potential Reproductive Effects of P-Nitroaniline (PNA) in the Rat". **The Toxicologist, Vol. 7, No. 1,** February 1987. (Presented by poster at the 26th Annual Meeting of the Society of Toxicology, February, 1987).

R.S. Nair, F.R. Johannsen and R.E. Schroeder. "Absence of Teratogenic Response in Rats and Rabbits Given a Detergent Builder". **The Toxicologist, Vol. 7, No. 1,** February 1987. (Presented by poster at the 26th Annual Meeting of the Society of Toxicology, February, 1987).

E.C. Robinson, R.D. Short, F.R. Johannsen and R.E. Schroeder. "A Teratology Study of a Mixture of Decyldodecyl Benzenes". **The Toxicologist, Vol. 7, No. 1,** February 1987. (Presented by poster at the 26th Annual Meeting of the Society of Toxicology, February, 1987).

R.E. Schroeder, I.W. Daly and V.J. Theodorides. "A Teratology Study in Rats with Virginiamycin". The Toxicologist, Vol. 7, No. 1, February 1987. (Presented by poster at the 26th Annual Meeting of the Society of Toxicology, February, 1987).

Biles, R.W., Schroeder, R.E. and Holdsworth, C.E., "Single Generation Reproduction Study of Methyl-T-Butyl Ether in Rats", **The Toxicologist** Vol. 6, No. 1, 1986. (Presented by poster at the 25th Annual Meeting of the Society of Toxicology, March, 1986).

Schroeder, R.E., Terrill, J.B., Lyon, J.P., Kaplan, A.M. and Kimmerle, G., "An Inhalation Teratology Study in the Rabbit with Nitrobenzene", The Toxicologist. Vol. 6, No. 1, 1986. (Presented by poster at the 25th Annual Meeting of the Society of Toxicology, March, 1986).

Raymond E. Schroeder, M.S.

PRESENTATIONS - continued:

Parker, J.A., MacGregor, J.A. and Schroeder, R.E., "Diet Restriction During Gestation in NZW Rabbits Does Not Adversely Affect Fetal Outcome", Teratology. Vol. 33, pp. 71 C 1986 (Presented by poster at the 26th Annual Meeting of the Teratology Society, July, 1986).

Nair, R.S., Johannsen, F.R., Levinskas, G.J., and Schroeder, R.E., "A Rat Teratology Study with Tetrathal", presented at the Federation of American Societies for Experimental Biology (FASEB), April 1984.

Lewis, S.C., Rinehart, W.E., Schroeder, R.E., Thackara, J.W. "Dominant Lethal Mutagenic Bioassay Dimethyl Formamide (DMF)"; Environmental Mutagen Society (abstract) (1979).

Schroeder, R.E., Vondruska, J.F. and Youkilis, L.J. "Teratological Evaluations of a Copper Containing Intrauterine Device (CuIUD) in Rats and Rabbits"; (abstract); Teratology. Vol. 3, (1975).

Bost, R.G., McConnell, R.G., Rao, K.S., Reno, F.E., Schroeder, R.E., and Vondruska, J.F. "An Evaluation of the Mutagenic Potential of Asparatame and SC-19192"; American College of Nutrition (abstract) (1974).

McConnell, R.G., Rao, K.S., Reno, R.F., Schroeder, R.E., Trutter, J. and Vondruska, J.F. "Fertility, Reproduction and Teratology Studies with Aspartame and SC-19192"; American College of Nutrition (abstract) (1974).

REPORTS ON PROPRIETARY COMPOUNDS SUBMITTED TO SPONSORS:

Numerous confidential preclinical toxicity reports in the areas of general toxicity, reproduction/fertility, developmental toxicity and mutagenicity (i.e., dominant-lethal studies). These reports are prepared for Pharmaceutical, Agricultural and Industrial Chemical, Food Additive and Cosmetic Industries for submission to regulatory agencies around the world.

I CERTIFY THAT THE ABOVE IS A TRUE AND ACCURATE ACCOUNT OF MY PROFESSIONAL HISTORY TO DATE.

SIGNED Raymond E Schroeder DATE: 1 Dec 95

CURRICULUM VITAE

NAME:	Ira W. Daly, Ph.D. Diplomate, American Board of Toxicology
PRESENT POSITION: (since November 1995)	Vice President, Agrochemical & Industrial Toxicology Huntingdon Life Sciences East Millstone, NJ 08875

Acts as the client representative on site. Responsible for maintaining communication with client without impeding direct communication between client and study management. Acts as program director for multidisciplinary projects to ensure that precise requirements of the client are fully understood and met and to be available to provide client with appropriate advice and consultancy. Develops appropriate protocols and programs to provide the most scientifically valid and cost-effective solution to the client's requirements which will be acceptable to regulatory agencies. Responsible to senior management for the quality of service provided to clients.

EDUCATION:

1977	St. John's University Jamaica, New York Ph.D. Pharmacology
1974	St. John's University Jamaica, New York M.S. Pharmacology

PROFESSIONAL EXPERIENCE:

1993-1995	Vice President, Business Development Operations
1989-1993	Sr. Vice President & Director of Toxicology Pharmaco LSR Inc., Toxicology Services Worldwide East Millstone, NJ 08875
1985-1989	Vice President and Director of Toxicology
1982-1985	Director of Toxicology
1980-1982	Associate Director of Toxicology/Operations
1978-1980	Staff Toxicologist/Study Director Bio/dynamics, Inc. East Millstone, NJ 08875
1979-1980	Adjunct Assistant Professor Graduate School of Allied Health Professions St. John's University, Jamaica, New York

Ira W. Daly, Ph.D.

PROFESSIONAL EXPERIENCE - continued:

1977-1978 — Supervisor, Applied Pharmacology/Consultant Toxicologist
Department of Biological Sciences and Clinical Affairs
American Chicle Division of Warner Lambert Company
Morris Plains, New Jersey

Conducted in-house toxicity studies, monitored contract toxicological investigations. Managed laboratory in the areas of basic and applied Gastrointestinal and Respiratory Pharmacology. Conducted clinical investigations in Gastrointestinal Pharmacology.

PROFESSIONAL ACCREDITATION:

Diplomate, American Board of Toxicology (1980)
Regulatory Affairs Certification (1992)

PROFESSIONAL APPOINTMENTS:

Technical Committee - Society of Toxicology (1984-1986)

PROFESSIONAL MEMBERSHIPS:

Society of Toxicology
European Society of Toxicology
Society of Toxicology Reproductive Specialty Section
Mid-Atlantic Chapter of the Society of Toxicology
Society of Environmental Toxicology and Chemistry

PUBLICATIONS AND PRESENTATIONS:

Carol S. Auletta, Lionel F. Rubin, Ira W. Daly, Ward R. Richter, Kazuhiro Hosoi, Hiroshi Suda and Toshimi Ikuse; "26-Week Ocular Toxicity Studies in Pigmented Rabbits Treated Topically with Bunazosin Hydrochloride Ophthalmic Solution"; **Atarashii Ganka (J.Eye)**, Vol. 12, No. 3, 1995.

Newton, P.E., Bolte, H.F., Daly, I.W., Pillsbury, B.D., Ben-Dyke, R., Drew, R.T. and Sheldon, A.W. "Subchronic and Chronic Inhalation Toxicity Studies of Antimony Trioxide in the Rat", **Fundamental and Applied Toxicology 22**, 561-576 (1994).

"Validation of a Neurotoxicity Screening Battery" (presented at the 33rd Annual Society of Toxicology Meeting, March 1994).

"Validation of a Developmental Neurotoxicity Screening Battery: Effects of Hydroxyurea, Methylmercuric Chloride and Diphenylhydantoin". K.E. Sloan, W.R. Richter, C.S. Auletta and I.W. Daly (presented at the 31st Annual Society of Toxicology Meeting, February 1992).

Ira W. Daly, Ph.D.

PUBLICATIONS AND PRESENTATIONS - continued:

"Oncogenicity Study of AO-128 in Mice", **Japanese Pharmacology and Therapeutics 19**, 131-142 (1991).

"One-Year Oral Gavage Toxicity Study of AO-128 in Rats", **Japanese Pharmacology and Therapeutics 19**, 233-260 (1991).

"One-Year Oral Toxicity Study of AO-128 in Beagle Dogs", **Japanese Pharmacology and Therapeutics 19**, 261-282 (1991).

"Subchronic Oral Toxicity of Cellulose Acetate in Rats", **Food and Chemical Toxicology 29**, 453-458 (1991).

"One-Year Oral Gavage Toxicity Study of Lansoprazole (AG-1749) in Rats", **Japanese Pharmacology and Therapeutics 18**, 59-91 (1990).

"One-Year Oral Gavage Toxicity Study of Lansoprazole (AG-1749) in Dogs", **Japanese Pharmacology and Therapeutics 18**, 93-118 (1990).

"Malignant Hyperthermia Induction in Susceptible Swine Following Exposure to Arduan" (presented at the International Anesthesia Research Society 64th Congress, March 1990, Honolulu, HI).

"A Subchronic Oral Toxicity Study with Cellulose Acetate" (presented at the 29th Annual Meeting of the Society of Toxicology, February 1990). **The Toxicologist 10**, #670 (1990).

"Subchronic Inhalation Toxicity of Ethylbenzene in Mice, Rats and Rabbits", **Fundamental and Applied Toxicology 13**, 399 (1989).

"Subchronic Inhalation and Oral Toxicity of Hydrogenated Terphenyls in Rats", **Fundamental and Applied Toxicology 13**, 559 (1989).

"Subchronic Inhalation Toxicity of Ethylbenzene in Mice, Rats and Rabbits" (presented at the 28th Annual Meeting of the Society of Toxicology, March 1989), **The Toxicologist, 9** (l) 1989.

"Human Safety Studies with Albendazole" (presented at the Proceedings of the American Association of Veterinary Parasitologists, 34th Annual Meeting, July 1989) Orlando, FL.

"Cardiac Performance and Hemodynamics in Malignant Hyperthermia Susceptible and Normal Pigs During Sevoflurane Anesthesia" (presented at the 42nd Post Graduate Assembly in Anesthesiology, December 1988).

"A Teratology Study in Rats with Virginiamycin" (presented at the 26th Annual Meeting of the Society of Toxicology, February 1987), **The Toxicologist** 7 (1) 1987.

Ira W. Daly, Ph.D.

PUBLICATIONS AND PRESENTATIONS - continued:

"A Two-Generation Reproduction Study in Rats with Virginiamycin" (presented at the 26th Annual Meeting of the Society of Toxicology, February 1987); **The Toxicologist** 7 (1) 1987.

"Chronic Toxicity and Oncogenicity Studies of LASSO (Alachlor) Herbicide in Rodents" (presented Fourth International Congress of Toxicology, July 1986); **Toxicology Letters, 31** (Suppl.) 56 (1986).

"Dose-Dependent Clearance of Antimony from Rat Lungs" (presented Society of Toxicology, March 1986); **The Toxicologist 6** (1) 36 (1986).

"Ectopic Pupil in Mice", **Laboratory Animal Science, 32** (1) 1982.

"Subchronic Toxicity of Chloropropanol in Rats and Dogs" (presented Society of Toxicology, San Diego, 1981).

"A Teratology Study of Topically Applied Linear Alkylbenzene Sulfonate in Rats", **Food and Cosmetic Toxicology,** Vol. 18, 1980.

"The Effect of Prostaglandins PGE2 and PGF2A on Spermatogenesis in Adult Male Sprague-Dawley Rats" (presented ASPET August 1977); **International Journal of Fertility,** 24 (3) 1979.

"Testicular Morphology Following Prostaglandin Administration" (presented ASPET, August 1977); **The Pharmacologist,** 19 (2) 523 (1977).

"Effects of d-Amphetamine Sulfate on Aggressive Behavior in Laboratory Mice", **Clinical Toxicology,** 8 (3) 1975.

REPORTS ON PROPRIETARY COMPOUNDS SUBMITTED TO SPONSORS:

Approximately 1500 confidential reports on acute, sub-chronic, chronic, reproduction/teratology and inhalation studies in rodent and higher animal species. These reports were issued to the pharmaceutical, agricultural, industrial chemical and food additive industries.

I CERTIFY THAT THE ABOVE IS A TRUE AND ACCURATE ACCOUNT OF MY PROFESSIONAL HISTORY TO DATE.

SIGNED _____ DATE 7 Dec 95

CURRICULUM VITAE

NAME: Teresa S. Kusznir, V.M.D.

PRESENT POSITION: Veterinarian, Toxicology Department
(since November 1995) Huntingdon Life Sciences Inc.
East Millstone, NJ 08875

Responsibilities include the development, implementation and maintenance of animal care standards for animals utilized (non-human primates, dogs, rodents, rabbits, birds and others with an average monthly census of approximately 9,000). Responsibilities also include assurance of compliance with local and federal laboratory animal regulations as well as AAALAC standards.

EDUCATION:

1993 University of Pennsylvania
Philadelphia, PA
V.M.D.

1982 Delaware Valley College of Science and Agriculture
Doylestown, PA
B.S. Animal Husbandry

PROFESSIONAL LICENSING: Pennsylvania

PROFESSIONAL EXPERIENCE:

1995 Veterinarian, Toxicology Department
Pharmaco LSR Inc., Toxicology Services Worldwide
East Millstone, NJ 08875

1973-1995 Post Doctoral Fellow, Laboratory Animal Science
SmithKline Beecham Pharmaceuticals
Swedeland, PA

Responsibilities included the complete medical and surgical care of various species of research animals within R&D, including non-human primates, dogs, rodents, swine and rabbits. Provided veterinary consultation and surgical services to investigators including, but not limited to, implantation of vascular and gastrointestinal access devices. Additional responsibilities included necropsy and clinical diagnostics. Facilitated technical training programs, including SB 1995 Investigator Training Seminar "Post-Operative Care and Monitoring of Lab Animals", and "Techniques in Anesthesia", L.A.S.T.S. Seminar.

1991-1991 Associate Scientist, Department of Investigative Pathology

Conducted hepatocellular proliferation studies in rats. Technical duties included dosing rats, surgical implantation of BrDU osmotic pumps, necropsy and terminal collection of liver samples. Further responsibilities included reading liver slides, assessing hepatic labeling indeces, correlating and graphing data and presenting results to senior staff.

Teresa S. Kusznir, V.M.D.

PROFESSIONAL EXPERIENCE:

1990-1990 Associate Scientist, Department of Immunology

Conducted experiments on murine models of endotoxin shock and effects of various inhibitors of shock, cyclo-oxygenase and lipoxygenase pathways. Performed ELISA assays to measure TNF levels of these models. Duties included media and drug preparation, dosing and bleeding. Utilized computer graphics and word processing software for data summarization. Assisted in experiments on the inhibition of rheumatoid arthritis in rats.

1987-1989 Associate Toxicologist, Investigative Toxicology Unit

Conducted pre-clinical in-vivo toxicity studies in various species of laboratory animals and evaluated the resulting data. Duties included drug preparation, delivery of test article to animals by standard and novel routes, phlebotomy, assessing non-invasive blood pressures and EKGs in dogs and primates, performing necropsies and identifying gross pathology. Various computer skills utilized in report production word processing and generation of graphics by RSE.

1984-1986 Research Technician, Toxicology
Wyeth Laboratories
Great Valley, PA

Responsible for carrying out drug safety evaluation studies in rats, mice, dogs and primates. Duties also included dosing, performing necropsies, maintaining accurate records, preparing drug and obtaining physiological specimens. Prepared Standard Operating Procedures for compliance with GLP regulations. Special projects included long term maintenance of drug induced diabetic monkeys and radiolabeled drug metabolism studies in dogs.

1983-1984 Veterinary Technician/Adoption Coordinator
Morris Animal Refuge
Philadelphia, PA

Provided general veterinary care to all shelter animals, including physical exams, treatments, vaccination, grooming, euthanasia and kennel duties. Also interviewed pet adopters and educated same in responsible pet ownership.

1976-1982 Veterinary Technician/Receptionist
Hopewell Veterinary Hospital
Philadelphia, PA

Responsible for assisting veterinarians with appointments, treatments, x-rays, surgeries and post-op care of animals. Performed routine lab work including heartworm tests, fecals, urinalyses, hematocrits and ultrasonic teeth cleaning. Additional responsibilities included clerical work, hiring, training and scheduling of part-time employees.

Teresa S. Kusznir, V.M.D.

I CERTIFY THAT THE ABOVE IS A TRUE AND ACCURATE ACCOUNT OF MY PROFESSIONAL HISTORY TO DATE.

SIGNED Teresa S. K DATE 4 Dec 95

CURRICULUM VITAE

NAME: Ward R. Richter, D.V.M., M.S.
Diplomate, American College of Veterinary Pathologists

PRESENT POSITION: Vice President & Scientific Director
(since 11/93) Huntingdon Life Sciences Inc.
East Millstone, NJ 08875

Responsible for all scientific and technical operations including toxicology, pathology, chemistry and computer operations. Interface with clients on scientific issues.

EDUCATION:

1962	Iowa State University, IA M.S. Pathology
1955	Iowa State University, IA D.V.M.
1951	University of Wisconsin, WI Pre-Veterinary

VETERINARY LICENSE:

Iowa and Illinois, inactive status

PROFESSIONAL EXPERIENCE:

7/91-11/93	Vice President & Director of Pathology

Responsibilities included organizing and directing the Department of Pathology (a department comprised of approximately 45 professional and technical staff) and interfacing with clients on matters of Pathology.

1990-7/91	President Kemdru International, Inc. (A Safety Assessment Consulting Firm) Evansville, IN
1989-1990	Coordinator, Pesticide Product Safety Chevron Environmental Health Center Richmond, CA
1983-1991	Vice President Toad Hall Systems, Inc. (A Software Development Firm specializing in Systems for Toxicology & Pathology Laboratories) Union Grove, WI

Ward R. Richter, D.V.M., M.S.

PROFESSIONAL EXPERIENCE - continued:

1985-1989	Chief of Pathology, Genetic Toxicology & Reproductive Toxicology Chevron Environmental Health Center Richmond, CA
1980-1985	Director, Pathology Division International Research and Development Corp. Mattawan, MI
1977-1985	Professor (Collaborator Basis) Department of Veterinary Pathology Iowa State University, IA
1973-1980	Director A.J. Carlson Animal Research Facility University of Chicago, IL
1973-1980	Professor, Department of Pathology University of Chicago, IL
1973-1980	Professor, The College University of Chicago, IL
1969-1972	Deputy Director A.J. Carlson Animal Research Facility University of Chicago, IL
1968-1973	Associate Professor of Comparative Pathology University of Chicago, IL
1964-1977	Associate Professor (Collaborator Basis) Department of Veterinary Pathology Iowa State University, IA
1967-1968	Clinical Associate Professor Department of Oral Biology Loyola University, Chicago, IL
1963-1968	Head, Cellular Pathology and Electron Microscopy (Pathology Department) Abbott Laboratories, North Chicago, IL

Ward R. Richter, D.V.M., M.S.

PROFESSIONAL EXPERIENCE - continued:

1961-1963	Research Associate (Pathology Department) University of Louisville School of Medicine Louisville, KY
1960-1963	Chief, Cellular Pathology Branch (Pathology Department), U.S. Army Medical Research Laboratory, Ft. Knox, KY
1958-1960	Instructor, Veterinary Pathology Iowa State University, IA

SOCIETY MEMBERSHIPS:

American College of Veterinary Pathologists
American College of Toxicology
Society of Toxicological Pathologists
United States & Canadian Academy of Pathology
American Society for Investigative Pathology
American Association for the Advancement of Science
American Veterinary Medical Association
International Society for the Study of Xenobiotics

HONORS AND HONOR SOCIETIES:

Alumni Merit Award (Iowa State University Alumni Association, 1971)
Alpha Zeta
Phi Zeta
Phi Kappa Phi
Sigma Xi
Outstanding Service Award (Charles Louis Davis, D.V.M. Foundation, 1981)

SPECIALTY BOARD:

Diplomate, American College of Veterinary Pathologists (1966)

SPECIAL CONSULTANTSHIPS AND COMMITTEE MEMBERSHIPS:

1957-1958	Faculty Advisor to Pre-Vet Club, Iowa State University

Ward R. Richter, D.V.M., M.S.

SPECIAL CONSULTANTSHIPS AND COMMITTEE MEMBERSHIPS - continued:

1958-1959	Admissions Committee, College of Veterinary Medicine, Iowa State University
1958-1959	Member Faculty Senate, Iowa State University
1965-1966	President, Midwest Society of Electron Microscopists
1966-1973	Special Lecturer and Consultant in Comparative Pathology, Resident Investigator Program in Laboratory Animal Medicine, V.A. Hospital, Hines, Illinois
1968-1969	Deputy Coroner, Lake County, Illinois
1969-1970	Director, Midwest Society of Electron Microscopists
1972-1973	Member of the Scientific Advisory Panel to Assistant Secretary of HEW on the safety of NTA as a detergent builder
1973	Meeting Arrangements Chairman for 1974 Meeting of American College of Veterinary Pathologists
1972-1973	Consultant to UAREP on long-term low level toxicity program development for the National Center for Toxicologic Research, Pine Bluff, Arkansas
1973	Member, Committee on Education, Society of Pharmacological and Environmental Pathologists
1973-1974	Consultant, Dental Research Institute Great Lakes Naval Training Station
1974-1975	Consultant, National Institute of Environmental Health Sciences
1974-1975	Consultant, Graduate Review Committee, Department of Pathology, University of Chicago

SPECIAL CONSULTANTSHIPS AND COMMITTEE MEMBERSHIPS - continued:

1974-1975	Consultant to HHHB Hospital Management Consultants, Chicago and San Francisco
1971-1978	Consultant, Argonne National Laboratories Argonne, Illinois
1974-1976	Member, Senior Scientific Advisory Board, National Center for Toxicologic Research, Jefferson, Arkansas
1976	Consultant, Medical Examiner Cook County, Illinois
1973-1979	Chairman, Committee on Animal Resources University of Chicago, Illinois
1969-1980	Representative to Council of National Society for Medical Research
1971-1980	Consulting Pathologist, Lincoln Park Zoo Chicago, Illinois
1973-1980	Midwest Program Director, Charles Louis Davis, D.V.M. Foundation
1974-1980	Consulting Pathologist, Berwyn Veterinary Medical Center, Berwyn, Illinois
1974-1980	Consultant, Carcinogenesis and Toxicology Illinois Institute of Technology Research Institute
1977-1981	Member. Editorial Board, Journal of Environmental Pathology and Toxicology
1979-1980	Vice President. Illinois Society for Medical Research Chicago. Illinois
1979-1980	Consultant to CAREP (FDA) for review and evaluation of Nitrite Carcinogenicity Studies
1979-1980	Consultant to Tracor-Jitco (NCI) for review of carcinogenic potential of Dioxanes
1973-1991	Faculty of Discussants, Charles Louis Davis, D.V.M. Foundation
1983	Advisory Board, Charles Louis Davis, D.V.M. Foundation

Ward R. Richter, D.V.M., M.S.

PUBLICATIONS:

Richter, W.R.: Tubular Adenomata of the Adrenal of the Goat, **Cornell Vet.** 47: 558-577, 1957.

Richter, W.R.: Adrenal Cortical Adenomata in the Goat, **American Jour. Vet. Res.** 19: 895-901, 1958.

Richter, W.R.: Observations on the Penile Development of the Angora Goat, **Am. Jour. Vet. Res.** 20: 603-606, 1959.

Pearson, P.T., Jensen, E.C. and Richter, W.R.: Urinary Calculi in a 2-Month Old Pup, **Jour. A.V.M.A.** 135: 329-331, 1959.

Thompson, J.H. and Richter, W.R.: Hematoxylin-Eosin Staining Adapted to Automatic Tissue Processing, **Stain Technology** 35: 145-148, 1960.

Richter, W.R.: The Electron Microscope and its Application to Medical Research, **Jour. A.V.M.A.** 140: 1304-1309, 1962.

Richter, W.R.: Electron Microscopic Observations on Transitional Epithelium of Dogs Infected with Canine Distemper, **U.S. Army Res. Lab. Report #556**, Fort Knox, Kentucky, 1962.

Richter, W.R.: Effects of RF Energy on Tissue Cultures, **U.S. Army Med. Res. Lab. Report #600**, Fort Knox, Kentucky, 1962.

Richter, W.R. and Moize, S.M.: Electron Microscopic Observations on the Collapsed and Distended Mammalian Urinary Bladder, **J. Ultrastructure Res.** 9: 1963.

Richter, W.R. and Yost, D.H.: A Morphological Study of Rat Testicular Tissue Exposed to RF Energy, **U.S. Army Med. Res. Lab. Report #601**, Fort Knox, Kentucky, 1964.

Richter, W.R., Shipkowitz, N.L. and Rdzok, E.J.: Oral Papillomatosis of the Rabbit, An Electron Microscopic Study, **Lab. Invest. 13**: 430-438, 1964.

Richter, W.R., Rdzok, E.J. and Moize, S.M.: Electron Microscopy of Virus-like Particles Associated with Duck Viral Hepatitis, **Virology** 24: 1964.

Cohen, A.I., Nicol, E.C. and Richter, W.R.: Nerve Growth Factor Requirement for Development of Dissociated Embryonic Sensory and Sympathetic Ganglia in Culture, **Proc. Soc. Exp. Bio. Med. 116**: 784-789, 1964.

Margoliash, E., Schenck, J.R., Hargie, M.P., Burokas, S., Richter, W.R., Barlow, G.H. and Mascona, A.A.: Characterization of Specific Cell Aggregating Materials from Sponge Cells. **Biochem. and Biophys. Res. Communications 20**: 383-388, 1965.

Richter, W.R., Stein, R.J., Rdzok, E.J., Moize, S.M. and Bischoff, M.B.: Ultrastructural Studies in Intranuclear Crystalline Inclusions in the Liver of the Dog. **Amer. J. Path.** 47: 587-599, 1965.

Ward R. Richter, D.V.M., M.S.

PUBLICATIONS - continued:

Smith, R.J., Richards, R.K., Richter, W.R., Hylton, R.R., McCabe, J.R. and Cullin, S.C.: Electrical Anesthesia Produced by Combining Direct and Alternating Currents: Electronmicroscopy of the Dog Brain. **Anesthesiology** 26: 607-611, 1965.

Rdzok, E.J., Shipkowitz, N.L. and Richter, W.R.: Rabbit Oral Papillomatosis, Ultrastructure of Experimental Infection. **Cancer Res. 26**: 160-165, 1966.

Richter, W.R., Bischoff, M.B. and Churchill, R.A. An Observation of Fine Tubules Within the Endoplasmic Reticulum in a Dog Liver. **Zeitschrift fur Zellforschung** 70: 180-184, 1966.

Stein, R.J., Richter, W.R., Zussman, R.A. and Brynjolfsson, G.: Ultrastructural Characterization of Daphnia Heart Muscle. **Jour. Cell Biol. 29**: 168-170, 1966.

Stein, R.J., Richter, W.R. and Brynjolfsson, G.: Ultrastructural Pharmacopathology: I. Comparative Morphology of the Livers of the Normal Street Dog and Purebred Beagle, A Baseline Study. **Exp. and Mol. Path.** 5: 195-224, 1966.

Richter, W.R., Stein, R.J. and Blockus, L.E.: Experimental Production of Intramitochondrial Filaments in Dog Liver. **Proceed. 6th Int. Cong. for Electron Microscopy**: 615-616, Kyoto, 1966.

Bischoff, M.B. and Richter, W.R.: Some Ultrastructural Features of the Pineal Organ in Japanese Quail. **Proceed. 6th Int. Cong. for Electron Microscopy**: 523-524, Kyoto, 1966.

Gerand, J., Richter, W.R., Fenters, J. and Holper, J.C.: Biophysical Studies with Rhinoviruses Using Zonal Ultracentrifuge Systems. **Jour. Virology** 2: 937-943, 1968.

Stein, R.J., Richter, W.R., Rdzok, E.J., Moize, S.M. and Brynjolfsson, G.: Comparative Hepatic Ultrastructure of Subhuman Primates: A Baseline Study of Rhesus, African Green and Squirrel Monkeys in: Use of Subhuman Primates in Drug Evaluation, ed. by Harold Vagtberg, **University of Texas Press**, Austin, 1968.

Bischoff, M.B., Richter, W.R. and Stein, R.J.: Ultrastructural Changes in Pig Hepatocytes During the Transitional Period from Late Foetal to Early Neonatal Life. **J. Cell Sci.** 4: 381-395, 1969.

Richter, W.R. and Moize, S.M.: Ultrastructural Nature of Canine Distemper Inclusions in the Urinary Bladder. **Path. Vet.** 7: 346-352, 1970.

Vesselinovitch, S.D., Mihailovich, N. and Richter, W.R.: The Induction of Malignant Melanomas in the Syrian White Hamster by Neonatal Exposure to Urethan. **Cancer Res. 30**: 2543-2547, 1970.

Ward R. Richter, D.V.M., M.S.

PUBLICATIONS - continued:

Port, C.D., Richter, W.R. and Moize, S.M.: An Ultrastructural Study of Tyzzer's Disease in the Mongolian Gerbil [**Meriones unguiculatus**]. **Lab. Invest.** 25: 81-87, 1971.

Richter, W.R., Zouhar, R.L., Tatsuno, J., Smith, R.H. and Cullen, S.C.: Electron Microscopy of the Macaca Mulatta Brain after Repeated Applications of Electrical Current. **Anesthesiology 36**: 374-377, 1972.

Churg, A. and Richter, W.R.: Histochemical Distribution of Carbonic Anhydrase after Ligation of the Pancreatic Duct. **Am. J. Path. 68**: 23-28, 1972.

Pine, J.H., Richter, W.R. and Esterly, J.R.: Experimental Bacterial Pneumonia. Ultrastructural, Autoradiographic and Histochemical Observations. **Amer. Jour. Path. 73**: 115-122, 1973.

Richter, W.R.: A Support Laboratory for Medical School Animal Research Facility. **Proceedings of National Conference on Research Animals in Medicine**. Washington, D.C., 1974.

Levin, S. and Richter, W.R.: A Systematic Method for the Detection of Experimentally Induced Hyperplasia and Early Neoplasia in the Urinary Bladder. **Toxicology and Applied Pharmacology** 27: 680-684, 1974.

Port, C.D., Baxter, D.W. and Richter, W.R.: The Mongolian Gerbil as a Model for Lead Toxicity I. Studies of Acute Poisoning. **Amer. Jour. Path. 76**: 79-94, 1974.

Torkelson, T.R., Leong, B.K.J., Kociba, R.R.J., Richter, W.R. and Gehring, P.J.: Lack of Manifestation of Toxicity in Rats Inhaling 111 ppm 1.4 Dioxane for Two Years. **Toxicology and Applied Pharmacology 30**: 1974.

Levin, S. and Richter, W.R.: Ultrastructure of Cell Surface Coat (Glycocalyx) in Rat Urinary Bladder Epithelium. **Cell Tissue Research 158**: 281-283, 1974.

Baxter, D.W., Port, C.D. and Richter, W.R.: Mongolian Gerbil as a Model for Chronic Lead Toxicity. **Jour. of Comparative Path. 85**: 1975.

Shybut, G.T., Richter, W.R. and Schuster, C.R.: Absence of Pathological Changes Following Intravenous Methamphetamine and IntraArterial Iothalamate Meglumine. **Research Communications in Chem. Path. and Path. 15**: 53-73, 1976.

Port, C.D. and Richter, W.R.: Eosinophilic Leukemia in a Syrian Hamster. **Vet. Path. 14**: 283-286, 1977.

Takata, A.N., Zaneveld, L. and Richter, W.R.: Laser-Induced Thermal Damage of Skin. USAF school of Aerospace Medicine, 1977.

Ward R. Richter, D.V.M., M.S.

PUBLICATIONS - continued:

Churg, A. and Richter, W.R.: Early Changes in the Exocrine Pancreas of the Dog and the Rat after Ligation of the Pancreatic Duct. A Light and Electron Microscopic Study. **Am. J. Path. 63**: 521-534, 1971.

Goyer, R.O., Falk, H., Feldman, Hogan, M. and Richter, W.R.: Renal Tumors in Rats Given Trisodium Nitrilotriacetic Acid in Drinking Water for Two Years. **JNCI 66**: 86-89, 1981.

Shefner, A.M., Dooley, L., Ficks, A., Grubbs, C.J., Rust, J.R. and Richter, W.R.: Carcinogenicity of Diesel Exhaust Particles by Intratrachael Instillation-Dose Range Study. **Proceedings of International Symposium on the Health Effects of Diesel Engine Emissions**, 1981.

Holmes, P.A., Rust, J.H., Shefner, A.M. and Richter, W.R.: Long-Term Effects of TCDD and HCDD in Mice and Rats. Health Effects of Halogenated Aromatic Hydrocarbons, **New York Academy of Sciences**, 1981.

Levine, B.S., Henry, M.C., Port, C.D., Richter, W.R. and Urbanek, M.A.: Nephrotoxic Potential of cis-Diaminechlorplatinum and Four Analogs in Male Fischer 344 Rats. **JNCI, 67**: 201-206, 1981.

Schoenig, G.P., Goldenthal, E.I., Geil, R.G., Frith, C.H., Richter, W.R. and Carlborg, F.W.: Evaluation of the Dose Response and in **utero** Exposure to Saccharin in the Rat. **Food and Chem. Toxicol. 23**: 475-490, 1985.

Kuncl, R.W. and Richter, W.R.: Prevalence and Ultrastructure of Sarcocystis in Rhesus Monkeys. **Jpn J Vet Sci. 50**: 519-527, 1988.

Cushman, J.R., Richter, W.R. and Duke, J.T.: Effects of Skin Sensitization Test Wrapping on Guinea Pigs. **Contact Dermatitis 21**: 279-280, 1989.

Magaw, R.I., Richter, W.R. and MacGregor, J.A.: A Reexamination of Liver Tumors in Mice Exposed to Wholly Vaporized Unleaded Gasoline. **Jour. American College of Toxicology 12**: 195-199, 1993.

MacGregor, J.A., Richter, W.R. and Magaw, R.I.: Uterine Changes in Female Mice Following Lifetime Inhalation of Wholly Vaporized Unleaded Gasoline: A Possible Relationship to Observed Liver Tumors? **Jour. American College of Toxicology 12**: 119-126, 1993.

Daughtrey, W.D., Duffy, J.S., Haddock, L.S., Keenan, T.H. and Richter, W.R.: Chronic Inhalation Study of Commercial Hexane in Mice. **The Toxicologist** 14, 1234, 1994.

Ward R. Richter, D.V.M., M.S.

PUBLICATIONS - continued:

Carol S. Auletta, Lionel F. Rubin, Ira W. Daly, Ward R. Richter, Kazuhiro Hosoi, Hiroshi Suda and Toshimi Ikuse; "26-Week Ocular Toxicity Studies in Pigmented Rabbits Treated Topically with Bunazosin Hydrochloride Ophthalmic Solution"; **Atarashii Ganka (J.Eye)**, Vol. 12, No. 3, 1995.

Richter, W.R.: Author or Co-author of several hundred reports on preclinical safety studies (IRDC, 1980-1985 and at CEHC, 1985-1987).

I CERTIFY THAT THE ABOVE IS A TRUE AND ACCURATE ACCOUNT OF MY PROFESSIONAL HISTORY TO DATE.

SIGNED: Ward R Richter DATE: 9 Nov 95

CURRICULUM VITAE

NAME: T. Bill Waggoner, Ph.D.

PRESENT POSITION: Senior Scientist, Chemistry
(since 11/93) Huntingdon Life Sciences
East Millstone, NJ 08875

Overall responsibility for the technical aspects of Department of Chemistry, overseeing the activities of over 9 professional and technical staff. Responsible for all of the Residue, Environmental, Analytical Chemistry and Metabolism/Pharmacokinetics work conducted by the organization.

Responsible for the preparation of protocols and chemistry cost estimates, and for the preparation of final reports for completed studies to support regulatory submissions.

Reviews analytical procedures submitted by Sponsors and the development of appropriate methods of analysis. Coordinates in-house studies between other departments and outside contracting with clients and other laboratories.

EDUCATION:

1964	Michigan State University East Lansing, MI Ph.D. Organic Chemistry
1959	Oklahoma State University Stillwater, OK M.S. Biochemistry
1957	Oklahoma State University Stillwater, OK B.S. Chemistry

PROFESSIONAL EXPERIENCE:

7/92-11/93	Director of Metabolism & Analytical Chemistry Pharmaco LSR Inc.

Overall responsibility for the Department of Metabolism and Analytical Chemistry, overseeing the activities of over 30 professional and technical staff. Responsible for all of the Residue, Environmental, Analytical Chemistry and Metabolism Pharmacokinetics work conducted by the organization.

T. Bill Waggoner, Ph.D.

PROFESSIONAL EXPERIENCE - continued:

1981-1984 — Pesticides Registration Manager
Rhone Poulenc Ag Company (formerly Rhone Poulenc Inc.)
Monmouth Junction, NJ

Primary contact with the EPA for registration of pesticides and establishing crop tolerances.

1979-1981 — Vice President
Analytical Development Corporation
Colorado Springs, CO

Head of technical staff of 30 providing support to Sponsor agrochemical and pharmaceutical companies - environmental fate and analytical chemistry.

1966-1978 — Manager Biochemical Research
Mobay Corporation, Agricultural Chemicals Division
Kansas City, MO

Head, staff of 35 professional and technical staff for development of new pesticide active ingredients: radiosynthesis, metabolism, residue chemistry - moderate contact with EPA.

1964-1966 — Senior Research Chemist
M&T Chemicals Incorporated, Electroplating Division
Detroit, MI

ACADEMIC EXPERIENCE:

1980 — Visiting Assistant Professor
Colorado College
Colorado Springs, CO
•Organic chemistry and inorganic course and laboratory course

1960 — Visiting Assistant Professor
Oklahoma State University
Stillwater, OK
•Advanced biochemical techniques; a course for graduate students and veterinary medicine students

PROFESSIONAL MEMBERSHIPS:

American Chemical Society

T. Bill Waggoner, Ph.D.

PUBLICATIONS:

"Spectrofluorometric Determination of Bay Vp 2674 Residues in Poultry Tissue", J.A.O.A.C., 70, 813-818 (1987) with M.C. Bowman.

"Problems and Pitfalls in Biochemical Studies for Pesticide Toxicology", The Pesticide Chemist and Modern Toxicology, S.K. Bandel et al, editors, ACS Symposium Series 160, ACS, Washington, D.C. (1981).

"New Aspects of Organophosphorous Pesticides; VII. Metabolism, Biochemical and Biological Aspects of Nemacur® and Related Phosphoramidate Compounds", Residue Reviews, Vol. 53 (1974)

PRESENTATIONS:

"Radiolabeled Compound Dosing Studies in Laboratory and Food Use Species for Tissue Residue Evaluations". Presented to The Association of Official Analytical Chemists Spring Workshop, Ottawa, Canada; Drug Residue Sessions Special Workshop (1981).

PATENTS:

Two patents from research at M&T Chemicals, Inc., Electroplating Division, Royal Oak, MI; topics were "plating on plastic materials" with E.J. Seyb (1967).

I CERTIFY THAT THE ABOVE IS A TRUE AND ACCURATE ACCOUNT OF MY PROFESSIONAL HISTORY TO DATE.

SIGNED: _____ DATE: 11/9/95

Huntingdon
Life Sciences

Summary of Relevant Publications

Paul E. Newton, Ph.D., DABT

J.B. Knaak, T.R. Barfknecht, L.W. Smith and P.E. Newton, "Subchronic Inhalation Toxicity Study of p-Chlorobenzoatrifluoride (PCBTF) in the Rat via Whole-Body Exposures", **The Toxicologist** 14, No. 1213, 1994.

P.E. Newton, W.L. Wooding and W.E. Rinehart, "An Inhalation Oncogenicity Study in Mice of Methylethyketoxime", **The Toxicologist** 14: No. 1220, 1994.

J.S. Duffy, L.S. Haddock, W.D. Daughtrey, T.H. Keenan and P.E. Newton, "Chronic Inhalation Study of Commercial Hexane in Rats", **The Toxicologist** 14: No. 1233, 1994.

Newton, P.E., Schroeder, R.E., Sullivan, J.B., Busey, W.M., and Banas, D.A. "Inhalation Toxicity of Phosphine in the Rat: Acute, Subchronic and Developmental", **Inhalation Toxicology**, 5 (2): 233-239 (1993).

Ward R. Richter, D.V.M., M.S., ACVP

Daughtrey, W.D., Duffy, J.S., Haddock, L.S., Keenan, T.H. and Richter, W.R.: Chronic Inhalation Study of Commercial Hexane in Mice. **The Toxicologist** 14, 1234, 1994.

Magaw, R.I., Richter, W.R. and MacGregor, J.A.: A Reexamination of Liver Tumors in Mice Exposed to Wholly Vaporized Unleaded Gasoline. **Jour. American College of Toxicology 12**: 195-199, 1993.

MacGregor, J.A., Richter, W.R. and Magaw, R.I.: Uterine Changes in Female Mice Following Lifetime Inhalation of Wholly Vaporized Unleaded Gasoline: A Possible Relationship to Observed Liver Tumors? **Jour. American College of Toxicology 12**: 119-126, 1993.

Raymond E. Schroeder, M.S., DABT

Biles, R.W., Schroeder, R.E. and C.E. Holdsworth; "Methyl Tertiary Butyl Ether Inhalation in Rats: A Single Generation Reproduction Study", **Toxicology and Industrial Health**, Vol. 3, No. 4, p. 519-534 (1987).

R.S. Nair, J.A. Barter, R.E. Schroeder, A. Knezevich and C.R. Stack, "A Two-Generation Reproduction Study with Monochlorobenzene Vapor in Rats", **Fundamental and Applied Toxicology** 9, 678-686 (1987).

F.R. Johannsen, G.J. Levinskas, G.M. Rusch and R.E. Schroeder, "Subchronic Inhalation Toxicity and Reproductive Assessment in Rats of Three Chlorinated Propanes", **J. Toxicol. Environ. Health 33** (3), 291-302 (1991).

Raymond E. Schroeder - continued:

R.A. Kuna, M.J. Nicolich, R.E. Schroeder and G.M. Rusch, "A Female Rat Fertility Study with Inhaled Benzene", **Journal of the American College of Toxicology**, Volume 11, Number 3, 275-282 (1992).

Conaway, C.C., Schroeder, R.E. and Snyder, N.K.; "Teratology Evaluation of Methyl Tertiary Butyl Ether in Rats and Mice", **Journal of Toxicology and Environmental Health 16**: 797-809 (1985).

Homan, E.R., Schroeder, R.E. "Inhalation Teratology Studies on Cyclohexanone", presented at "A Symposium on an Industry Approach to Chemical Risk Assessment-Caprolactam and Related Compounds as a Case Study"; (Abstract) published in the Proceedings of a Symposium on An Industry Approach to Chemical Risk Assessment, May, 1984, pp. 206-218.