90-DAY INHALATION STUDY IN RATS, USING G7-25 CIGARETTES

STUDY PROTOCOL

This inhalation study will examine the toxicological responses observed in rats exposed to smoke from cigarettes formulated with 2 different inclusions of an additive ("test cigarettes"). Responses will be compared with those in animals exposed to smoke from cigarettes without the additive ("reference cigarettes"). The study will use 7 groups each containing 32 male and 32 female Sprague-Dawley rats, plus a sentinel group comprising 50 animals. Three concentrations of smoke from the each of the test and reference cigarettes will be used. The comparisons of test and reference will be made on the basis of the amounts of wet total particulate matter (WTPM) presented to the animals. There will also be a sham-exposure group ("controls") and a group of sentinels. Animals will be exposed one hour per day, 5 days per week for 14 consecutive weeks (total of 65 exposures). The experiment will include a study of the reversibility of any changes observed. End-points will include histopathology, plasma nicotine and cotinine, organ and body weights, feed consumption, CO-oximetry, and measurements of respiratory physiology.

OBJECTIVE

The objective of this study (TOX-43) is to compare toxicological responses of rats exposed to mainstream smoke from cigarettes containing 0 or 30% of G7-25 sheet, in a sub-chronic nose-only inhalation study using Sprague-Dawley rats.

EXPERIMENTAL DESIGN

The experimental design is based on the OECD guideline No. 413, "Sub-chronic Inhalation Toxicity: 90-day study", adopted 12 May 1981.

Three groups of animals will be exposed to smoke from the test cigarette (30% G7-25) and three groups to smoke from the reference cigarette (0% G7-25). The actual dilutions of the smoke will be adjusted to provide for each comparison a smoke with comparable concentrations (low, medium, high) of WTPM at the animal ports. In addition there will be a sham-exposure group, placed in restraint tubes but exposed only to dilution air. A group of non-exposed animals will serve as sentinels for disease. Each inhalation group will consist of 32 animals per sex; there will be 25 animals per sex in the sentinel group.

Animals will be exposed to smoke for one hour per day, 5 days per week for 14 consecutive weeks (excluding holidays), to give a total of 65 exposure days. Sub-groups of surviving animals groups will be kept for an additional 14 weeks without treatment, as a reversibility study.

DATA MANAGEMENT / QUALITY ASSURANCE

Specialist computer software produced by Xybion Medical Systems (Cedar Knolls, NJ) will be used for scheduling and for data acquisition and analysis.

Any records that would be required to reconstruct the experiment will be maintained. This study will not be listed as a regulated study and the results will not be submitted to any regulatory agency. In general, the spirit of *Good Laboratory Practice* (21 CFR, Part 58, 1987) will be followed. Data will be reviewed for accuracy of reporting, using staff experienced in Quality Assurance (QA) review of toxicology studies. Original laboratory notebooks and specimens will be stored at the evaluation facilities.

EXPERIMENTAL ANIMALS

This section was written with reference made to the *Format for preparation of protocols and ACUP's*, prepared by the Chairman of the RJRT Institutional Animal Care and Use Committee (IACUC) on July 26, 1991.

Animal Selection and Justification for Test System

The study requires the use of animals, as there are no alternative systems for examining the inhalation toxicology (if any) of the addition of G7-25 sheet into cigarettes.

The Sprague-Dawley rat (Crl: CD/BR, VAF/Plus) is chosen as the experimental animal, because it has frequently been used in inhalation studies and there is a large amount of background data available in the scientific literature.

The number of animals to be used is the minimum associated with meaningful statistical analyses of the data, based on the

variation seen in previous studies (Coggins et al., 1989a, b).

There are currently no data available on the toxicology of the addition of G7-25 sheet to cigarettes: the present study thus is not a duplicate of any other work.

Animal husbandry

A minimum of 520 (260♂, 260♀) animals weighing 126-150 g will be ordered, to provide the required number of animals for testing. A reputable vendor (e.g. Charles River Raleigh, NC will be used). Animals will be housed individually in transparent polycarbonate cages and acclimated to laboratory conditions for 2-3 weeks prior to the first exposure.

Two days after delivery, but before group allocation (see below), 5 animals per sex will be randomly chosen and killed (see "Euthanasia", below) for collection of sera. Sera will be tested for the following antibodies to disease: Reovirus Type 3, cilia associated respiratory bacillus, Kilham's rat virus, Toolan's H-1 virus, pneumonia virus of mice, Sendai, rat coronavirus / sialodacryoadenitis virus, lymphocytic choriomeningitis virus, *Mycoplasma pulmonis* . Antibody testing will again be made on sera obtained from 5 animals per sex at the beginning, mid-point and end of the inhalation part of the experiment, and again at reversibility (a total of 25 animals per sex for serology testing). The lungs from the sentinel animals will be taken and examined histopathologically, to ascertain health status.

The start of the inhalation part of the study is dependent upon negative serology data being obtained on the pre-study samples, and upon a histopathology statement on the animals killed at delivery, releasing the animals from quarantine.

Any animals with questionable health (as assessed by a veterinarian or experienced assistant) will be excluded from the following procedure. One week after delivery the animals will be allocated by sex into 7 groups (32 animals per sex per group, giving a total of 224 animals per sex), such that the body weights in the groups are as homogeneous as possible. A record will be kept of quarantine number, weight at group allocation and permanent identification number.

Of the animals discarded from the above process, 20 per sex will be allocated to the sentinel group, thus minimizing the total usage of animals in the experiment. Surplus animals thereafter will be removed from the study and made available for methods development work.

During the week after allocation into groups, animals will be tail-tattooed (Animal Identification & Marking Systems, Piscataway, NJ) with their permanent identification number. The animals will be returned to cages with cards attached, recording the study number, animal number, sex, pre-study number, and Study Director.

The following animal numbers will be used: sham ♂ 101-132 ♀ 151-182, sentinel ♂ 201-220 ♀ 251-270, test low ♂ 301-332 ♀ 351-382, medium ♂ 401-432 ♀ 451-482, high ♂ 501-532 ♀ 551-482, reference low ♂ 601-632 ♀ 651-682 medium ♂ 701-732 ♀ 751-782 high ♂ 801-832 ♀ 851-832.

The animals will be housed and cared for in accordance with the Animal Welfare Act of 1970 and amendments (Public Law 91-579), as set forth in CFR Title 9, Part 3 Sub-part E, *Specifications for the humane handling, care, treatment, and transportation of warm-blooded animals other than dogs, cats, rabbits, hamsters, guinea pigs and non-human primates* . Reference will also be made to the DHHS document *Guide for the Care and Use of Laboratory Animals* (NIH 86-23), and to the Society of Toxicology's *Code of Ethics.*

The animals will be housed in a vivarium and inhalation suite with controlled lighting (12 hours of darkness, from 6 p.m.), temperature (20-24°C), and humidity (40-60% relative humidity, RH). Seven-day continuous recordings will be kept of RH and temperature. When empty, the animal rooms will be certified as being Class 100 (less than 100 particles per cubic meter).

Animals will be housed individually in polycarbonate cages with Alpha-Dri cellulose bedding (Shepherd Specialty Papers, Kalamazoo, MI); cages will be cleaned thrice weekly and refilled with clean bedding.

Animals will have unrestricted access to certified feed (Purina Rodent Chow # 5002, presented as pellets) and distilled water. No feed or water will be available during inhalation exposures. Chemical analyses of feed, water or bedding will not be performed, because it is unlikely that contaminants would adversely affect the experiment.

Invasive Techniques

The only invasive techniques to be used in the experiment (other than necropsy: see below) are related to tattooing and blood sampling. Tail-tattooing will be performed under light anesthesia with 70% carbon dioxide (CO_2) in air, by certified operators.

Blood samples are required from animals at the end of their daily inhalation exposures, to verify the blood concentrations of carboxyhemoglobin (COHb). The latter are required to ensure that animals do not die from hypoxia (Ayres et al., 1989). The blood samples obtained are also used to assess dosimetry, by providing plasma concentrations of nicotine and cotinine.

The procedure involves light anesthesia with 70% CO_2 in air, followed by blood sampling from the retro-orbital sinus. Only skilled personnel approved by the laboratory veterinarian will be allowed to perform this technique. There are no known side-effects of this procedure.

Survival Surgery

No surgical interventions are planned during the in-life phase of the experiment, and so no survival surgery is scheduled.

Any animals that die during the experiment will not be replaced, nor will any attempts be made to treat any moribund animals (see "Viability checks" and "Euthanasia", below).

Pain/distress

An integral part of the study is the restraint of the animals in nose-only exposure tubes and in whole-body plethysmographs (see below). This restraint is inevitably stressful to the animals. Previous work has shown that this stress is minimal, and that animals continue to gain weight throughout a 90-day study similar to that described here (Coggins et al., 1989 a, b). The restraint tubes have a number of features which minimize stress, such as ventilation holes and channels for the removal of urine and feces (Baumgartner & Coggins, 1980). The tail of the animal is not restrained, which is thought to reduce elevated temperatures in the tubes.

Animals are only placed in plethysmograph tubes (where ventilation, drainage channels and unrestrained tails are not possible: see Coggins et al., 1981) for 1-2 exposures during the study.

Euthanasia

Animals will be weighed and then killed by first anesthetizing with 70% CO_2 in air and then exsanguination prior to cessation of heartbeat. Only personnel approved by the laboratory veterinarian will be allowed to perform this procedure.

Hazardous Materials

There is no evidence to suggest that the test compound is in any way hazardous and consequently there are no special requirements for animal care personnel. Disposal of soiled bedding and animal carcasses need only follow routine procedures.

EXPOSURE REGIMEN

Cigarettes

The test and reference cigarettes will be uniquely identified, and will be stored under conditions recommended by the G7-25 research team.

Smoke Dilutions

Smoke dilutions will be confirmed by assays made in the breathing zones of the inhalation chamber before any animals are exposed to smoke. In particular, the carbon monoxide (CO) concentrations will be compared with the known toxicity for this smoke component (Ayres et al., 1989).

The dilution air will be passed through a Del-Monox compressed air purification system (Deltech Engineering, New Castle, DE) and humidified before being presented to the animals. The exposure atmospheres will have an RH of 40-60%, with a temperature of 20-24°C.

The approximate WTPM exposures to be used in the study, expressed as milligrams per liter of air presented, are as follows : low exposure, 0.16, medium exposure, 0.32, and high exposure, 0.64. These latter exposures have been shown to be tolerated in earlier experiments (Coggins et al., 1989 a, b).

Exposure Duration.

Each exposure group will be exposed to smoke for 60 minutes, once per day, 5 days per week, for 14 consecutive weeks. The total number of exposures will be 65 for each of the groups. The low, medium and high exposures will be "stagger-started" by one day. A portion of the surviving animals at the end of the 90-day inhalation study will be kept for an additional 14 weeks without treatment. These animals will be used to evaluate the reversibility of any changes observed after 90 days.

Calibration of Air Sampling Instruments

The measurement of flow rates is of critical importance in inhalation toxicology. The inhalation exposure system will use several different instruments to measure flow rates.

Two primary standards will be used : soap bubble flow meters (Series 823, Mast Development Co., Davenport, IA) for flow rates up to 5 liters per minute (LPM), and certified dry gas meters (Singer DTM 115, American Meter Company, Philadelphia, PA) for flow rates above 5 LPM. A variety of secondary measuring instruments, including pressure differential devices such as the CME (MIE Corp., Manassas, VA) will be used for routine measurements; these instruments will be calibrated against the primary standards.

Smoke generation.

The smoking machine has been described previously (Baumgartner & Coggins, 1980); it operates under standard conditions of a 2-second puff with a volume of 35 ml, taken once per minute. A number of modifications have recently been incorporated into the basic design of the machine (Ayres et al., 1990), modifications which have been included in the latest models (CH Technologies, Westwood, NJ)

Separate exposure devices will be used for test and reference cigarettes, and for sham exposures (total of three devices). Each exposure device will consist of three 24-port circular chambers. The flow rates of the diluting air streams will be adjusted to ensure flow rates at each of the animal ports of 350 ml/min (Coggins et al., 1982).

Pre-exposure characterization

Before the animal exposures begin, satisfactory achievement of uniformly distributed concentrations at or near the targets will be documented, for test and reference cigarettes. The characterization of each chamber will be made for WTPM, CO and nicotine, along with a measurement of particle size distribution and oxygen.

Daily characterization of inhalation exposures.

The puff volume produced by the pump at the cigarette ports will be measured using a soap bubble flow meter, before each 60-minute exposure.

Each 30-port smoke generator requires 10-15 minutes to reach equilibration. During the calibration period, the diluted smoke will be directed to equilibration chambers until a steady concentration is achieved. Then the diluted smoke stream will be directed to the inlet manifold on the animal exposure chamber and the exposures will begin. Animals will be placed on the exposure trays during the equilibration of the smoke generator; during this time animals will be exposed to an adequate supply of dilution air.

During animal exposures, several of the ports will be used for monitoring the aerosol presented. Two ports will be used for collection of WTPM on 44 mm glass fiber filter pads (Model 1021215P1, Cambridge Filter Corp., Syracuse, NY). Filter pads will be pre-conditioned at 40-60% RH before use. Nicotine on pads will be estimated using standard analytical techniques. WTPM will also be monitored on line from a third port, using the output from the RAM-1 instrument (GCA Corp., Bedford, MA). The output from the RAM-1 will be displayed on a chart recorder.

Ports will be used for monitoring CO and oxygen concentrations. The analytical instrument used for CO will be the Horiba PIR-2000 CO-analyzer (Horiba Instruments Inc., Irvine, CA), calibrated daily with certified gas mixtures of CO in nitrogen (AIRCO Welding Supply, Greensboro, NC). The output from the Horiba will be displayed as voltage on a chart recorder and logged on the printer of the computer-control unit as voltage and calculated parts per million (ppm, based on prior calibration). Oxygen concentrations (%) will be monitored by a Horiba PMA-200 instrument, also calibrated with a certified gas mixture.

One port will be used for the measurement of particle size distribution, using a cascade impactor (In-Tox Products, Albuquerque, NM) similar to that described in the literature (Mercer et al., 1970). The impactor will have cut-off diameters in the range of 0.4-2.5 µm under the conditions of use; calculations of mass median aerodynamic diameter (MMAD) and geometric standard deviation will be made by the use of probit analysis. The cover slips (22 mm; Fisher Products, Raleigh, NC) used to collect the aerosol will be weighed using Cahn C-31 microbalances (Cahn, Cerritos, CA).

Measurement of particle size will be made once every two weeks for each of the groups.

Temperature and RH measurements will be made once every two weeks. RH will be determined by comparing the dry bulb temperature and the dew point temperature, using an I-100DP Dew Point Hygrometer (General Eastern Instruments Company, Waterstown, MA). The RH of the aerosols will be controlled by regulating a portion of the air dilution stream through an air humidifier and then joining a non-humidified air stream prior to mixing with the smoke aerosol.

IN-LIFE MEASUREMENTS AND OBSERVATIONS

Gross Observations

Animals will be observed for signs of toxicity on each exposure day as they are being transferred from their cages to the restraint tubes, and when being transferred back to their cages. In addition, more detailed clinical observations will be recorded on each animal once every four weeks, before each exposure and within 2 hours of the end of the exposure (once per day for sentinels).

Early Deaths / Viability Checks

Viability checks will be made twice daily at intervals of at least 4 hours (5 days per week, with only a single check each day at weekends and holidays). Necropsies with tissue collection for any subsequent histopathology will be performed by a veterinary pathologist (or experienced assistant) on animals found dead or in a moribund condition. If necropsy cannot be performed immediately, the carcass will be stored at 4°C to minimize tissue autolysis. Animals will thus be necropsied no later than 16 hours after the discovery of death.

Body Weights; Feed consumption

Individual non-fasted body weights will be determined within 48 hours of receipt, at group allocation, and weekly thereafter. Body weight data will be acquired using Mettler PM 2000 balances (Mettler Instrument Corporation, Hightstown, NJ). Feed consumption will be measured over 72 hours in exposure week 13, using the same equipment as described for body weights.

Blood CO-Oximetry

Blood for determination of COHb and other CO-Oximetry parameters will be drawn from the retro-orbital sinus, using anesthesia with 70% CO_2 in air and heparinized micropipettes (American Hospital Supply, McGaw Park, IL), after 50-60 minutes of exposure. Blood samples will be collected in Eppendorff micro centrifuge vials (Brinkmann Instruments, Westbury, NJ) containing the sodium salt of ethylene diamine tetra acetic acid (Na_2-EDTA) and stored on ice.

Blood COHb concentrations will be determined using a Model 482 CO-Oximeter (Instrumentation Laboratories, Hartford, CT). Each animal will be sampled once during the experiment, with samples collected in exposure weeks 1, 4, 7, 10 and 13.

Plasma nicotine and cotinine

At the same time as blood samples are obtained for CO-Oximetry (see above) an additional blood sample will be taken from the micropipette into Vacutainer tubes (Becton-Dickinson, Rutherford, NJ) containing Na_2-EDTA. Plasma samples will be obtained immediately by centrifugation, and samples stored at -70°C until subsequent analysis for plasma nicotine and cotinine. Additional plasma samples will be obtained from plethysmograph animals: see below.

Either gas chromatography (Davis, 1986) or a newly-developed ELISA method will be used to measure the plasma concentrations of nicotine and cotinine.

Respiratory Physiology

Tidal volume, respiratory rate and minute ventilation will be measured in 4 animals per sex per group, during exposure weeks 2, 3, 5, 6, 8, 9, 11 and 12. The measurements will be derived from respiratory flow measurements, using whole body plethysmography (Coggins et al., 1981), a pulmonary mechanics analyzer (Buxco Electronics Inc, Sharon, CT), and pulmonary physiology software (Branch Technologies, Dexter, MI). Measurements will be made throughout the 60 minutes of exposure, plus 10-15 minutes pre-exposure.

At the end of the 60 minutes of exposure the animals will have blood samples drawn for assays of plasma nicotine and cotinine (see above). Each plethysmograph animal will be sampled twice during the experiment, with maximized temporal separations between samplings.

NECROPSY AND HISTOPATHOLOGY

Standard necropsy techniques will be used (Feldman & Seely, 1988) for each animal in the study. The first 22 surviving animals per group will be killed on the day following the final inhalation exposure; the remaining animals in each group will be kept without any further treatment for an additional 14 weeks.

Necropsy

Feed will be removed from the animals during the day prior to necropsy. At each necropsy, animals will be weighed and then killed by first anesthetizing with 70% CO_2 in air and then exsanguination prior to cessation of heartbeat.

Animals will be subjected to a complete examination in the presence of a board-certified veterinary pathologist, with special attention paid to the lungs and upper respiratory tract. Animals from comparable groups will be killed on the same day.

Organ Weights

The lungs (complete with trachea and associated mediastinal tissue), liver, kidneys (pair), brain, testes (pair, with epididymides attached), adrenals (pair), spleen and heart (excluding major vessels) will be weighed at necropsy, using Mettler PM 460 balances. Organ weights and the terminal (fasted) body weight at death will be used to calculate organ : body weight and organ : brain weight ratios. The time from removal of the organ until weighing will be minimized and tissues will be kept in saline until weighing. No measurements will be made of organ weights in "early death" animals.

Tissue Collection

Tissues will be removed from each animal and fixed in 10% neutral buffered formalin (NBF), at an appropriate volume dilution. The NBF will contain 20 ml of 1% eosin per 20 liters of 37% formalin, to identify the fluid as fixative.

After weights of the lungs have been obtained, lungs will be infused with NBF using gravity filling (25 cm water pressure). The trachea will be ligated after lung infusion. The larynx, with the tongue attached, will be separated from the trachea prior to weighing and infusion of lungs. The following tissues will be collected:

Adrenals, Aorta, Bone (marrow: femur), Bone (sternum), Brain, Cecum, Colon, Cranium, Duodenum, Epididymides, Esophagus, Eyes/optic nerve, Heart, Ileum, Jejunum, Kidneys, Larynx, Liver, Lungs, Lymph Nodes (thymic, mesenteric and peribronchial), Mammary glands, Nasopharynx, Nose/turbinates, Ovaries, Pancreas, Parathyroid, Pituitary, Prostate, Rectum, Salivary gland (and associated lymph nodes), Seminal vesicle, Skeletal muscle (thigh), Skin (abdominal), Spinal Cord (lumbar), Spleen, Stomach, Tail, Testes, Thymus, Thyroid, Trachea, Urinary bladder, Uterus, Zymbal's gland.

Additionally, organs showing gross lesions will be collected. The carcass and remaining tissues will be incinerated. Any remaining sentinel animals will be killed and discarded.

Histopathology

Respiratory tract tissues (nasal passages, larynx, trachea, conducting airways, deep lung), heart and related lymph nodes (thymic and peribronchial) will be examined in each animal, along with any gross changes. The study pathologist will decide on the work to be performed in any "early death" animals. The nasal tissues will be cut at four different locations to obtain representative sections of the different epithelia (Young, 1981). The lungs will be sectioned so as to provide a section along the main stem bronchus of each lung lobe. A precise anatomical site for cutting the larynx is required (Sagartz et al., 1991). Three sections of trachea will be prepared.

In the high exposure groups and in controls, the following tissues will also be examined in each animal: brain, liver, kidneys, spleen, adrenals and gonads.

Sections will be stained with hematoxylin and eosin (H&E); duplicate slides of nasal tissues (level III only), larynx, lung and trachea will be stained with Periodic - Acid - Schiff / Alcian Blue (PAS-AB) to facilitate evaluations of goblet cells.

Tissues will be read by a board-certified veterinary pathologist, initially with knowledge of the treatment groups.

STATISTICAL ANALYSES

In-life, organ weights.

Statistical evaluations will be made using Bartlett's test of homogeneity of variance, followed by Fisher's least significant difference (LSD) test.

Histopathology

The statistical evaluation of incidence and severity data will be made by the Kolmogorov-Smirnov test (Siegel, 1956).

Significance

Statistical tests will be carried out to 5%, two-sided criteria.

QUALITY ASSURANCE

A rigorous procedure of QA will be maintained, with contract staff using on-line access (read only) to the data stored on the computer. A a review will be made of 15% of the raw data generated in the study.

REPORTING

Draft Interim Report

An interim report will be prepared as soon as possible after the final necropsy. The report will be a summary of in-life observations, body weight changes, absolute and relative organ weights, any completed data on plasma nicotine and cotinine, and gross pathology. The report will also include CO-Oximetry and respiratory physiology data; a section on the major inhalation parameters will also be included.

Gross Pathology, Histopathology.

A separate schedule will be established for the preparation of pathology reports.

Final Report

A final report will be prepared in draft form when data collation is complete. The QA unit will prepare and sign a statement to be included in the final report which shall specify the dates inspections were made and findings reported to management and to the Study Director. The report will include :

- objectives and procedures, as stated in the protocol
- description of the smoking machines and their operating conditions
- performance of the smoking machines, including chemical and physical data
- tabulation of response data
- a separate pathology report, including tabulated gross and microscopic pathology
- a separate section on physiology data
- results of serology assays
- deviations from the approved protocol or from SOPs

PROJECTED TIMING

Attempts will be made to achieve the following 1991 target dates. A time-line is attached.

Delivery of Animals : Tuesday 13 August
Randomization : 15 August

Exposure Starting Dates:

Sham Control : Tuesday 3 September
Low exposure : 4 September
Medium exposure : 5 September
High exposure : 3 September

Necropsies: 90-day

Sham Control : 4 December
Low exposure : 5 December
Medium exposure : 6 December
High exposure : 4 December

Necropsy : Reversibility

All : 11 March, 1992

STATEMENT BY STUDY DIRECTOR

The Study Director is responsible for ensuring that the work will be performed as described in the protocol. Every attempt will be made by the Study Director to perform the study as described above.

Any amendments to the approved protocol will be documented as such; amendments involving significant modifications in the usage of animals will be referred to the IACUC prior to any such modification being made.

The Study Director certifies that this study does not involve any unnecessary duplication, and that the study will follow all regulations, NIH guidelines, Animal Welfare Act, Society of Toxicology Code of Ethics and IACUC policies.

Study Director

C.R.E. Coggins, PH.D., DABT Date

Vice-President

A.W. Hayes, PH.D., DABT Date

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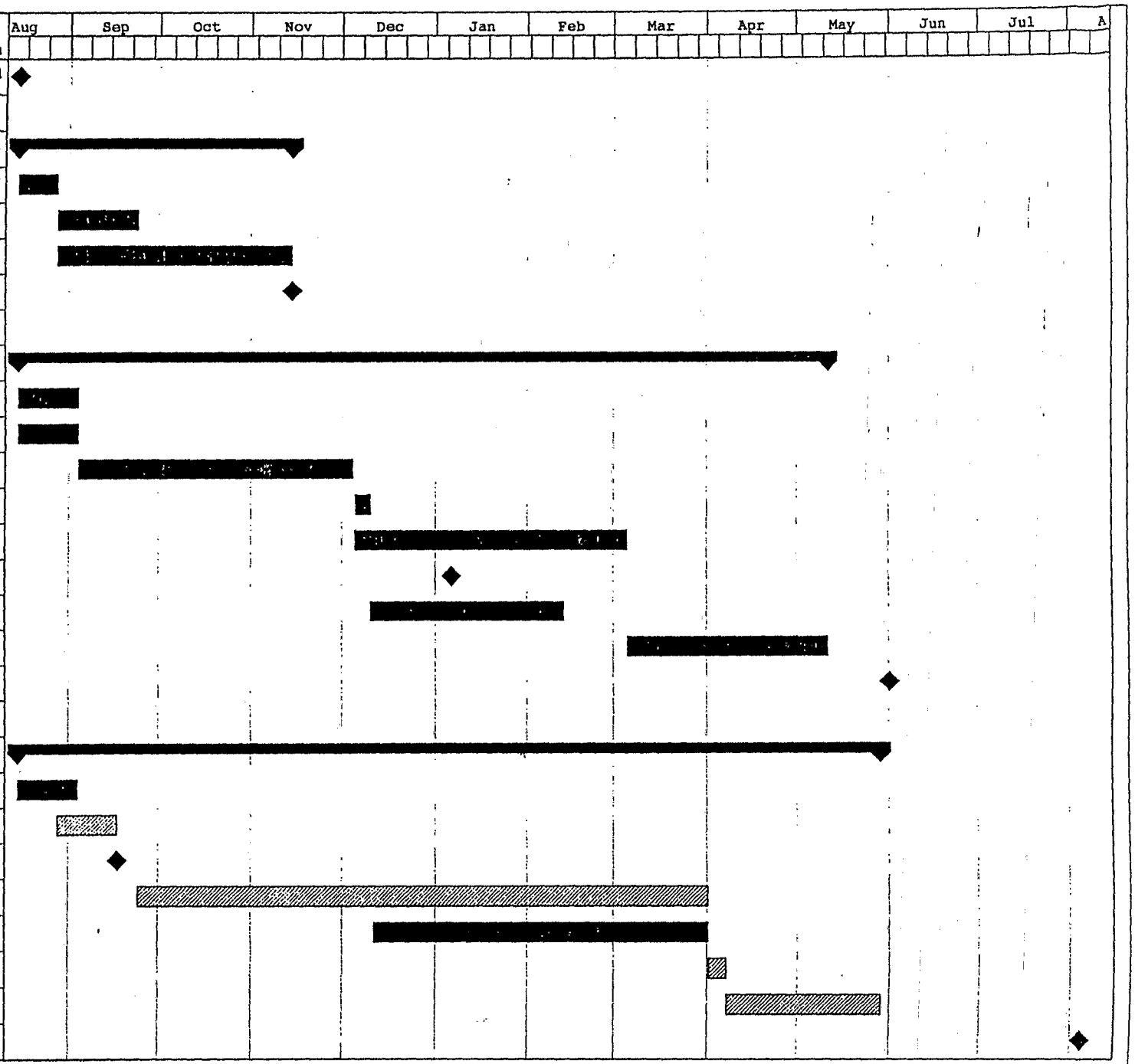
ID	Name	Duration
1	CIGARETTE DELIVERY	0d
2		
3	GENETIC TOXICOLOGY	91.31ed
4	CSC Preparation	2w
5	Ames	4w
6	SCE/CA	12w
7	Final Report	0d
8		
9	INHALATION	270.33ed
10	Characterization	3w
11	Quarantine	3w
12	Exposures	14w
13	Necropsy	4d
14	Reversibility	14w
15	Verbal Pathology	0d
16	Resp. Tract	10w
17	Reversibility	10w
18	Final Report	0d
19		
20	30-WEEK MSP STUDY	288.04ed
21	CSC characterization	3w
22	CSC reserve	3w
23	Initiation	0d
24	CSC Application	29w
25	"Growth-counts"	17w
26	Necropsy	4d
27	Pathology	8w
28	Final Report	0d



G7-25 TOX TESTING

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