

Hoechst

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Hoechst Corporation
P.O. Box 4915
Warren, N.J. 07060-4915

November 14, 1997
GL-048-97

8EHQ - 1197 - 0406

PDCM: 888/00000003

Attn: TSCA Section 8(e) Coordinator
Document Processing Center (TS-790)
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

Contains No CBI
No CBI

Subject: 8EHQ-0781-0406

Dear Sir or Madam:

With this letter, Hoechst Celanese Corporation is providing additional inhalation toxicity information for glycerol polyglycidyl ether (GPE), CAS no. 25038-04-4.

This information refers to an inhalation oncogenicity study of GPE in the Sprague Dawley Rat. The study was conducted using groups of 70 animals per sex which were exposed 6 hours/day, 5 days/week for 2 years. The exposure concentrations were 0 (control), 1, 3 and 10 mg/m³. The draft report (Study No. 94-6069, An Inhalation Oncogenicity Study of 70055 in the Rat) has recently been received and is being reviewed. A copy of the draft narrative sections, Introduction, Materials and Methods, Results and Discussion, and Conclusion are attached. A copy of the final report will be forwarded once it is completed.

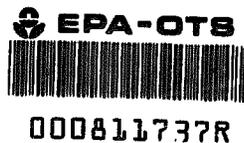
Please be advised that Hoechst Celanese has not processed this chemical since December 1993.

This submission contains no confidential business information.

If any further information is required, please do not hesitate to contact me at 908-231-4482.

Sincerely,

Gordon V. Loewengart
Gordon V. Loewengart, Ph.D.
Vice President, Health Sciences



Attachment
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I. INTRODUCTION:

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A series of whole-body inhalation exposures was performed for Hoechst Celanese Corporation to assess the potential oncogenic effects of 70055 when administered to Sprague-Dawley CD® rats (110/sex/group). The test material was generated into the breathing zone of the animals as a liquid aerosol for 6 hours per day, 5 days per week, for a major portion of the test animals life span at target concentrations of 1, 3 and 10 milligrams per cubic meter of air (mg/m³). Air control animals (110/sex) were exposed to clean air only. Recovery animals (20/sex/group) were predesignated and were exposed for three months and then allowed a three-month recovery period prior to sacrifice.

The test procedures followed guidelines as described in the Health Effects Test Guidelines; Office of Pesticides and Toxic Substances; United States Environmental Protection Agency, Volume 50 of the Federal Register, Number 188, September 17, 1985, and V Volume 52, Number 97, May 20, 1987, Part 798.3300.

This study was conducted following Good Laboratory Practices as set forth in 40 CFR Part 792 (EPA/TSCA). This study was conducted at Huntingdon Life Science, Mettlers Road, P.O. Box 2360, East Millstone, New Jersey 08875-2360. This Testing Facility was operated in accordance with the Animal Welfare Act regulations: 9 CFR Parts 1, 2 and 3 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.

All raw data, specimens, the original study protocol, the original final report as well as a sample of the test material are stored in the Archives at the Testing Facility for at least one year after submission of the final report. The Sponsor will determine the final disposition of these materials.

II. MATERIALS AND METHODS:

A. Study Dates:

Study Initiation Date: 11 January 1994
(Date Study Director signed the protocol)

Initiation of Exposures: 21 July 1994
(Experimental Start Date)

Termination of Exposures: 29 July 1996

Necropsy:

3 Month Interim Sacrifice: 21 October 1994
Recovery Sacrifice: 20 January 1995
12 Month Interim Sacrifice: 21 July 1995
18 Month Interim Sacrifice: 26 January 1996
Terminal Sacrifice: 19, 22, 23 July 1996

Study Completion Date: Date final report is signed by the Study Director. (Experimental Termination Date)

B. Test Material:

70055

Supplier:

Hoechst Celanese Company
P.O. Box 2500
Somerville, NJ 08876-1258

The test material was prepared as described in Appendix A from the Sponsor-supplied components (received 18-22 March 1994). The testing facility supplied the distilled/ demineralized water.

Component: 13015

Lot No.: FL-298

Purity: Not provided.

Description: Clear liquid.

Expiration Date: 31 March 1997

II. MATERIALS AND METHODS (cont.):

B. Test Material (cont.):

Component: 13394
Lot No.: WI4A034986
Purity: 93%
Description: Amber liquid.
Expiration Date: 31 March 1997

Component: 11793.5
Lot No.: 882814
Purity: 100% Active Ingredient
Description: White, granular powder.
Expiration Date: 31 March 1997

Component: Distilled/demineralized water
Source: Prepared by the Testing Facility from tap water supplied by Elizabethtown Water Company, Westfield, New Jersey.

Description: Clear, colorless liquid.

Characterization: The identity, strength, purity, composition, methods of synthesis, fabrication, and/or derivation of the test material components are the responsibility of the Sponsor.

Physical Properties: The documentation of the nature of the test material components, their solubility, melting/boiling point, vapor pressure and flammability are the responsibility of the Sponsor.

Storage: 60-85°F.

II. MATERIALS AND METHODS (cont.):

B. Test Material (cont.):

Sample Retention: An archival sample of approximately 10 grams of each test material component is stored in the Archives of the Testing Facility.

Disposition: All remaining containers of the test material and components will be returned to the Sponsor after issuance of the final report.

C. Test Animal:

Albino Rat

Strain: Sprague-Dawley CD®
[Cr1: CD® BR]

Justification for Animal Selection: The Sprague Dawley rat is a standard laboratory animal for inhalation toxicity studies. The Sprague-Dawley rat was used due to its availability and due to the existing historical data base for comparative evaluation.

Number of Animals

Received: 998 total (499 males, 499 females)

Placed on Test: 880 total (440 males, 440 females)

Supplier: Charles River Laboratories.
Kingston, New York 12484

Date of Birth: 9 June 1994

Date Received: 7 July 1994

Age at Receipt (approx.): 4 weeks

II. MATERIALS AND METHODS (cont.):

C. Test Animal (cont.):

Age at Initiation of Exposure (approx.):

6 weeks (the females were nulliparous and non-pregnant).

Weight at Initiation of Exposure (grams):

Males:
Females:

<u>Mean</u>	<u>Range</u>
163	140 - 185
138	119 - 159

Acclimation Period:

Animals were acclimated for 2 weeks prior to the initiation of the study. All animals were examined by the staff veterinarian during the acclimation period.

D. Selection and Group Assignment:

More animals than required for the study were purchased and equilibrated. Animals considered suitable for the study on the basis of pretest physical examinations, ophthalmology and body weight data were randomly assigned, by a computerized sort program, into 4 groups of 110 animals per sex so that body weight means for each group were comparable. Disposition of all animals not utilized in the study is maintained on file at the Testing Facility.

E. Animal Identification:

Each animal was assigned a temporary identification number upon receipt. After selection for study, each animal was tattooed with a number assigned by the Testing Facility. The number assigned plus the study number comprised the unique animal number for each animal. Each non-exposure cage was provided with a cage card which was

II. MATERIALS AND METHODS (cont.):

E. Animal Identification (cont.):

color-coded for exposure-level identification and contained the animal number.

F. Experimental Outline:

Group	Exposure Level ^a mg/m ³	Number of Animals							
		Initial		Clinical Laboratory Studies ^b		Necropsy		Microscopic Pathology ^c	
				Month 12 and 24	Month 18	Month 3, 6(rec), 12, 18	Month 24	Month 3, 6(rec), 12, 18	Month 24
		M	F	M/F	M/F	M/F	M/F	M/F	M/F
I	0	110	110	10/10	4/1	AS	AS	10/10	AR
II	1	110	110	10/10	1/5	AS	AS	AR	AR
III	3	110	110	10/10	3/3	AS	AS	AR	AR
IV	10	110	110	10/10	4/4	AS	AS	10/10	AR

^aExposures were 6 hours/day, 5 days/week for 523 exposures over 105 weeks.

^bClinical studies included nematology and clinical chemistry.

^cAS = all survivors of predesignated animals. AR = As required: lungs, trachea, larynx, nasal turbinates in all animals. All tissues from Groups I and IV animals and from animals found dead or euthanized in moribund condition during the study.

mg/m³ = milligrams per cubic meter of air; M = male; F = female.

G. Justification of Target Exposure Level Selection:

Sponsor selected on the basis of available toxicity data.

H. Husbandry:

During Non-Exposure Periods:

Currently acceptable practices of good animal husbandry were followed, e.g., Guide for the Care and Use of Laboratory Animals: DHHS Publication No. (NIH) 86-23 Revised 1985. The Testing Facility is accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

II. MATERIALS AND METHODS (cont.):

H. Husbandry (cont.):

During Non-Exposure
Periods (cont.):

Housing:

Animals were doubly housed in suspended, stainless steel, wire mesh cages during the first week of the acclimation period and individually housed during the remainder of the acclimation period and all other non-exposure periods.

Food:

Certified Rodent Diet, No. 5002; (PMI Feeds, Inc., St. Louis, MO) *ad libitum*. Fresh food presented as required.

Analysis of Feed:

Analysis of each feed lot used during this study was performed by PMI Feeds, Inc. Results are maintained on file at the Testing Facility.

Water:

ad libitum; by automated watering system (Elizabethtown Water Company).

Analysis of Water:

Monthly analysis of water supplied to this facility was provided by the Elizabethtown Water Company, Westfield, New Jersey (Raritan-East Millstone Plant). Results are maintained on file at the Testing Facility.

Biannual chemical and microbiological analysis of water samples collected from representative rooms in this facility were conducted to assure that water met standards specified under the EPA National Primary Drinking Water Regulations (40 CFR Part 141). Results are maintained on file at the Testing Facility.

II. MATERIALS AND METHODS (cont.):

H. Husbandry (cont.):

During Non-Exposure
Periods (cont.):

Contaminants:

There were no known contaminants reasonably expected to be found in the food or water which would interfere with the results of this study.

Environmental
Conditions:

Approximately 12 hour light/dark cycle (6 AM to 6 PM/6 PM to 6 AM) via automatic timer. Temperature was monitored and recorded twice daily, humidity was monitored and recorded once daily. They were maintained, to the extent possible, within the following ranges:

Temperature:

Desired: 18-26°C
Actual: 19-26°C

Relative Humidity:

Desired: 40-60%
Actual: 12-89%

During Exposure Periods:

Housing:

Animals were individually housed in wire mesh, stainless steel cages within 10m³ glass and stainless steel exposure chambers (Figure 1).

Food:

None

Water:

None

Environmental
Conditions:

Chamber temperature and humidity were monitored and recorded every half hour during exposure and maintained, to the extent possible, within the ranges presented on the next page.

II. MATERIALS AND METHODS (cont.):

I. Test Material Administration and Chamber Operation (cont.):

Chamber Operation:

The exposure chambers were operated dynamically under slight negative pressure. The chamber's airflow rate, total flow rate, time for air change and 99% equilibrium time (T99) for each group are summarized as follows:

Group	Airflow Rate (Lpm)	Air Change (min.)	T99 (min.)
I	2082	4.8	22
II	2042	4.9	23
III	2016	5.0	23
IV	2019	5.0	23

This chamber size and airflow rates were considered adequate to maintain the animal loading factor below 5% and oxygen above 19%. The chambers were exhausted through a system consisting of a coarse filter, a HEPA filter and an incinerator.

Recordings of chamber temperature, relative humidity, airflow rate and static pressure were made every half-hour during exposure. See Figure 1 and Appendix A for equipment details.

Test Material Preparation:

The test material was prepared on a daily basis. See Appendix A for procedure.

Exposure Procedure: Group I:

The control animals were exposed to House-supply air only, for 6 hours/day plus an additional 30 minutes to simulate clearing of the chamber.

II. MATERIALS AND METHODS (cont.):

I. Test Material Administration and Chamber Operation (cont.):

Exposure Procedure (cont.):
Groups II-IV:

The exposure atmosphere was generated using a spray atomizer (see Figure 2). The test material was delivered to the atomizer by either a syringe pump (Groups II and III) or a fluid metering pump (Group IV). The test material was fed from the pumps directly into the liquid inlet of an air atomizing nozzle. Houseline air was delivered, at a constant backpressure of 50 pounds per square inch, into the air inlet of the atomizer to generate the aerosol. The test atmosphere was directed into the exposure chamber which housed the animals. The animals remained in the chamber for 30 minutes following the exposure to allow the chamber to clear, using clean air at the same airflow rate used during exposure. See Figures 1 and 2 and Appendix A for equipment details.

Pump settings were selected in order to achieve the target exposure levels and were adjusted during the exposures as necessary. The initial settings for each group on Day 1 were as follows:

<u>Group</u>	<u>Initial Pump Setting</u>	<u>Size</u>
II	1.7mL/hr	10 mL syringe
III	7.2 mL/hr	20 mL syringe
IV	25% ^a	1/8" piston

^aA 100% pump setting provides 0.4 mL/min.

II. MATERIALS AND METHODS (cont.):

J. Exposure Chamber Sampling:

Chamber Sampling:

Samples for determination of the exposure levels were withdrawn from the breathing zone in the exposure chambers through glass fiber filters mounted open-faced in a filter holder. Samples were withdrawn four times per chamber per exposure from the normal sampling portal (designated H-1 in Figure 1). The uniformity of distribution of the test atmospheres was evaluated on a quarterly basis. The filter papers were weighed before and after sample collection, and the gravimetric concentration in mg/m^3 was calculated by dividing the weight difference in milligrams by the volume of air sampled in cubic meters. See Appendix A for equipment details.

Sampling flowrates and duration were as follows:

Group	Sample	
	Flowrate (Lpm)	Duration (min)
I	20	30
II	20	30
III	20	20
IV	20	5

One sample per chamber per day was also analyzed using UV/Visual Spectrophotometry. The analytical exposure concentrations in mg/m^3 were calculated by dividing the quantity of test material detected in milligrams by the volume of air sampled in cubic meters. See Appendix B for Analytical Method.

II. MATERIALS AND METHODS (cont.):

J. Exposure Chamber Sampling (cont.):

Particle Size

Distribution Analysis:

Particle size distribution measurements were performed pretest, daily for weeks 1 and 2 and weekly thereafter for each chamber using a TSI Aerodynamic Particle Sizer equipped with a diluter. The samples were drawn for 20 seconds at a rate of 5 liters per minute. The mass median aerodynamic diameter, geometric standard deviation and percent of particles ≤ 2.0 microns were calculated. A computer was used to program the system to the appropriate settings prior to sampling. The particle size distributions were calculated by the computer and printed. See Appendix A for equipment details.

Nominal Concentration:

The nominal concentrations (mg/m^3) were determined by weighing the generation apparatus containing the test material before and after the exposure and dividing the difference in these weights (mg) by the total volume of air (m^3) used during exposure (volumetric flow rate times total exposure time).

K. In-Life Observations:

For Mortality and Gross Signs of Toxicologic or Pharmacologic Effects:

Twice daily.

For Abnormal Signs:

Daily. Animals were observed as a group, in cage, once during each exposure.

II. MATERIALS AND METHODS (cont.):

K. In-Life Observations (cont.):

Detailed Physical
Examinations:

Pretest and weekly thereafter.
See Appendix A for general
physical examination,
methodology and references.

L. Ophthalmoscopic Examination: Ophthalmoscopic evaluations
were performed on all animals
pretest and just prior to the
scheduled sacrifices.

Time Intervals

Pretest - 14 July 1994
Month 3 - 20 October 1994
Recovery - 17 January 1995
Month 12 - 19 July 1995
Month 18 - 17 January 1996
Month 24 - 16 July 1996

M. Body Weight:

Twice pretest, weekly during
the first 13 weeks of the
study period, monthly
thereafter, and prior to
scheduled sacrifice. See
Appendix A for body weight
collection methodology and
references.

N. Laboratory Studies:

Blood was obtained via
venipuncture of the orbital
sinus (retrobulbar venous
plexus) under carbon
dioxide/oxygen (CO₂/O₂)
anesthesia. Animals were
fasted overnight prior to
blood collections.

Number of Animals:

Performed on 10 animals/
sex/group at the 12 and 24
month intervals; up to 5
animals/sex/group at the 18
month interval. The actual
numbers of animals per group
bled at the 18 month interval
were reduced from the intended
10/sex/group based on animal
survivorship concerns.

II. MATERIALS AND METHODS (cont.):

N. Laboratory Studies (cont.):

<u>Time Intervals</u>
Month 12 - 21 July 1995
Month 18 - 26 January 1996
Month 24 - 19 July 1996

Parameter Evaluated:

Hematology:

hemoglobin concentration
hematocrit
erythrocyte count
reticulocyte count
platelet count
mean corpuscular volume
mean corpuscular hemoglobin
mean corpuscular hemoglobin concentration
prothrombin time
activated partial thromboplastin time
total and differential leukocyte counts
erythrocyte morphology

Clinical Chemistry:

aspartate aminotransferase
alanine aminotransferase
alkaline phosphatase
blood urea nitrogen
fasting glucose
total protein
albumin
globulin (calculated)
albumin/globulin ratio (calculated)
creatinine
total bilirubin
sodium
potassium
chloride
calcium
inorganic phosphorus

O. Postmortem:

Macroscopic Postmortem
Examination:

Performed on all animals found dead, sacrificed in a moribund condition or killed at the scheduled sacrifice intervals. Examinations included the external surface, all orifices, the cranial cavity

II. MATERIALS AND METHODS (cont.):

0. Postmortem (cont.):

Macroscopic Postmortem Examination (cont.):

and the external surfaces of the brain and spinal cord, the nasal cavity and paranasal sinuses, the thoracic, abdominal and pelvic cavities and their viscera, cervical tissues and organs, and the remaining carcass.

Necropsy:

3 Month Interim Sacrifice: 21 October 1994
Recovery Sacrifice: 20 January 1995
12 Month Interim Sacrifice: 21 July 1995
18 Month Interim Sacrifice: 26 January 1996
Terminal Sacrifice: 19, 22, 23 July 1996

Number of Animals:

3 Month Interim Sacrifice: 40 males, 40 females
Recovery Sacrifice: 39 males, 40 females
12 Month Interim Sacrifice: 37 males, 37 females
18 Month Interim Sacrifice: 12 males, 13 females
Terminal Sacrifice: 67 males, 98 females

Sacrifice Method: Exsanguination following carbon dioxide inhalation.

Order of Sacrifice: Scheduled sacrifice animals were necropsied in an order that assured that approximately equal numbers of males and females from each group were examined on each day of a scheduled sacrifice interval. Examinations of animals of both sexes were performed at similar times of day throughout the necropsy period.

II. MATERIALS AND METHODS (cont.):

O. Postmortem (cont.):

Organs Weighed, Organ/Body
and Organ/Brain Weight
Ratios Calculated:

The following organs were weighed for all animals at the scheduled sacrifice intervals. Prior to weighing, the organs were carefully dissected and properly trimmed to remove adipose and other contiguous tissues in a uniform manner. Organs were weighed as soon as possible after dissection in order to avoid drying. Paired organs were weighed together.

adrenal glands
brain
kidneys
liver
lungs
ovaries
spleen
testes/epididymides

Tissues Preserved¹:

The following tissues were preserved for all animals.

adrenal glands (2)
aorta (thoracic)
bone (sternum)
bone marrow (sternum)²
brain (medulla/pons, cerebrum
and cerebellum)
epididymides (2)
esophagus
eyes with optic nerve (2)
femur
heart
kidneys (2)
larynx (2)
large intestine (cecum, colon
and rectum)
liver (2 lobes)
lungs with mainstem bronchi(2)
lymph nodes (mesenteric and
mediastinal)
mammary gland

¹The number in parentheses indicates the number of sections preserved and examined.

²Qualitative examination only.

II. MATERIALS AND METHODS (cont.):

0. Postmortem (cont.):

Tissues Preserved (cont.): muscle (*Biceps femoris*)
nasopharyngeal tissues
nerve (sciatic)
ovaries (2)
pancreas
pituitary gland
prostate gland
salivary glands, mandibular(2)
seminal vesicles (2)
skin
small intestine (duodenum,
jejunem and ileum)
spinal cord (cervical,
thoracic and lumbar)
spleen
stomach
testes (2)
thymic region
thyroid/parathyroid glands (2)
trachea
urinary bladder
uterus (body/horns) with
cervix
macroscopic lesions

Preservative: 10% neutral buffered formalin (NBF). The lungs, urinary bladder and nasal turbinates were perfused with 10% NBF prior to immersion in the fixative.

Tissues Examined Histopathologically: The preserved tissues were examined for all animals in Groups I and IV and for all animals which died while on test. The lungs, trachea, larynx, and nasal turbinates were also examined for all animals in Groups II and III.

Peer Review: Dr. Henry Bolte, D.V.M., Ph.D. performed the microscopic evaluations. Dr. Peter C. Mann, D.V.M. reviewed all slides of the nasal turbinates, larynx, lung and neoplasms; and all tissues from 10% of the animals in Groups I and IV.

II. MATERIALS AND METHODS (cont.):

P. Statistical Analysis:

Body weight, change in body weight, hematology and clinical chemistry parameters, organ weights, organ/body and organ/brain weight ratios were analyzed. Mean values of all exposure groups were compared to control at each time interval. Statistically significant differences from control are indicated on mean tables in the appendices. See Appendix A for further details.

In addition, statistical analyses were performed for survivorship and tumor data. Benign and malignant tumors analyzed were those that met any of the following criteria: tumors in the target organs, tumors seen in at least 5% of the animals examined in any group, or tumors which caused death. Analyses were performed for all benign and malignant tumors combined and individually by organ for the target organs.

Q. Protocol Deviations:

The following deviations occurred. They were not considered to have had an adverse effect upon the conclusions drawn from this study:

1. The test material stability analysis was not performed during Month 3 of the study. The Month 4 stability analysis was performed earlier in the month than scheduled to compensate for the lack of the Month 3 determination.

II. MATERIALS AND METHODS (cont.):

- Q. Protocol Deviations (cont.):
2. The humidity of the animal room was not recorded for two days of the study. The temperature of the animal room was recorded once for one day of the study.

III. RESULTS AND DISCUSSION:

This two year study included 10 predesignated animals/sex/group which were exposed to control or test material atmospheres for three months and were then held for a three month recovery period. These animals were sacrificed at the six month study interval. There were no in-life findings evident in these exposure/recovery animals which were attributed to exposure to the test material. The macroscopic and microscopic pathology data for these animals are contained in Appendix L.

A. Chamber Monitoring (Appendix C):

Chamber monitoring results are presented in Appendix C. Prestudy as well as quarterly chamber distribution analyses demonstrated that the test material was evenly distributed within each chamber. The target and actual mean (\pm SD) gravimetric, analytical and nominal chamber concentrations were as follows:

Group	Target Concentration (mg/m ³)	Gravimetric Concentration (mg/m ³)	Analytical Concentration (mg/m ³)	Nominal Concentration (mg/m ³)
I	0	0.016 \pm 0.025	<LOQ	N/A
II	1	1.0 \pm 0.13	0.84 \pm 0.16	15 \pm 2.0
III	3	3.0 \pm 0.45	2.7 \pm 0.56	58 \pm 8.1
IV	10	10 \pm 0.92	9.5 \pm 1.2	113 \pm 11

<LOQ = less than the limit of quantification.

The achieved mean exposure concentration (gravimetric and analytical) for each group was very close to the desired target concentration. The analytical results consistently confirmed the gravimetric results. The differences between measured and nominal concentrations were typical for this type of exposure and were considered related to test material depositing on surfaces within the exposure chambers. Chamber environmental conditions averaged 20°C and 48% relative humidity.

III. RESULTS AND DISCUSSION (cont.):

A. Chamber Monitoring (cont.):

Mean particle size distribution measurements for the test material exposures were as follows:

Group	Mass Median Aerodynamic Diameter(μm)	Geometric Standard Deviation	Percent of Particles $\leq 2.0\mu\text{m}$
II	1.9	1.8	56
III	1.5	1.8	68
IV	2.0	1.8	52
Mean	1.8	1.8	59

These particle size results indicate that the aerosol profile for all three test material exposure groups were reasonably similar and highly respirable to the rat.

B. Bulk Test Material Analysis (Appendix B):

Spectrophotometric analysis of 13015, the principal component of the test material, was performed on the stored bulk test material on a monthly basis during the 24 months of the study. The results consistently averaged close to 100% of expected. These results confirmed the stability of this component of the test material for the duration of the study.

C. Mortality and Survivorship (Figure 3; Appendices D and M):

After 12, 18 and 24 months of exposure to the test material the percent survivorship in the control and treated groups was as follows:

SEX	MALE				FEMALE			
	I air control	II 1 mg/m ³	III 3 mg/m ³	IV 10 mg/m ³	I air control	II 1 mg/m ³	III 3 mg/m ³	IV 10 mg/m ³
12 MONTHS	98%	91%	89%	83%	97%	96%	100%	99%
18 MONTHS	80%	66%	81%	57%	79%	71%	84%	80%
24 MONTHS	23%	30%	25%	12%	37%	25%	39%	29%

III. RESULTS AND DISCUSSION (cont.):

C. Mortality and Survivorship (cont.):

Life table analyses were performed for survival. Various analyses for relating proportions to dose were performed using procedures outlined in Thomas, Breslow, and Gart. For each data set, life table curves were computed using both Kaplan-Meier and standard methods. Homogeneity of curves were computed using Cox's test for life table data and the Gehan-Breslow generalized Kruskal-Wallis tests. For both tests, exact and conservative approximations were reported and pairwise comparisons of groups were given. Finally, for each data set, unadjusted and time-adjusted tests for linear trend in the proportions were performed.

There was a statistically significant decrease in survivorship for the high-exposure males when compared to the control group. Chronic purulent inflammation was the most frequently observed cause of death (17 of the 68 unscheduled deaths) in the high-dose males. There was no statistically significant difference in survival between controls and low or mid-exposure groups for males. There was no statistically significant differences in survivorship for females from any of the compound exposed groups when compared to the control group.

When comparing the homogeneity of curves for the high-exposure group with the control group, deaths occurred earlier and there were more deaths among high-exposure animals. The difference between these two curves was statistically significant by the Cox test ($p < 0.05$) and the Kruskal-Wallis test ($p < 0.01$). The greater significance seen in the Kruskal-Wallis test is the result of the greater emphasis this test places on the early part of the curve. The Cox test for unadjusted and adjusted trend was significant ($p < 0.05$) and the Kruskal-Wallis test for unadjusted and adjusted trend was significant ($p < 0.01$).

III. RESULTS AND DISCUSSION (cont.):

D. Physical Observations (Appendix E):

Physical observations were unremarkable. Observations commonly seen in laboratory rats were seen in control and treated groups at generally similar incidences or occurred sporadically. No effect of test material exposure was evident in the physical observation data during the two year study period.

E. Ophthalmoscopic Examinations (Appendix F):

No ophthalmological changes, attributed to test material exposure, were seen during the 3, 6, 12, 18 or 24 month ophthalmoscopic examinations.

F. Body Weights (Figure 2; Appendix G):

The mean body weights and body weight gains of the control and test material exposed animals were unremarkable throughout the two year exposure period. No adverse effects attributable to test material exposure were evident. On many occasions statistically significant differences were observed between the control and test material exposed animals. However, in these cases the treated animals were slightly greater in body weight than the controls and the data failed to exhibit a dose response. Increased body weights are not usually considered indicative of toxicity. There was no evidence of toxicity evident in the body weight data of the test material exposed animals during this two year study.

III. RESULTS AND DISCUSSION (cont.):

G. Clinical Laboratory Studies:

1. Hematology (Appendices H and I):

No effect of test material exposure on hematology parameters was evident after 12, 18 or 24 months of treatment. Values for control and treated groups were comparable. Very few animals were examined in each of the groups at 18 months due to concern regarding animal survivorship.

2. Clinical Chemistry (Appendix J):

No clear effect of test material administration on clinical chemistry parameters was evident during this study. At 12 months, mean total protein and albumin levels were slightly lower than control for females in the high-exposure group. In addition, mean calcium levels were slightly lower than control in the female mid and high-exposure groups. These differences from control were not evident in the males at any bleeding interval nor in the females at termination. Only one control female was evaluated at 18 months due to overall survivorship concerns and the availability of only one surviving animal, pre-selected for bleeding, from this group. The mean albumin levels of the females in each of the test material exposure groups were lower than the individual control female examined at 18 months. However, these data did not exhibit a dose response and the value in the single control animal appeared slightly elevated.

None of the slight differences from control noted in the total protein, albumin or calcium levels of the test material exposed females were considered indicative of significant toxicity.

III. RESULTS AND DISCUSSION (cont.):

H. Terminal Organ and Body Weights, Organ/Body Weight and Organ/Brain Weight Ratios (Appendix K):

At 18 Months, the high-exposure males exhibited a statistically significant increase in mean lung to brain weight ratio. This increase is consistent with the pulmonary pathology observed in these animals and was considered treatment-related.

A statistically significant increase, compared to control, was observed in the mean spleen to body weight ratios of the high-exposure females at 12 months. This increase was attributed to two of the nine animals which had greater body weights and spleen weights than the other animals in the high-exposure group. There were no macroscopic or microscopic findings evident in the spleens of these two animals which were associated with these increased organ to body weight ratios.

The organ weights, organ to body weights and organ to brain weight ratios for animals sacrificed after 3, 12, 18 and 24 months of test material exposure and for animals sacrificed after 3 months of exposure followed by 3 months of recovery (Month 6 sacrifice) were generally consistent with body weight differences or exhibited normal variability. With the exception of the high-exposure male lungs at 18 months, there were no indications of toxicity exhibited in these data.

I. Pathology (Appendix L):

Findings related to the whole body exposure to 70055 were seen in the larynx, nasoturbinal tissues and lungs. These were considered to be a direct local effect of whole body exposure to 70055. The magnitude of the changes in the larynx was considered to be greater than that seen in the nasoturbinal tissues or in the lung.

III. RESULTS AND DISCUSSION (cont.):

I. Pathology (cont.):

For reporting purposes, microscopic findings in the different levels of the nasoturbinal tissues and in the different levels of the larynx were consolidated under the headings "nasoturbinates" and "larynx", respectively. The respective incidence and severity values for individual animals were based on a compilation of findings in each of the four levels of the nasoturbinal tissues or the three levels of the larynx. When a comparable finding was observed in more than one section of the nasoturbinal tissues or larynx, it was counted only once to arrive at an incidence value and the highest severity rating was assigned to the finding. A summary-incidence of findings in each of the levels of the larynx and nasoturbinates is presented in Appendix L.

Exposure-related microscopic findings are presented in the in-text tables as follows: overall (excluding the recovery rats) and for the 24-, 18-, 12- and 3-Month intervals and for the 3-Month post exposure recovery period following 3 months of exposure. For practical purposes findings in rats sacrificed at the scheduled intervals and the associated decedents are considered together.

LARYNX:

Macroscopic Findings:

No macroscopic findings were noted for the larynx.

Microscopic Findings (summary of data on pages 42-43):

Neoplastic Findings: Ten rats had epithelial neoplasms. Nine had squamous cell papilloma (three from Group II, four from Group III and two from Group IV) and one from the mid-exposure group had a squamous cell carcinoma. The squamous cell carcinoma was seen in a mid-exposure unscheduled death at Week 56.

III. RESULTS AND DISCUSSION (cont.):

I. Pathology (cont.):

LARYNX (cont.):

Microscopic Findings (cont.):

The first squamous cell papilloma was seen in a high-exposure unscheduled death at Week 23. The other papillomas were seen between Weeks 77 and 105 (terminal sacrifice).

Non-neoplastic Findings: Squamous/squamoid metaplasia/hyperplasia (minimal to severe) of the pseudostratified columnar epithelium in the area of the ventral seromucous gland and keratinization (minimal to moderately severe), were seen in numerous males and females from the exposure groups and in a small number of controls. The incidence of metaplasia/hyperplasia had no dose-related pattern. Incidence of keratinization, in the males, was greatest in the mid and high-exposure groups (similar incidence); In the females there was a dose-related increase. Both findings, in both sexes, had a dose-related increase in severity. Atypia (minimal to moderately severe) of the metaplastic epithelium and cysts (minimal to moderate) in the metaplastic epithelium and overlying keratin were seen only in the exposure groups. Atypia, in both sexes, exhibited a dose-related increase in incidence and severity. Cysts were most frequent in males from the mid and high-exposure groups (similar incidence) and in females from the mid-exposure group; in both sexes the greatest severity was in the mid-exposure group. Keratinocysts (minimal to moderate) occurred only in the high-exposure group: five males and two females.

Squamous/squamoid metaplasia/hyperplasia and keratinization (both minimal to moderately severe) of the cuboidal/columnar epithelium lining the ventral diverticulum were seen most frequently in the exposure groups. Both had

III. RESULTS AND DISCUSSION (cont.):

I. Pathology (cont.):

LARYNX (cont.):

Microscopic Findings (cont.):

a dose-related increase in incidence. The severity was greatest in the high-exposure group (both sexes). Atypia (minimal to moderate) of the metaplastic epithelium was seen in a small number of rats from the mid and high-exposure groups.

Hyperplasia (minimal to moderately severe) and hyperkeratosis (minimal to moderate) of the stratified squamous epithelium normally lining portions of the larynx were seen in numerous rats from the exposure groups and in a small number of controls. Both findings, in both sexes, exhibited dose-related increases in incidence and severity.

Atrophy of seromucous glands (minimal to moderately severe) was seen in a number of rats from all groups. Incidence and severity were greatest in the high-exposure group followed by the mid-exposure group. This may be an age related finding exacerbated by exposure to the test material. Squamous/squamoid metaplasia of glandular epithelium; an exposure-related finding, occurred most frequently in the mid and high-exposure. Both findings in both sexes exhibited a dose-related increase in severity.

Mixed inflammatory cells (minimal to moderately severe), primarily lymphoid cells admixed with a small and variable number of neutrophils and/or eosinophils, were seen in the laryngeal mucosa of all rats examined. When present in small numbers, they are a normal component of the mucosa. In both sexes, the severity in the exposure groups was slightly greater than in the controls; there were no remarkable dose related differences. In conjunction with

III. RESULTS AND DISCUSSION (cont.):

I. Pathology (cont.):

LARYNX (cont.):

Microscopic Findings (cont.):

this, intraluminal inflammatory cells/cell debris (minimal to moderately severe) were seen in numerous rats from all groups. Incidence and severity were greatest in the high-exposure group. Intraluminal keratin (minimal to moderately severe), in rats from the exposure groups only, occurred most frequently in males and females from the high-exposure followed by the mid-exposure group. The greatest severity was in the high-exposure group (both sexes).

Except for intraluminal keratin, atrophy of seromucous glands and atypia in the ventral diverticulum, all of the aforementioned findings were first seen at 3-Months. Atrophy of seromucous glands and intraluminal keratin were first seen at 18-Months and atypia in the ventral diverticulum was first seen at 24-Months.

Stratified squamous epithelium normally lines the upper portion of the larynx whereas the remainder is lined by cuboidal and pseudostratified columnar (ciliated/non-ciliated) epithelium. The pseudostratified columnar epithelium on the ventral floor of the larynx at the base of the epiglottis, cranial to the ventral diverticulum and overlying the seromucous glands, is especially sensitive to inhaled materials. Therefore, this is considered to be a target site for histopathological evaluations following inhalation exposure to particulates, vapors and aerosols¹. Squamous/squamoid metaplasia of the

¹John W. Sagartz, et al., "Histological Sectioning of the Rodent Larynx for Inhalation Toxicity Testing," Toxicologic Pathology 20, No. 1 (1992), pp. 118-121.

III. RESULTS AND DISCUSSION (cont.):

I. Pathology (cont.):

LARYNX (cont.):

Microscopic Findings (cont.):

pseudostratified columnar epithelium covering the ventral floor of the larynx is considered to be a normal adaptive response to inhaled foreign materials. In this study, the high incidence and dose-related increase in severity of squamous/squamoid metaplasia accompanied by keratinization and atypia was considered to be an adverse response at all exposure levels. With respect to these findings, there was no NOEL (No Observed Effect Level).

At the end of the 3-Month recovery period the incidence and severity of comparable findings were compared to those seen at the end of the 3-Month exposure period. Decreases in both indicated that reversibility had occurred but was incomplete. One male from the high-exposure group, found dead during the first week of the post-exposure recovery period did not live long enough for any degree of reversibility to occur.

III. RESULTS AND DISCUSSION (cont.):

I. Pathology (cont.):

LARYNX (cont.):

Microscopic Findings (cont.):

Selected Larynx Findings - All Animals

SEX	MALE				FEMALE			
	0	1	3	10	0	1	3	10
DOSE (mg/m ³)	0	1	3	10	0	1	3	10
NUMBER EXAMINED	99	99	100	100	99	100	99	100
squamous cell papilloma, benign ^a	0	1	1	1	0	2	3	1
squamous cell carcinoma, malignant ^b	0	0	1	0	0	0	0	0
mucosa:pseudostratified columnar epithelium-squamous/squamoid metaplasia/hyperplasia								
total	10	93	99	98	7	97	97	98
minimal	5	15	1	1	4	48	20	1
slight	3	40	28	15	3	37	41	16
moderate	2	36	61	54	0	11	36	67
marked	0	2	9	26	0	1	0	14
severe	0	0	0	2	0	0	0	0
mucosa:metaplastic epithelium-keratinized								
total	2	69	98	98	1	54	77	96
minimal	2	33	19	8	1	34	20	7
slight	0	25	48	31	0	18	42	44
moderate	0	9	31	54	0	2	15	44
marked	0	2	0	5	0	0	0	1
mucosa:metaplastic epithelium-atypia								
total	0	8	37	46	0	5	30	43
minimal	0	6	22	22	0	3	16	23
slight	0	2	12	16	0	2	14	15
moderate	0	0	3	7	0	0	0	5
marked	0	0	0	1	0	0	0	0
mucosa:metaplastic epithelium-cyst								
total	0	4	12	11	0	0	10	3
minimal	0	1	1	1	0	0	0	1
slight	0	3	6	7	0	0	5	1
moderate	0	0	5	3	0	0	5	1
mucosa:epithelium-keratinocyst(s)								
total	0	0	0	5	0	0	0	2
minimal	0	0	0	1	0	0	0	1
slight	0	0	0	3	0	0	0	0
moderate	0	0	0	1	0	0	0	1

^aanimal 2077, found dead on Day 660; animal 3018, terminal sacrifice on Day 730; animal 4051, found dead on Day 160; animal 2534, terminal sacrifice on Day 733; animal 2566, found dead on Day 539; animal 3532, found dead on Day 682; animal 3514, found dead on Day 536; animal 3515, terminal sacrifice on Day 733; animal 4542, found dead on Day 611.
^banimal 3035, found dead on Day 390.

III. RESULTS AND DISCUSSION (cont.):

I. Pathology (cont.):

LARYNX (cont.):

Microscopic Findings (cont.):

Selected Larynx Findings - All Animals (cont.)

SEX	MALE				FEMALE				
	DOSE (mg/m ³)	0	1	3	10	0	1	3	10
NUMBER EXAMINED	99	99	100	100	99	100	99	100	
ventral diverticulum:pseudostratified cuboidal/columnar epithelium-squamous/squamoid metaplasia/hyperplasia	total	0	6	44	86	1	7	24	74
	minimal	0	3	14	4	0	3	10	6
	slight	0	1	21	40	1	4	11	24
	moderate	0	2	9	35	0	0	5	42
	marked	0	0	0	7	0	0	0	2
ventral diverticulum:metaplastic epithelium-keratinized	total	0	4	31	85	0	2	15	69
	minimal	0	0	10	5	0	1	6	11
	slight	0	1	10	39	0	1	6	30
	moderate	0	3	9	33	0	0	3	25
	marked	0	0	2	5	0	0	0	3
ventral diverticulum:metaplastic epithelium-atypia	total	0	0	1	4	0	0	0	2
	minimal	0	0	0	1	0	0	0	0
	slight	0	0	0	2	0	0	0	0
	moderate	0	0	0	1	0	0	0	0
mucosa:stratified squamous epithelium-(normal)-hyperplasia	total	4	21	58	80	4	23	40	64
	minimal	0	6	13	3	1	12	15	7
	slight	3	15	33	40	3	12	27	39
	moderate	1	0	12	36	0	0	1	18
	marked	0	0	0	1	0	0	0	0
mucosa:stratified squamous epithelium-(normal)-hyperkeratosis	total	1	17	57	80	2	16	39	63
	minimal	0	1	29	28	2	14	32	32
	slight	1	5	26	45	0	2	7	29
	moderate	0	0	2	7	0	0	0	2
mucosa:seromucous gland(s)-atrophy	total	7	3	13	22	11	10	15	19
	minimal	6	2	3	1	6	6	3	2
	slight	1	1	4	1	3	2	11	6
	moderate	0	0	5	16	2	0	0	6
	marked	0	0	1	4	0	0	1	9
mucosa:glands-squamous/squamoid metaplasia	total	0	2	12	10	0	1	6	4
	minimal	0	2	7	5	0	1	2	0
	slight	0	0	5	2	0	0	4	3
	moderate	0	0	0	2	0	0	0	1
mucosa:mixed inflammatory cell infiltrate	total	99	99	100	100	99	100	99	100
	minimal	9	10	2	2	11	12	7	4
	slight	80	66	59	66	80	68	74	68
	moderate	10	23	38	32	8	20	18	28
	marked	0	0	1	0	0	0	0	0
lumen:inflammatory cells/cell debris	total	23	22	25	49	17	25	23	32
	minimal	14	11	15	15	11	14	15	17
	slight	8	10	9	23	6	9	5	13
	moderate	1	1	1	11	0	3	3	1
	marked	0	0	0	0	0	0	0	1
lumen:keratin	total	0	2	12	19	0	4	6	32
	minimal	0	0	8	3	0	3	3	7
	slight	0	1	2	8	0	1	3	18
	moderate	0	0	2	8	0	0	0	7
	marked	0	1	0	0	0	0	0	0

III. RESULTS AND DISCUSSION (cont.):

I. Pathology (cont.):

NASOTURBINAL TISSUES:

Macroscopic Findings:

No macroscopic findings were noted for nasoturbinal tissues.

Microscopic Findings (summary of findings on page 47):

Neoplastic Findings: Neoplasms of the nasal mucosa were seen in three rats from the mid-exposure group; two males had an adenoma and one female had a squamous cell carcinoma. The squamous cell carcinoma was seen in an unscheduled death at week-77. The first adenoma was seen in an unscheduled death at week-83; the other was seen at terminal sacrifice. Spontaneous neoplasms of the nasal mucosa are considered to be rare. In recent NTP studies using the F344 rat, only six were identified in nearly 4000 control males and none occurred in a similar number of control females².

Five females from the exposure groups had a squamous cell carcinoma of the nasolacrimal duct(s); one each in the low and high-exposure groups and three in the mid-exposure group. The first squamous cell carcinoma of the nasolacrimal ducts was seen in a mid-exposure unscheduled death at Week 80. The remaining four carcinomas were seen between Weeks 83 and 105 (terminal sacrifice). Neoplasms of the nasolacrimal ducts in the rat have not been a common occurrence in comparable long term studies conducted in this facility. In this study, the occurrence of squamous cell carcinoma in the nasolacrimal duct may be related to the test material. It is quite likely that lacrimal secretions carried the test material into the nasolacrimal ducts. Squamous cell hyperplasia was seen in

²Boorman, Gary A. and Morgan, Kevin T. "Nose, Larynx and Trachea", Pathology of the Fischer Rat, Academic Press, Inc., New York, New York, 1990, 315-337.

III. RESULTS AND DISCUSSION (cont.):

I. Pathology (cont.):

NASOTURBINAL TISSUES (cont.):

Microscopic Findings (cont.):

several of the rats from the exposure groups. Therefore, it is not unlikely that some animals may have developed squamous cell carcinoma.

Non-neoplastic Findings: Hypertrophy/hyperplasia of goblet cells in the respiratory mucosa and intracytoplasmic eosinophilic material in epithelial cells of respiratory and/or olfactory mucosa (both minimal to moderately severe) were seen in numerous rats from all groups. The incidence of goblet cell hypertrophy/hyperplasia was comparable in all groups. In males, severity exhibited a slight dose-related increase while in females the greatest severity was in the mid-exposure group followed by the high-exposure group and then the low-exposure group. Intracytoplasmic eosinophilic material exhibited dose-related increases in incidence and severity (both sexes). Intracytoplasmic eosinophilic material (minimal to moderate) was also seen in the glandular epithelium at the junction of the respiratory and olfactory mucosa in a small number of rats. The highest incidence was in the high-exposure group (males and females). The incidences in the control, low- and mid-exposure groups were comparable. Severity had no dose-related pattern.

At 3-Months, severity of goblet cell hypertrophy/hyperplasia in rats from the exposure groups was greater than in the comparable controls. Also, at 3-Months, intracytoplasmic eosinophilic material was seen in the respiratory and/or olfactory mucosa of rats from the exposure groups.

The increased severity of hypertrophy/hyperplasia of goblet cells in the respiratory mucosa of the nasal septum was most pronounced in the anterior

III. RESULTS AND DISCUSSION (cont.):

I. Pathology (cont.):

NASOTURBINAL TISSUES (cont.):

Microscopic Findings (cont.):

region of the nasoturbinal tissues (Level I). This was considered to be a localized adaptive response to the irritant effect associated with liquid aerosols rather than an adverse toxicological response to the test material in the nasal passages. Similar responses have been seen in rats exposed to mild irritants from other studies conducted in this facility.

Dose-related increases in incidence and severity of intracytoplasmic eosinophilic material in the epithelial cells of the respiratory and/or olfactory mucosa, and in glandular epithelium, as seen in this study, have been observed in rats from other subchronic and chronic inhalation studies of irritants conducted in this facility. The exact nature of this material, presumed to be secretory, and reasons for its increase following exposure to a variety of test materials are uncertain but presumably represent a nonspecific response to the inhalation of the test material³.

The incidence and severity of goblet cell hypertrophy/hyperplasia in the respiratory mucosa and of intracytoplasmic eosinophilic material in epithelial cells of the respiratory and/or olfactory mucosa, at the end of the 3-Month exposure and 3-Month post-exposure recovery periods, were compared. At the end of the recovery period, neither finding had decreased in incidence or severity; reversibility had not occurred.

³Monticello, Thomas M.; Morgan, Kevin T. and Uriah, Linda; Non-Neoplastic Nasal Lesions in Rats and Mice, Environmental Health Perspectives, Vol 85, pp 249-274, 1990.

III. RESULTS AND DISCUSSION (cont.):

I. Pathology (cont.):

NASOTURBINAL TISSUES (cont.):

Microscopic Findings (cont.):

Selected Nasal Turbinate Findings - All Animals

SEX	MALE				FEMALE				
	DOSE (mg/m ³)	0	1	3	10	0	1	3	10
NUMBER EXAMINED	100	100	100	100	100	99	100	99	
nasal mucosa:adenoma, benign ^a	0	0	2	0	0	0	0	0	
nasal mucosa:squamous cell carcinoma, malignant ^b	0	0	0	0	0	0	1	0	
nasolacrimal duct:squamous cell carcinoma, malignant ^c	0	0	0	0	0	1	3	1	
nasal mucosa(respiratory):goblet cell hypertrophy/hyperplasia	total	95	96	91	93	83	84	83	82
	minimal	12	13	7	10	35	26	9	14
	slight	39	32	28	23	33	31	36	39
	moderate	44	45	56	52	15	26	34	28
	marked	0	6	0	8	0	1	4	1
nasal mucosa(respiratory/olfactory):epithelium-intracytoplasmic eosinophilic material	total	82	92	97	99	81	91	95	97
	minimal	25	6	5	0	26	12	5	0
	slight	31	27	11	9	37	26	8	4
	moderate	25	58	58	31	18	52	60	56
	marked	1	1	23	59	0	1	22	37
nasal mucosa:(junction between respiratory/olfactory):glandular epithelium-intracytoplasmic eosinophilic material	total	7	9	8	15	3	3	8	12
	minimal	4	8	5	8	2	2	7	10
	slight	3	1	3	7	0	1	1	2
	moderate	0	0	0	0	1	0	0	0
nasal lacrimal duct: squamous epithelium-hyperplasia	total	16	7	8	16	6	11	8	8
	minimal	3	1	3	6	1	1	2	2
	slight	8	5	5	10	3	6	1	5
	moderate	5	1	0	0	1	4	5	1
	marked	0	0	0	0	1	0	0	0

^aanimal 3047, terminal sacrifice on Day 733; animal 3030, found dead on Day 578.
^banimal 3549, found dead on Day 538.
^canimal 2511, found dead on Day 680; animal 3526, terminal sacrifice on Day 734;
 animal 3529, found dead on Day 560; animal 3568, terminal sacrifice on Day 734;
 animal 4514, found dead on Day 582.

LUNG:

Macroscopic Findings (summary of findings on page 48):

Tan discoloration of the lung was the only macroscopic finding considered related to the whole body exposure to 70055. The highest incidence was in the high-exposure group followed by the mid-exposure group; incidence in the control and low-exposure groups were comparable. The tan discolorations correlated with the presence of alveolar/intraalveolar macrophages (either with or without interstitial inflammation) seen microscopically.

III. RESULTS AND DISCUSSION (cont.):

I. Pathology (cont.):

LUNG (cont.):

Macroscopic Findings (cont.):

Macroscopic Lung Discoloration: Tan

SEX	MALE				FEMALE				
	DOSE (mg/m ³)	0	1	3	10	0	1	3	10
3 Months	0/11	0/11	0/11	0/11	0/12	0/10	0/10	0/10	0/10
12 Months	0/12	1/18	0/14	0/21	0/11	0/14	0/11	1/11	1/11
18 Months	2/17	0/25	1/16	4/30	2/20	1/23	2/20	4/20	4/20
24 Months	4/60	3/47	8/59	13/39	7/57	7/52	17/59	18/59	18/59
Total	6/100	4/100	9/100	17/100	9/100	8/99	19/100	23/100	23/100
Recovery Group	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10

Microscopic Findings (summary of data on page 50):

Neoplastic Findings: There were no neoplastic findings in the lung which were considered to be related to the whole body exposure to 70055. Three broncho-alveolar neoplasms were seen in this study. The incidence did not exhibit a dose-related pattern.

Non-Neoplastic Findings: Alveolar-intraalveolar macrophages, a normal component of the lungs, were seen in all rats with a dose-related increase in their number based on an assessment of severity (minimal to essentially moderately severe). Their function is to engulf exogenous and endogenous debris. In this study, increases in the number of macrophages were considered to be a localized adaptive response to the presence of a liquid aerosol rather than an adverse toxicological response to the test material. Similar responses have been seen in rats exposed to a variety of foreign materials from other studies conducted in this facility.

III. RESULTS AND DISCUSSION (cont.):

I. Pathology (cont.):

LUNG (cont.):

Microscopic Findings (cont.):

Subacute-chronic interstitial inflammation was seen in numerous males and females from all groups and the incidence was slightly greater in the exposure groups than in the comparable controls. Severity (minimal to moderately severe) was slightly greater in the high-exposure group males and females than in comparable controls; severity in the control, low- and mid-exposure groups were similar. In this study, the slight increase in the severity of the inflammatory response in the high-exposure group was considered to be a low grade adverse response.

Edema (minimal to moderately severe), seen in a number of rats from all groups, had the greatest incidence and severity in the high-exposure group males. Incidence and severity in males from the control and low and mid-exposure groups and in females from the control and all exposure groups were similar. The incidence of purulent inflammation/abscesses (minimal to moderately severe) was greatest in the high-exposure group males; severity in all groups was similar. Incidence and severity of microgranulomas (minimal to moderate) was greatest in the high-exposure group females. The toxicological significance, if any, of these findings is not known.

At 3-Months, severity of alveolar/intraalveolar macrophages in rats from the exposure groups was greater than in the comparable controls. At the end of 12-Months, incidence and severity of subacute/chronic interstitial inflammation in males from the exposure groups was greater than in the comparable controls. Also, at 12-Months, edema, purulent inflammation/abscesses and/or

III. RESULTS AND DISCUSSION (cont.):

I. Pathology (cont.):

LUNG (cont.):

Microscopic Findings (cont.):

microgranulomas were first seen in one or more rats from the exposure groups.

Incidence and severity of alveolar-intraalveolar macrophages and subacute-chronic interstitial inflammation in the lungs at the end of the 3-Month exposure and 3-Month post-exposure recovery periods were compared. In the recovery rats, a slight decrease in the number of macrophages (based on severity) indicated that some reversibility had occurred. The incidence and severity of subacute-chronic interstitial inflammation at the end the 3-Month exposure and recovery periods were similar.

Selected Lung Findings - All Animals

SEX		MALE				FEMALE			
		0	1	3	10	0	1	3	10
DOSE (mg/m ³)		0	1	3	10	0	1	3	10
NUMBER EXAMINED		100	100	100	100	100	100	100	100
alveolar/intraalveolar macrophages	total	100	100	100	100	100	100	100	100
	minimal	28	7	6	2	45	11	7	5
	slight	62	78	67	35	45	69	63	53
	moderate	9	12	23	46	6	17	24	39
	marked severe	1 0	3 0	4 0	17 0	4 0	2 1	6 0	3 0
interstitium:subacute/chronic inflammation	total	35	50	52	56	33	55	54	62
	minimal	22	34	25	17	18	29	33	26
	slight	7	7	18	26	8	17	10	21
	moderate	5	7	7	13	5	8	8	15
	marked	1	1	2	0	2	1	3	0
edema	total	13	13	17	31	8	10	8	11
	minimal	0	1	0	0	0	1	0	2
	slight	10	9	12	10	1	4	4	7
	moderate	3	3	4	18	6	3	2	2
	marked	0	0	1	3	1	2	2	0
purulent/chronic purulent inflammation/abscess(es)/chronic abscess(es)	total	4	1	2	28	7	6	5	7
	slight	0	0	0	4	2	4	3	5
	moderate	2	0	2	12	4	2	1	2
	marked	2	1	0	12	1	2	1	0
	total	5	3	0	7	3	3	5	16
microgranulomas	minimal	4	2	0	1	2	1	2	8
	slight	0	1	0	4	1	2	4	
	moderate	1	0	0	2	0	1	4	
	total	5	3	0	7	3	3	5	
broncho-alveolar adenomas		0	1	1	0	0	0	0	0
broncho-alveolar carcinomas		0	0	0	0	0	1	0	0

III. RESULTS AND DISCUSSION (cont.):

I. Pathology (cont.):

OTHER FINDINGS:

Macroscopic Findings:

Other macroscopic findings in the lungs and in other tissues and organs occurred with comparable incidence and severity in rats from the exposure and control groups or they occurred sporadically; they were not considered to be related to the whole-body exposure to 70055. These incidental findings have been seen in control rats of similar strain and age used for other studies in this facility.

Microscopic Findings:

Neoplastic Findings: Except for the neoplasms in the larynx and nasoturbinal tissues, whole body exposure to 70055 for up to 105 weeks did not have an oncogenic effect in any other tissues and organs. Overall, the total number of primary neoplasms, malignant and benign, and the number of tumor bearing males and females in the exposure and control groups were comparable. Various neoplasms were seen at the end of 24-, 18- and 12-Months. At the end of 3-Months one control female had a mammary carcinoma. No neoplasms were seen in the recovery rats.

Non-Neoplastic Findings: Other microscopic findings in the larynx, nasoturbinal tissue and lungs and in other tissues and organs occurred with comparable incidence and severity in rats from the exposure and control groups or they occurred sporadically; they were not considered to be related to the whole-body exposure to 70055. These incidental findings have been seen in control rats of similar strain and age used for other studies in this facility.

III. RESULTS AND DISCUSSION (cont.):

I. Pathology (cont.):

STATISTICAL ANALYSIS OF TUMOR DATA:

There was no statistical difference for homogeneity of life table data for trend or in pairwise comparisons of control versus treated groups for the following parameters. Thus there was no statistically significant increase in the incidence of any of these tumor categories among treated animals.

Males	Females
All malignant tumors combined	All malignant tumors combined
All malignant nasal turbinate tumors	All malignant nasal turbinate tumors
All malignant tumors of the teeth	All malignant lung tumors
All malignant larynx tumors	All benign tumors combined
All benign tumors combined	All benign larynx tumors
All benign larynx tumors	
All benign nasal turbinate tumors	
All benign lung tumors	

For all benign tumors in the male, there was a statistically significant difference ($p < 0.05$) in homogeneity of life table data using the Kruskal-Wallis test but not when using the Cox test. The Kruskal-Wallis test also indicated a statistically significant ($p < 0.05$) adjusted trend. The difference between the two tests, Cox and Kruskal-Wallis is attributed to the fact that the Kruskal-Wallis test places more emphasis on the earlier part of the life table curve where the difference between control and high dose curves was

III. RESULTS AND DISCUSSION (cont.):

I. Pathology (cont.):

STATISTICAL ANALYSIS OF TUMOR DATA:

greater than toward the end of the study.

However, while the trend test indicated statistical significance, the pairwise Chi square comparisons of the control animals with each of the treated groups were all negative. The Kruskal-Wallis tests for homogeneity of life table curves demonstrated a statistically significant difference for the high dose group when compared to the control group ($p < 0.05$). The Cox tests for homogeneity did not demonstrate statistically significant differences in pairwise comparisons. Because the pairwise incidence was negative and the Cox tests did not indicate a statistically significant difference in life table homogeneity in any of the pairwise comparisons, it is concluded that there was no compound related increase in all benign tumors for males.

In summary, the target tissues were the larynx, nasolacrimal ducts, nasoturbinial tissues and the lung. The test material was oncogenic in the larynx and nasoturbinates. The oncogenic effect was seen at the low-exposure level in the larynx and nasolacrimal ducts. Based on microscopic findings in the larynx and nasolacrimal duct, there was no NOEL (No Observable Effect Level).

IV. CONCLUSION:

In conclusion, whole-body exposure of Sprague-Dawley rats to 70055 for two years resulted in increased mortality in the high-exposure group males. Target organ toxicity was observed in the larynx, nasolacrimal ducts, nasal turbinal tissues and lungs. Neoplastic findings in the larynx and nasolacrimal ducts at the lowest exposure concentration suggest that 70055 is oncogenic at the lowest exposure concentration evaluated (1 mg/m^3). A no observed effect level (NOEL) was not observed in this study.

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