In vivo and *in vitro* Studies of Mainstream Smoke from a New Cigarette Prototype

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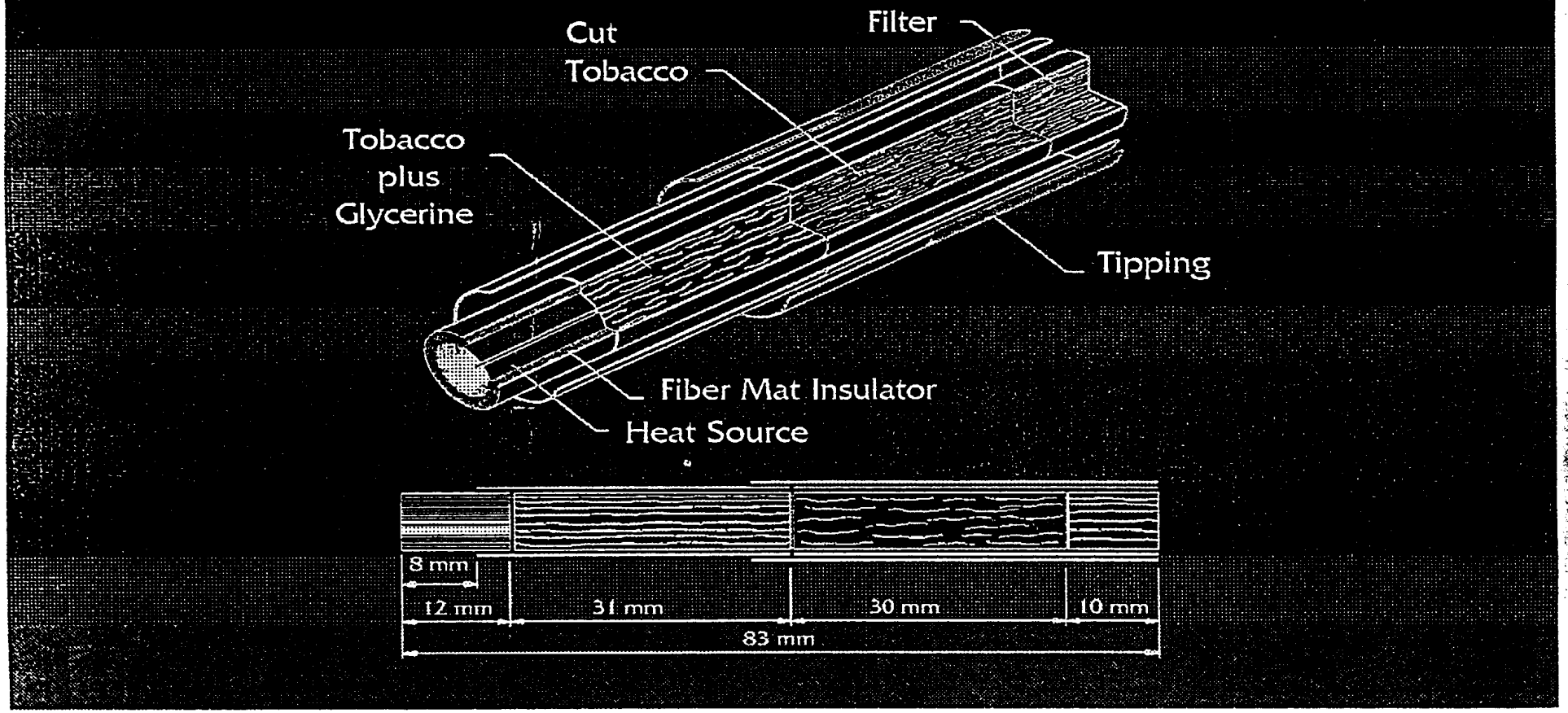
Background

- A new cigarette has been developed that primarily heats tobacco.
- Only a small amount of tobacco in the heat source is burned (less than 25mg).
- Biological activity of the new cigarette was compared to the University of Kentucky 1R4F reference cigarette.

Cigarette That Primarily Heats Tobacco



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Experimental Systems

In vitro

- Analyses - tobacco smoke condensate
  - » Ames bacterial mutagenicity
  - » Sister chromatid exchange assay (Chromosome re-arrangement)
  - » Cytotoxicity

In vivo

- Sprague-Dawley Rat - Subchronic nose-only inhalation study conducted at R. J. Reynolds Tobacco Company

- Syrian Golden Hamster - Subchronic nose-only inhalation study conducted at Battelle Northwest Laboratories

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Experimental Design

Comparative Approach:

- Biological changes observed in smoke exposed laboratory animals were compared to sham exposed animals to determine smoke related effects.

- Smoke related effects were then compared between the TEST and REFERENCE cigarette groups.

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Experimental Design

- A new prototype cigarette that primarily heats tobacco - TEST cigarette
- Kentucky 1R4F cigarette - REFERENCE cigarette
- Equivalent wet total particulate matter (WTPM) concentration exposures were conducted
- Biological changes with the TEST cigarette were compared to the REFERENCE cigarette

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Cigarette Yields - FTC

TEST

- 6.7 mg WTPM
- 4.5 mg Tar
- 0.2 mg Nicotine
- 11.9 mg CO

1R4F - REFERENCE

- 10.8 mg WTPM
- 9.2 mg Tar
- 0.8 mg Nicotine
- 9.8 mg CO

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Results : *In vitro* studies

Ames Bacterial Mutagenicity - TA98, TA100, TA1537, TA1538
+/- S9 activation; 0-250 ug WTPM/plate

TEST cigarette - negative

1R4F cigarette - positive + S9 as low as 50 ug WTPM/plate

Sister Chromatid Exchange (CHO cells) +/- S9; 0-250 ug WTPM/mL

TEST cigarette - negative

1R4F - positive +/- S9 as low as 25 ug WTPM/mL

Cytotoxicity Neutral Red Assay (CHO cells); 0-150 ug WTPM/mL

TEST cigarette - negative

1R4F cigarette - positive as low as 25 ug WTPM/mL

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Subchronic Inhalation Studies

- Sprague-Dawley rats
  - » 32 juvenile male and female rats per group
  - » 22 rats of both sexes killed at 90 days
  - » 10 rats of both sexes remained on study for a 13 week reversibility period without exposure

- Syrian Golden hamsters
  - » 32 juvenile male and female hamsters per group
  - » 22 hamsters of both sexes killed at 90 days
  - » 10 hamsters of both sexes remained on study for a 13 week reversibility period without exposure

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Exposure Conditions

- Cigarettes smoked under FTC conditions
- Smoke from cigarettes was diluted and controlled to target concentrations of WTPM
- Concentrations of WTPM used were:
  - » 0.00 mg WTPM/L - SHAM exposure
  - » 0.16 mg WTPM/L - LOW exposure
  - » 0.32 mg WTPM/L - MID exposure
  - » 0.64 mg WTPM/L - HIGH exposure
- Rats and hamsters were exposed nose-only 1 hour per day, 5 days per week, during a 13 week period.

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Exposure Characterization - Summary Data
Rat Subchronic Inhalation Study

	TEST			REFERENCE		
	LOW	**MID**	**HIGH**	**LOW**	**MID**	**HIGH**
WTPM mg/L cv < 7 %	0.16	0.33	0.64	0.16	0.32	0.64
CO ppm cv < 15 %	358	707	1317	186	377	762
Nicotine ug/L cv < 25 %	4.9	11.1	24.1	7.9	18.6	41.9
Particle size µm MMAD (gsd)	0.74 (1.12)	0.75 (1.12)	0.76 (1.12)	0.72 (1.14)	0.71 (1.14)	0.72 (1.14)

The same type of equipment was used for the hamster study.
Similar results were obtained.

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Methods - Biological Endpoints

- Measures of Exposure
  - » Inhaled Mass of WTPM
  - » Carboxyhemoglobin
  - » Serum nicotine
- In-life observations
  - » Clinical observations
  - » Body weights
- Pathology
  - » Gross changes, organ weights, organ weight ratios
  - » Histopathology

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Measure of Exposure
Inhaled Mass of WTPM

- Minute volume - the product of respiratory rate and tidal volume
- Normalized inhaled mass
  - » minute volume x exposure concentration x duration of exposure; normalized by body weight

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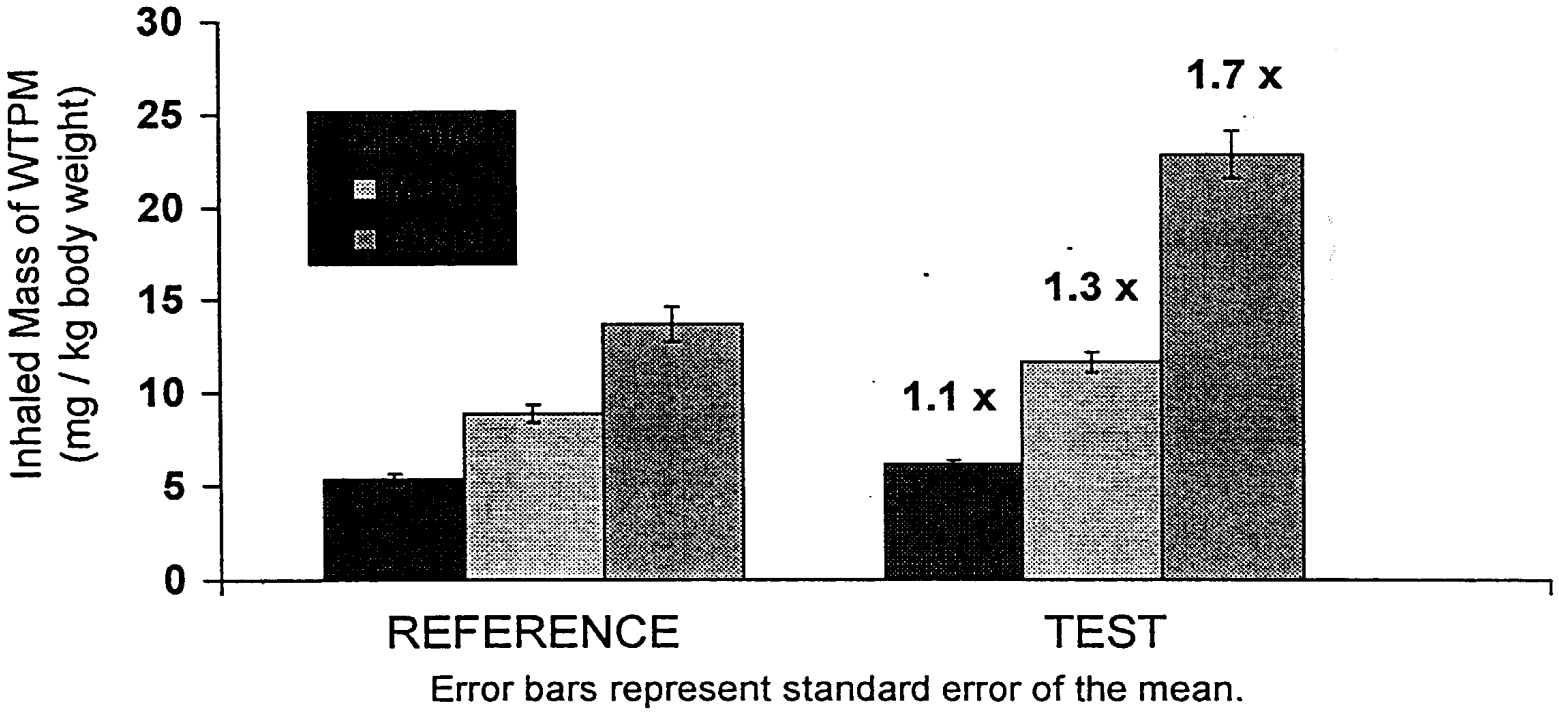
Measure of Exposure
Inhaled Mass of WTPM in Rats



Error bars represent standard error of the mean.

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Measures of Exposure
Rat and Hamster

- Inhaled mass of WTPM was higher in TEST exposed rats compared to REFERENCE
- Inhaled mass of WTPM was similar in TEST and REFERENCE exposed hamsters
- Carboxyhemoglobin - related to exposure in a concentration dependent manner for both TEST and REFERENCE exposed rats and hamsters
- Serum Nicotine - related to exposure in a concentration dependent manner for both TEST and REFERENCE exposed rats and hamsters.

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Clinical Observations
Body Weights

Clinical Observations

- No significant smoke related clinical observations were observed in either rats or hamsters for either the TEST or REFERENCE cigarette.

Body Weight

- Rat - No significant differences in body weights between comparable TEST and REFERENCE smoke exposed groups
- Hamster - In male hamsters, body weights of the mid and high exposure groups of the REFERENCE cigarette were depressed relative to the TEST cigarette.

Results

- [redacted]
  - [redacted]
  - [redacted]
  - [redacted]
- [redacted]
  - [redacted]
  - [redacted]
- Pathology
  - » Gross observations, organ weights, organ weight ratios
  - » Histopathology

Findings at Necropsy- Rats and Hamsters

- There were no significant findings at 90 days in either the TEST or REFERENCE groups of rats or hamsters in terms of :
  - » Gross observations
  - » Organ weights
  - » Organ weight ratios

Comparative Approach
Statistical Analysis of Histological Findings

- Kolmogorov-Smirnov test - incidence and degree of change are compared between groups; $p < 0.05$
- Smoke-related changes compared on exposure basis for affected organs
  - » Low TEST compared to low REFERENCE
  - » Mid TEST compared to mid REFERENCE
  - » High TEST compared to high REFERENCE

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Smoke Related Histological Changes
Rat and Hamster

- Rat
  - » Nasal 1 section- epithelial hyperplasia
  - » Ventral larynx - squamous metaplasia
  - » Lung - brown/gold pigmented macrophages

- Hamster
  - » Nose, turbinates - hyperplasia of respiratory epithelium
  - » Larynx, epiglottis - squamous metaplasia, hyperplasia, hyperkeratosis
  - » Larynx, protuberance - squamous metaplasia, hyperplasia, and hyperkeratosis
  - » Larynx, ventral pouch - hyperplasia
  - » Larynx, dorsal lateral walls - squamous metaplasia
  - » Lung - pigmented macrophages

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Significant Findings -Rat Study
TEST Responses Relative to REFERENCE

- Nasal 1, epithelial hyperplasia (turbinate) - low, mid, and high TEST of both sexes (except low female) reduced
- Ventral larynx, squamous metaplasia - low TEST reduced (males only); mid and high TEST not different
- Lungs, pigmented macrophages - low, mid, and high TEST of both sexes reduced

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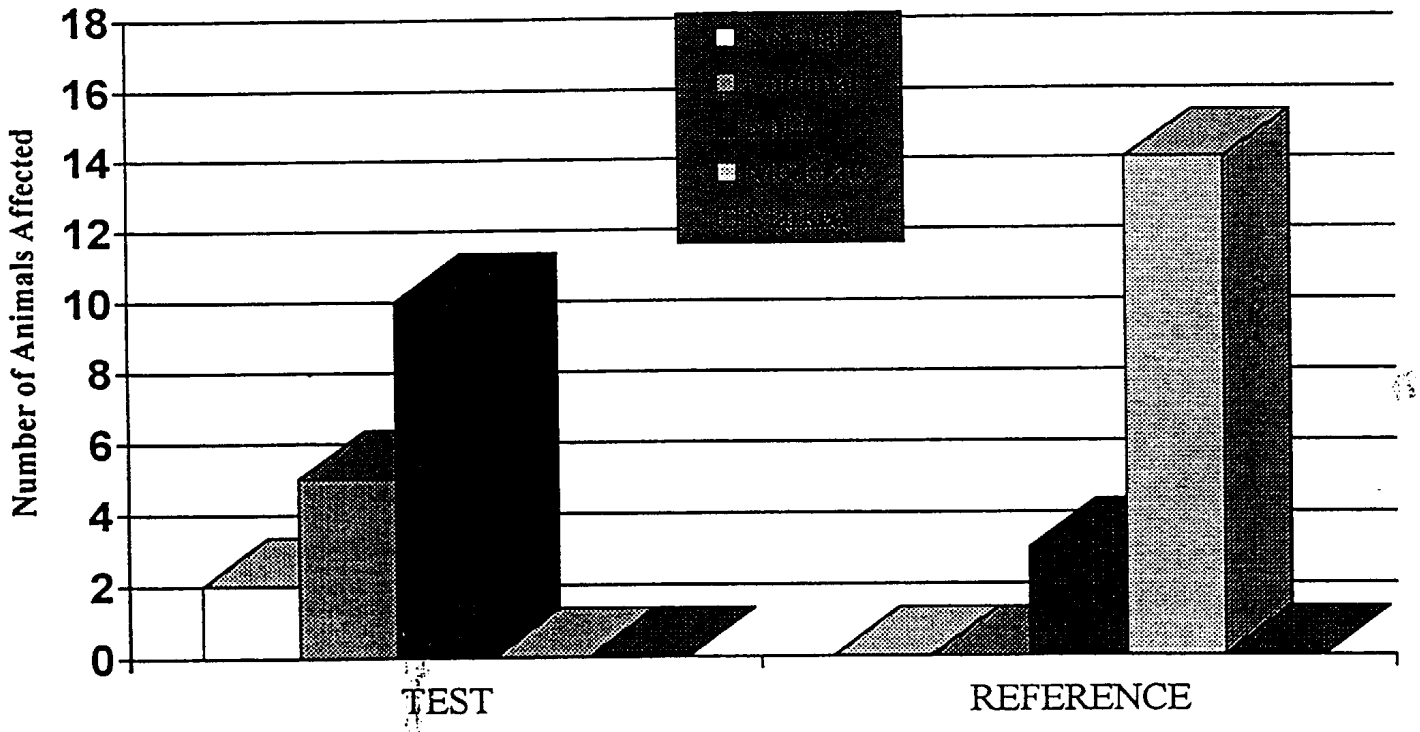
Distribution of Changes- High Exposure
Male Rats - Nasal 1 Epithelial Hyperplasia - 90 Day Findings



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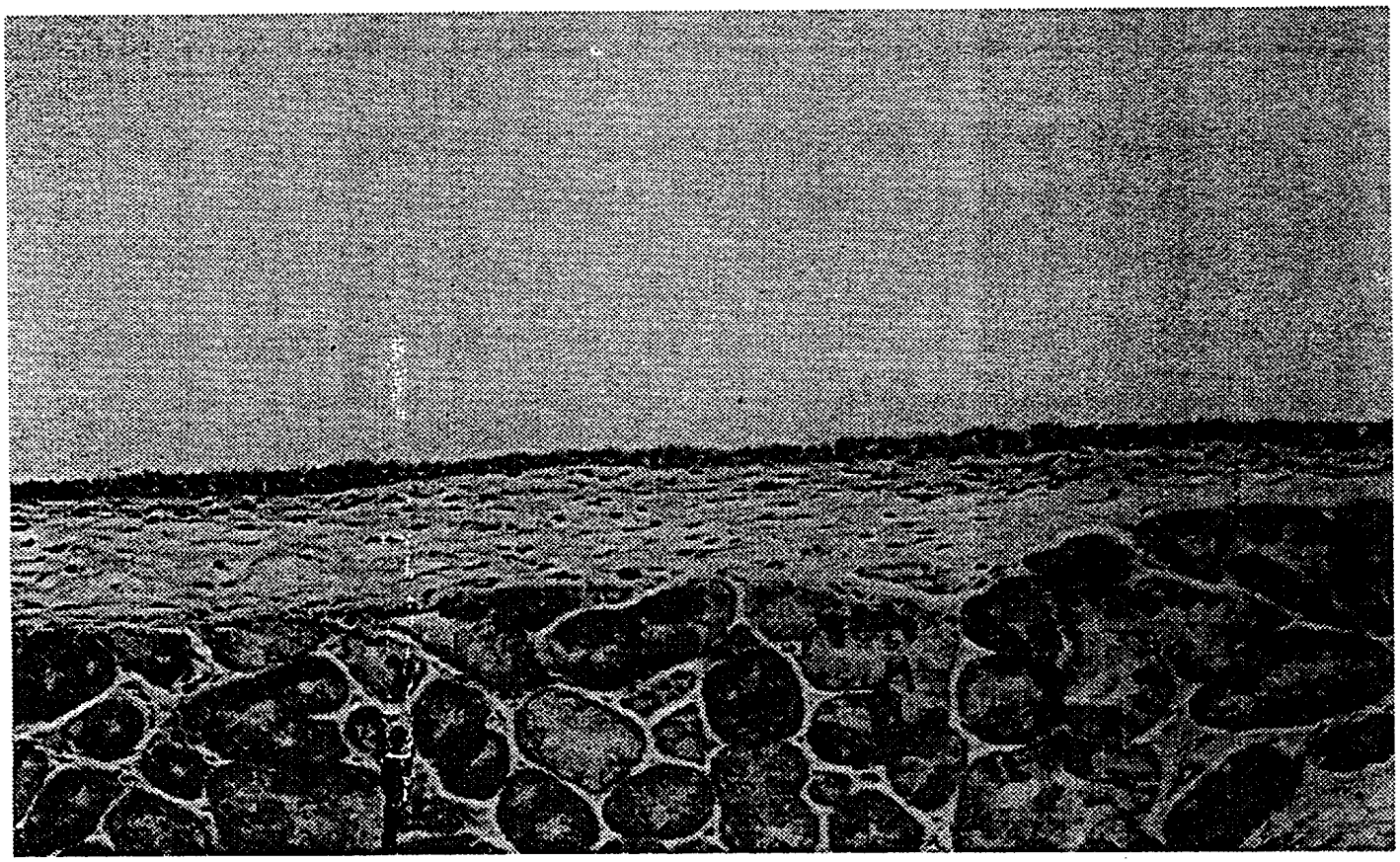
Significant Findings -Rat Study
TEST Responses Relative to REFERENCE

- Nasal 1 epithelial hyperplasia [illegible] TEST of both sexes (except low female) reduced
- Ventral larynx, squamous metaplasia - low TEST reduced (males only); mid and high TEST not different
- Lungs, pigmented macrophages - low, mid, and high TEST of both sexes reduced

Ventral Larynx of Control Rat



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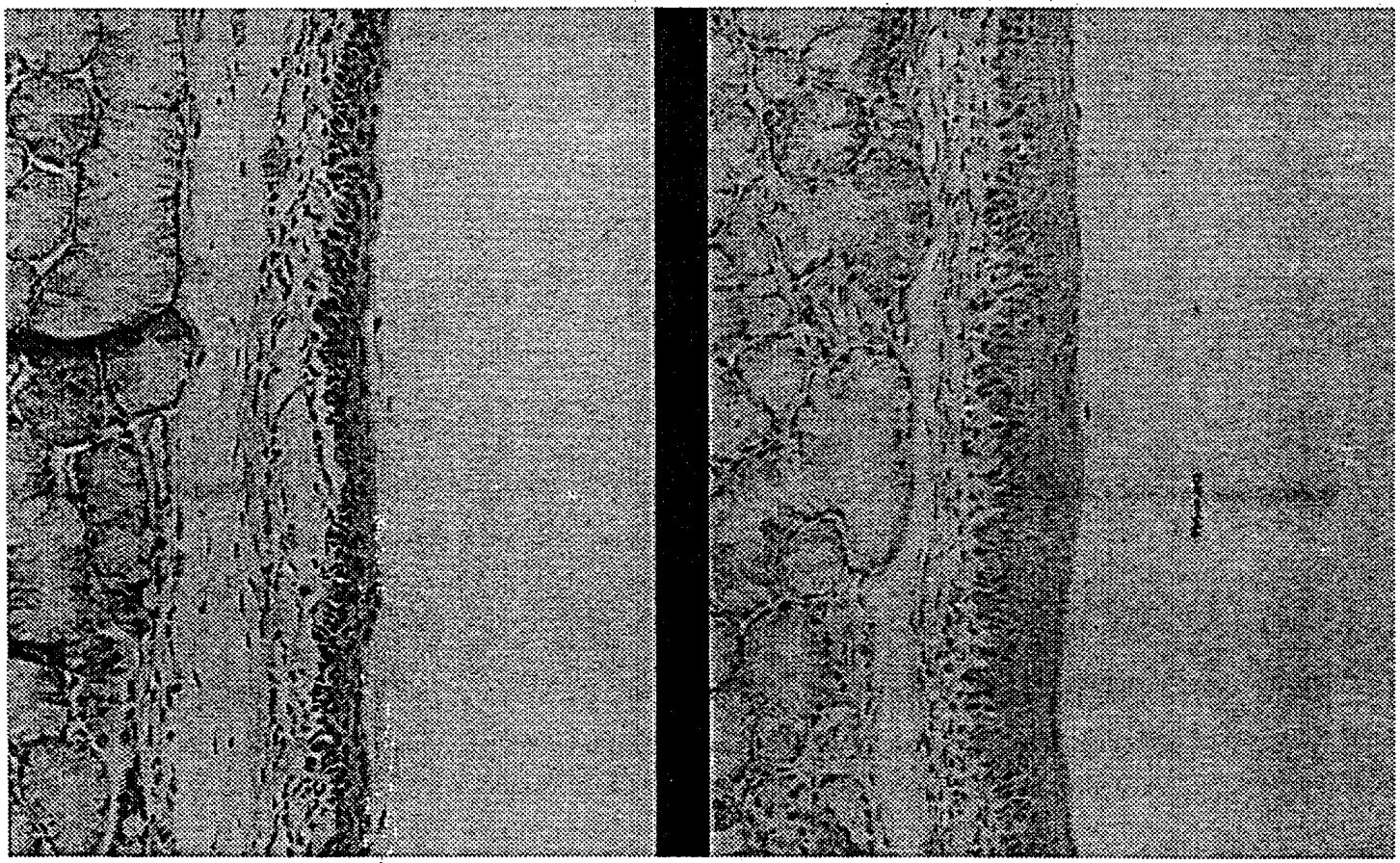
Ventral Larynx of TEST Low Exposure Group

Ventral Larynx of REFERENCE Low Exposure Group



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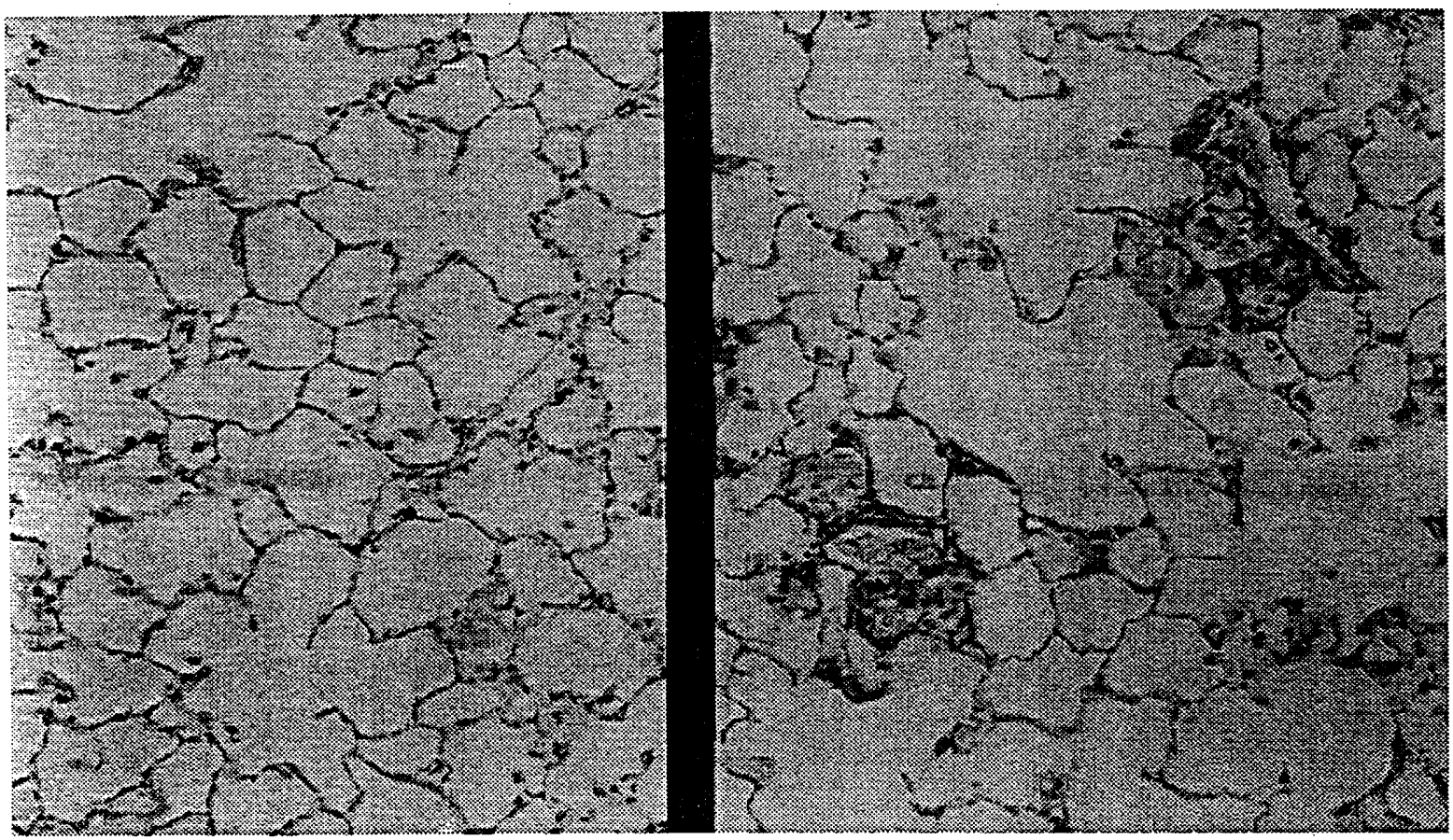
Significant Findings -Rat Study
TEST Responses Relative to REFERENCE

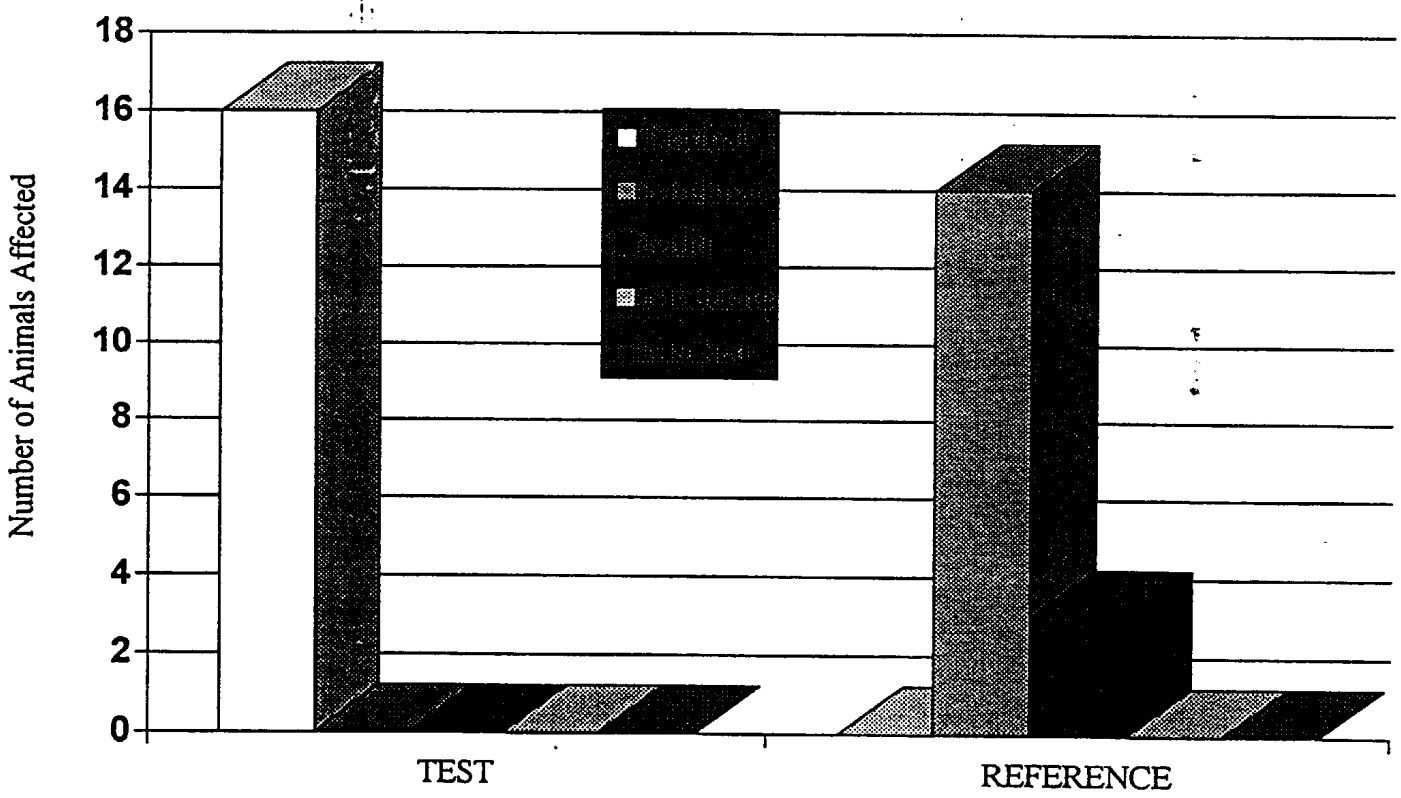
- [illegible]
- [illegible]
- Lungs, pigmented macrophages - low, mid, and high TEST of both sexes reduced

Lung Tissue of TEST
High Exposure

Lung Tissue of REFERENCE
High Exposure



Distribution of Changes- High Exposure Male Rats - Lung Intermediate Lobe; Brown and Gold Macrophages-90 Day Findings



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Significant Findings - Hamster Study
TEST Responses Relative to REFERENCE

- Nose, turbinates, hyperplasia respiratory epithelium - high and mid TEST of both sexes reduced; no response at low
- Larynx, epiglottis, squamous metaplasia and hyperplasia - high TEST of both sexes reduced; mid and low TEST not different
- Larynx, protuberance, squamous metaplasia, hyperplasia, and hyperkeratosis - low, mid, and high TEST of both sexes reduced
- Larynx, ventral pouch, hyperplasia - high TEST of both sexes reduced
- Larynx, dorsal lateral walls, squamous metaplasia - high TEST of both sexes reduced, mid TEST not different, no response at low

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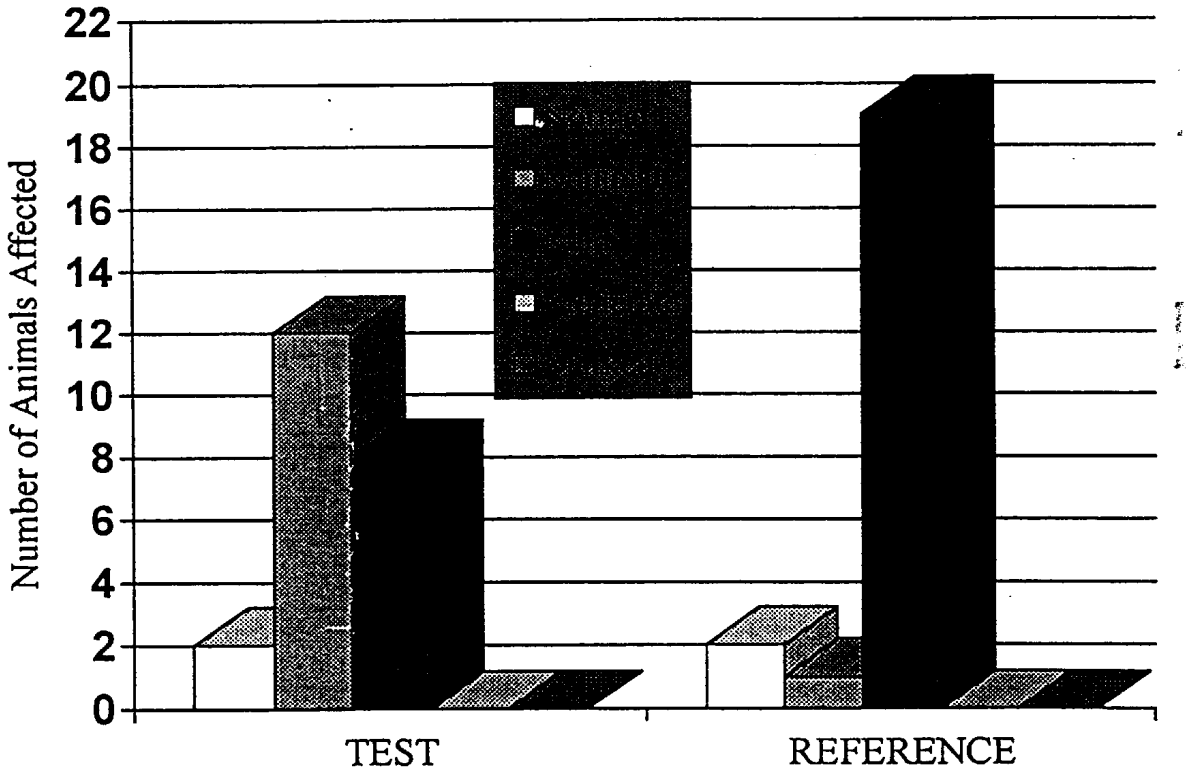
Distribution of Changes- High Exposure Male Hamsters - Nose, Hyperplasia of Respiratory Epithelium- 90 Days



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Significant Findings - Hamster Study
TEST Relative to REFERENCE Responses

- [illegible]
  both sexes reduced; mid and low TEST not different
- Larynx, epiglottis, squamous metaplasia and hyperplasia - high TEST of both sexes reduced; mid and low TEST not different
- [illegible]
  low, mid and high TEST of both sexes reduced
- Larynx, ventral pouch, hyperplasia - high TEST of both sexes reduced
- Larynx, dorsal lateral walls, squamous metaplasia - high TEST of both sexes reduced, mid TEST not different, no response at low

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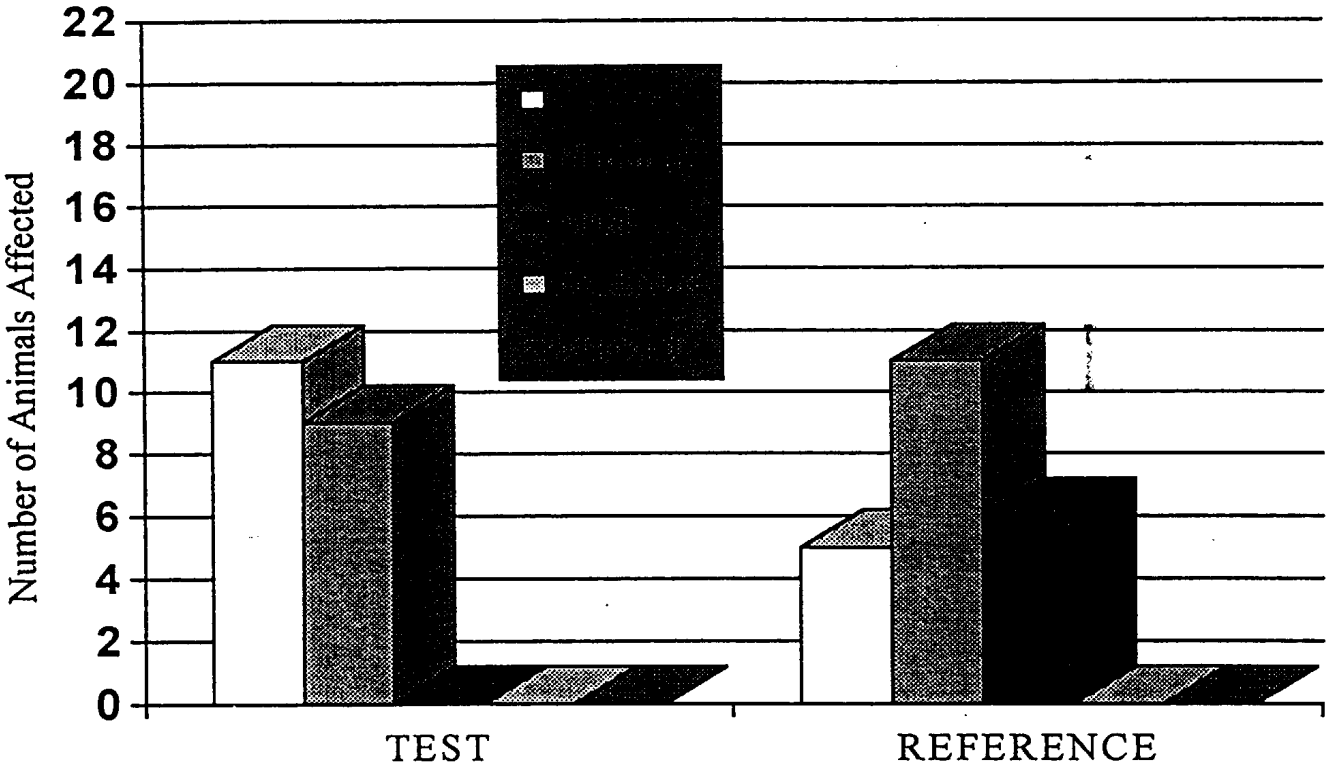
Distribution of Changes- High Exposure
Male Hamsters - Larynx, Epiglottis
Squamous Metaplasia- 90 Days



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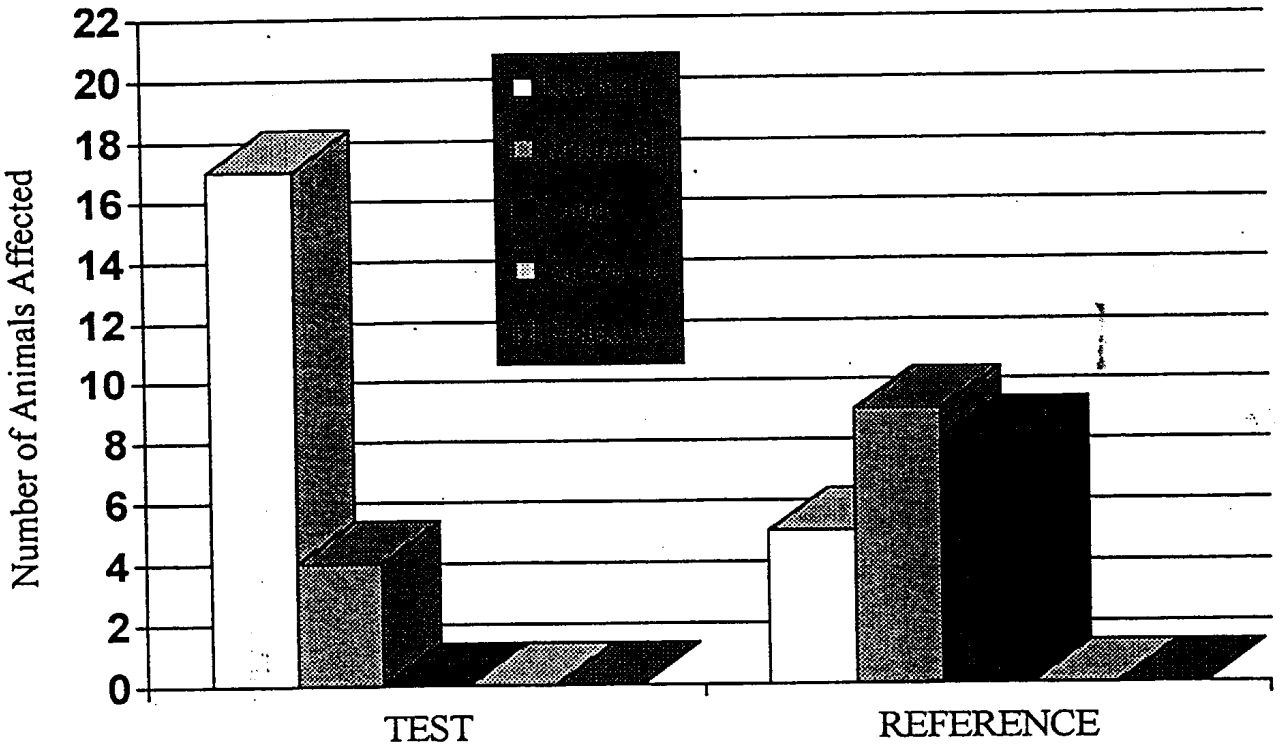
Distribution of Changes - High Exposure
Male Hamsters - Larynx, Epiglottis
Hyperplasia - 90 Days



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Significant Findings - Hamster Study
TEST Responses Relative to REFERENCE

- [illegible]
  both sexes reduced, no response at low

- Larynx, laryngeal squamous metaplasia and hyperplasia [illegible]
  sexes reduced, mid and low TEST not different

- Larynx, protuberance, squamous metaplasia, hyperplasia, and hyperkeratosis - low, mid, and high TEST of both sexes reduced

- Larynx, ventral pouch, hyperplasia - high TEST of both sexes reduced

- Larynx, dorsal lateral walls, squamous metaplasia - high TEST of both sexes reduced, mid TEST not different, no response at low

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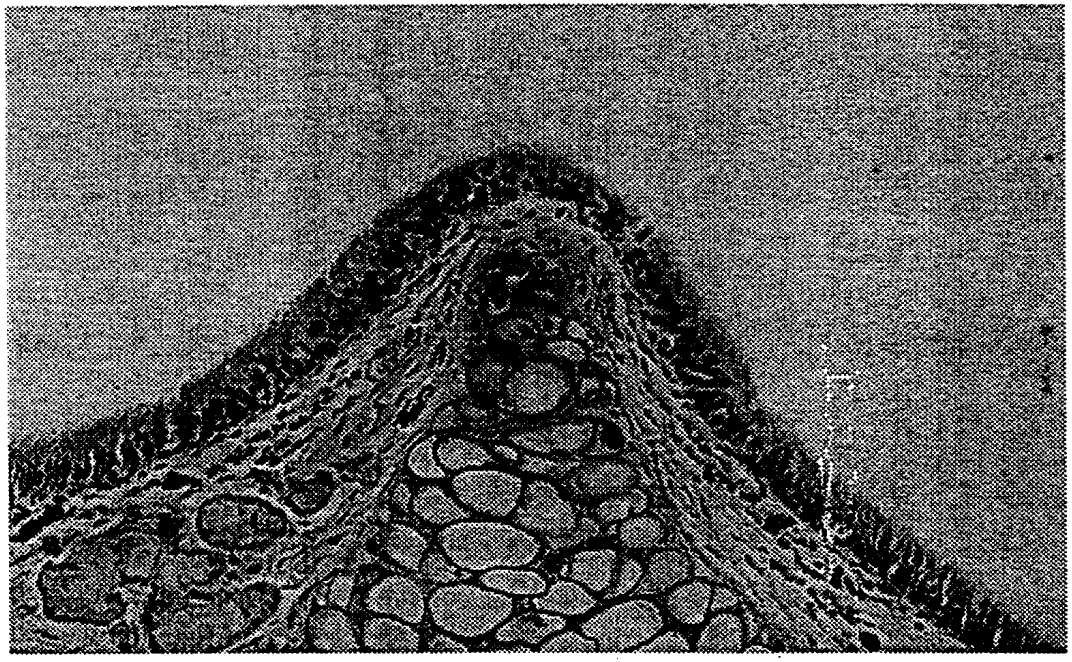
Larynx Protuberance Control Hamster



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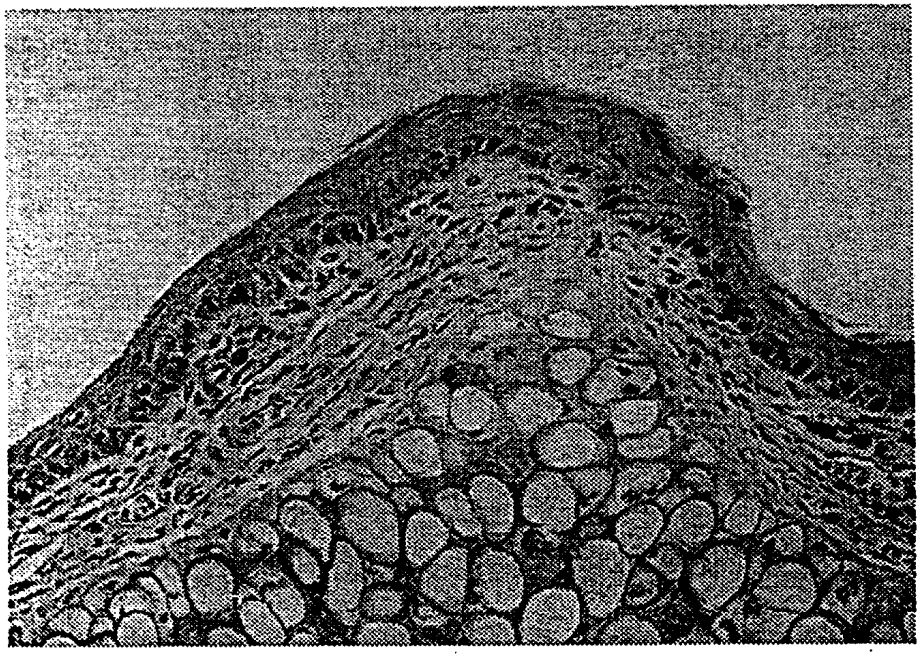
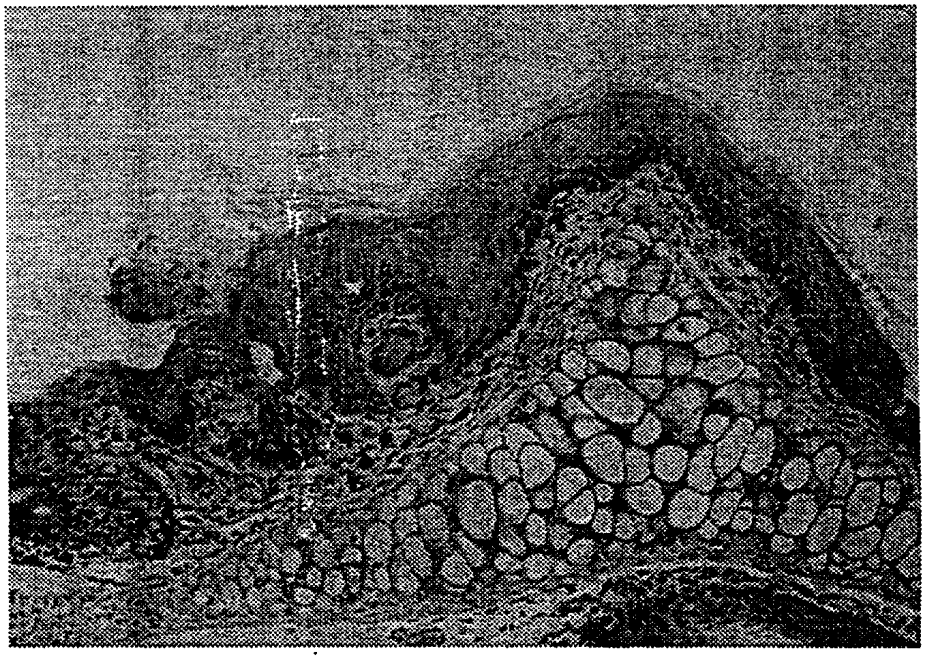
Larynx Protuberance of High TEST Hamster



Larynx Protuberance of High REFERENCE Hamster



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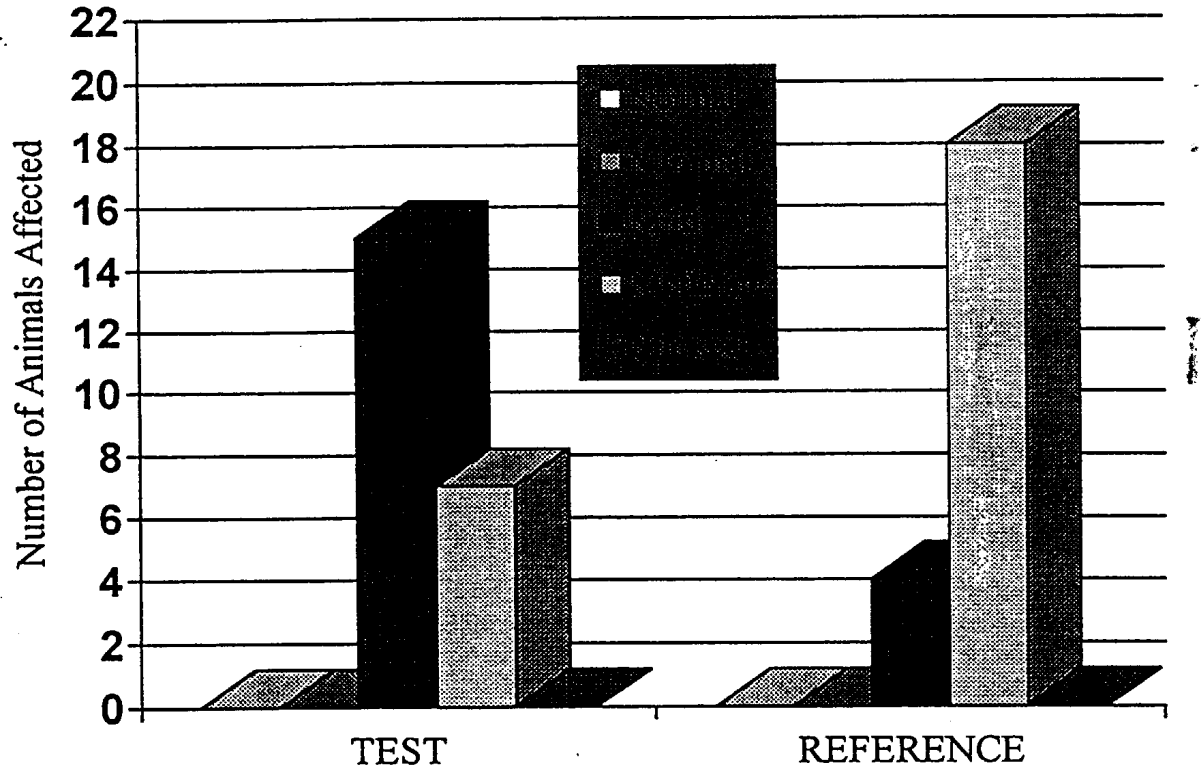
Distribution of Changes- High Exposure Male Hamsters - Larynx, Protuberance Squamous Metaplasia- 90 Days



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Significant Findings - Hamster Study
TEST Responses Relative to REFERENCE

- [illegible]
- [illegible]
- [illegible]

- Larynx, ventral pouch, hyperplasia - high TEST of both sexes reduced
- Larynx, dorsal lateral walls, squamous metaplasia - high TEST of both sexes reduced, mid TEST not different, no response at low

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Summary of Results

- ***In vitro*** studies of tobacco smoke condensate - TEST cigarette was negative; REFERENCE cigarette was positive in all assays.
- Rat - TEST cigarette responses were reduced in nasal tissues, larynx, and lung compared to REFERENCE, even though rats exposed to TEST smoke inhaled more smoke than REFERENCE exposed rats.
- Hamster - TEST cigarette responses were reduced in nasal tissues and four sites of the larynx compared to REFERENCE exposed hamsters.

Conclusions

- Based on *in vitro* assays and subchronic inhalation studies in two species, the TEST cigarette that primarily heats tobacco was shown to have reduced biological activity compared to the REFERENCE cigarette.

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