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National Toxicology Program

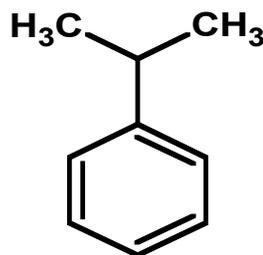
# **TOXICOLOGY AND CARCINOGENESIS STUDIES OF CUMENE IN F344/N RATS AND B6C3F1 MICE**

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## Introduction



- Cumene occurs in petroleum, coal tar, food, tobacco
- Used in the production of phenol and acetone; as a solvent for fats and resins
- Released into environment during processing and use
- People are exposed to cumene via inhalation, food, and cigarette smoking





## ADME

- Well absorbed
- Oxidized to:
  - 2-phenyl-2-propanol
  - 2-phenylpropanoic acid
  - 2-hydroxy-2-phenylpropanoic acid
  - 2-phenyl-1,2-propanediol
  - Phenyl lactic acid
- Excreted in urine in conjugated forms



## Genotoxicity

- Nonmutagenic in *Salmonella typhimurium* tester strains with or without metabolic activation
- Other *in vitro* and *in vivo* genetic toxicity test systems generally indicated no mutagenic or clastogenic activity





## NTP Genotoxicity Tests

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<u>In Vitro</u>	<u>In Vivo</u>	
Salmonella gene mutation assay	Rat acute MN assay (i.p. injection)	Mouse subchronic MN assay (inhalation)
	Males	Males/Females
Negative	Positive	Negative/Negative

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## Study Rationale

- High production volume
- Presence in gasoline and other fuels
- Potential for human exposure
- Lack of carcinogenicity data
- Inhalation route of exposure chosen as humans are exposed mainly by inhalation



## 2-Week Rat Studies

- Exposure concentrations: 0, 250, 500, 1,000, 2,000, or 4,000 ppm
- All rats exposed to 4,000 ppm died
- Surviving 2,000 ppm rats had lower body weights; lethargic
- Liver and kidney weights of exposed groups increased
- Accumulation of hyaline droplets in renal tubular cortex in all exposed males



## 3-Month Rat Studies

- Exposure concentrations: 0, 62.5, 125, 250, 500, or 1,000 ppm
- All rats survived
- Mean body weights of all exposed groups similar to controls
- Kidney and liver weights of exposed males greater than controls
- Liver weights of 1,000 ppm females greater than controls
- $\alpha$ 2u-Globulin, medullary granular casts, and regeneration in male kidneys





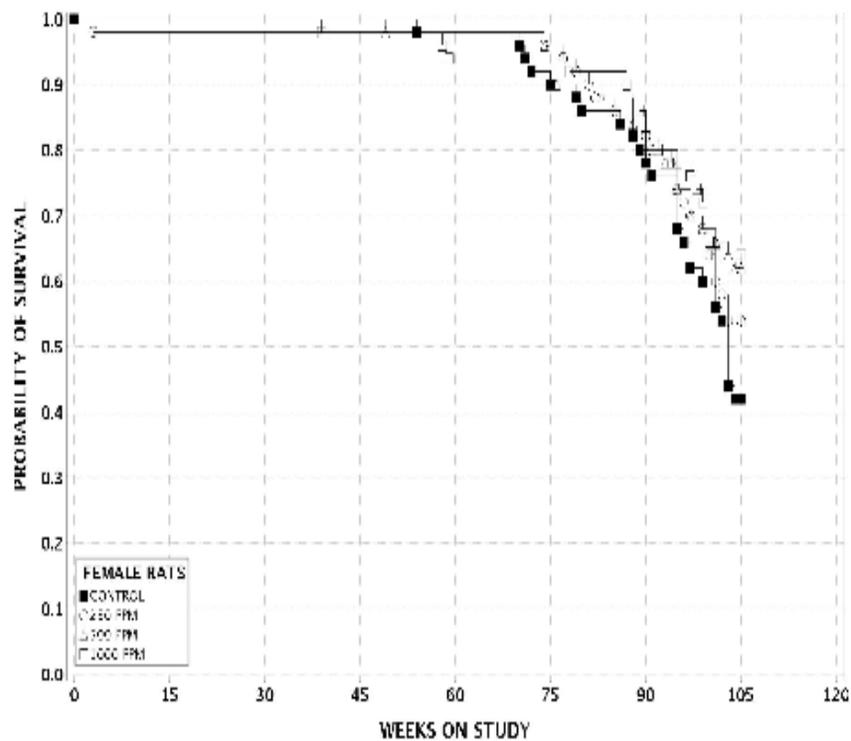
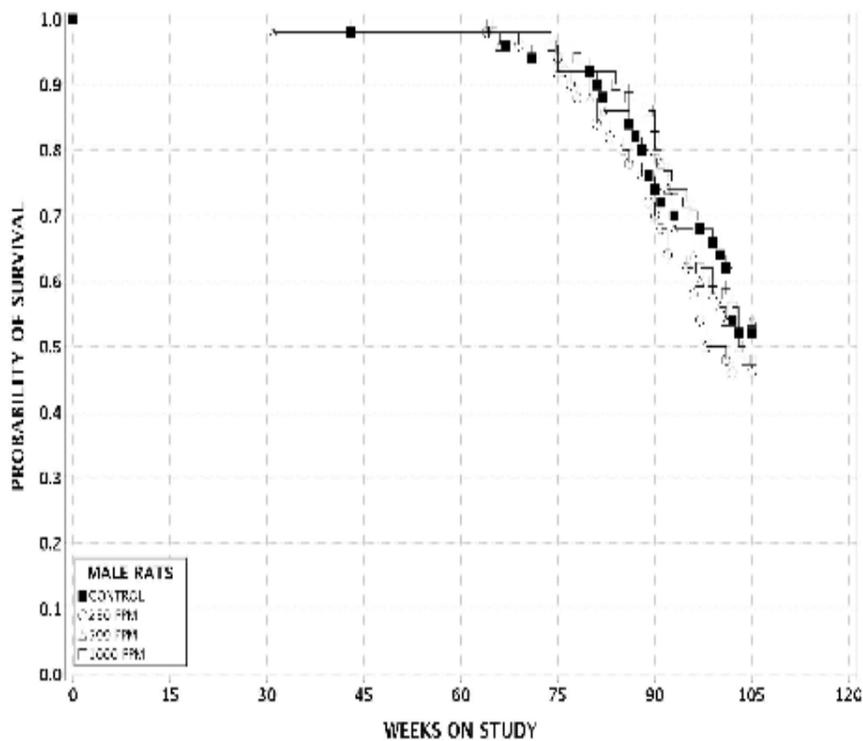
## **2-Year Rat Study**

- Groups of 50 male and 50 female rats were exposed at 0, 250, 500, or 1,000 ppm
- Exposure concentrations were selected based on no changes in survival, body weights, and lack of histopathology changes other than those observed in male kidneys



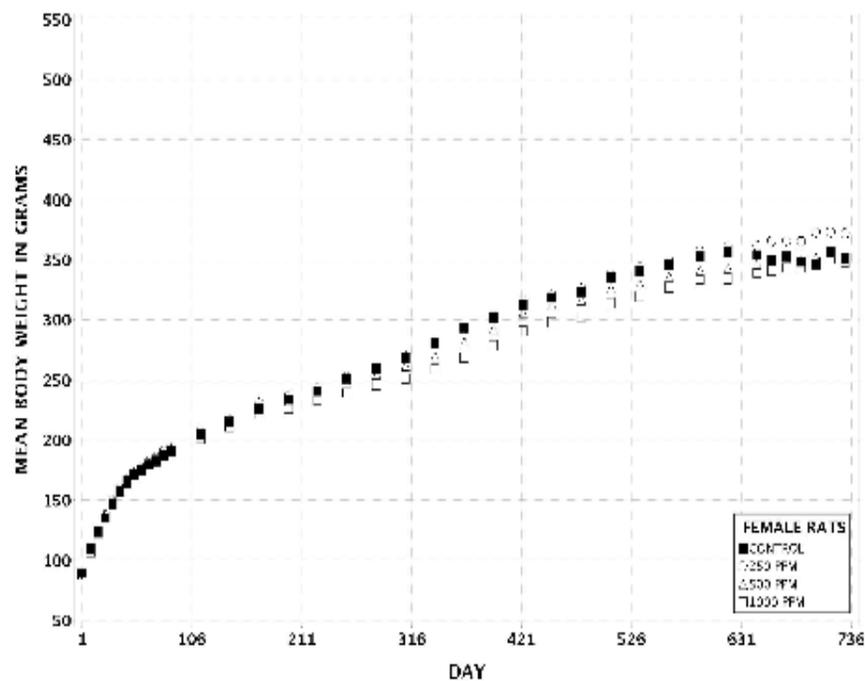
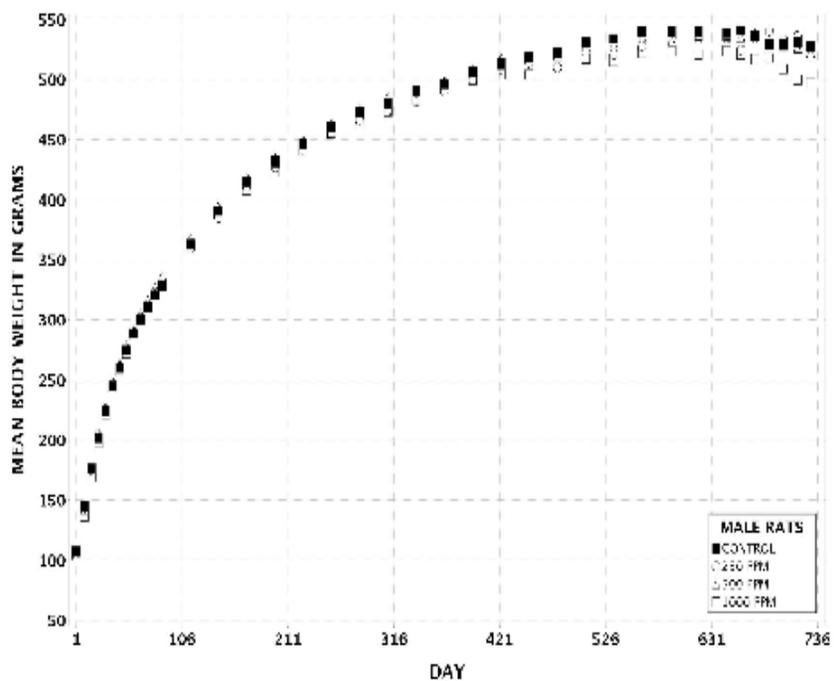
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## Incidences of Lesions of the Nose in Male Rats

	0	250	500	1,000
	(ppm)			
Number Examined	50	50	49	50
Olfactory Epithelium Hyperplasia	0	19**	27**	26**
Respiratory Epithelium Hyperplasia	0	15**	16**	23**
Goblet Cell, Hyperplasia	3	11*	7	5
Respiratory Epithelium Adenoma	0	7**	18**	10**

\*P<0.05

\*\*P<0.01



## Incidences of Lesions of the Nose in Female Rats

	0	250	500	1,000
	(ppm)			
Number Examined	50	48	50	50
Olfactory Epithelium Hyperplasia	0	14**	25**	31**
Respiratory Epithelium Hyperplasia	0	0	4	6*
Respiratory Epithelium Adenoma	0	5*	4	3

\*P<0.05

\*\*P<0.01



## Incidences of Kidney Lesions in Rats

	0 ppm	250 ppm	500 ppm	1,000 ppm
Number examined	50	50	50	50
		Male		
Nephropathy	47 (2.3) <sup>a</sup>	47 (2.6)	47 (2.9)	50 (2.7)
Papilla, mineralization	5 (1.0)	35** (1.7)	44** (2.1)	41** (2.1)
Renal tubule, hyperplasia	0	3 (3.3)	8** (2.6)	6* (2.2)
Pelvis Transit. Epith. Hyperplasia	3 (1.7)	5 (1.8)	14** (2.4)	15** (2.0)
Renal Tubule Adenoma	1	4	5	4
Renal Tubule Carcinoma	1	1	3	3
Renal Tubule Ad. Or Ca.	2	5	8**	7*
		Female		
Nephropathy	38 (1.4)	37 (1.5)	41 (1.9)	44 (1.9)

\*P<0.05    \*\*P<0.01

<sup>a</sup>Average severity grade: 1=minimal, 2=mild, 3=moderate, 4=marked



## **2-Week Mouse Study**

- Exposure concentrations: 0, 250, 500, 1,000, 2,000, 4000 ppm
- All mice exposed to 2,000 and 4,000 ppm died
- Body weights among survivors in either sex similar
- Ataxia and lethargic
- Kidney weights increased in exposed groups in both sexes
- No histopathology changes in liver or kidney
- Thymus weights decreased in 1,000 ppm males





## **3-Month Mouse Study**

- Exposure concentrations: 0, 62.5, 125, 250, 500, 1,000 ppm
- All mice survived except eight of 1,000 ppm females
- Body weights of males were lower than controls at >250 ppm
- Ataxia and lethargy in 1,000 ppm males
- Relative liver weights increased in male and female groups at >125 ppm



## Incidences of Lesions in Mice in 3-Month Study

	0 ppm	62.5 ppm	125 ppm	250 ppm	500 ppm	1,000 ppm
<b>Male</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>
Liver Necrosis	0	1 (1.0)	1 (2.0)	1 (1.0)	1 (1.0)	5* (1.2)
<b>Female</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>
Liver Inflammation	1 (1.0)	10**(1.0)	10**(1.0)	9**(1.0)	7**(1.0)	2 (1.0)
Thymus Necrosis	0	0	0	0	0	8**(4.0)

\* P<0.05

\*\*P<0.01



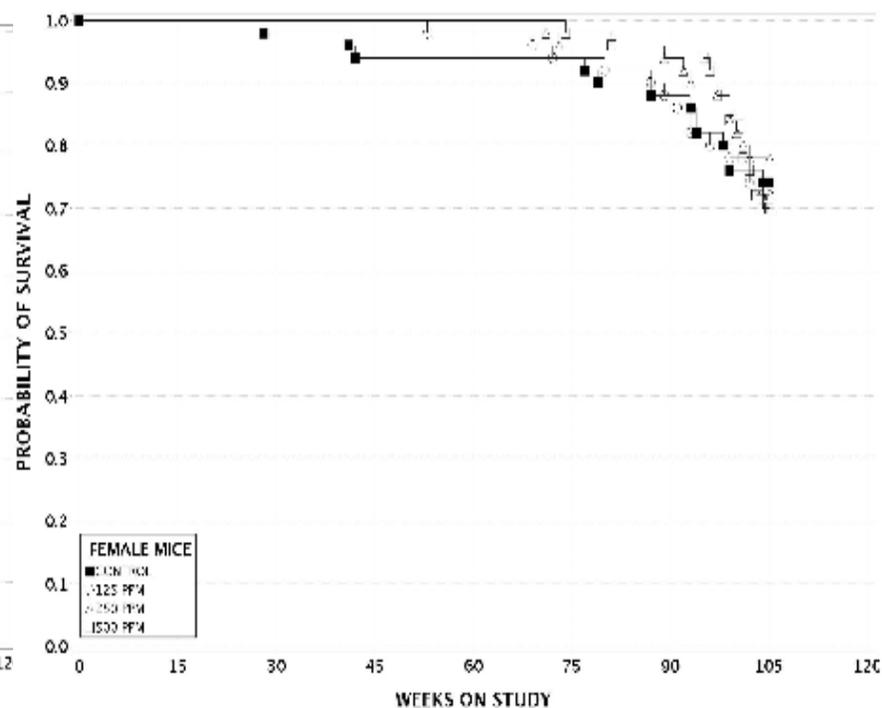
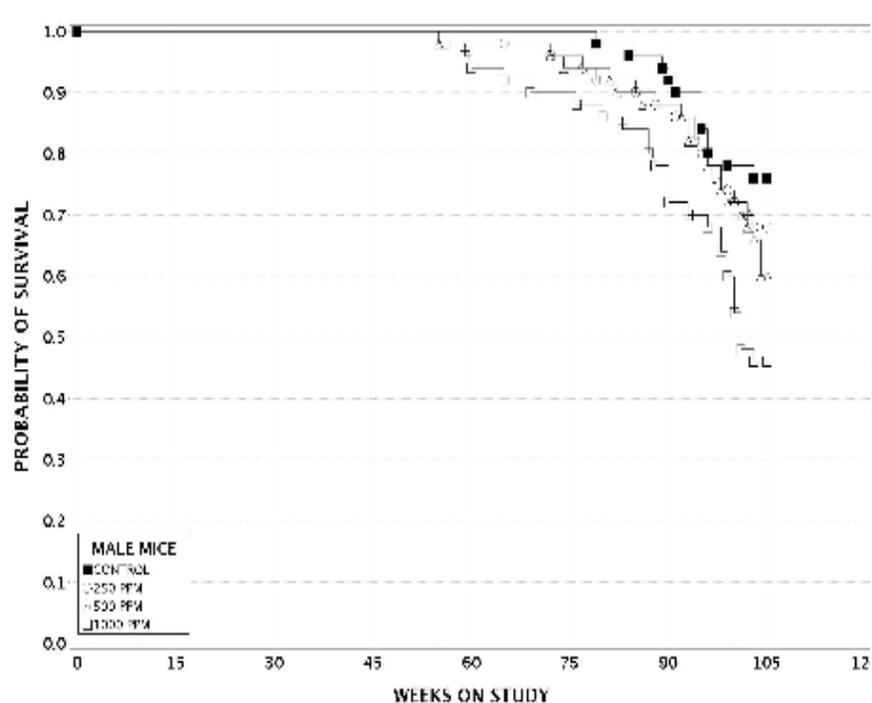


## 2-Year Mouse Study

- Exposure concentrations: Males: 0, 250, 500, 1,000 ppm  
Females: 0, 125, 250, 500 ppm
- In males exposure concentration selection was based on slight decreases in body weights and organ weights, and minimal incidences of lesions in the 3-month study
- In females exposure concentration selection was based on lower survival rates and thymic necrosis at 1,000 ppm

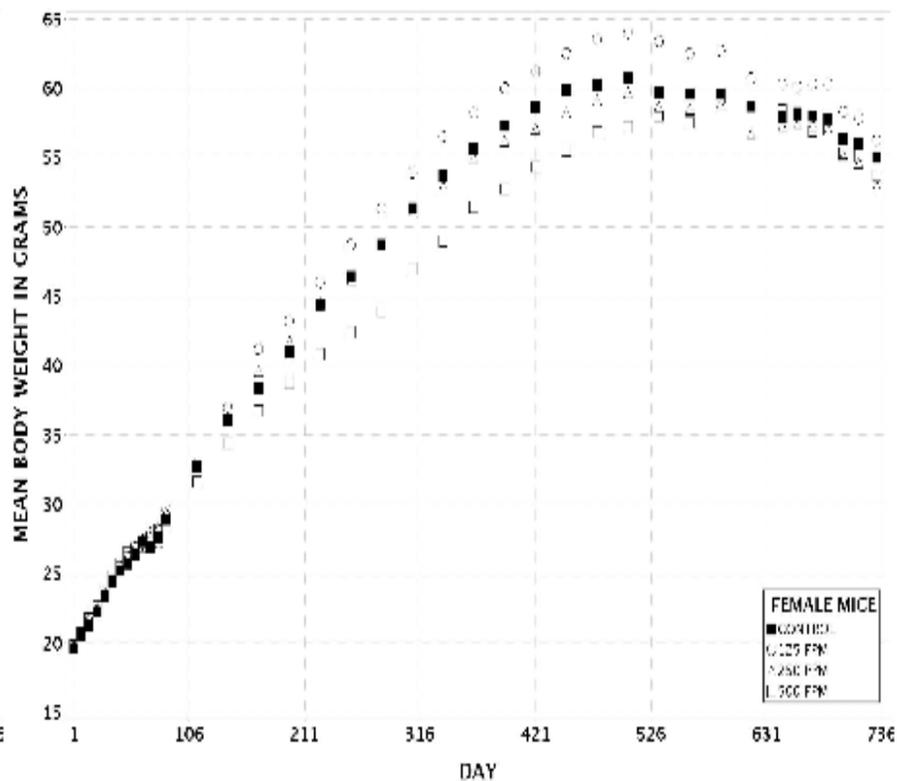
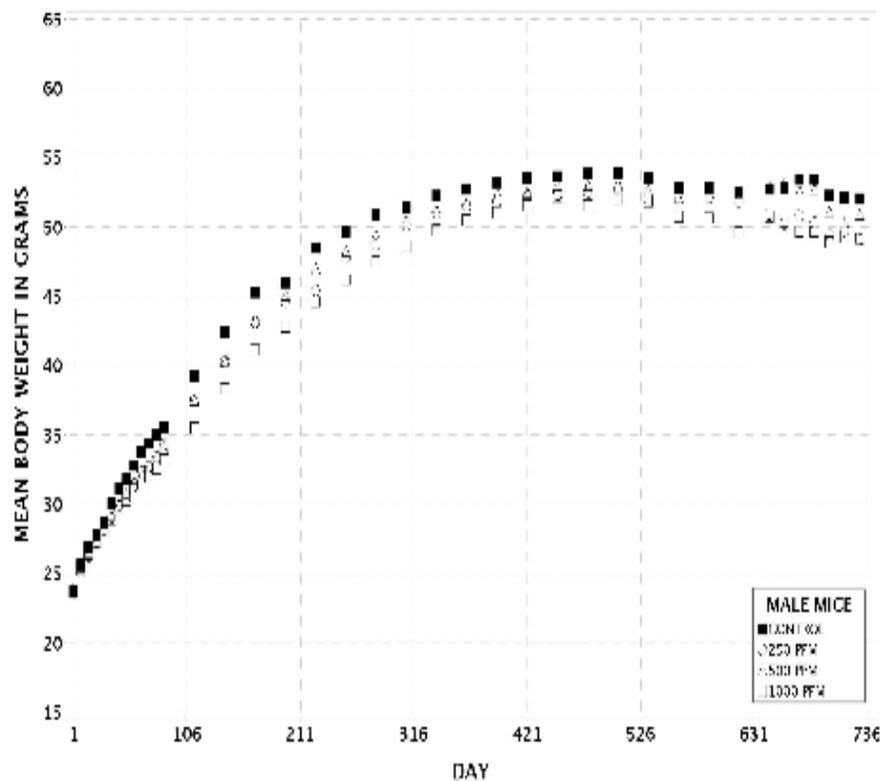


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## Incidences of Lesions of the Lung in Male Mice

	0 ppm	250 ppm	500 ppm	1,000 ppm
Number Examined	50	50	50	50
Alveolar Epith., Bronchiole, Metaplasia	5 (1.4)	43**(2.9)	42**(3.1)	39**(3.0)
Bronchiole Hyperplasia	0	11**(2.1)	17**(3.2)	18**(2.8)
Alveolar/bronchiolar Ad. Multiple	1	12**	15**	20**
Alveolar/bronchiolar Ad. (incl. multiple)	13	31**	31**	29**
Alveolar/bronchiolar Ca. Multiple	0	8**	20**	17**
Alveolar/bronchiolar Ca. (incl. multiple)	9	19**	32**	33**
Alveolar/bronchiolar Ad. or Ca.	19	38**	42**	43**

\*\*P<0.01



## Incidences of Lesions of the Lung in Female Mice

	0 ppm	125 ppm	250 ppm	500 ppm
Number Examined	50	50	50	50
Alveolar Epith., Bronchiole, Metaplasia	0	42**(2.6)	49**(2.9)	47**(3.3)
Bronchiole, Hyperplasia	0	17**(2.7)	10**(2.8)	14**(2.8)
Alveolar/bronchiolar Ad. Multiple	0	13**	20**	30**
Alveolar/bronchiolar Ad. (incl. multiple)	1	26**	36**	38**
Alveolar/bronchiolar Ca. Multiple	0	6*	7**	19**
Alveolar/bronchiolar Ca. (incl. multiple)	3	16**	20**	34**
Alveolar/bronchiolar Ad or Ca	4	31**	42**	46**

\*P<0.05

\*\*P<0.01



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## **Non-neoplastic lesions of the Nose in Mice**

### **Olfactory Epithelium (male and female)**

Atrophy

Basal Cell hyperplasia

Atypical hyperplasia

Glandular hyperplasia

Suppurative inflammation (males only)

### **Respiratory Epithelium (female)**

Squamous metaplasia

Suppurative Inflammation





## Incidences of Liver Lesions in Female Mice

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	0 ppm	125 ppm	250 ppm	500 ppm
Number Examined	50	50	50	50
Eosinophilic focus	8	11	7	14
Hepatocellular Ad. (incl. multiple)	18	23	27	29*
Hepatocellular Ca. (incl. multiple)	10	7	6	12
Hepatocellular Ad. or Ca.	25	26	29	36*

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\*P<0.05



## Incidences of Forestomach Lesions in Male Mice

	0 ppm	250 ppm	500 ppm	1,000 ppm
Number Examined	50	50	50	49
Epithelium, Hyperplasia	2 <sup>a</sup> (2.0)	7 (2.1)	8* (2.3)	13**(2.1)
Ulcer	1 (3.0)	4 (2.8)	6 (2.8)	6* (2.8)
Inflammation	0	2 (2.0)	1 (2.0)	5* (2.0)

\*P<0.05 by the Poly-3 test

\*\*P<0.01



## Incidence of testicular lesions in male rats

Cumene	0 ppm	250 ppm	500 ppm	1,000 ppm
Number examined	50	50	50	50
Interstitial cell adenoma	36 (72%)	38 (76%)	40 (80%)	46 (92%)
Poly-3 test	P=0.006	P=0.370	P=0.311	P=0.007

Historical control incidence: All routes: 1242/1449 (85.7%), range: 66-98%

Inhalation: 345/449 (76.9%), range: 66-84%





## Discussion

- Cumene is a multisite carcinogen inducing neoplasms in nose and kidney in rats and in lung and liver in mice
- Cumene was not genotoxic in Ames tests and was negative in micronuclei induction in mice via inhalation exposure
- Cumene was positive in MN induction in male rat bone marrow cells following I.p. injection and significantly increased frequencies of K-ras and p53 mutations in mouse lung neoplasms, suggesting genetic mechanism
- No activation of cumene to an intermediate capable of reacting with protein or DNA has been identified



## Conclusion

- Clear evidence of carcinogenic activity of cumene in male F344/N rats based on increased incidences of respiratory epithelial adenoma in the nose and renal tubule adenoma or carcinoma
- Some evidence of carcinogenic activity of cumene in female F344/N rats based on the incidences of respiratory epithelium adenoma in the nose
- Clear evidence of carcinogenic activity of cumene in male B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms
- Clear evidence of carcinogenic activity of cumene in female B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms and incidences of hepatocellular adenoma or carcinoma