Rodent Lung Tumors in NTP Studies

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Overview

- NTP studies with increased lung tumor incidence
- Examples
  - Styrene, Naphthalene, Coumarin
  - Ethylbenzene, Cumene
- Potential predisposing factors in pulmonary carcinogenesis

Summary of Lung Lesions in NTP Studies

- Common non-neoplastic lesions
  - Hyperplasia, inflammation, infiltrate, hemorrhage
- Alveolar/Bronchiolar adenomas/carcinomas are the most common lung tumors in mouse and rat
- NTP calls for carcinogenicity
  - Clear Evidence (CE)
  - Some Evidence (SE)
  - Equivocal Evidence (EE)
  - No Evidence (NE)

Dixon et al., Toxicologic Pathology, 36: 428-439, 2008

Background Lung Tumors

(NTP Historical controls, 2013)

All routes/All vehicles

Tumor Type	Male Rat	Female Rat	Male Mouse	Female Mouse
AB Adenoma	2.4	1.3	15.3	5.7
AB Carcinoma	1.3	0.1	13.9	4.0
AB Ad/Carc	3.6	1.4	27.7	9.5

Rat (n=700/sex) = F344/NTac; Mouse (n=950/sex) = B6C3F1;
All numbers are in percentages (%)

- No incidence of spontaneous cystic keratinizing epithelioma and squamous cell carcinoma in controls
  - Only seen with treatment

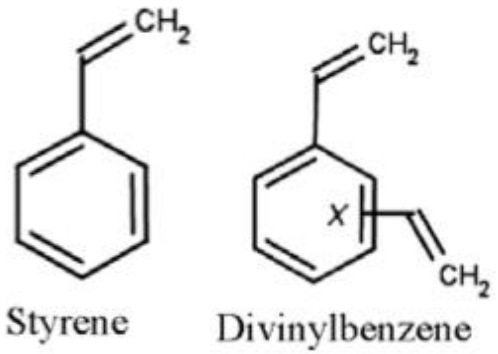
Chemically Induced Lung Tumors

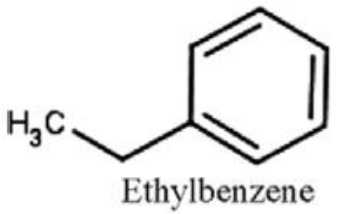
(67/580 NTP studies where same chemical was tested in rats and mice)

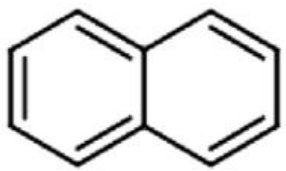
Animal Species and Sex	Carcinogenic including all organs (CE and SE) n=67	Carcinogenic including all organs (including EE) n=67	Studies with Lung tumor response (CE and SE) n=67	Studies with Lung tumor response (including EE) n=67
Rat Male	69% (46)	81% (54)	21% (14)	24% (16)
Rat Female	70% (47)	84% (56)	21% (14)	24% (16)
Mouse Male	63% (42)	78% (52)	51% (34)	60 % (40)
Mouse Female	76% (51)	82% (55)	60% (40)	64 % (43)

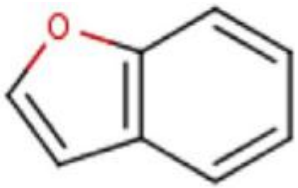
- Tumors in multiple sites - 87% (58/67)
- Positive lung tumor response
  - Both Rats and Mice: 21% (14/67)

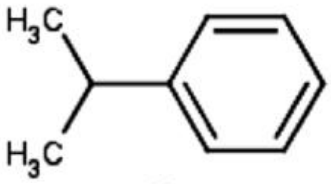
NTP Data on Select Chemicals

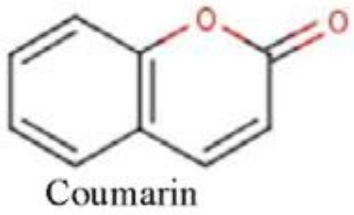












Cruzan et al., 2009

TR #	Chemical	Ames	Route	Male Rat	Female Rat	Male Mouse	Female Mouse	Multiple sites
TR-185	Styrene	-	Gavage	NE	NE	(EE)	NE	**No**
TR-410	Naphthalene	-	Inhal'n	CE	CE	NE	(SE)	**No**
TR-422	Coumarin	+	Gavage	SE	EE	(SE)	(CE)	Yes
TR-466	Ethylbenzene	-	Inhal'n	CE	SE	(SE)	SE	Yes
TR-542	Cumene	-	Inhal'n	CE	SE	(CE)	(CE)	Yes
TR-534	Divinylbenzene	-	Inhal'n	EE	NE	NE	(EE)	Yes
TR-370	Benzofuran	-	Gavage	NE	SE	(CE)	(CE)	Yes

Styrene (NCI, 1979)

TR #	Chemical	Ames	Route	Male Rat	Female Rat	Male Mouse	Female Mouse	Multiple sites
TR-185	Styrene	-	Gavage	NE	NE	(EE)	NE	No

- Positive cytogenetic effects (SCE, ChrAb, micronucleus) in human lymphocytes and mammalian cells in vitro
- Mice were exposed (300, 150 mg/kg) for 78 weeks and unexposed for 13 weeks before euthanasia
- Mouse
  - Male: Alveolar/Bronchiolar Adenoma/Carcinoma (A/B Ad/Carc)

Naphthalene (NTP, 1992, 2000)

TR #	Chemical	Ames	Route	Male Rat	Female Rat	Male Mouse	Female Mouse	Multiple sites
TR-410, TR-500	Naphthalene	-	Inhal'n	CE	CE	NE	**(SE)**	No

- Positive cytogenetic effects (SCE, ChrAb)
- Mouse
  - Female: Alveolar/Bronchiolar Adenoma/Carcinoma
- Rat
  - Male and female: Olfactory neuroblastoma and nasal epithelial adenoma (NTP 2000)

Coumarin (NTP, 1993)

TR #	Chemical	Ames	Route	Male Rat	Female Rat	Male Mouse	Female Mouse	Multiple sites
TR-422	Coumarin	+	Gavage	SE	EE	(SE)	(CE)	Yes

- Positive cytogenetic effects (SCE, ChrAb)
- Mouse
  - Male and Female: Alveolar/Bronchiolar Adenoma/Carcinoma
  - Male and Female: Forestomach SCC
  - Female: Hepatocellular adenoma/carcinoma
- Rat
  - Male: Renal tubule adenoma

Ethylbenzene (NTP, 1999)

TR #	Chemical	Ames	Route	Male Rat	Female Rat	Male Mouse	Female Mouse	Multiple sites
TR-466	Ethylbenzene	-	Inhal'n	CE	SE	**(SE)**	SE	Yes

- Negative for genotoxicity
- Mouse
  - Male: Alveolar/Bronchiolar Adenoma/Carcinoma
  - Female: Hepatocellular adenoma/carcinoma
- Rat
  - Male and Female: Renal tubule adenoma

Cumene (NTP, 2009)

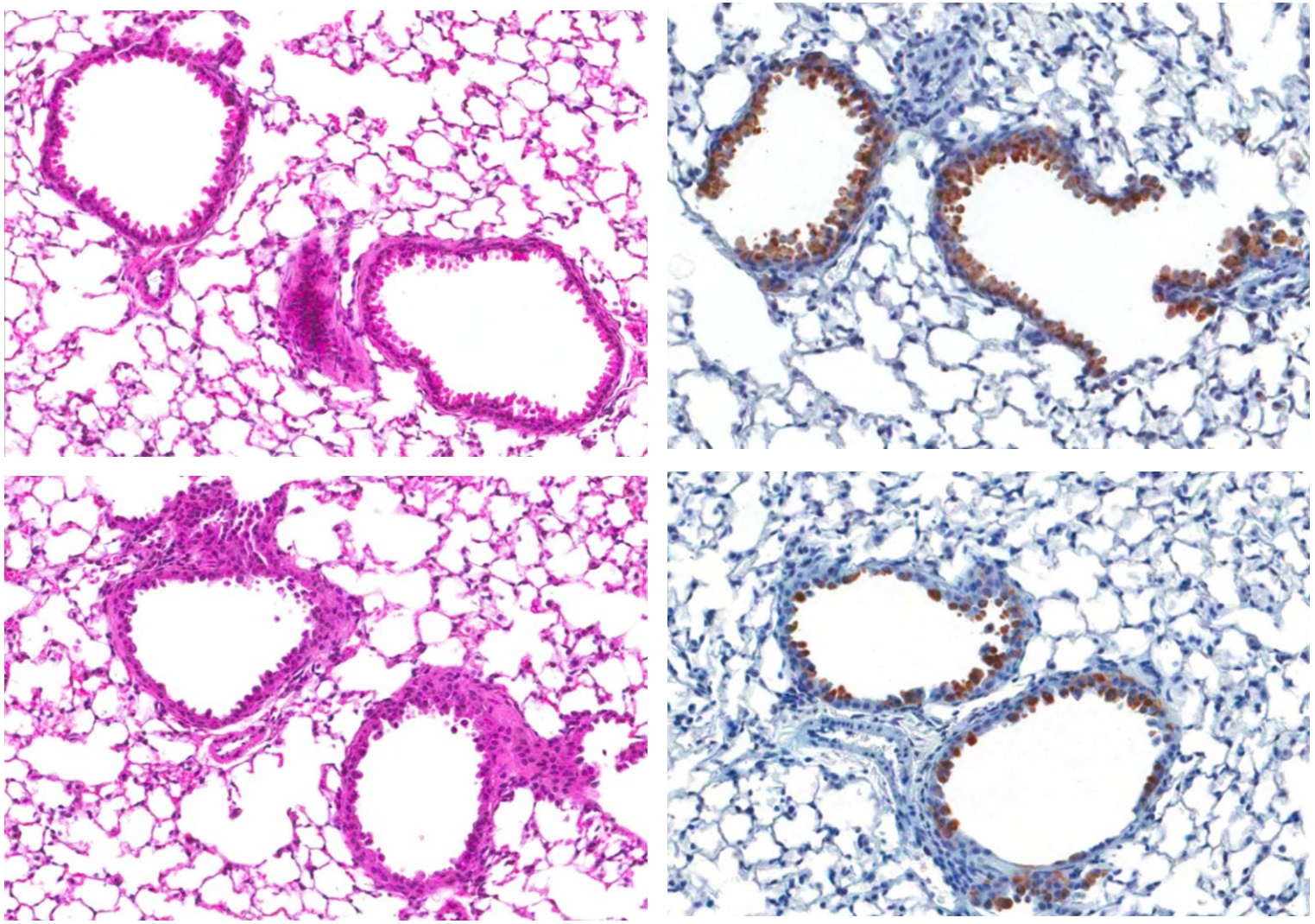
TR #	Chemical	Ames	Route	Male Rat	Female Rat	Male Mouse	Female Mouse	Multiple sites
TR-542	Cumene	-	Inhal'n	CE	SE	(CE)	(CE)	Yes

- Negative for genotoxicity
- Mouse
  - Male and Female: Alveolar/Bronchiolar Adenoma/Carcinoma
  - Female: Hepatocellular Adenoma/Carcinoma
- Rat
  - Male and Female: Nose, respiratory epithelium adenoma
  - Male: Renal tubule adenoma

Potential Predisposing Factors in Pulmonary Carcinogenesis

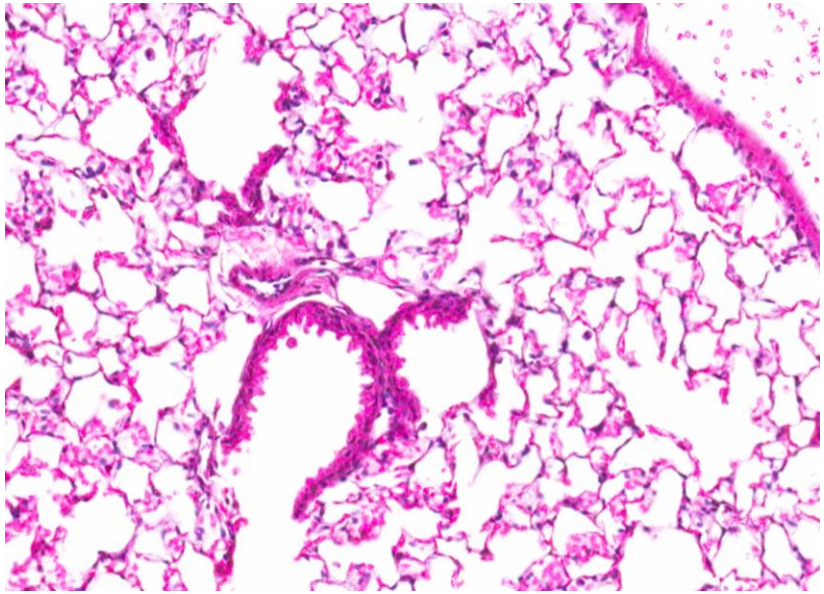
- Cytotoxicity
  - degeneration, necrosis, inflammation
- Cell proliferation
  - Hyperplasia
- Mutations in cancer genes
- Target sites in early time points
  - Terminal bronchioles, alveolar duct, alveoli
- Target cells for tumorigenesis
  - Type II cells
  - Clara (club) cells

Styrene, 13-week Mouse Gavage Study
Loss of Clara (club) cells

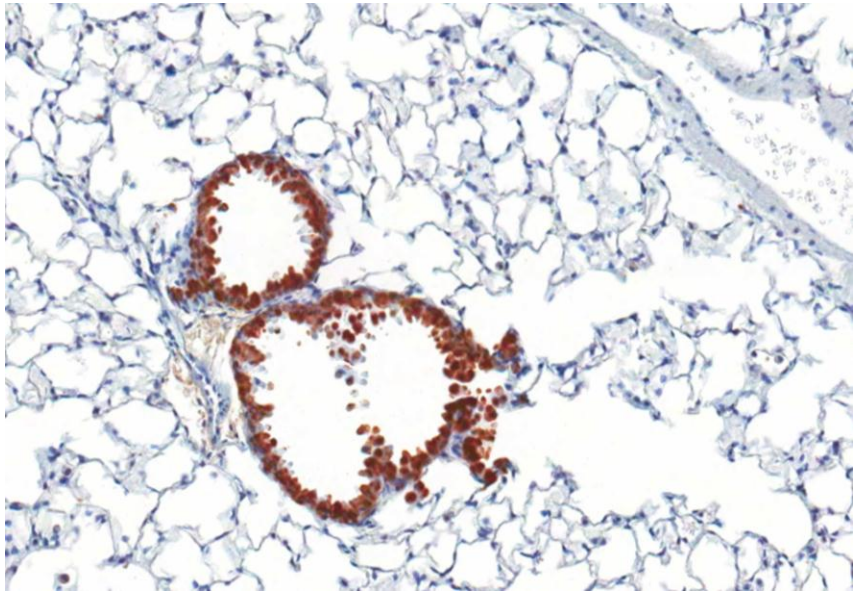


Ethylbenzene, 13-week Mouse Inhalation Study

Ethylbenzene



H & E

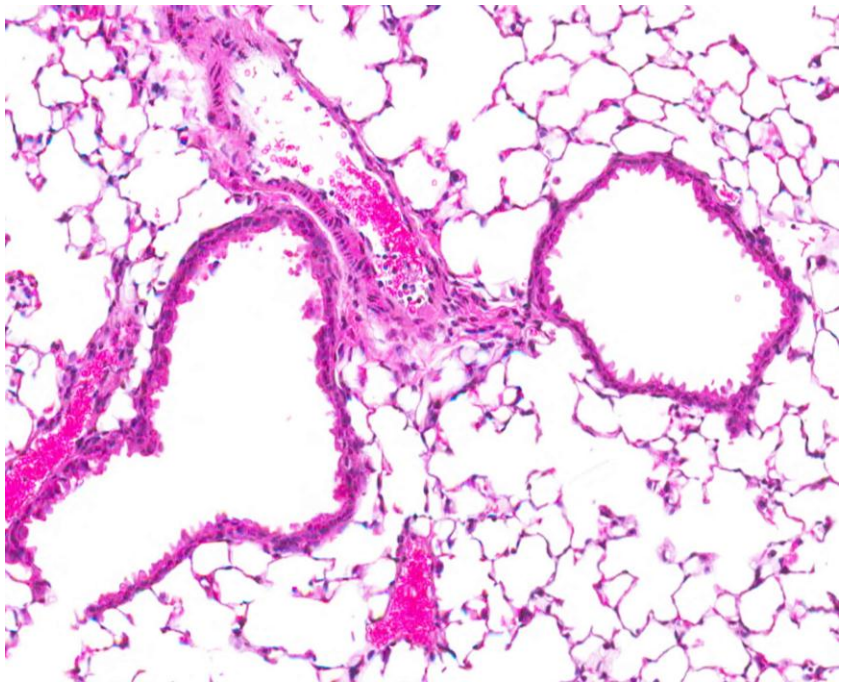


CC10

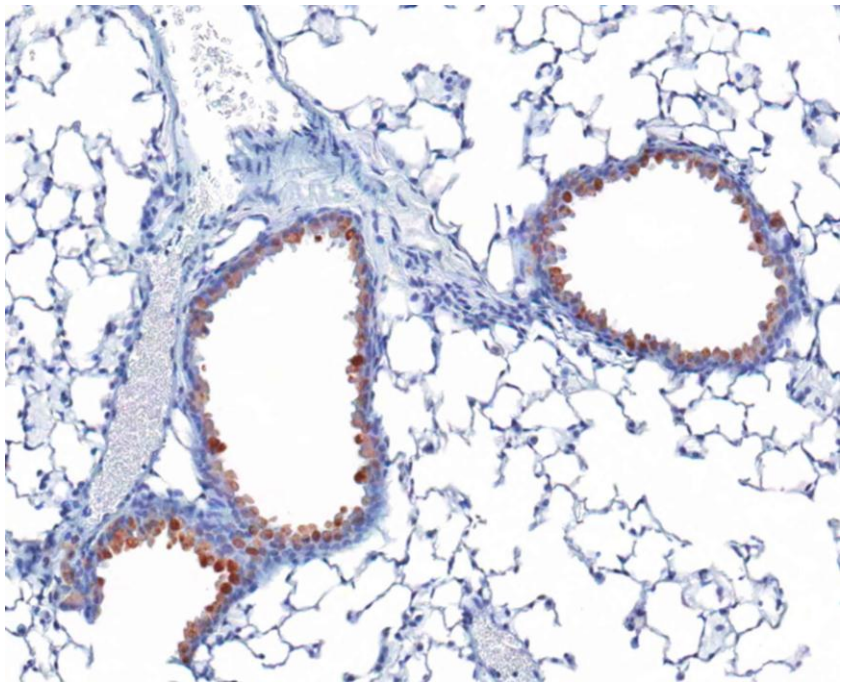
- No pulmonary histologic lesions were noted
- Clara (club) cells unaffected as demonstrated by CC10 stain

Cumene, 13-week Mouse Inhalation Study

Cumene



H & E



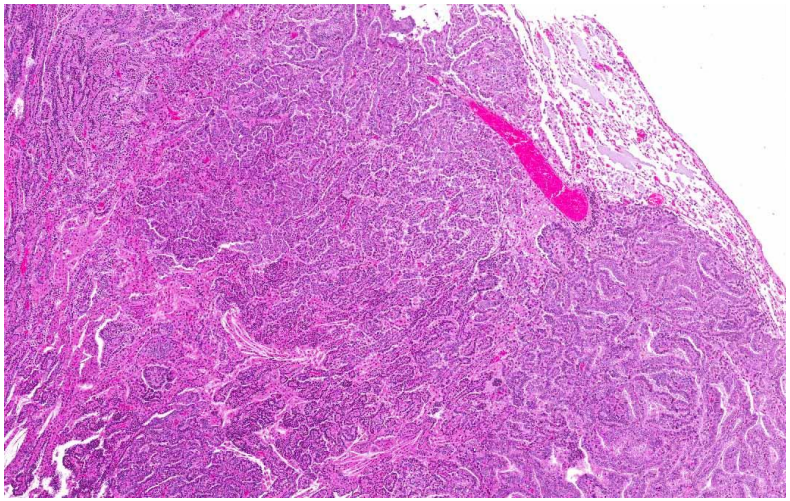
CC10

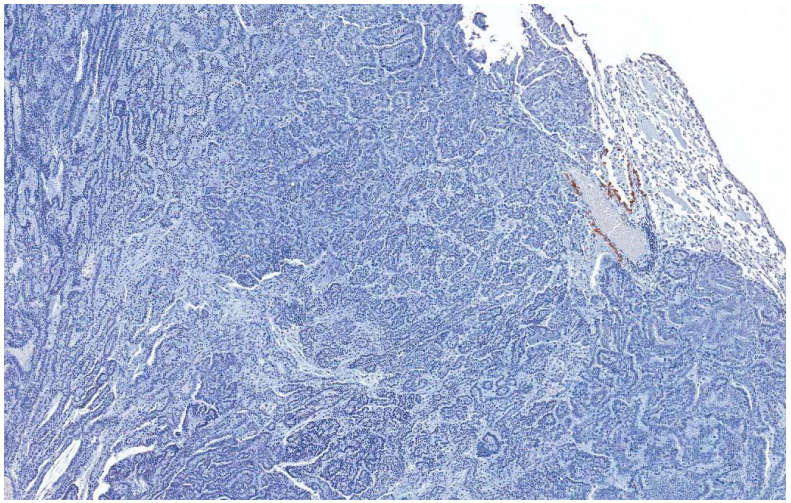
- No histologic lesions in the nose or lung
- Clara (club) cells unaffected as demonstrated by CC10 stain

Ethylbenzene-induced Mouse Lung Tumors:

Type II Cell Phenotype (+ve for SPC and –ve for CC10)

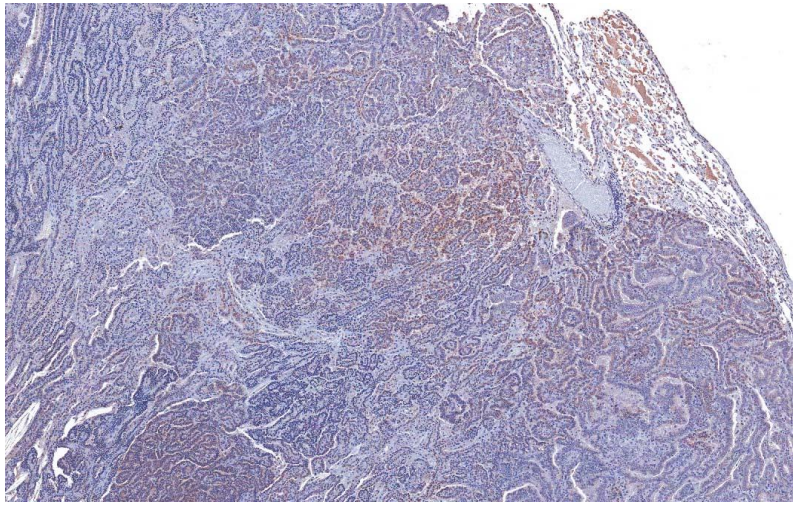
H&E

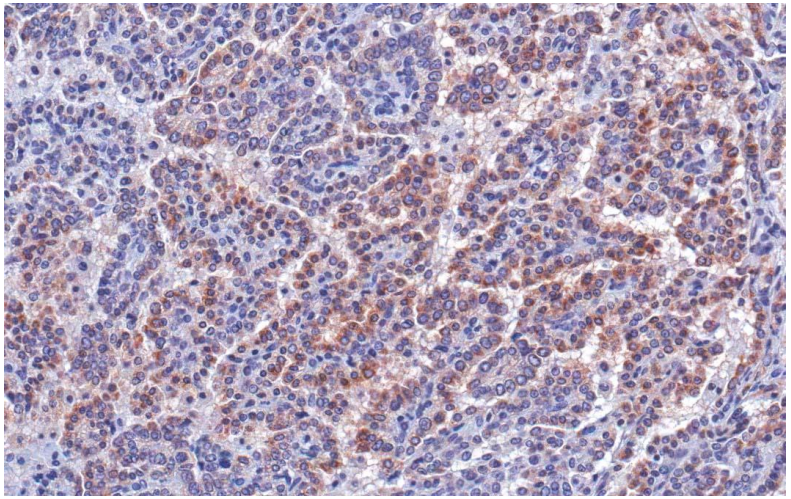




CC10

SPC





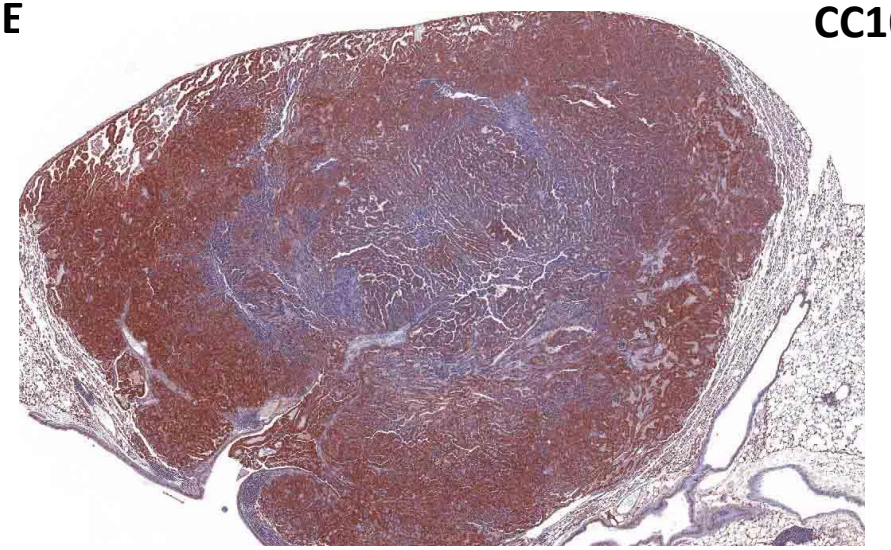
SPC

Cumene-induced Mouse Lung Tumors:

Type II Cell Phenotype (+ve for SPC and –ve for CC10)

H & E

CC10

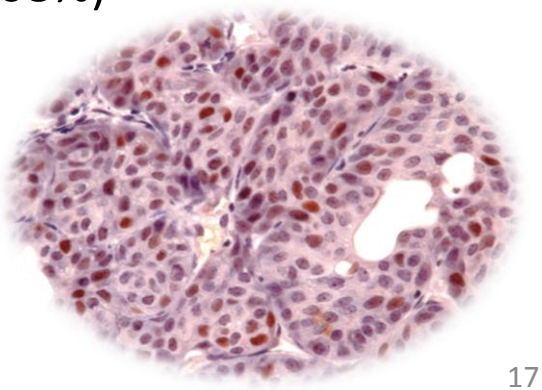


SPC

Mutational Analysis of Mouse Lung Tumors: Cumene Study

Cumene (ppm)	n	*Kras*	*Tp53*
Historical controls	117	33 (28%)	No data
Chamber Controls	7	1 (14%)	0
125	4	1 (25%)	0
250	13	10 (77%)	5 (38%)
500	18	17 (94%)	11 (61%)
1,000	17	17 (100%)	11 (65)
Cumene Total	52	45 (87%)	27 (52%)

- Predominant K*ras* mutations (Cumene *vs* Spontaneous)
  - Codon 12 (GTT): G to T transversions (21% *vs* 0.008%)
- Predominant *Tp53* mutations
  - Exon 5: 89% *vs* 0%
  - *Tp53* protein expression **(IHC)**: 56% *vs* 14%



Hong et al., Toxicologic Pathology, 36: 720-726, 2008

Summary and Discussion

- Lung tumor incidences in NTP studies are higher in the mouse than in the rat
- Mouse lung tumors are usually associated with tumors in multiple sites, with some exceptions like Styrene and Naphthalene
- Structurally similar compounds may cause lung tumors through different mechanisms
  - Mechanism: cytotoxicity vs genotoxicity
  - Cellular target: Type II cells vs Clara cells vs ???
  - Tumor type: Type II predominantly but possible Clara cells
  - Distribution: Lung only vs multiple organ sites
  - Comparison to human disease (morphological & molecular)

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Variation in Clara cell numbers across species

TABLE 2. *Comparison of numerical density and percentage of Clara cells in the bronchiolar epithelial population of adults.*

Species	Bronchiolar epithelium density (#/mm^2)	Clara cells	
		Density (#/mm^2)[a]	% of cells
Mouse[b]	9,759 ± 1,700	8,730 ± 1,966	89.5
Hamster[b]	14,238 ± 2,794	8,248 ± 2,106	57.9
Rat (Sprague-Dawley)[b]	18,813 ± 2,722	14,028 ± 2,918	82.2
Rat (Fisher 344)[c]	17,070 ± 791	4,336 ± 201	25.4
Rabbit[d]	15,073 ± 706	9,261 ± 434	61.44
Cat[e]	19,532 ± 383	19,532 ± 383	100
Bonnet monkey[f]	9,565 ± 304	8,800 ± 280	92

Concordance of Rat and Mouse lung tumor incidence

- Positive lung tumor incidence response in both Rats and Mice: 21% (14/67)
- **Studies with lung tumors - Rat only**: 6 chemicals
  - Diphenhydramine HCl; Dimethyl hydrogen phosphite; Talc; 1,2-Epoxybutane; Nickel subsulfide; Gallium arsenide)
- **Studies with lung tumors - Mouse only**: 7 chemicals
  - Styrene; Trifluralin; Ozone; Dimethyl terephthalate; Estradiol mustard; N-Methylolacrylamide; bis (2-chloro-1-methylethyl) ether)

Styrene-7,8-oxide (SO)

- Indirect exposure via styrene (~90% metabolized)
- SO genotoxic (Ames and Cytogenetic assays)
- SO by gavage induced forestomach Squamous cell carcinomas in mice and rats; Hepatocellular carcinomas in male mice – *No lung tumors* (Lijinsky, 1986)
- A metabolite of 4-vinylphenol, a minor (0.1%) styrene urinary metabolite is potent pneumo- and hepato-toxicant (10x Styrene and 5x SO)
- Inhibitors of Cyp2E1, 2F1 and other Cyps reduced toxicity (Carlson, 2002)

Divinyl Benzene

- Negative for mutagencity and clastogenicity
- Mouse - ABA/ABC
  - Female: 6/50, 12/50, 8/50, 13/49
  - Male: 16/49, 10/49, 8/49, 8/49
- No tumors in male rats only hyperplasia and metaplasia in nose and lung
- Male rat:
  - Kidney: renal tubule carcinoma 0/50, 0/49, 2/50, 3/49)
  - Brain: oligodendroglioma or astrocytoma 0/49, 1/50, 3/50, 0/50

Benzofuran

- **Male mouse:**
  - ABA/ABCA: 10/49, 9/39, 19/4
  - HCAd/HCC/HBA: 12/49, 31/39, 40/48
  - Forestomach tumors: 2/49, 11/39, 13/48
- **Female mouse:**
  - ABA/ABCA: : 2/50, 9/48, 14/47
  - HCAd: 1/50, 9/48, 21/47
  - Forestomach tumors: 2/50, 9/50, 5/50
- **Rat, Female- Renal tubular cell adenoma**