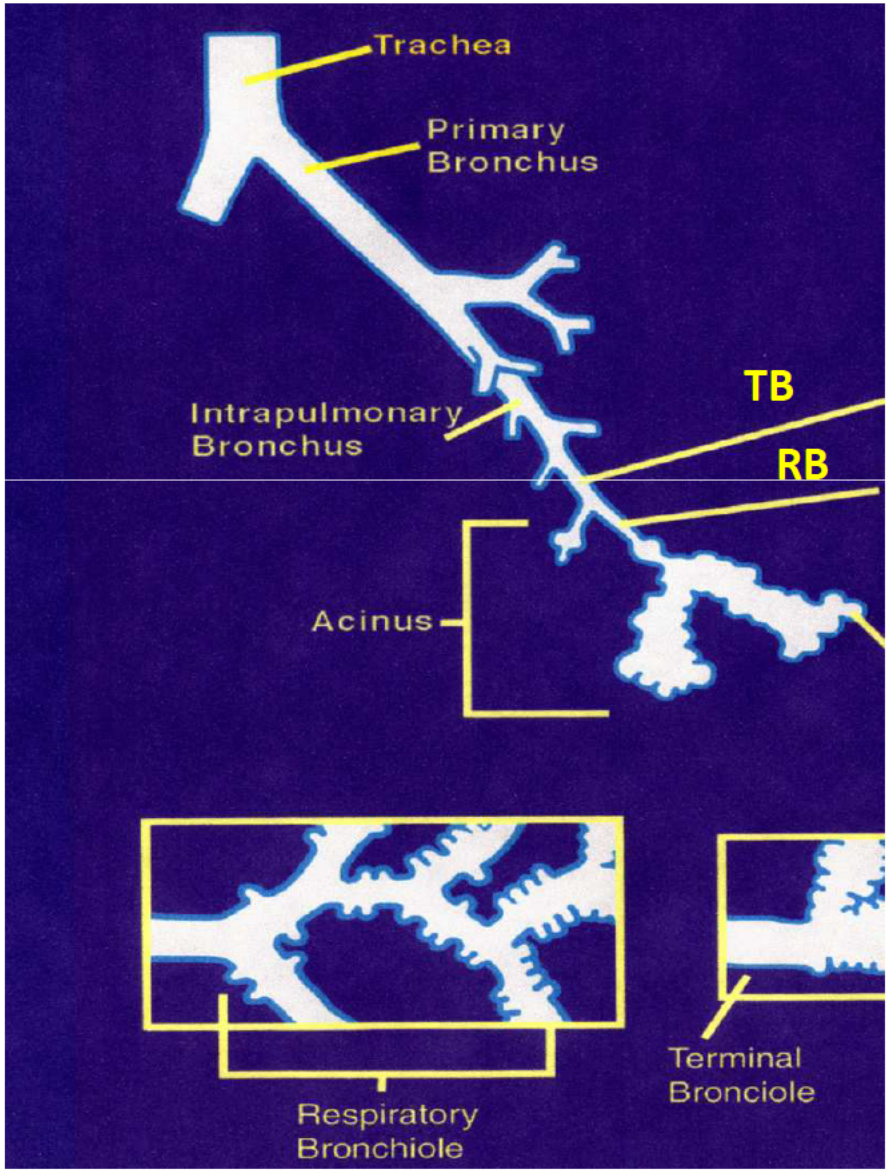
Species Difference in Response and Cell of Origin

Laura S. Van Winkle, PhD DABT
Center for Health and the Environment
Department of Anatomy, Physiology & Cell Biology
School of Veterinary Medicine
University of California at Davis

Anatomy and Airway Cell types vary by Species



The following vary position in the tracheo-bronchiolar airway tree:

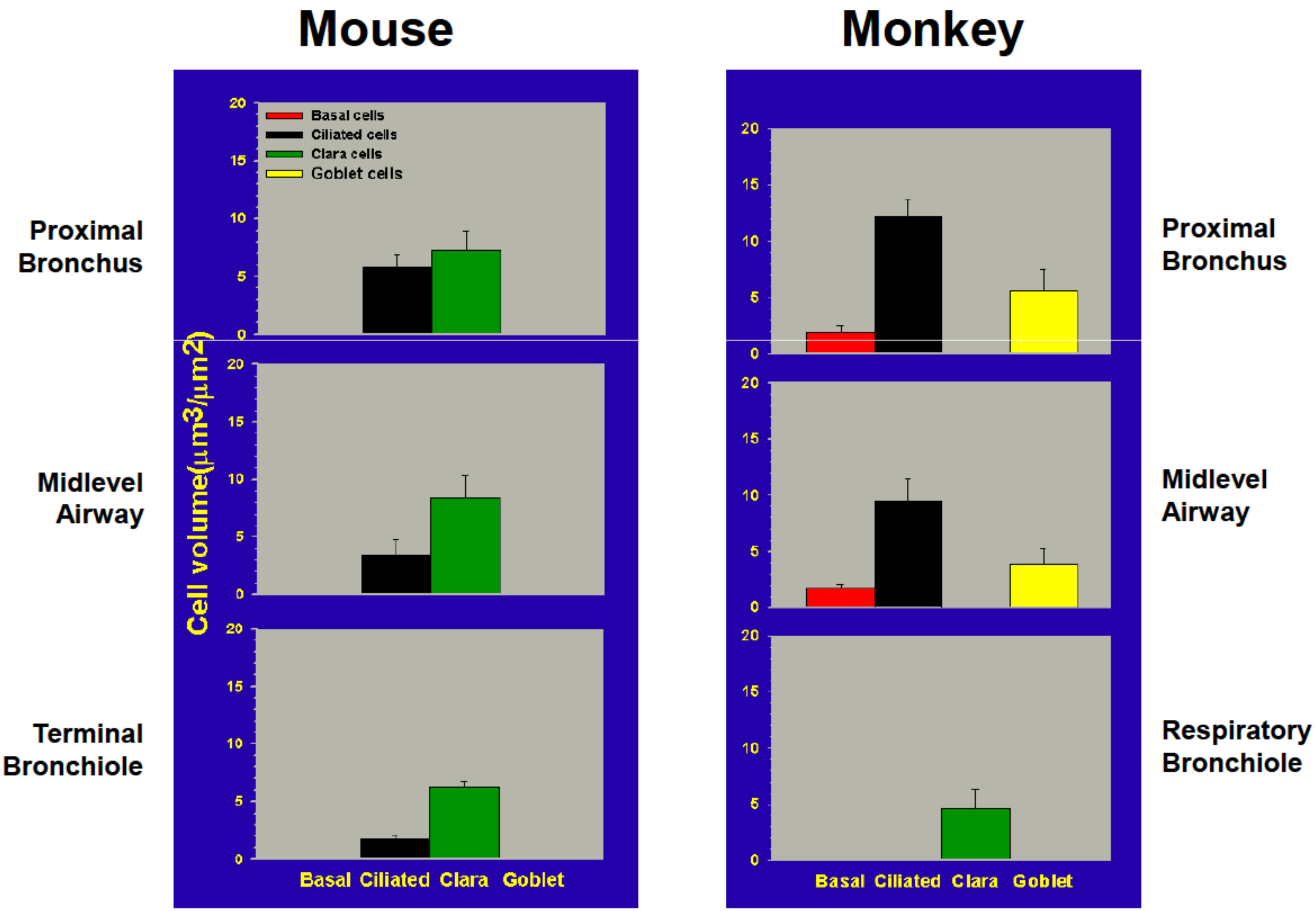
cell types

susceptibility to injury

local dose (route of exposure)

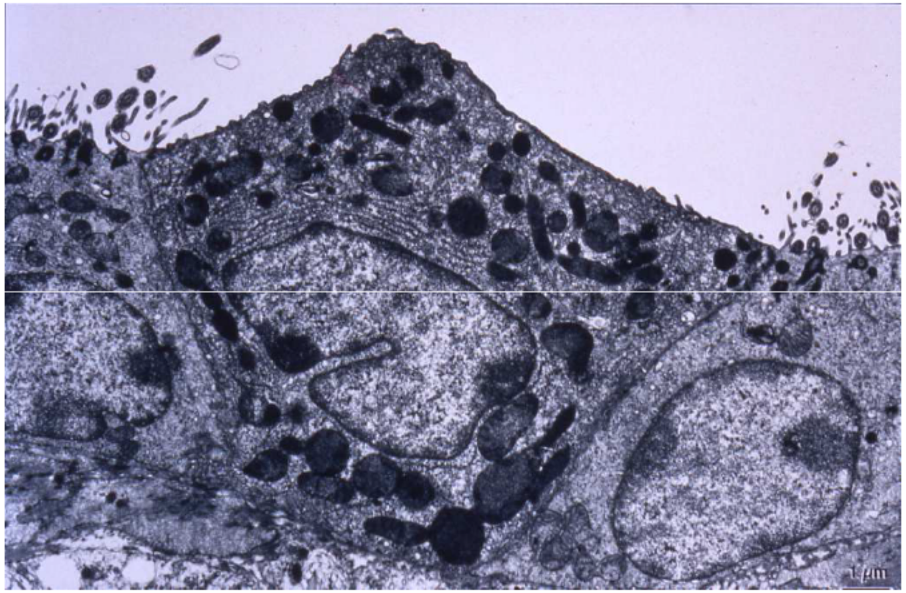
capability to repair

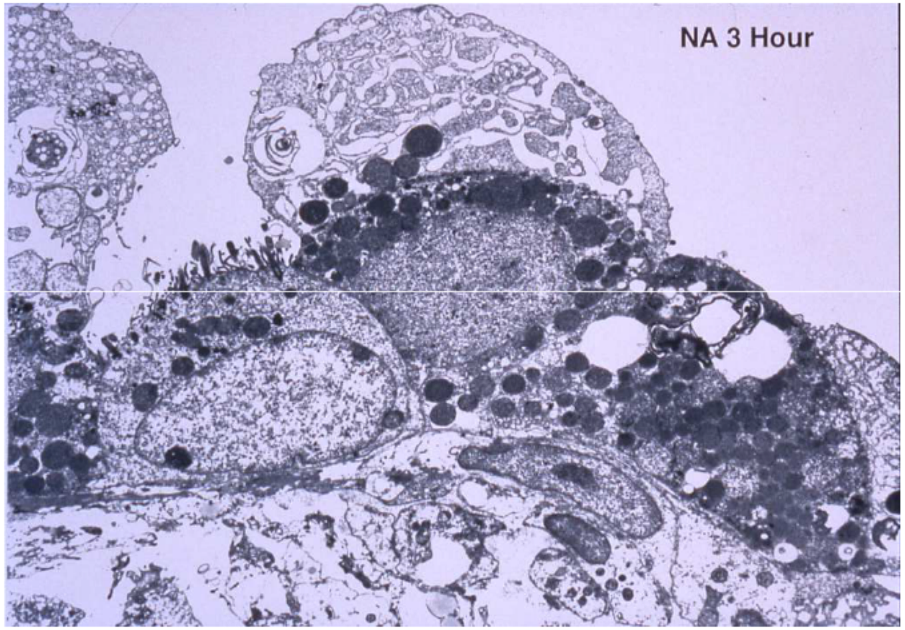
Comparison of Epithelial Composition in Conducting Airways of Mice and Rhesus Monkeys



Naphthalene

Naphthalene is toxic to Club (Clara) cells regardless of route of exposure





Images from Van Winkle et al 1999

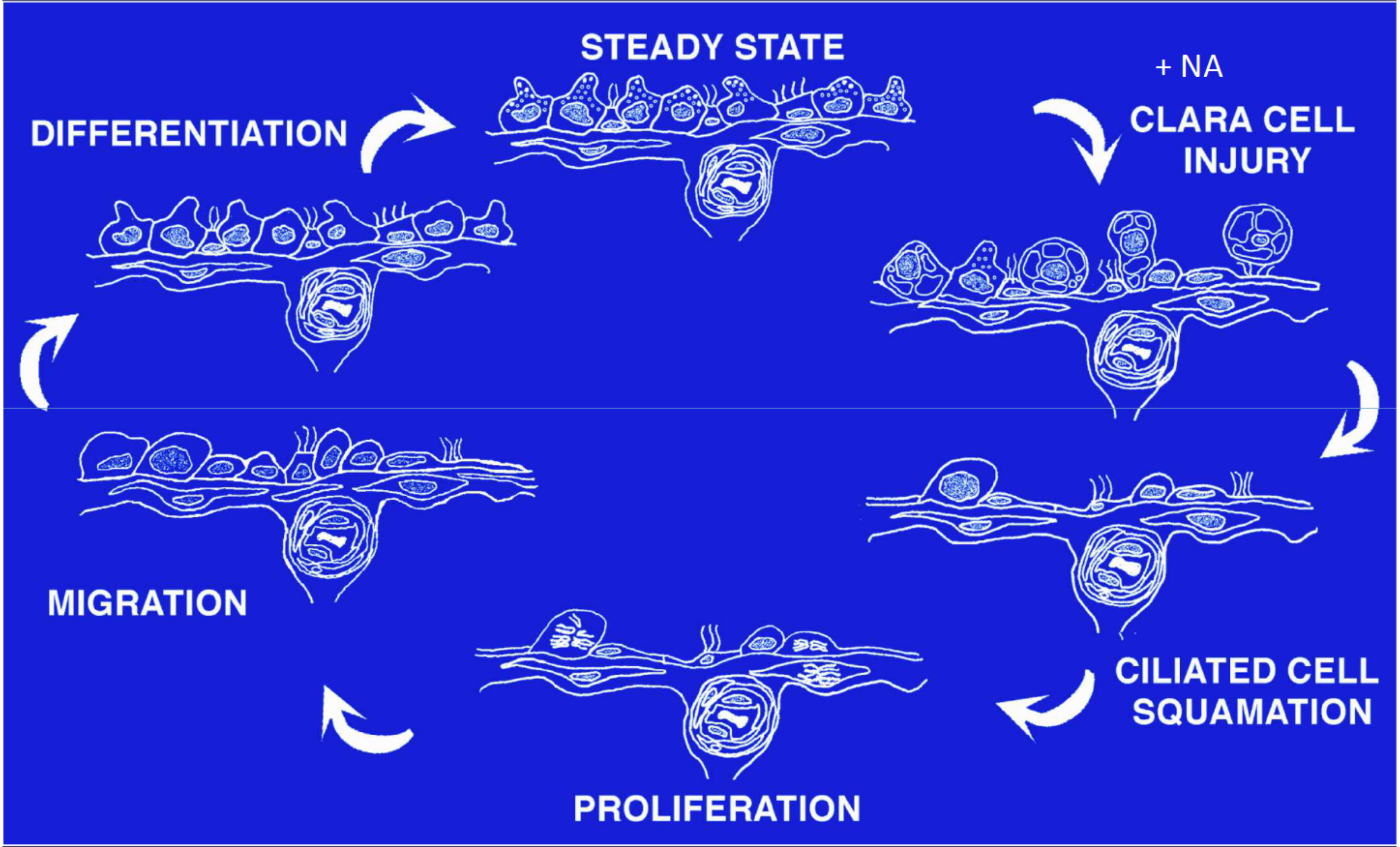
Species and Site Selective Toxicity of Naphthalene in Adult Animals- 24 hrs post exposure

			Distal		Nasal	Epithelium
Species	Dose	Trachea	Bronchiole	Parenchyma	Olfactory	Respiratory
Mouse	50	0	+	0	0	0
	100	0	++	0	0	0
LD_{50}=380 mg/kg	200	+	+++	0	0	0
	400	++	++++	0	++	0
inhalation	2-5 ppm	+	+		+	
	10 ppm	+++	++		++	++
Rat	200	0	0	0	++	0
	400	0	0	0	+++	0
LD_{50}=1600 mg/kg	800	0	0	0	+++	0
	1600	0	0	0	+++	0

Current OSHA exposures are 10 ppm TWA, 15 ppm STEL

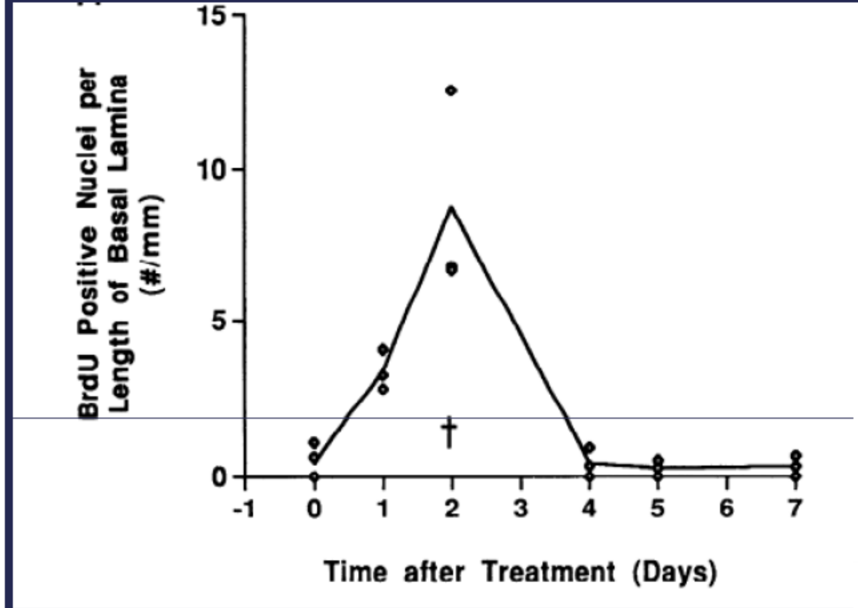
Plopper et al., 1992; 1993; West et al, 2001; Lee et al., 2005; Dodd et al, 2012.

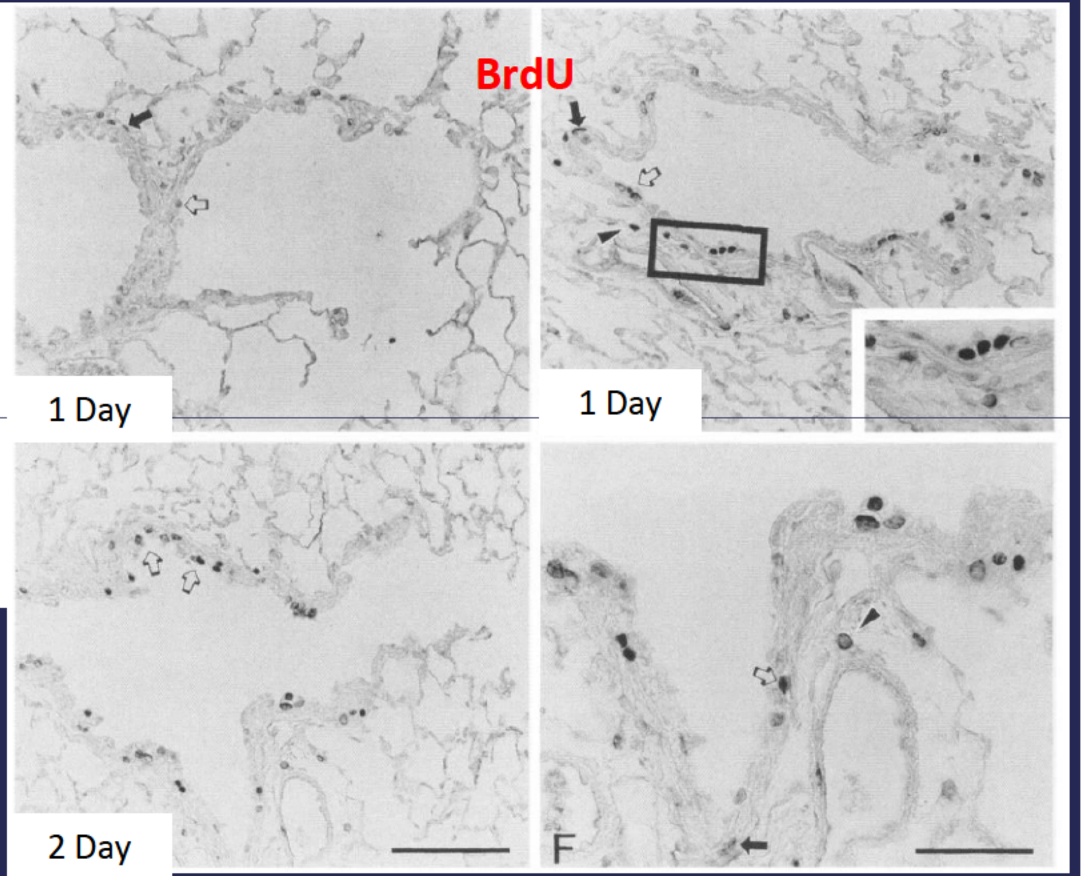
Acute Naphthalene and the Cycle of Injury and Repair

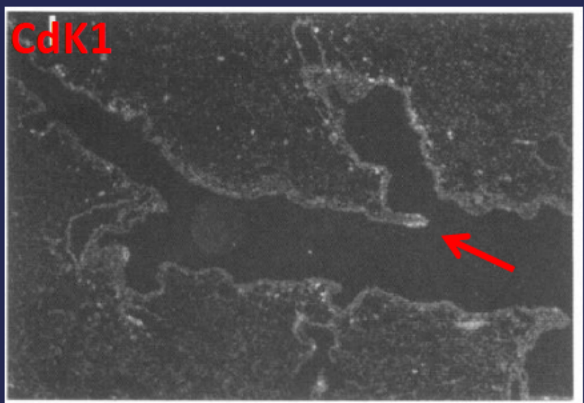


from Van Winkle et al . Am J Pathol. 151 (443-459) 1997

Cell Proliferation following Acute i.p. NA Exposure

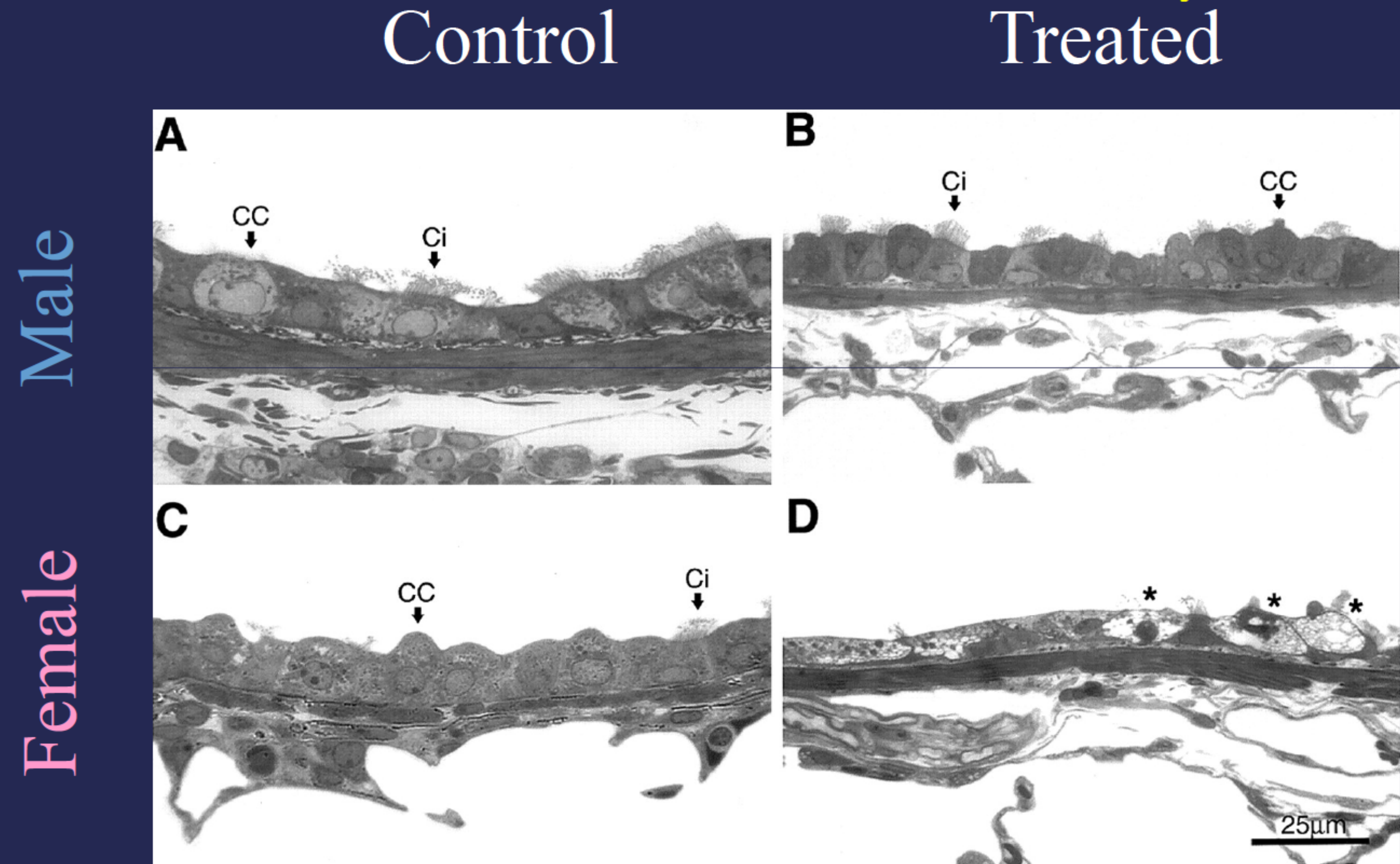






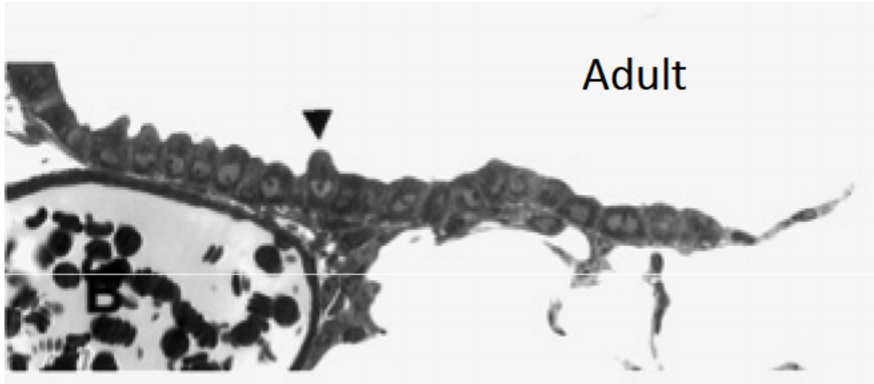
Van Winkle et al AJP:Lung 1995
Stripp et al AJP: Lung 1995
Lawson et al Am J Pathol 2002

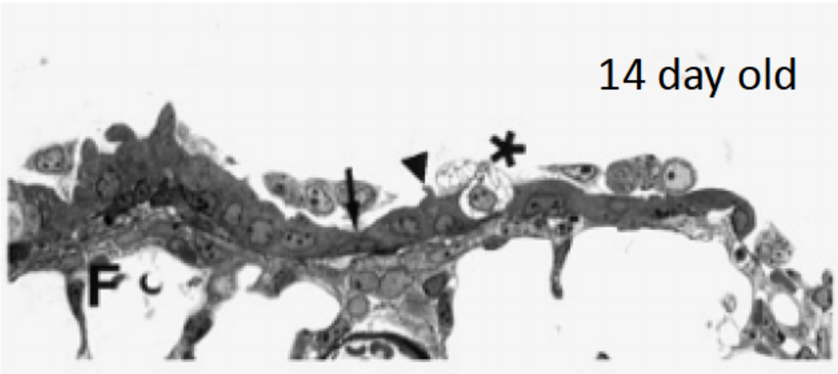
Female Mice are more susceptible than Male mice to NA toxicity

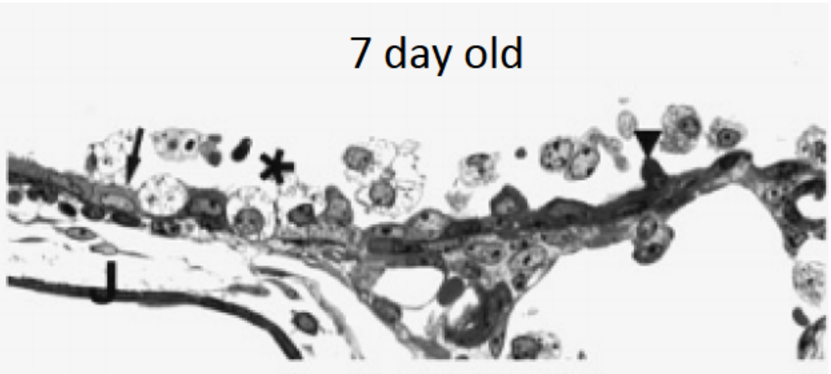


200 mg/kg ip from Van Winkle et al 2002 AJP: Lung p L1122-34

Neonatal mice are more susceptible than adult mice to NA toxicity
25 mg/kg ip







Fanucchi et al TAAP 1997 144(1):96-104

Repeated Inhalation or Injection of Naphthalene causes "Tolerance"

Tolerance is resistance to a high challenge dose following a week or more of exposure to repeated doses well below the LD50

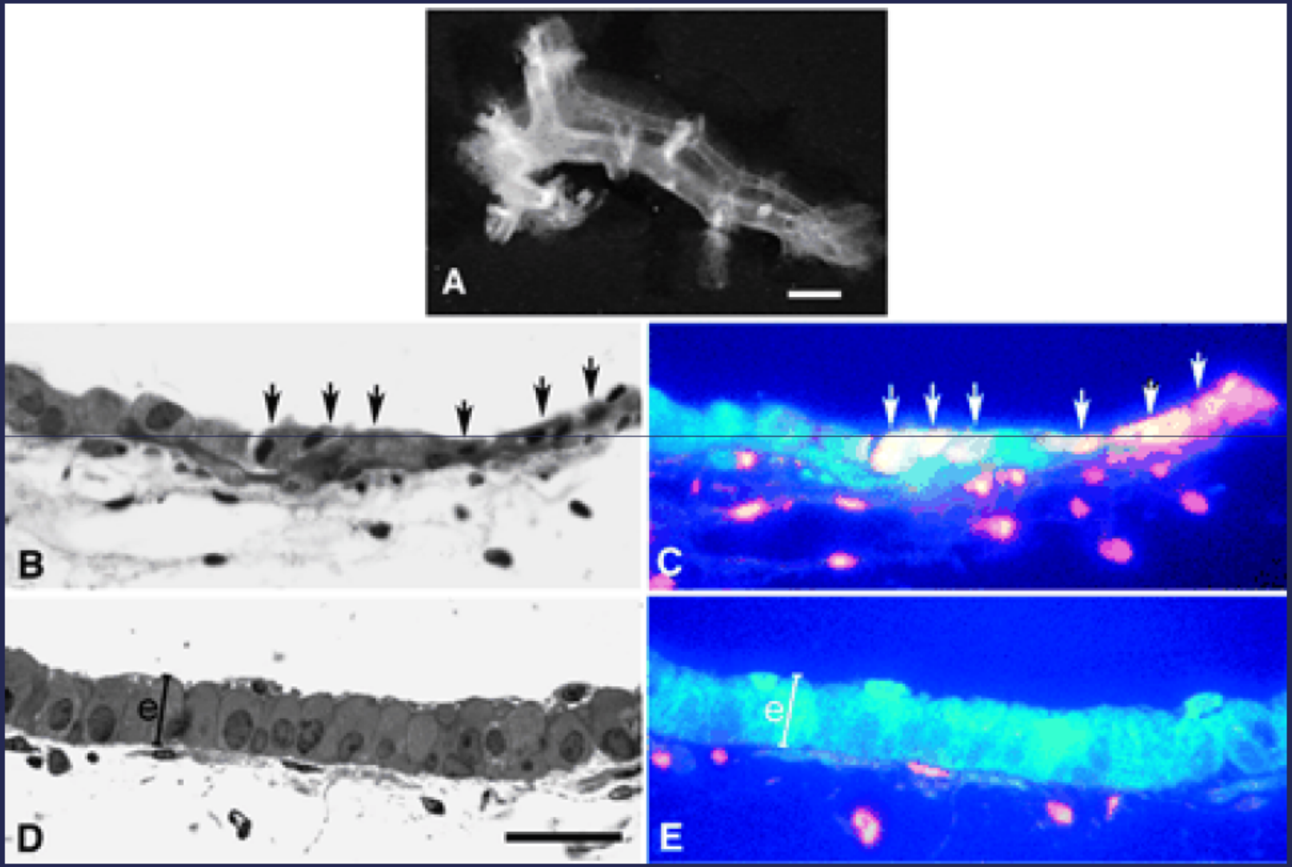
-NA i.p. tolerance *Lakritz et al 1996; O'Brien et al 1989*

-NA inhalation tolerance *West, Van Winkle et al 2003*

- incomplete tolerance i.p. in females Sutherland et al 2012

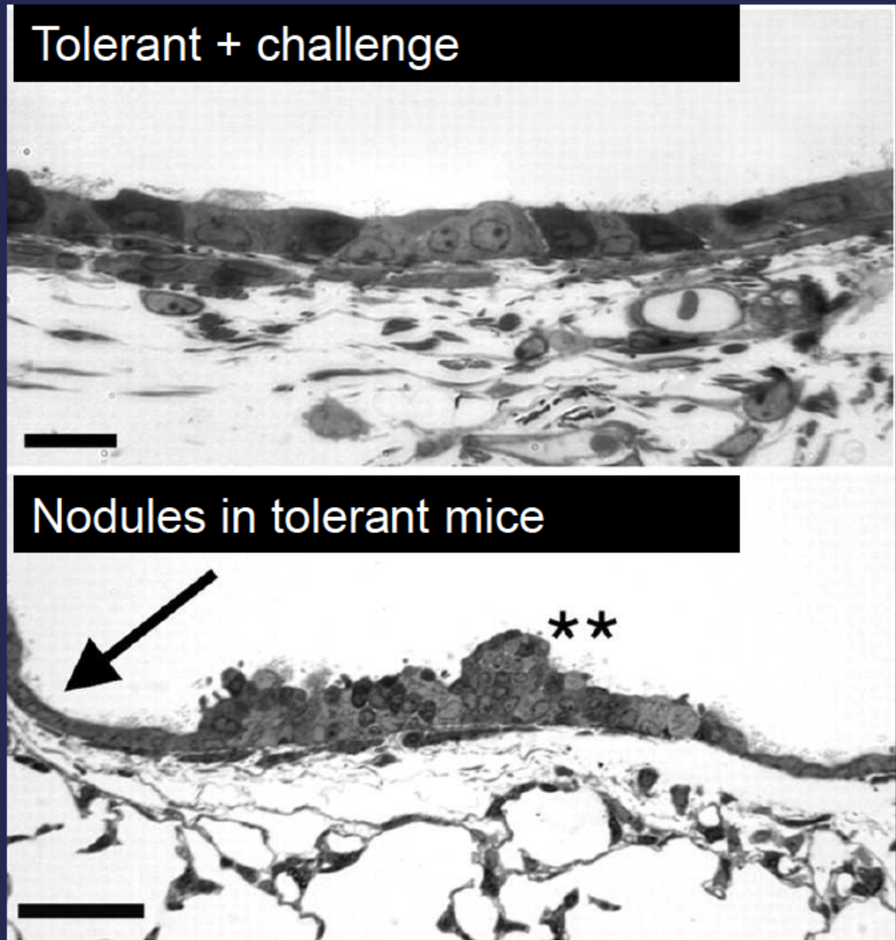
- tolerance is due to induction of gamma GCS West et al 2002

A property intrinsic to the airway epithelium makes it "tolerant"



Repeated Inhalation Exposures to the Bioactivated Cytotoxicant Naphthalene (NA) Produce Airway-Specific Clara Cell Tolerance in Mice Jay A. A. West*,1, Laura S. Van Winkle*, Dexter Morin , Chad A. Fleschner*, Henry Jay Forman and Charles G. Plopper* Toxicological Sciences 75, 161-168 (2003)

Morphology of Epithelium in NA Tolerance (inhaled NA)



West J A A et al. Toxicol. Sci. 2003;75:161-168

Other info re: Mode of Action

- Glutathione depletion occurs early, before tox
- P450 required
- Protein binding of reactive metabolites
- Naphthalene epoxide and downstream metabolites are toxic to Clara cells (Chichester et al studies)
- CYP2F2 contributes to mouse lung Clara cell toxicity- lessons from the knockout mouse
- Female mice are more susceptible than male mice to acute toxicity

Ethylbenzene

Ethylbenzene

- Information concerning the carcinogenicity of ethylbenzene in animals comes from an NTP-sponsored bioassay in male and female rats and mice exposed to 0, 75, 250, or 750 ppm ethylbenzene for up to 2 years (NTP 1999).
- NTP (1999) concluded that ethylbenzene showed **some evidence of carcinogenic activity in male mice based on increased incidence of alveolar/bronchiolar neoplasms**(NTP 1999).
- Lung: alveolar/ bronchiolar adenoma (5/50, 9/50, 10/50, 16/50); alveolar/ bronchiolar adenoma or carcinoma (7/50, 10/50, 15/50, 19/50)

Evaluation of Potential Modes of Action of Inhaled Ethylbenzene in Rats and Mice

TABLE 4
Treatment-related Effects in B6C3F1 Mice in the One-week Study

	Males			Females		
Exposure (ppm)	**0**	**75**	**750**	**0**	**75**	**750**
Relative liver weight	6.07 (0.46)	5.88 (0.29)	6.45 (0.44)*	5.39 (0.16)	5.44 (0.25)	6.24 (0.40)*
Relative lung weight	0.706 (0.045)	0.724 (0.047)	0.680 (0.051)	0.793 (0.079)	0.786 (0.022)	0.747 (0.064)
Liver S-phase DNA synthesis-LI%						
Centrilobular	1.89 (1.58)	2.77 (2.06)	23.11 (11.45)*	8.14 (3.45)	8.68 (4.32)	24.40 (7.24)*
Midzonal	1.87 (1.71)	4.26 (2.25)	11.00 (7.05)*	8.20 (2.76)	9.01 (3.20)	17.40 (6.44)*
Periportal	1.05 (1.05)	2.14 (1.77)	2.82 (2.20)	4.38 (1.27)	7.39 (3.88)	6.30 (3.11)
Lung S-phase DNA synthesis-LI%						
Small airways	3.47 (1.85)	NA	9.73 (5.80)*	5.11 (3.89)	NA	12.74 (10.73)*
Alveoli	6.63 (4.08)	NA	7.80 (4.51)	5.53 (3.96)	NA	5.33 (2.41)

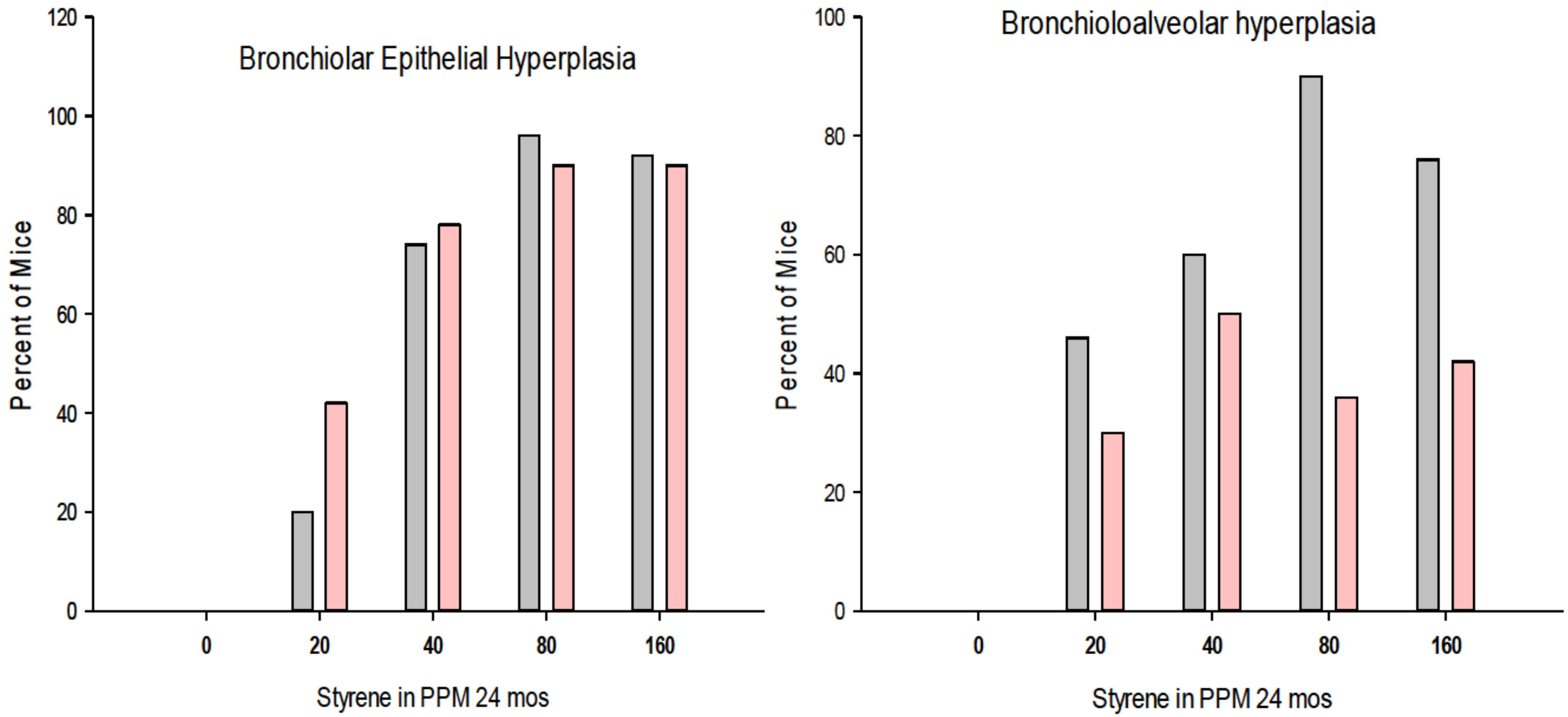
Mice in the Four-week

	Males		Females	
Exposure (ppm)	**0**	**750**	**0**	**750**
Relative liver weight	5.66 (0.49)	6.06 (0.26)	5.31 (0.15)	6.00 (0.20)*
Relative lung weight	0.693 (0.040)	0.699 (0.082)	0.741 (0.039)	0.731 (0.040)
Centrilobular	2.09 (1.21)	9.48 (5.03)*	12.35 (5.23)	19.29 (8.34)
Midzonal	3.24 (1.85)	10.11 (5.66)	13.97 (6.83)	17.99 (6.69)
Periportal	3.34 (2.08)	7.81 (3.87)*	12.52 (5.45)	14.76 (4.90)
Small airways	3.99 (1.11)	7.27 (3.27)	4.93 (1.70)	10.62 (5.47)
Alveoli	8.00 (1.27)	4.92 (1.94)*	8.43 (2.67)	9.60 (3.80)

Stott, WT et al Toxicol Sci. 2003 Jan;71(1):53-66

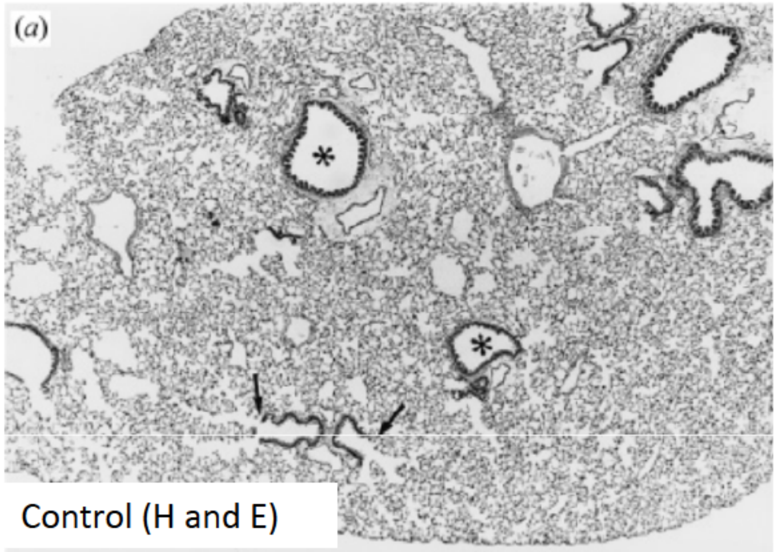
Styrene

24 mos Styrene Oxide vapor in Male/Female Mice

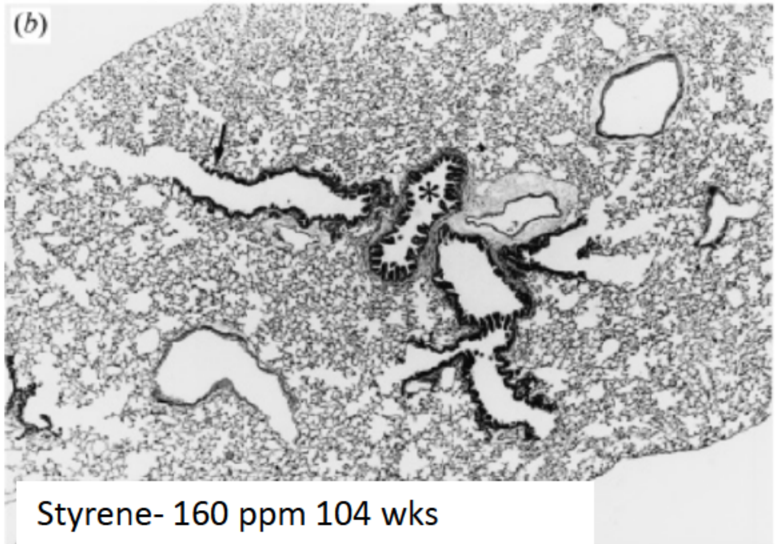


Cruzan et al 2002 Reg Toxicol and Pharm 35, 308-319

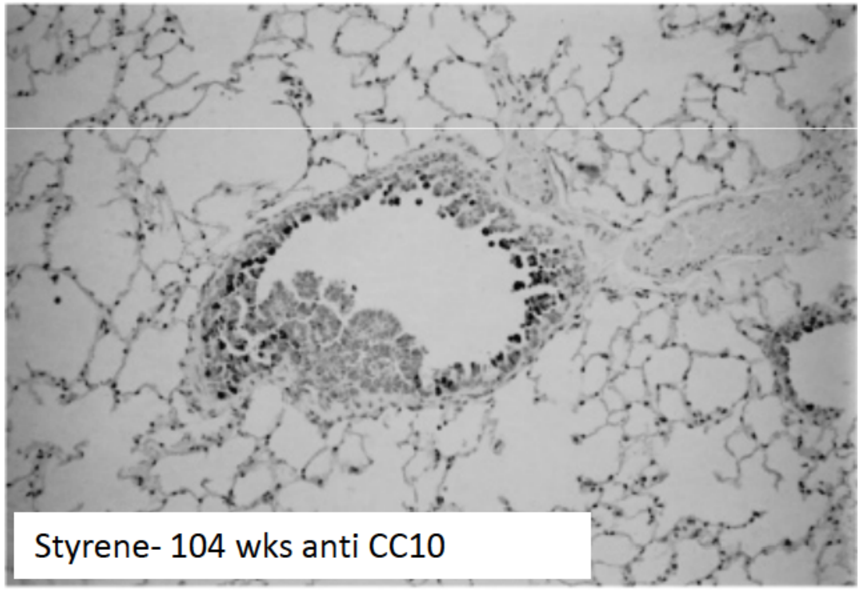
Is the Club (Clara) cell a target?



Control (H and E)



Styrene- 160 ppm 104 wks



Styrene- 104 wks anti CC10

Cruzan, G et al (2001) Journal of Applied Toxicology 21:185-198

Lung cell fractions enriched for CC have enhanced styrene metabolism- but is it the target?

TABLE 4
Metabolism of Styrene to Styrene Oxide by Mouse and Rat Isolated Lung Cells

% Clara	% Type II	R enantiomer [a]	S enantiomer [a]	R/S
Mouse				
18.3 ± 3.5[b]	33.5 ± 4.9[b]	19.4 ± 4.1	6.9 ± 2.2	3.62 ± 1.09
55.8 ± 8.0[b]	6.5 ± 2.5[b]	83.3 ± 27.7	23.0 ± 8.2	3.98 ± 0.75
Rat				
12.8 ± 3.2[c]	42.3 ± 4.1[c]	3.7 ± 1.1	8.0 ± 2.6	0.47 ± 0.01
37.3 ± 9.0[c]	4.0 ± 1.0[c]	11.2 ± 3.6	11.0 ± 3.2	1.02 ± 0.09

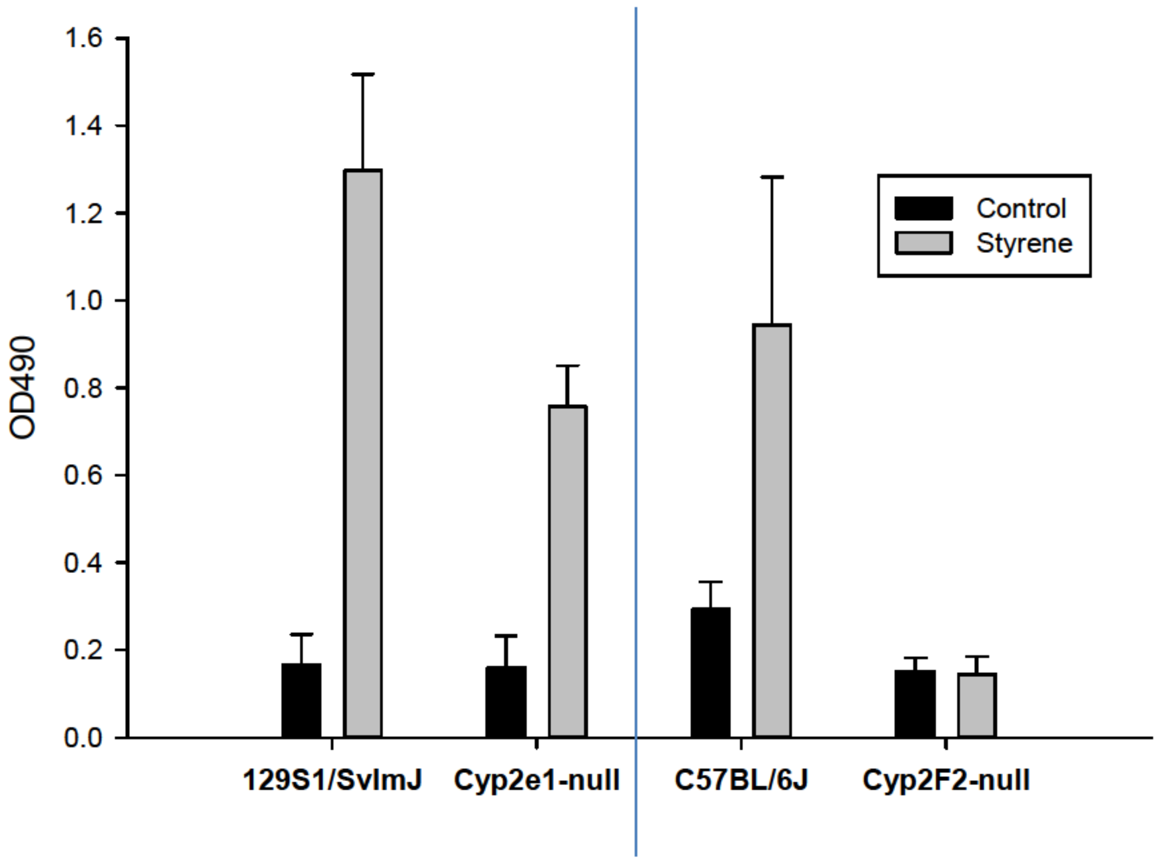
Note. R and S enantiomer values in pmols/10^6cells/min.
[a] Calculated on basis of total number of nucleated cells.
[b] Percent is mean ± SE for 4 experiments.
[c] Percent is mean ± SE for 3 experiments.

Hynes et al Tox Sci 51, 195-201 (1999)

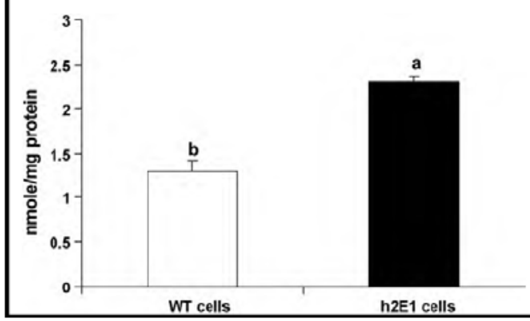
Decrease in labelling index of terminal bronchioles of Cyp2F2 null mice exposed to either styrene or styrene oxide for 5 days (Cruzan et al 2012) compared to styrene exposed WT indicates involvement of CYP2F2 in toxicity. Note that dosing was ip.

CYP2E1-null and Cyp2F2-null mice LDH in BALF- is it CYP2F?

Mice were given 6nmol/kg styrene ip BALF was assessed for LDH activity (Shen, S et al [Chem Res Toxicol.](#) 2013 Dec 19. Epub)



Increased protein covalent binding in cells with increased cyp2E



Yuan, W et al(2010) Chem-Biol Interactions 186:323-330.

What is the role of the liver?

Table 2
Toxicity of styrene in wild-type and hepatic cytochrome P450 reductase knockout mice.

Strain	Treatment	BALF			
		N	Cells[b]	*N*	Protein[c]
WT	Control	7	32 ± 9[f]	7	336 ± 32[f]
WT	Styrene[a]	9	633 ± 97[g]	9	740 ± 83[g]
KO	Control	6	43 ± 11[f]	6	379 ± 68[f]
KO	Styrene[a]	8	61 ± 15[f]	8	429 ± 68[g]

Within each column values with different superscripts (f, g) are significantly different ($p < 0.05$).

[a] 600 mg/kg ip 24 h prior to sacrifice.

[b] Cells per microliter.

Carlson. Toxicology *294 (2012) 104– 108*

Summary Questions:

- **Is there clear morphologic evidence of club (Clara) cell cytotoxicity?**
  - Naphthalene- yes
  - Styrene – not in vivo, some evidence from in vitro biochemical studies with isolated cells
  - Ethylbenzene - no
- **Is there a clear temporal distinction between cytotoxicity (from EM or histopath) and proliferation in terminal bronchiolar epithelial cells?**
  - Naphthalene- yes, acutely. Not clear that these are separate under conditions of repeated exposure and likely overlaps.
  - Styrene – no, cytox not well defined on a cellular basis in intact tissue
  - Ethylbenzene – no, cytox not well defined on a cellular basis in intact tissue
- **Are there species differences in response in the lung?**
  - Naphthalene- yes for both cytotoxicity and tumors in lungs of mice (female) and not rats
  - Styrene – tumors in mice but not rat lungs. Cytox unclear
  - Ethylbenzene- tumors in mice (male) but not rat lungs. Cytox unclear