# Pharmacokinetics and Pharmacodynamics of Naphthalene

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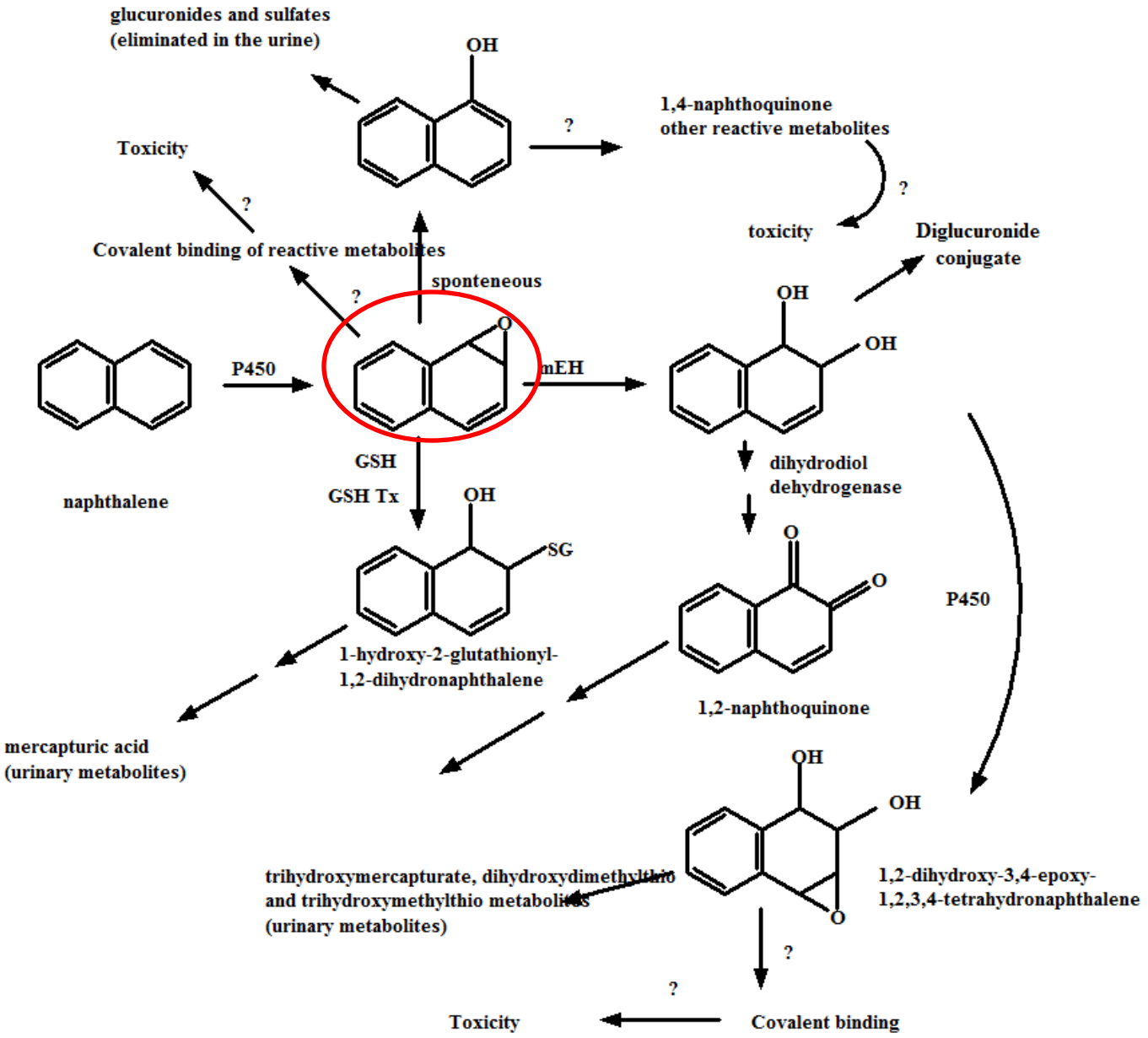
# Naphthalene Metabolism



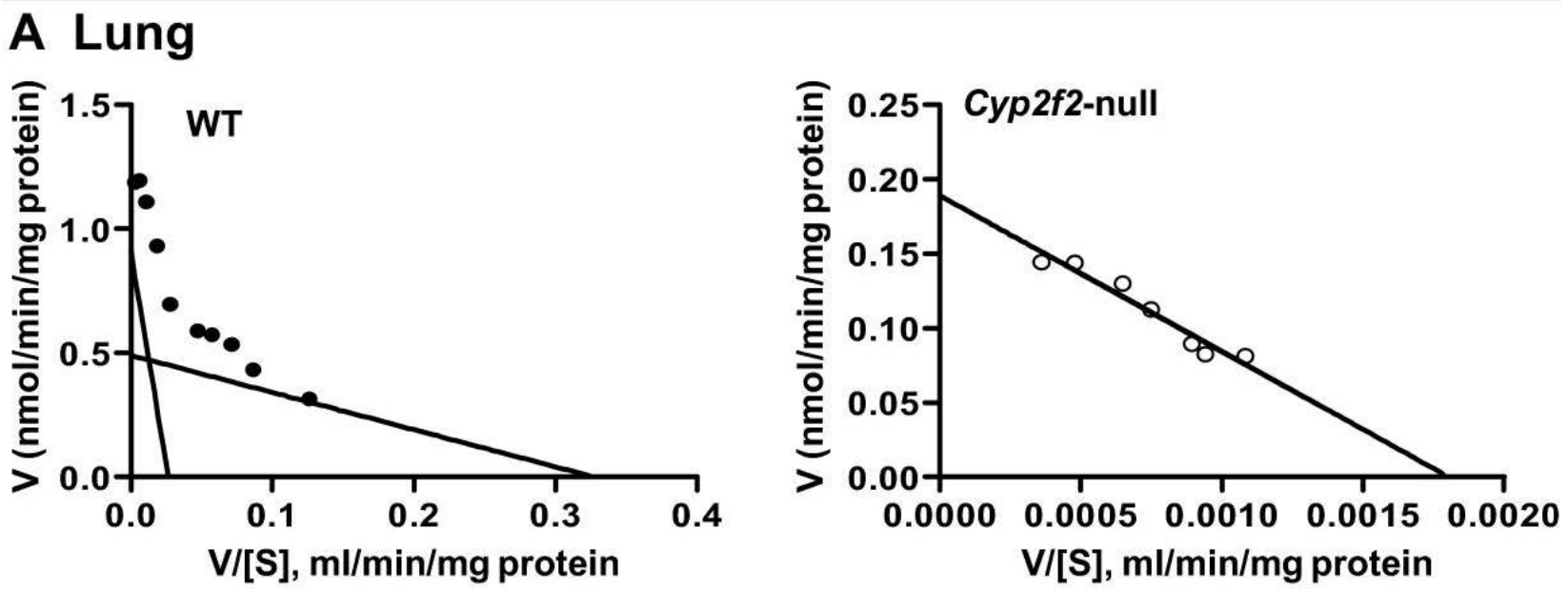
# Evidence for the involvement of reactive, P450 generated, GSH depleting metabolites in mouse lung toxicity

| Pretreatment | Airway epithelial Injury |
|---|---|
| None | -- |
| Piperonyl Butoxide | ↓ ↓ ↓ ↓ |
| Diethyl maleate | ↑ ↑ ↑ ↑ |

Warren et al., 1982

# Importance of CYP2F2 in mouse lung NA metabolism

Microsomal NA metabolism in lung. Cyp2F2 null mice have ~ 160 fold decreased catalytic efficiency compared to WT




Li, L et al. JPET 2011. 339 (1):62-71

# Correlation of Lung Covalent Binding with Toxicity

| Pretreatment | Airway epithelial Injury | Whole Lung Covalent Binding |
|---|---|---|
| None | -- | -- |
| Piperonyl Butoxide | ↓ ↓ ↓ ↓ | ↓ ↓ ↓ ↓ |
| Diethyl maleate | ↑ ↑ ↑ ↑ | ↑ ↑ ↑ ↑ |

**Warren et al., 1982**

# Significance of Protein Adducts

- Overall reactive metabolite binding correlates with toxicity
  - Dose responsive, binding precedes earliest signs of toxicity
  - Binding much higher in airway epithelium than in residual lung
  - Pretreatments which alter severity of toxicity, alter binding levels
- Binding to critical proteins thought to be common mechanism for toxicities associated with acetaminophen, 4-ipomeanol and others

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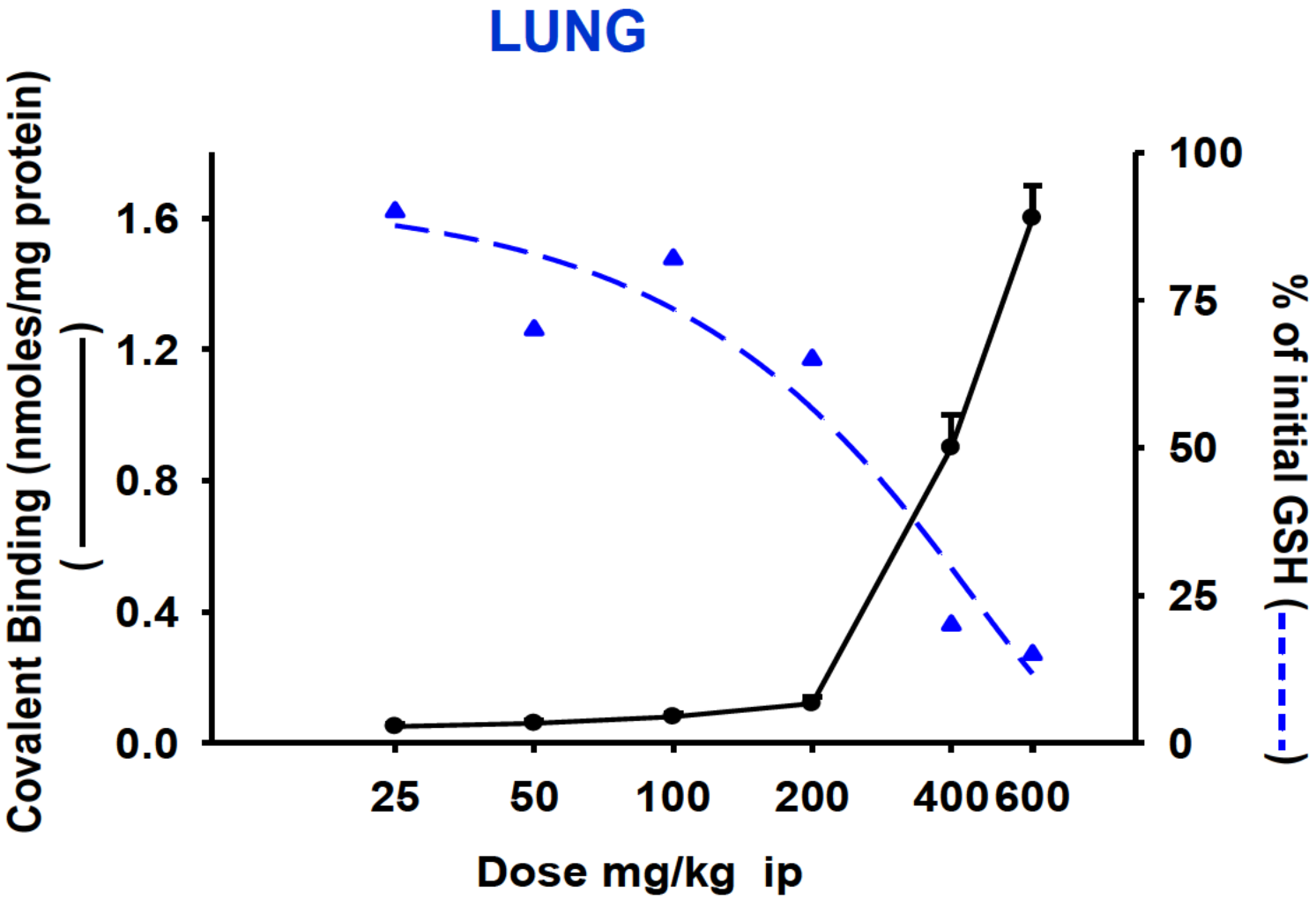
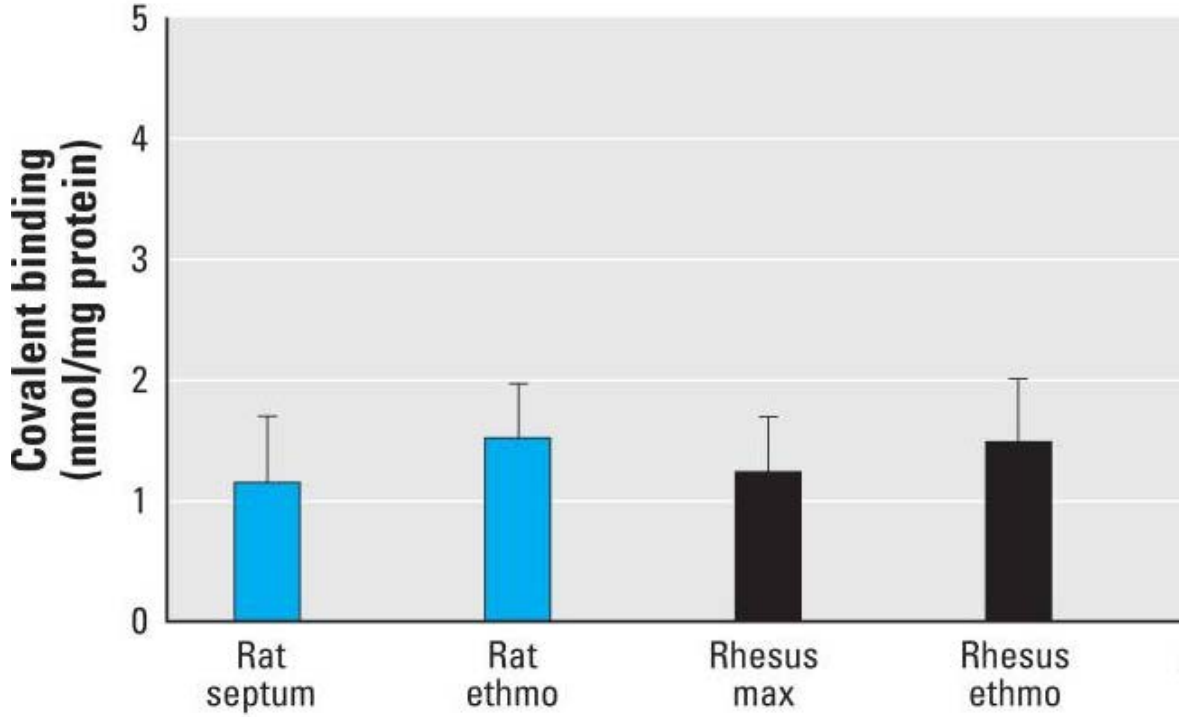
DNA adducts in lung following in vivo or ex vivo treatment with naphthalene have not been reported

# Formation of reactive naphthalene metabolites in vivo-dose threshold-mouse




Warren et al., 1982

# Reactive NA Metabolite Binding Species comparison

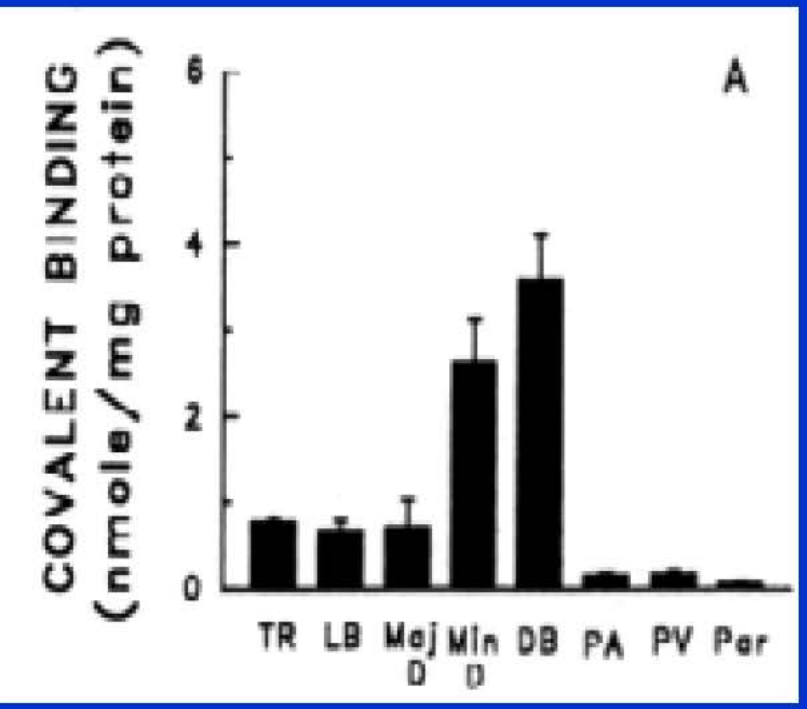



Above incubations are 250 uM 2 hr in DeStefano-Shields et al Environ Health Perspect. 2010 May; 118(5): 647–652.

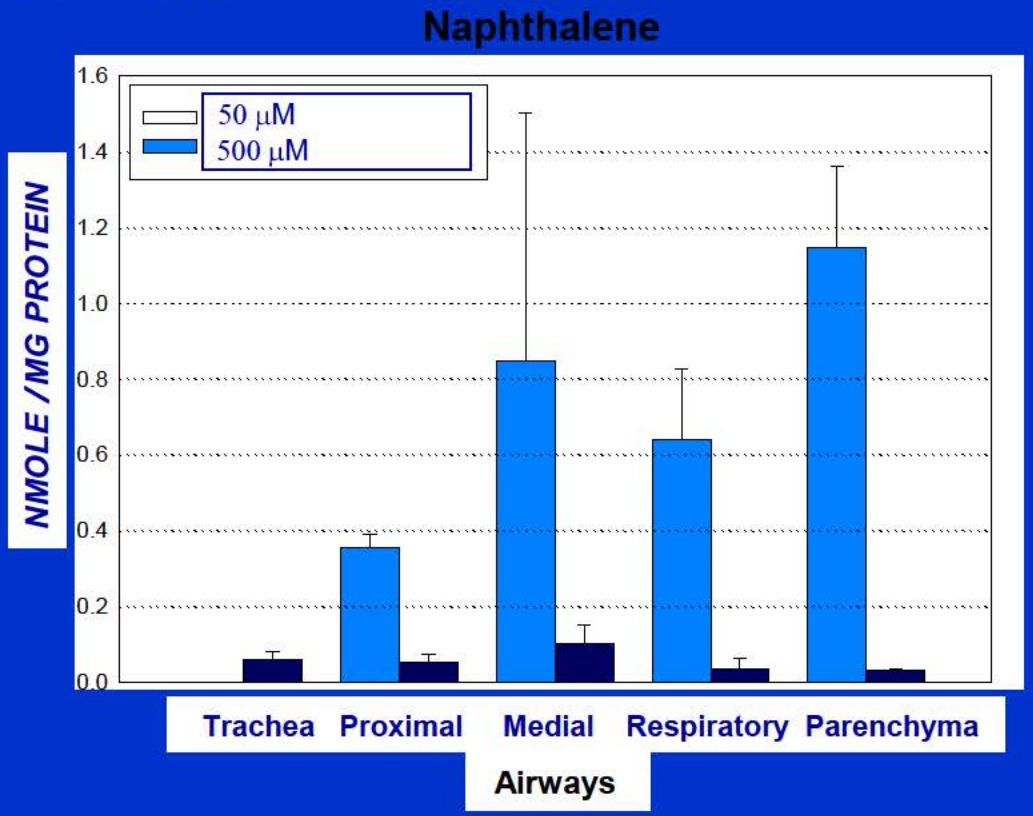
Exposed Rhesus Macaques Incub
and 50 M-10000dpm/nmole **Napl**

# Total reactive metabolite formation in dissected airways: mice vs. rhesus macaques

**1 : ~ 0.3**

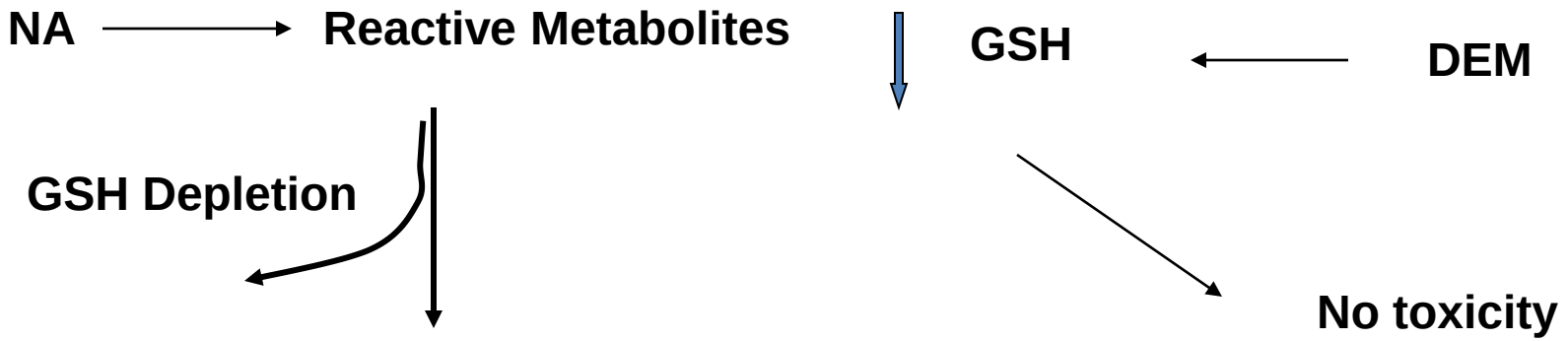



**A.** Total reactive metabolite formation in dissected mouse airways incubated with NA (500 µM). Cho et al., 1994




Total reactive metabolite formation in dissected monkey airways incubated with NA (500 µM and 50 µM). Boland et al JPET 2004 Aug;310(2):546-54.

# Possible sequence of events leading to cytotoxic injury




**Cell is left without normal protective mechanisms protein thiol oxidation ensues leading to protein unfolding. The cell attempts to correct this but critical proteins involved in protein folding are adducted.**

**Toxicity**

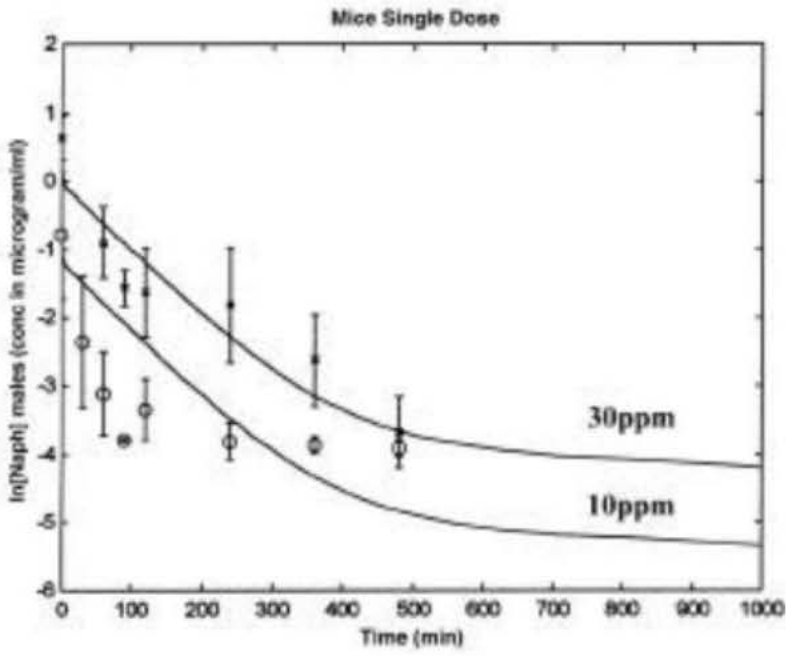
**Glutathione depletion is a necessary but not sufficient event for toxicity**

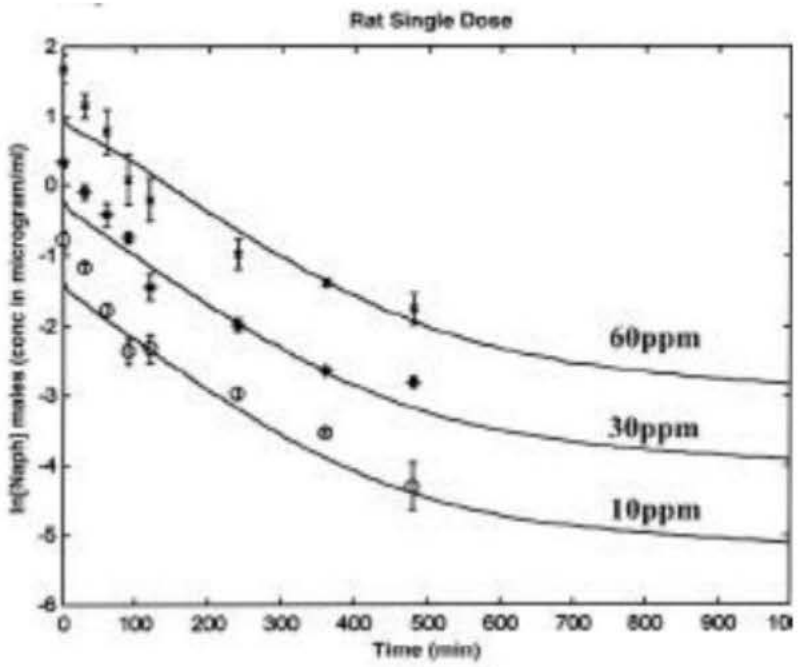
# Which metabolites adduct proteins?

- NAoxide, NAdiolepoxide, both NQ, all form protein adducts with model peptides, with NAoxide adducting fewer sites.(Pham et al 2012 Chem Biol Interact 199 (2):120-128 and Pham et al 2012 PLOS One 7(8):e42053 )
- Epoxide binding to sulfur nucleophiles was minor relative to binding by the 1,2-naphthoquinone in isolated Clara cell incubations with naphthalene (Antibody based method in Zheng, J et al 1997 Chem Res Toxicol 10:1008-1014)

# Pharmacokinetics

- Blood levels of naphthalene were measured acutely for male rats and male mice from the NTP study (TR500 Appendix) shown below.




- Isolated perfused lung treated with naphthalene generated dihydrodiol and NA-GSH conjugates as 70% of total metabolites in perfusate. Thus circulating NA is metabolized in the lung *(Kanekal et al JPET 1991 256 (1): 391-401)*

# Liver metabolizes NA

Human liver microsomes metabolize naphthalene to a cytotoxic, nongenotoxic, protein reactive (reduced by addition of GSH) metabolite *Tingle, M et al Biochemical Pharm 46 (9) 1529-1538*

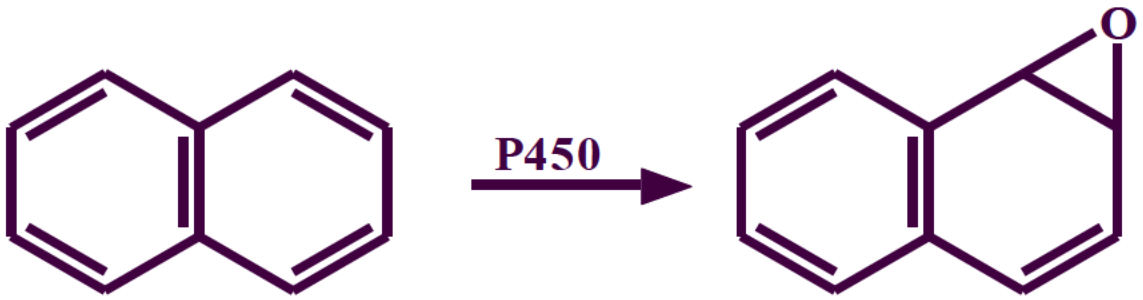
Pooled human liver microsomes metabolize NA resulting in diol and naphthol. *Cho, T et al. Drug Metab. Dispos., 34 (2006), pp. 176–183*

Mouse liver also metabolizes naphthalene at a similar total rate than mouse lung microsomes *Buckpitt et al 1987 Drug Metab Dispos 15(4):491-8*

# The role of the liver in lung tox?

- **The metabolites of naphthalene are stable enough to travel through the circulation and impact the lung.** NA oxide can escape hepatocytes and diffuse from hepatocytes. NA oxide in presence of protein has T1/2 of 11 min. *(Richieri and Buckpitt 1987, Buonarati el al 1989, Kanekal et al 1991)*
- **All metabolites cause changes in isolated perfused mouse lung , but differ in potency** *(Kanekal et al JPET 1990 252 (1): 428-37 and JPET1991 256(1):391-401)*
  - NA: decreased GSH, CC toxicity, increased reactive metab
  - NAoxide: decreased GSH, CC toxicity
  - NQs, and dihydrodiol did cause CC toxicity as an increase in vacuolated cells but this was much less than the oxide or NA
  - 1 naphthol did not cause Clara cell toxicity
- **Liver is <u>not required</u> for lung tox,** Clara cells in lung still a target in ex vivo systems. (*Kanekal et al above*)
- **Tolerance is due to a change intrinsic to the airway.** *(West et al Toxicol Sci 2003 Sep;75(1):161-8).*
- Elimination of liver P450 in HRN mouse increases circulating NA, but did not decrease circulating NA-GSH metabolites so **liver also has a key role in detox but the lung is very capable of metabolizing circulating NA in the absence of significant liver metabolism.** *(Li et al JPET 2011 Oct;339(1):62-71)*

# Importance of Kinetics of Metabolism




naphthalene

naphthalene epoxide

- Without conversion to the intermediate epoxide, naphthalene is toxicologically inert
- Exposure levels are likely to be low-therefore enzymes with high μM or low mM Km are unlikely to be important
- Overall P450 levels in primate lung are low-therefore high catalytic efficiency is essential if enzyme is to be important (high $V_{max}/K_m$ )
- Amounts of catalytically active protein present per cell are important

# Human cytochrome P450 genes expressed in different areas of the respiratory tract

| Tissue | CYPs detected |
|---|---|
| Nasal mucosa | 2A6, 2A13, 2B6, 2C, 2J2, 3A |
| Trachea | 2A6, 2A13, 2B6, 2S1 |
| Lung | 1A1, 1A2, 1B1, 2A6, 2A13, 2B6, 2C8, 2C18, 2D6, 2E1, 2F1, 2J2,2S1, 3A4, 3A5, 4B1 |
| Esophagus | 1A1, 1A2, 2A, 2E1, 2J2, 3A5 |

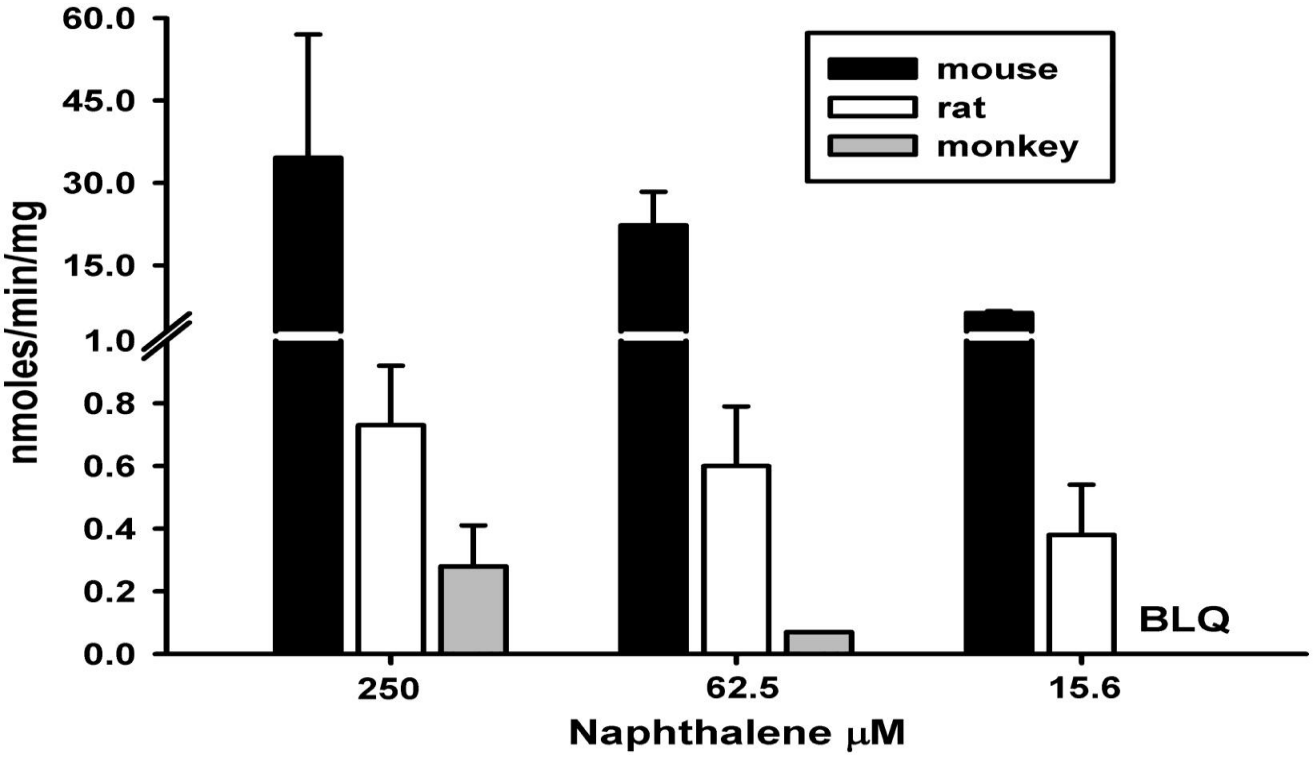
from Ding and Kaminsky, ARPT, 2003

# Comparison of kinetics of naphthalene metabolism in recombinant human P450s

| P450 isoform | $V_{max}$ pmoles/pmoles/min | $K_m$ μM |
|---|---|---|
| 1A1 | 9.1 | 111 |
| 1A2 | 35.8 | 73 |
| 2B6 | 20.2 | 58.6 |
| 2E1 | 8.4 | 10.1 |
| 3A4 | 8.1 | 60.7 |
| 2F2 (mouse) | 107 | 3 |

Cho, Rose and Hodgson, DMD, 2006

# Comparison of the rates of naphthalene metabolism in lung airway microsomes from mouse, rat and monkey.




Buckpitt et al 2013 Toxicology and Applied Pharmacology Volume 270, Issue 2 97 - 105

# Summary

- CYP2F2 (mouse) has the highest catalytic activities with naphthalene; the catalytic activities of CYP2F4 (rat) are identical
- CYP2F1 (human), 2F5 (Rhesus) are difficult to express as catalytically active proteins
- Rat vs mouse differences in metabolism and susceptibility can be accounted for, in part, by substantial differences in quantities of CYP2F protein present *(Baldwin et al JPET 2004 309(1):127-36)*

# Summary

- High susceptibility of mouse lung to naphthalene appears driven by CYP2F2.
- Excellent correlation between the catalytic efficiency of naphthalene metabolism in microsomes from different species and in toxicity of selective portions of the respiratory tract of rodents. In non human primates catalytic efficiencies are low.
- Formation of covalent protein adducts correlates with toxicity but whether this is a key step is not clear. Monkeys have a higher than expected level of covalent binding in the lung in comparison to the measured rate of metabolism.
- GSH depletion is a necessary but not sufficient step to cause lung toxicity (e.g. just depleting GSH does not cause Clara cell necrosis)
- The role of the liver in toxicity, and possibly carcinogenesis, is not well defined.
- The relative abundance of metabolites in intact systems (not microsomes), and following repeated exposures, is not well understood.