

# Using Biomarkers to Characterize Human Benzene Metabolism

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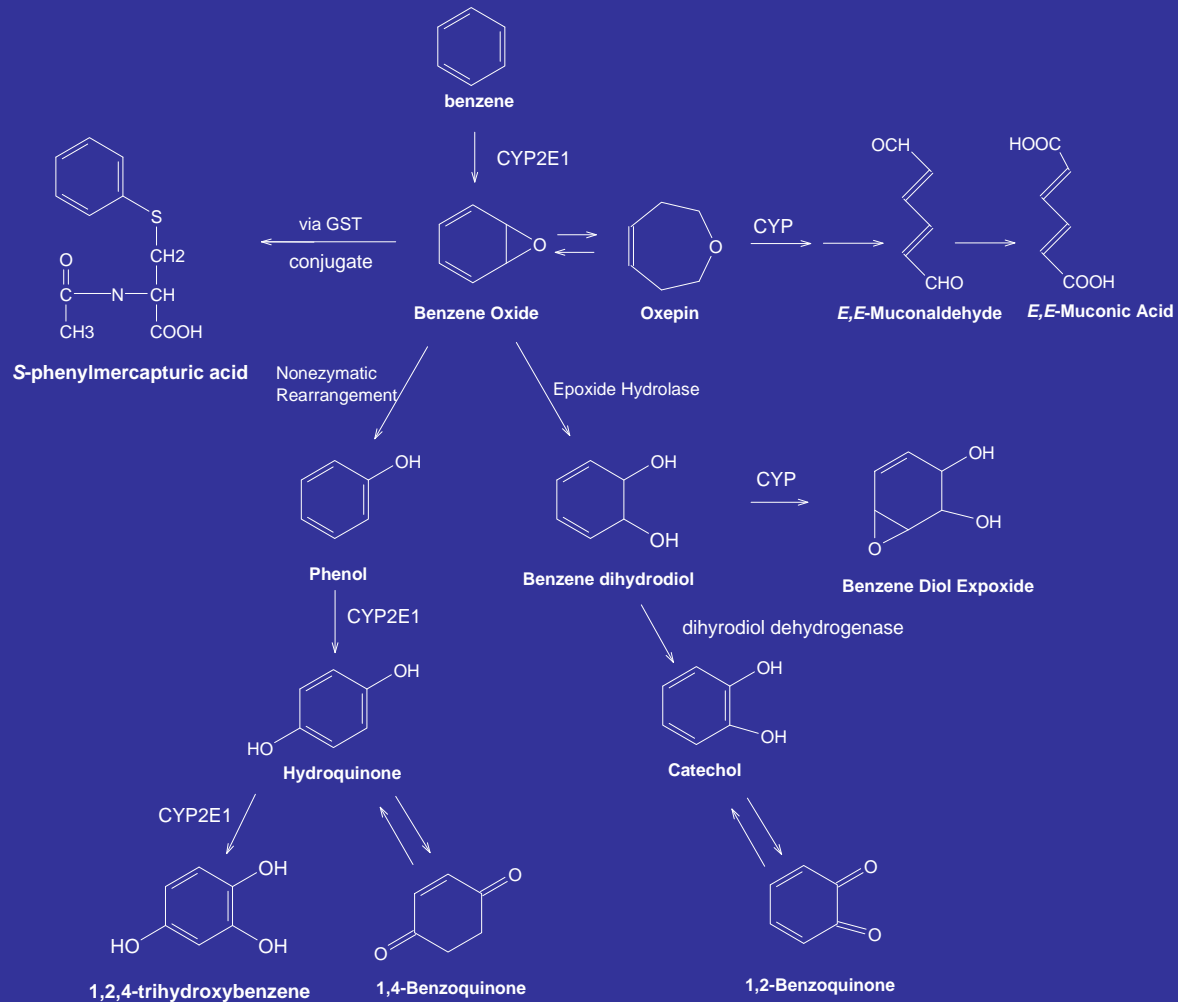
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# Benzene

- Human carcinogen
  - First linked with bone marrow toxicity in 1896 (Santesson, C. Arch Hyg Berl 31: 337) and with leukemia in 1928 Delore, P. and Borgomano, C. J Med Lyon 9: 227)
  - Dose-response poorly defined
  - Uncertain risks, particularly at low exposures
- Mechanism not completely understood
  - Linked to metabolism
- Metabolism is complex
  - Qualitatively similar in all mammals
  - Important quantitative differences among species
  - Can be affected by genetics (SNPs)
- Dose-related metabolism poorly characterized in humans

# Benzene Metabolism



# Benzene Biomarkers Among Chinese Workers

STUDY #1 (NCI, UC-Berkeley, UNC)

44 Exposed subjects and 44 controls in Shanghai, China

- Very high exposure (med. = 31 ppm)
- Focus on hematology and cytogenetics
- Developed and validated biomarkers of exposure (protein adducts, urinary benzene, and urinary metabolites)
- Evidence of saturable metabolism

Rothman *et al.*, *PNAS*, 1995

Rothman *et al.*, *AJIM*, 1996

Rothman *et al.*, *EHP* 1996

Rothman *et al.*, *Cancer Res*, 1997

Rothman *et al.*, *OEM*, 1998

Smith *et al.*, *Cancer Res*, 1998

Smith *et al.*, *PNAS*, 2000

Yeowell-O'Connell *et al.*, *Carcinogenesis*, 1998

Yeowell-O'Connell *et al.*, *CEBP*, 2001

Waidyanatha *et al.*, *Carcinogenesis*, 2001

Waidyanatha *et al.*, *Analyt Biochem*, 2004

Rappaport *et al.*, *J Chromatog B*, 2002



# Benzene Biomarkers Among Chinese Workers

## STUDY #2

134 Exposed subjects and  
51 controls in Tianjin,  
China

Lower exposure (med. = 3.1  
ppm)

- Focus upon hematology  
and biomarkers of  
exposure

Qu *et al.*, *AJIM*, 2000

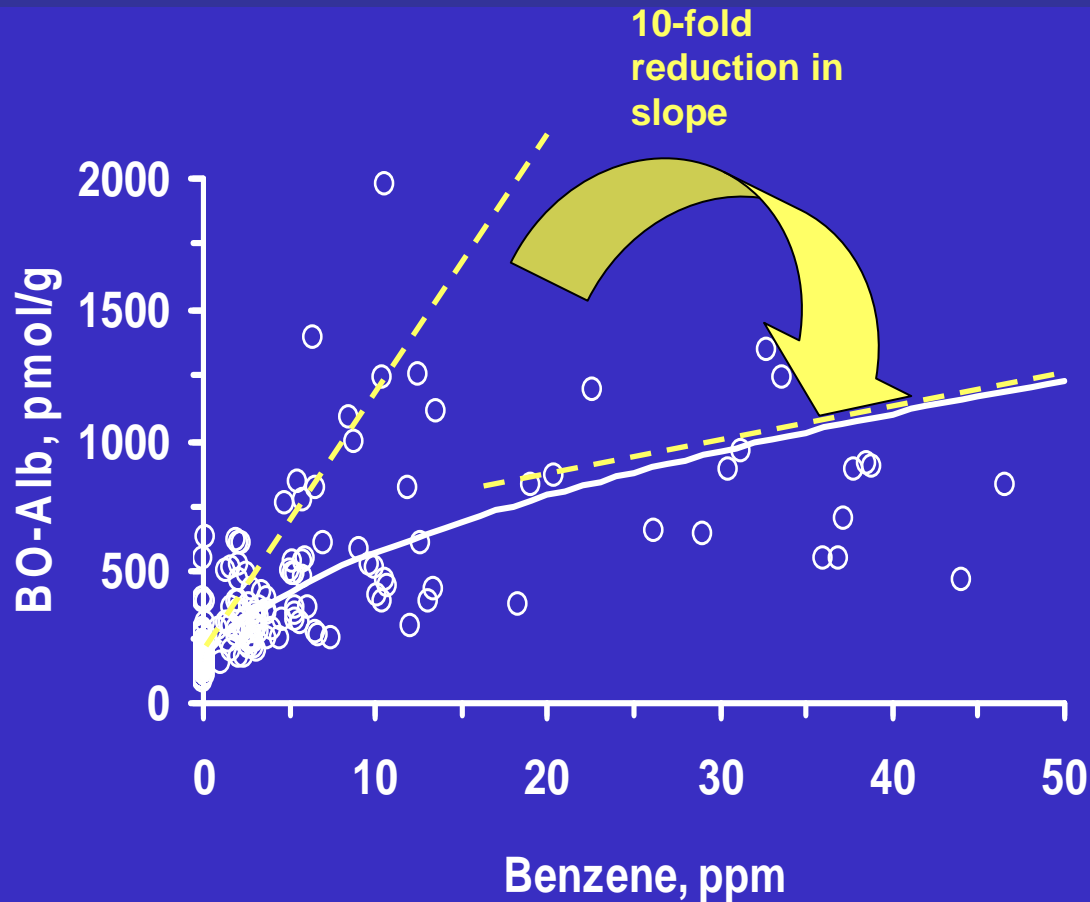
Qu *et al.*, *AJIM*, 2002

Melikian *et al.*, *J Chromatog (B)*, 2002

Rappaport *et al.*, *Cancer Res*, 2002



# Benzene in Air and BO-Alb Adducts (STUDY #2)



Saturation of benzene metabolism beginning at about 1 ppm (much lower than previously suggested)

Greater unit risk at low exposure levels

Motivated EPA to reduce benzene content in gasoline (2007)

CAN THIS FINDING BE VERIFIED?

# Benzene Biomarkers Among Chinese Workers

STUDY #3 (NCI, UC-Berkeley, UNC)

250 Exposed subjects and 140 controls in Tianjin, China

- Similar exposures (med. = 3.7 ppm)
- Focus on exposure-biomarker relationships and mechanism

*Vermeulen et al., Ann Occup Hyg, 2004*

*Lan et al., Science, 2004*

*Lan et al., Zhang et al., Chem-Biol Interact, 2005*

*Lan et al., Cancer Res, 2005*

*Vermeulen et al., PNAS, 2005*

*Chen et al., Carcinogenesis, 2006*

*Kim et al., Carcinogenesis, 2006*

*Kim et al., CEBP, 2006*

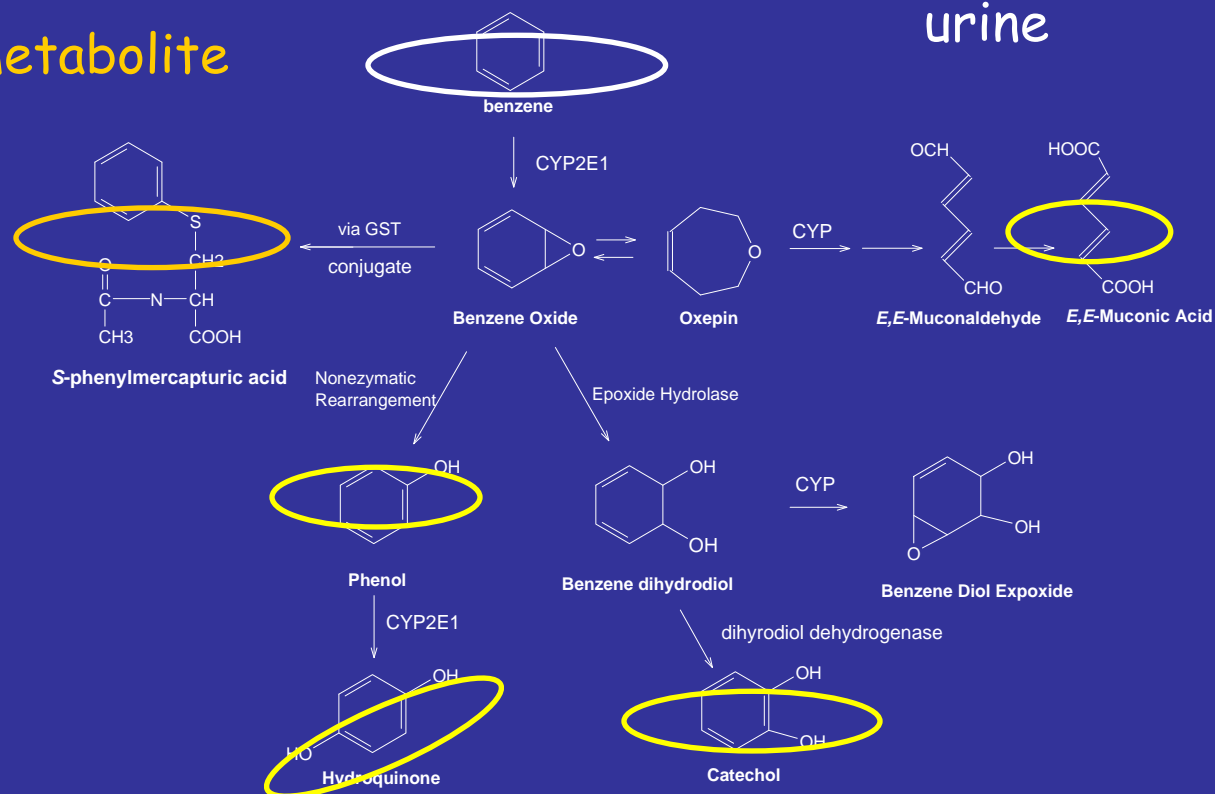
*Kim et al., PGEN, in press*



# Air Samples (n=2783) and Urinary Analytes (n=620)

Air; Unmetabolized benzene in urine

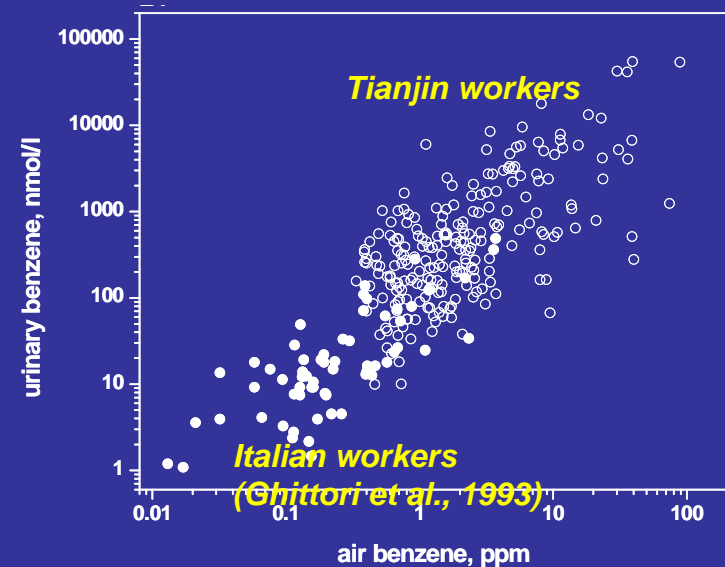
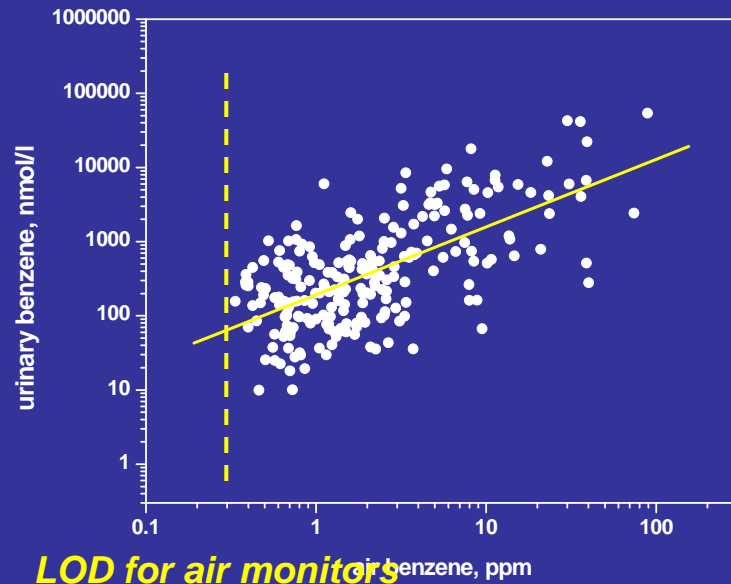
Minor metabolite



Major metabolites  
(99% of absorbed  
dose)

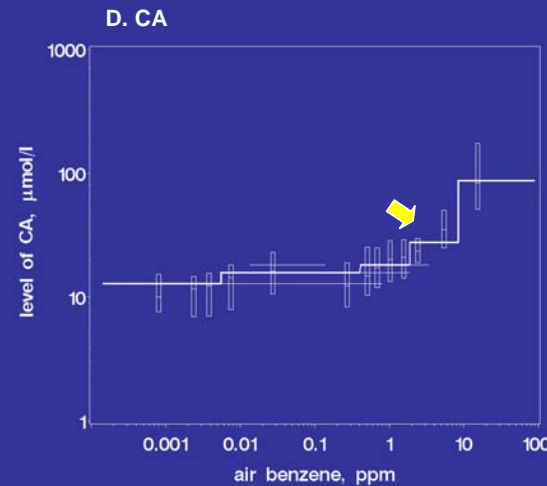
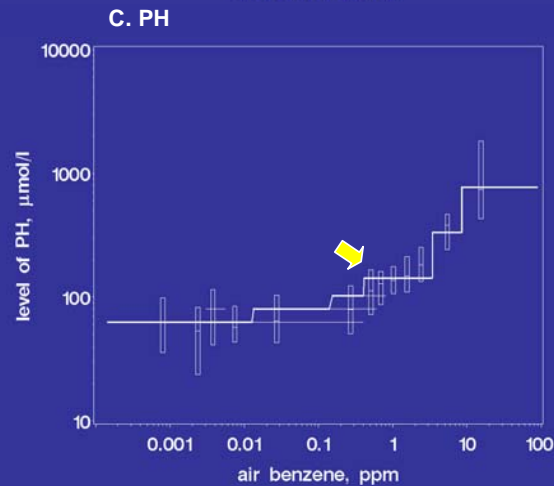
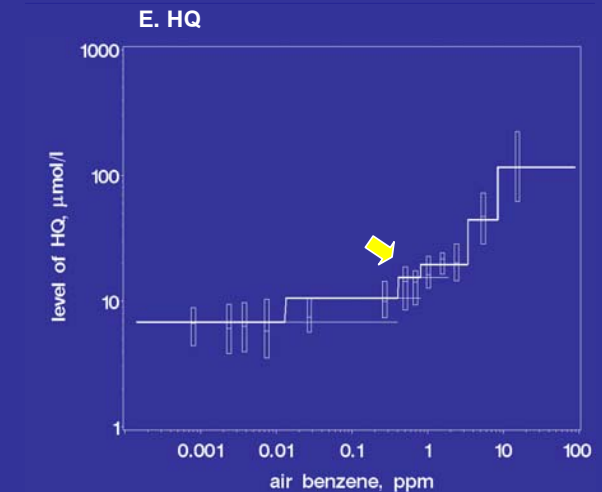
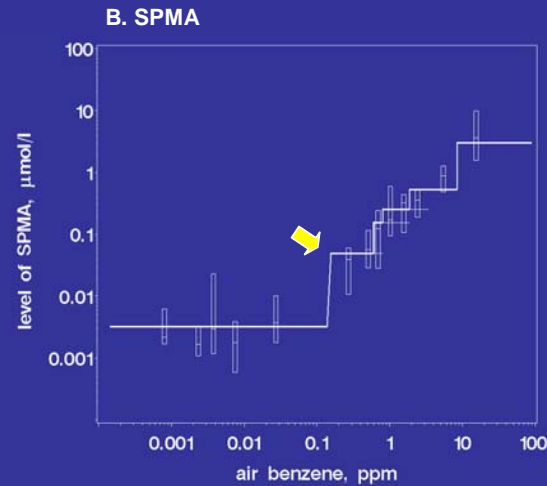
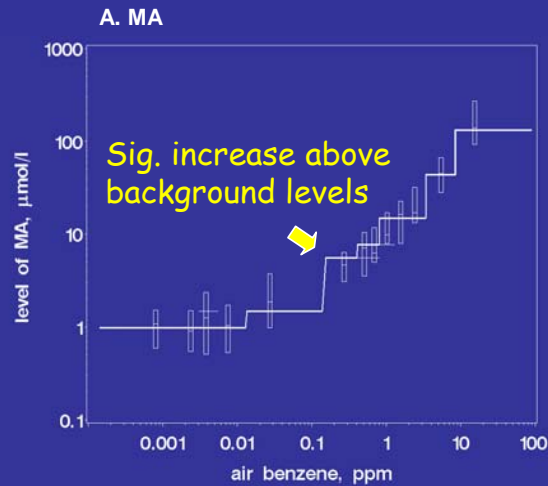


# Urinary Benzene vs. Exposure



Urinary benzene detected in all exposed AND control subjects - Used to predict benzene exposures in controls

# Metabolites vs. Exposure (Groups of 30 Workers)

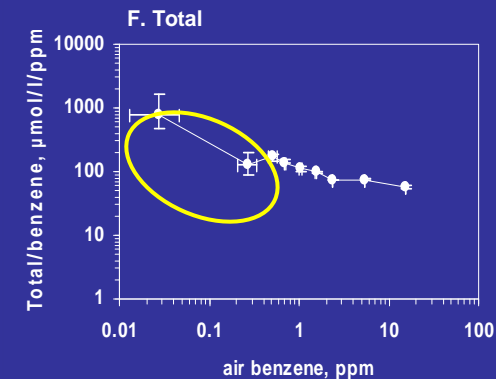
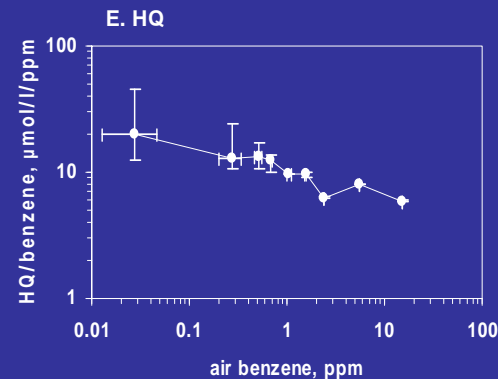
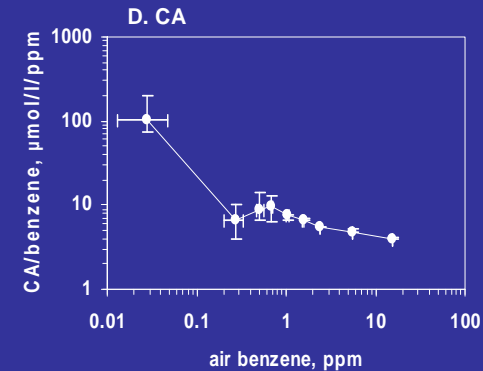
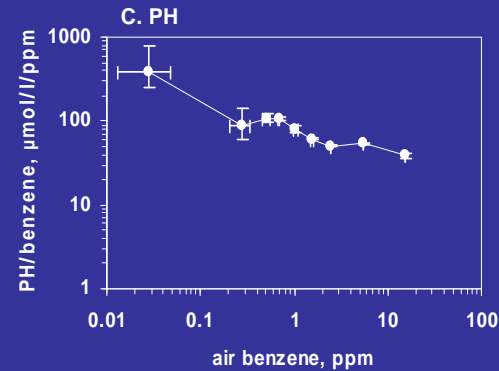
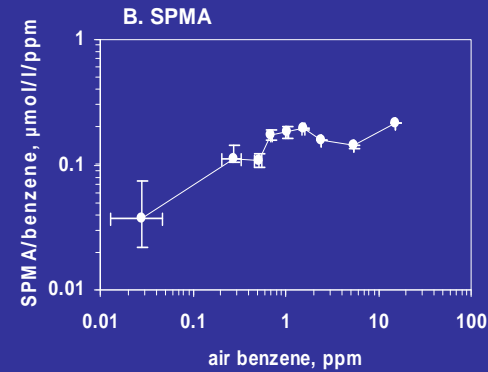
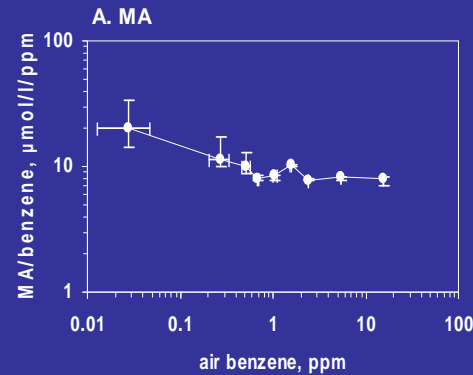


Urinary metabolites not useful for monitoring environmental exposures to benzene due to background sources (other than benzene)

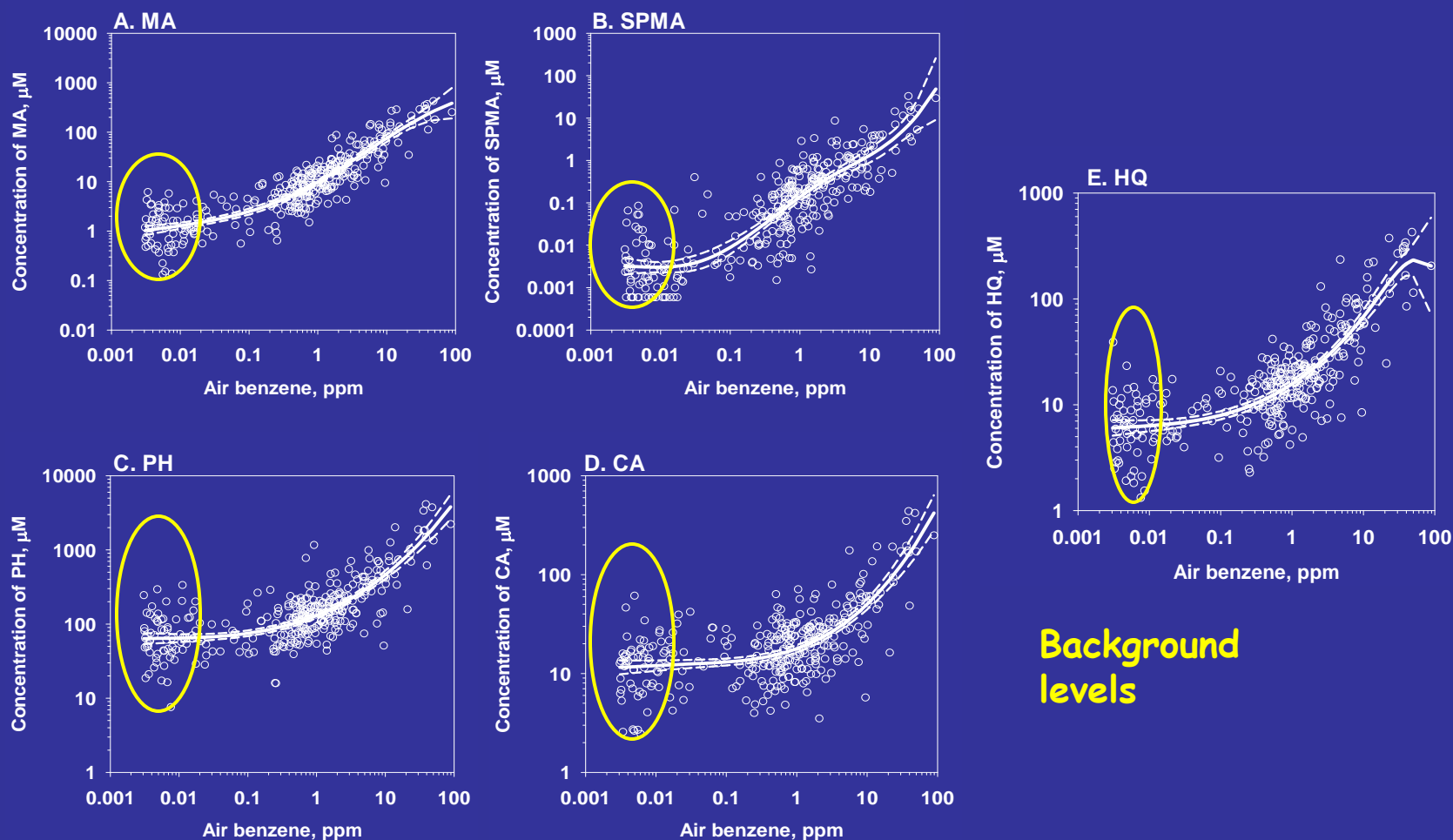
# Dose-specific Metabolism

Groups ( $n = 30$ ) after adjustment for background levels

• Reduced production of major & total metabolites at low exposures (0.01 - 1 ppm, never reported previously)



# Modeling Metabolite Levels (Natural Splines)



# Effects of Covariates [GLM+NS]

Metabolite	Adj.R <sup>2</sup>	Covariate	Parameter	
			Estimate	p-value
MA	0.812	Intercept	1.15	<0.0001
		Age	-0.016	0.001
		Sex (male)	-0.215	0.013
SPMA	0.744	Intercept	-6.04	<0.0001
		Age	-0.016	0.086
		Sex (male)	-0.347	0.032
PH	0.608	Intercept	4.22	<0.0001
		Age	-0.011	0.016
		Sex	-0.198	0.009
CA	0.506	Intercept	2.66	<0.0001
		BMI	-0.022	0.028
		Sex (male)	-0.243	0.007
		Smoking	0.323	0.001
HQ	0.689	Intercept	1.96	<0.0001
		Age	-0.013	0.003
		Sex (male)	-0.221	0.019
		Smoking	0.338	0.001

Age: metabolite production reduced 1-2%/year of life

Sex: females produce more metabolites than males

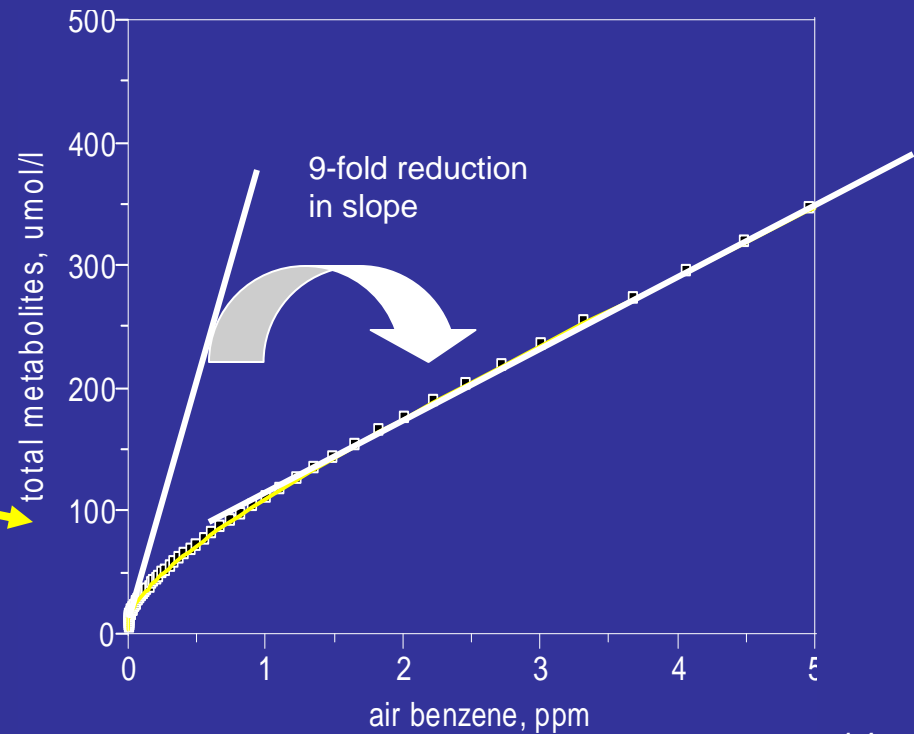
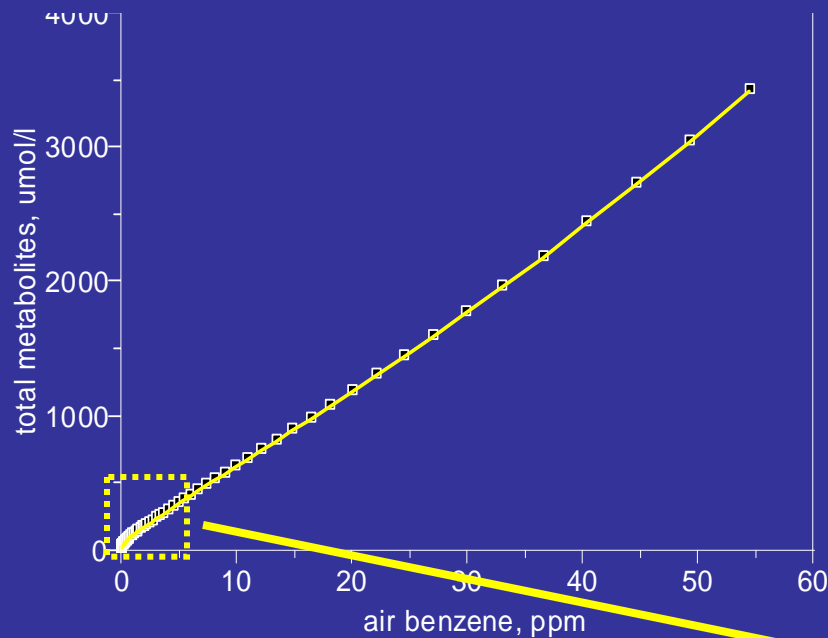
Smoking: Cigarette smoke contains HQ and CA

No effect of:

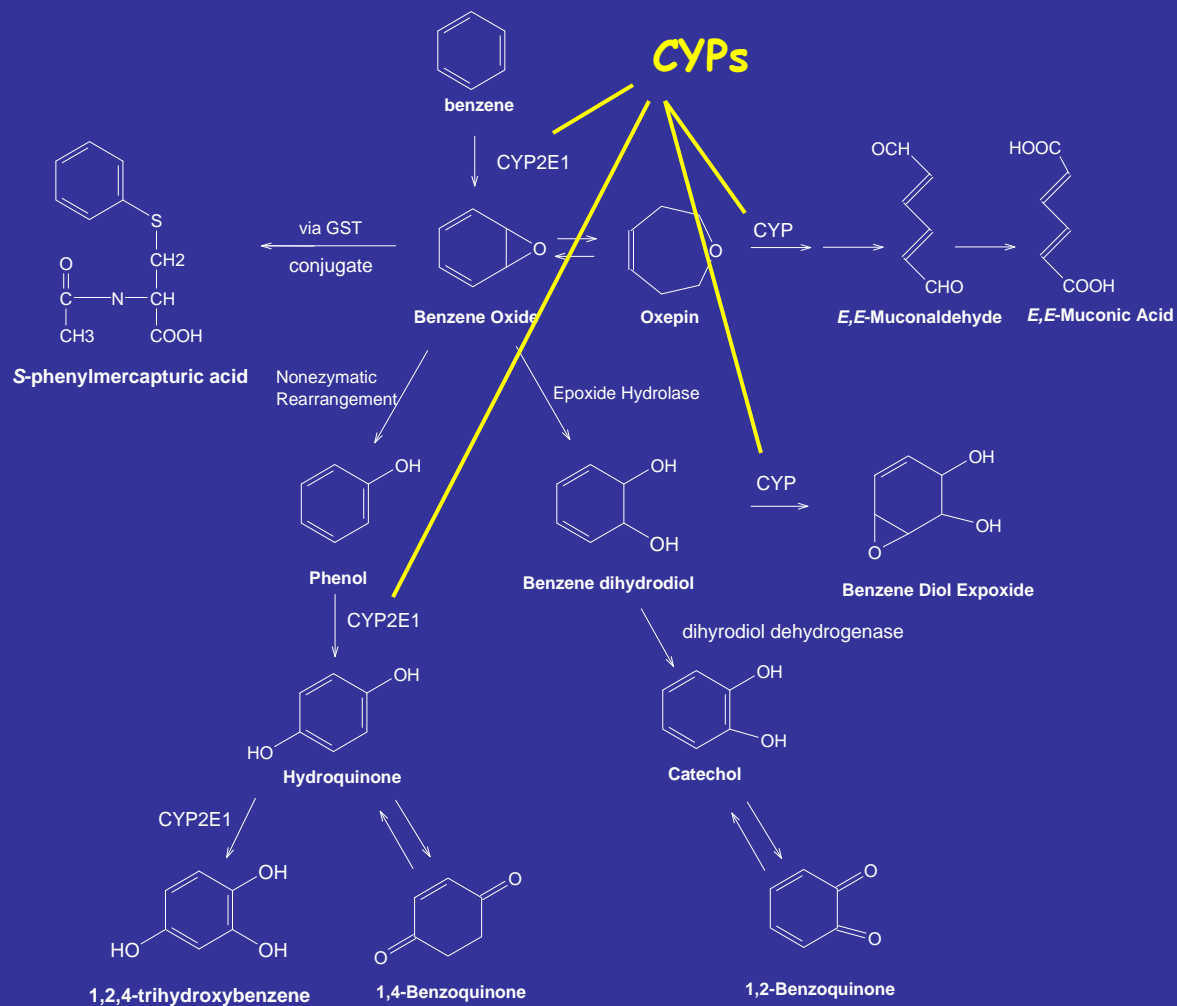
- Alcohol
- Toluene co-exposure<sub>3</sub>

# Dose-Related Metabolism of Benzene

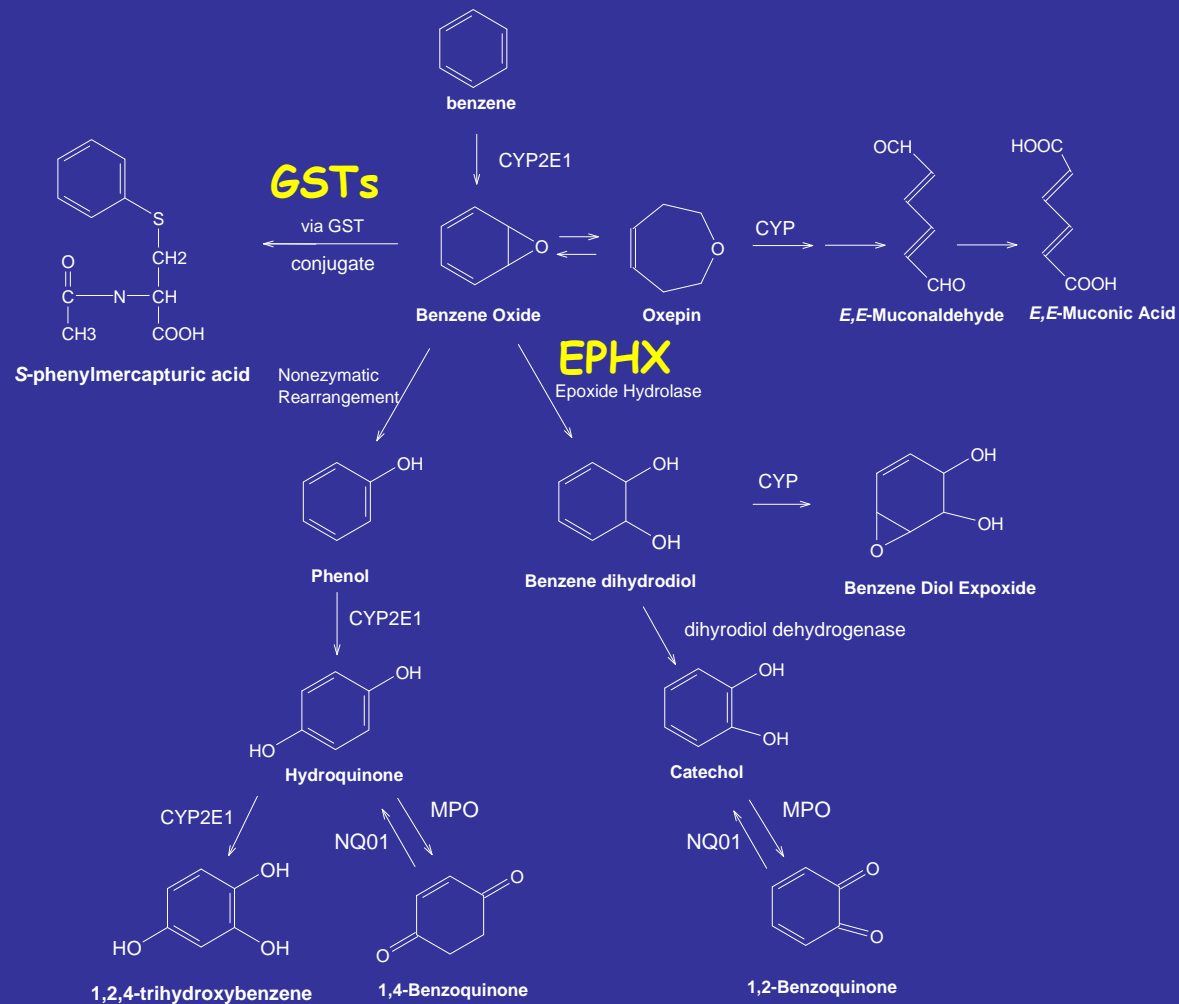
Predicted levels of total metabolites (background adjusted,  
female subject, 29 y of age)



# Effects of Metabolism Genes

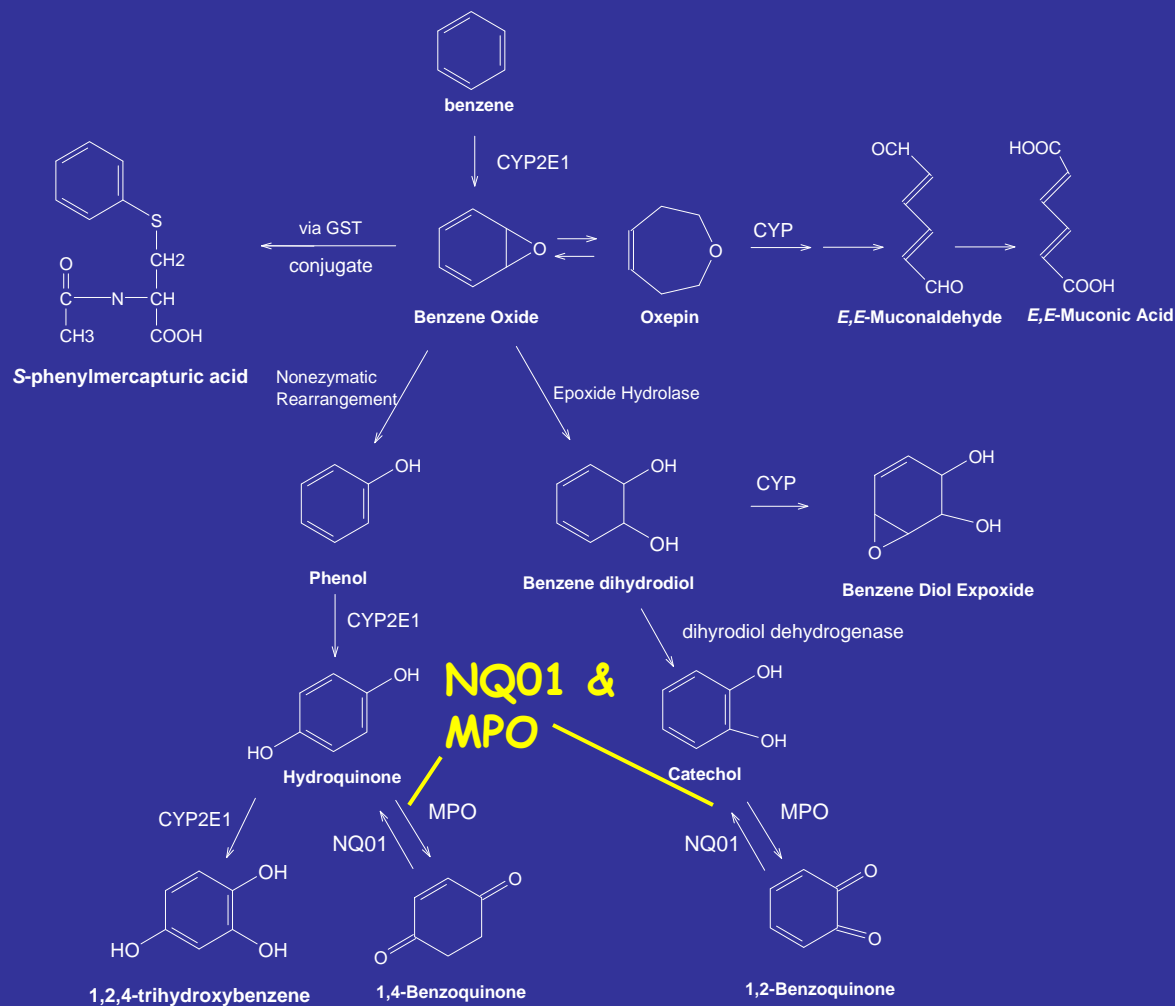


# Effects of Metabolism Genes





# Effects of Metabolism Genes



# Magnitudes of Genetic Effects and Gene-Environment Interactions

## SNP EFFECTS ON BENZENE METABOLITES

Ratio of (Var:Var)/(Wild:Wild)

Metabolite	SNP	Air concentration (ppm)			
		0.1	1	10	100
MA	<i>CYP2E1</i>	0.941	0.666	0.472	0.333
	<i>NQO1</i> *2	0.976	0.832	0.708	0.603
SPMA	<i>CYP2E1</i>	0.675	0.619	0.567	0.519
	<i>EPHX1</i>	1.00	1.26	1.59	2.01
	<i>GSTT1</i>	0.328	0.236	0.17	0.122
	<i>GSTM1</i>	0.554	0.554	0.554	0.554
	<i>NQO1</i> *2 (Nonsmokers)	0.614	0.524	0.448	0.382
	<i>NQO1</i> *2 (Smokers)	1.74	1.48	1.27	1.08
	<i>CYP2E1</i>	0.75	0.542	0.392	0.283
PH	<i>NQO1</i> *2 (Nonsmokers)	0.714	0.714	0.714	0.714
	<i>NQO1</i> *2 (Smokers)	1.22	1.22	1.22	1.22
	<i>EPHX1</i>	1.15	1.15	1.15	1.15
CA	<i>NQO1</i> *2	0.902	0.789	0.69	0.603
	<i>EPHX1</i> (Nonsmokers)	1.29	1.10	0.938	0.8
	<i>EPHX1</i> (Smokers)	0.685	0.584	0.498	0.425
HQ	<i>CYP2E1</i>	0.692	0.518	0.389	0.291
	<i>NQO1</i> *2	0.849	0.849	0.849	0.849

Several significant effects, most in expected directions

Most effects are small (< 3-fold)

# Magnitudes of Genetic Effects and Gene-Environment Interactions

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Most effects vary with conc. due to saturable metabolism and gene-environment interactions

# Magnitudes of Genetic Effects and Gene-Environment Interactions

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Evidence of gene-smoking interactions

# Conclusions

Benzene is metabolized more efficiently at air concentrations below 1 ppm (about 9-fold for total metabolites)

- Observed in two independent Chinese studies using two different biomarkers of exposure (protein adducts and urinary metabolites)

Risk assessments based upon linear models may *underestimate* risks at low exposure levels

Metabolism varies with age and differs between males and females

Metabolizing genes and gene-environment interactions have detectable but small effects on metabolite production

# Conclusions

Biomarkers can be used in observational studies to elucidate effects of metabolism and other phenomena of interest

IF exposures are carefully measured in the same subjects

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