Using Biomarkers to Characterize Human Benzene Metabolism

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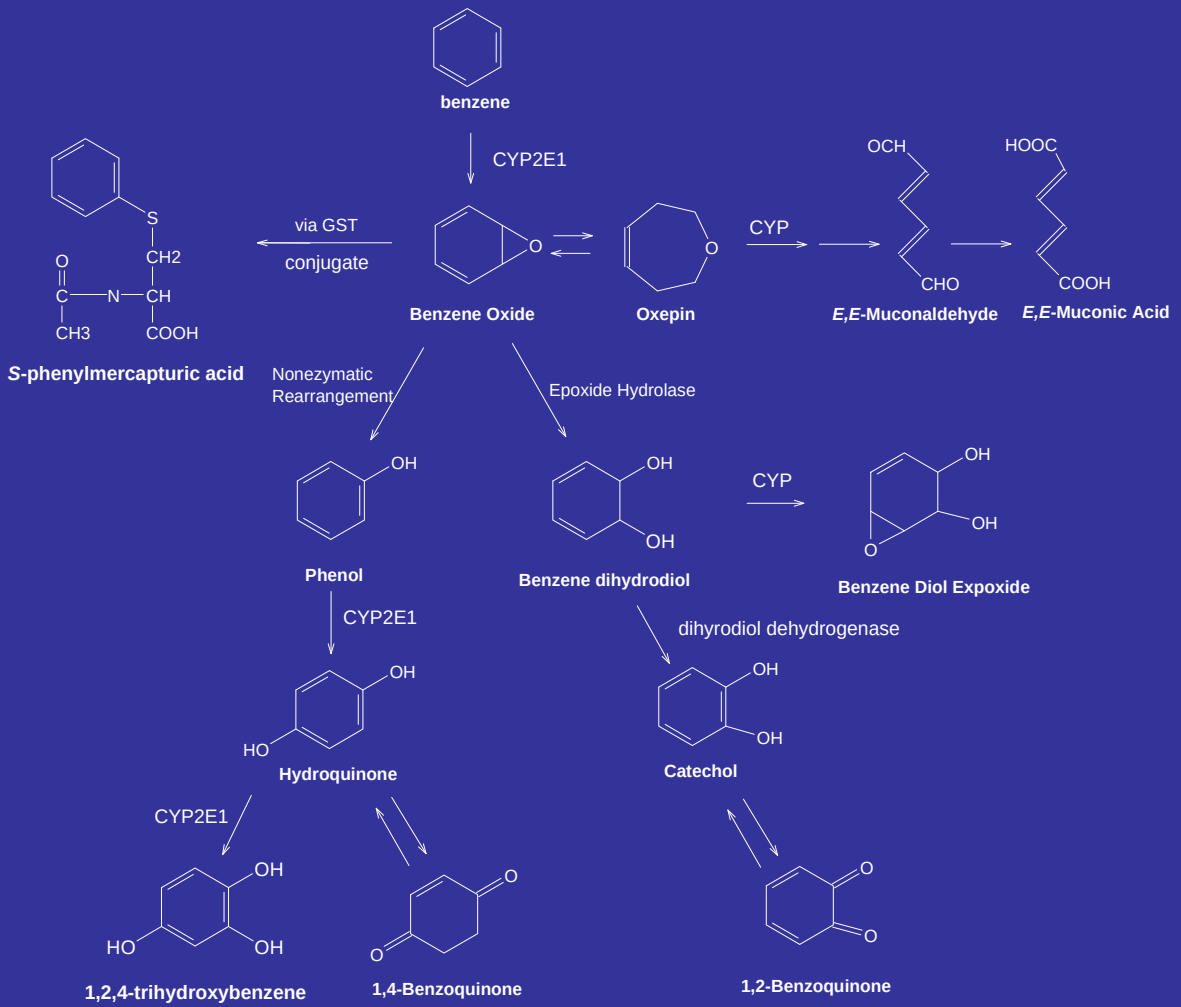
Benzene

- 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

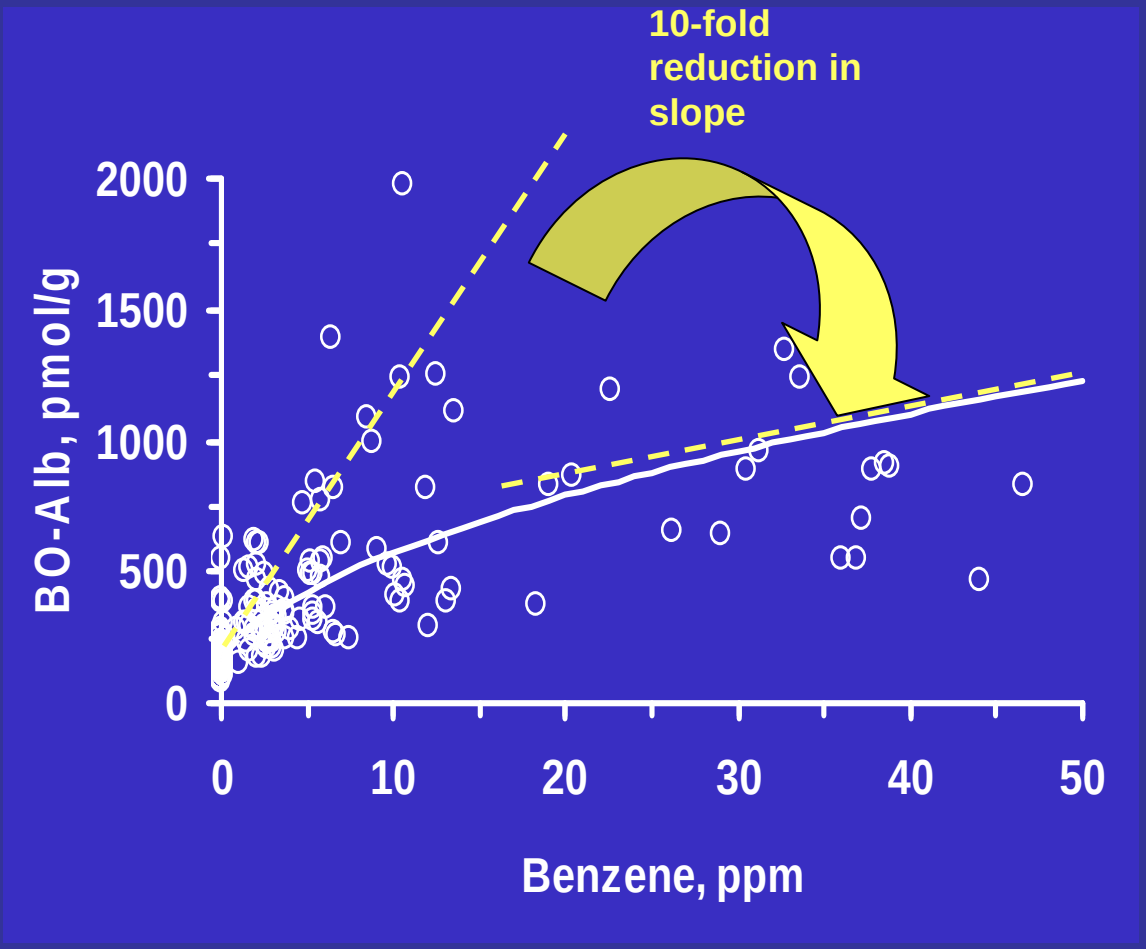
134 Exposed subjects and 51 controls in Tianjin, China

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?

Rappaport, *et al.*, *Cancer Res*, 62: 1330-1337 (2002).

Benzene Biomarkers Among Chinese Workers

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

250 Exposed subjects and 140 controls in Tianjin, China

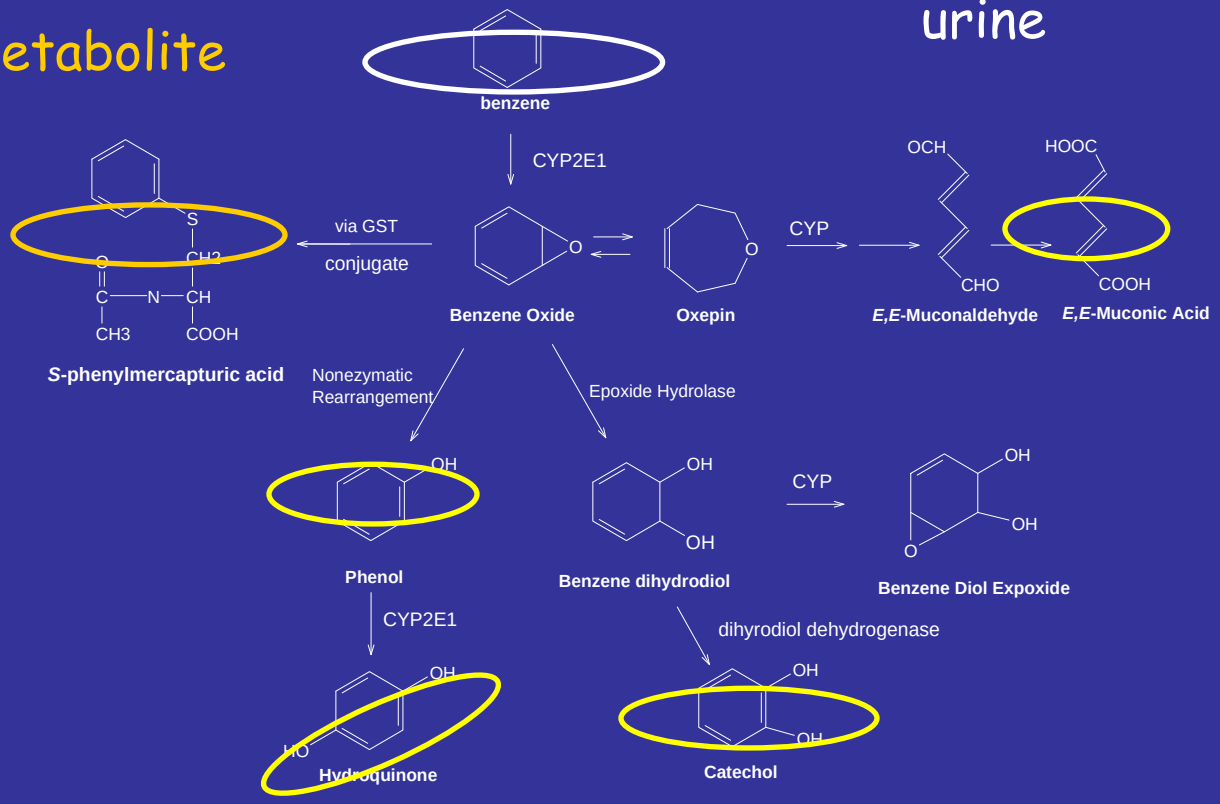
- **Similar exposures (med. = 3.7 ppm)**
- **Focus on exposure-biomarker relationships and mechanism**

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)

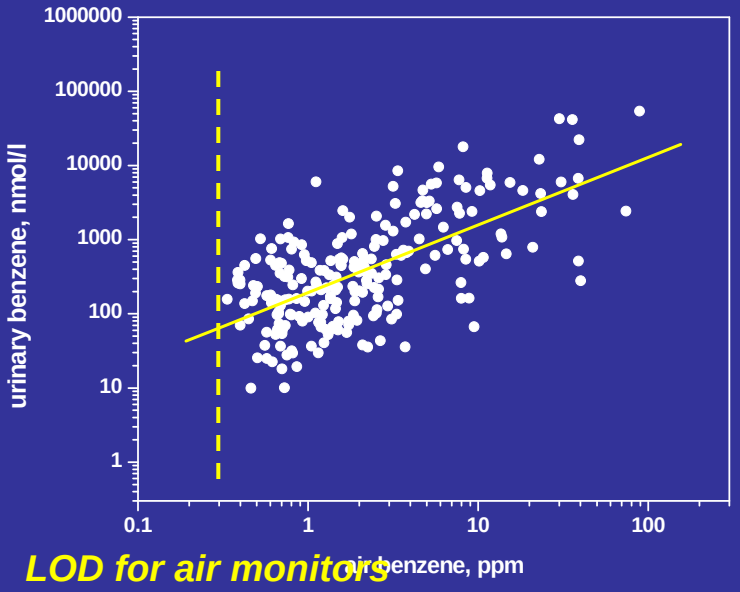
Air; Unmetabolized benzene in urine

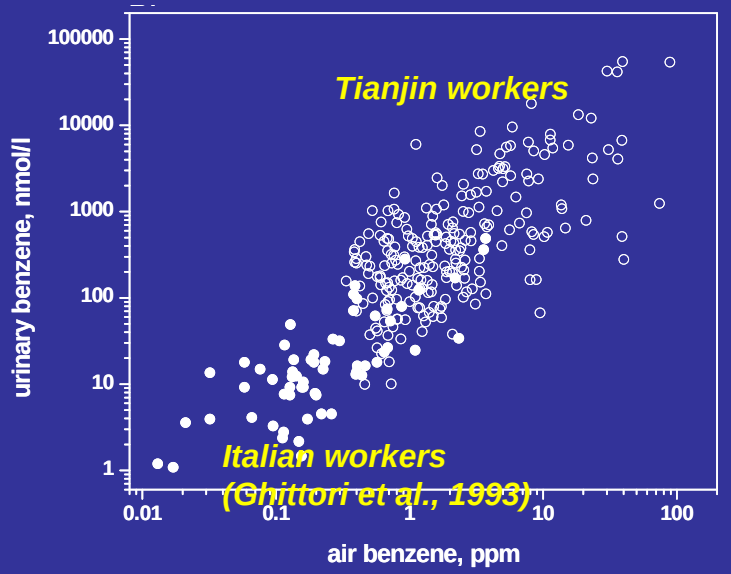
Minor metabolite



Major metabolites (99% of absorbed dose)

Urinary Benzene vs. Exposure

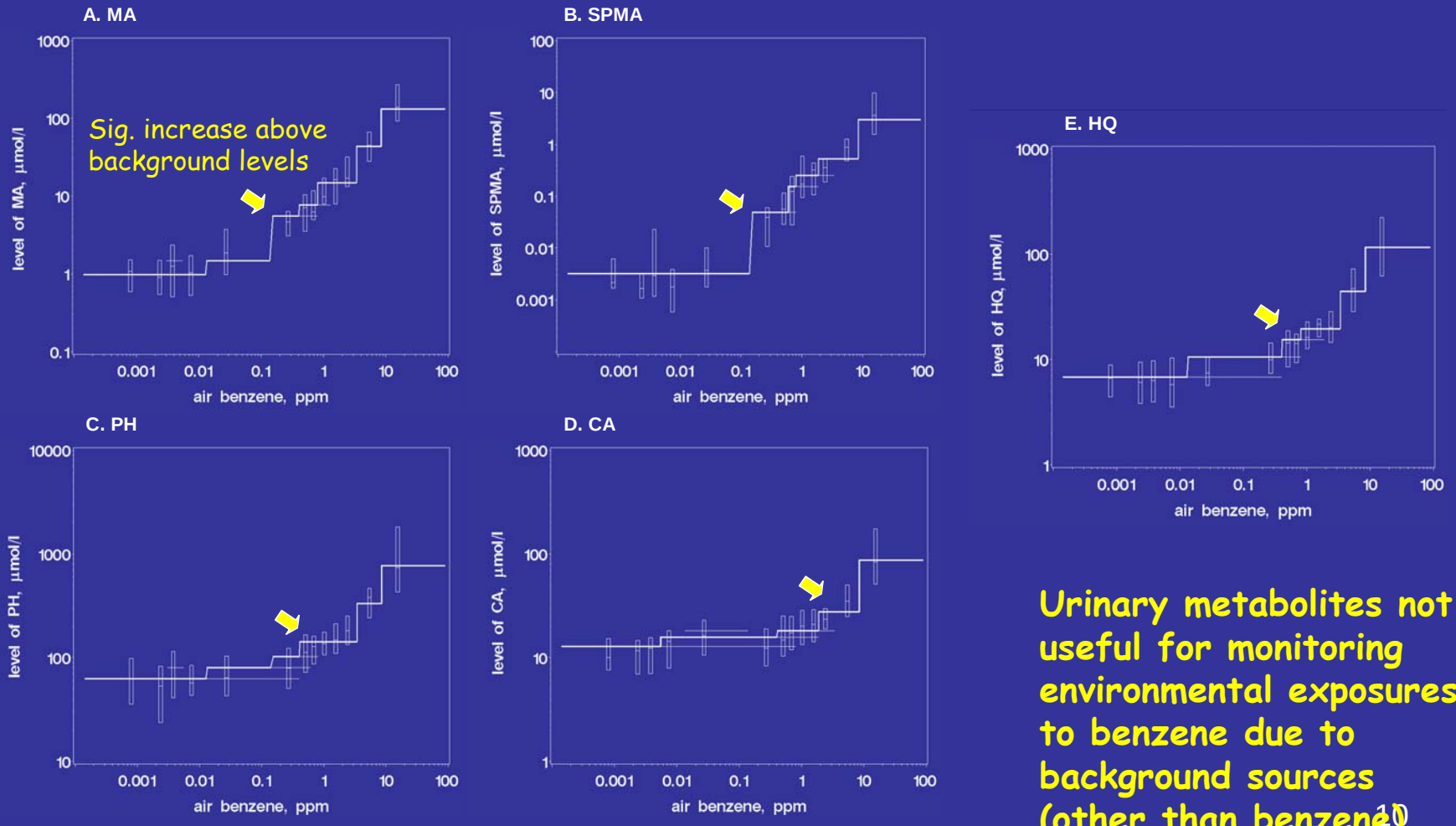




Urinary benzene detected in all exposed AND control subjects – Used to predict benzene exposures in controls

Kim et al., Carcinogenesis, 27:772 (2006)

Metabolites vs. Exposure (Groups of 30 Workers)



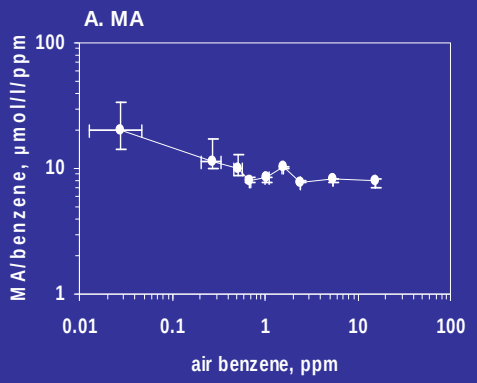
Urinary metabolites not useful for monitoring environmental exposures to benzene due to background sources (other than benzene)

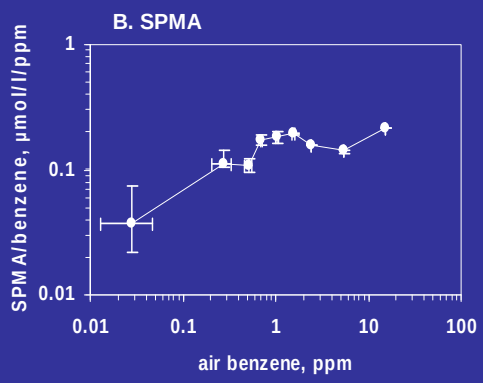
Kim et al., Carcinogenesis, 27:772 (2006)

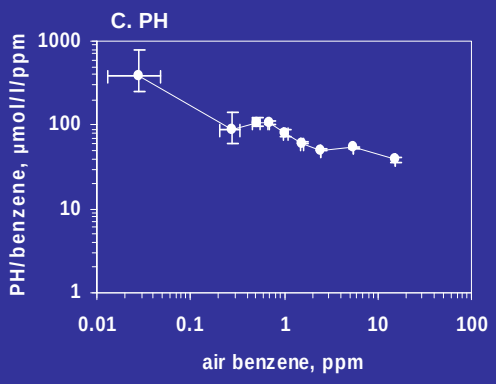
Dose-specific Metabolism

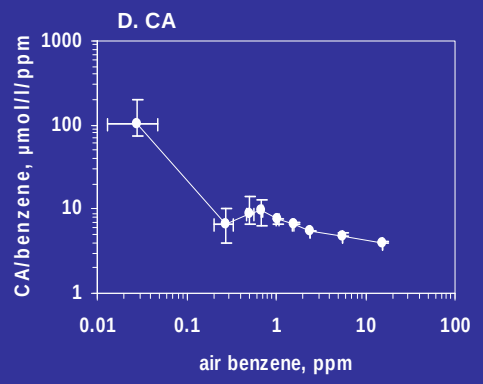
Groups (n = 30) after adjustment for background levels

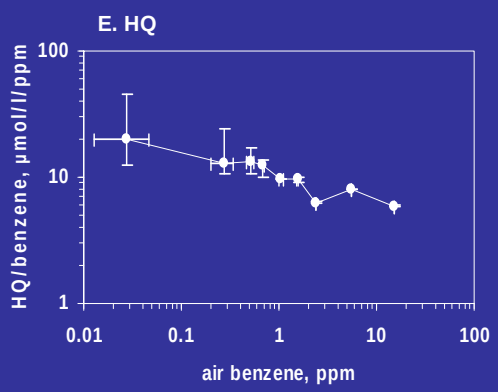
- Reduced production of major & total metabolites at low exposures (0.01 – 1 ppm, never reported previously)

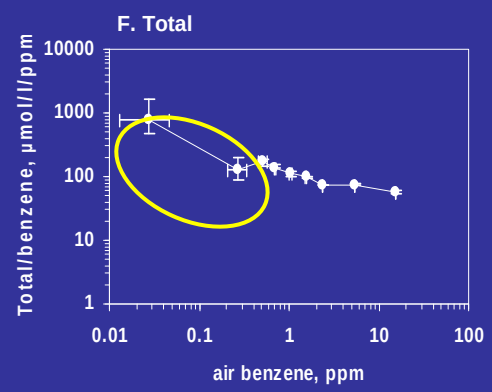






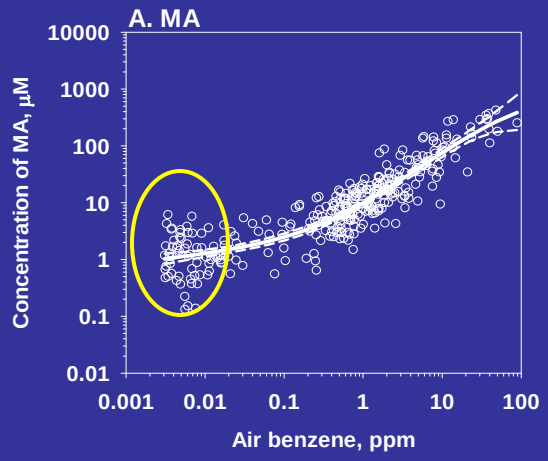


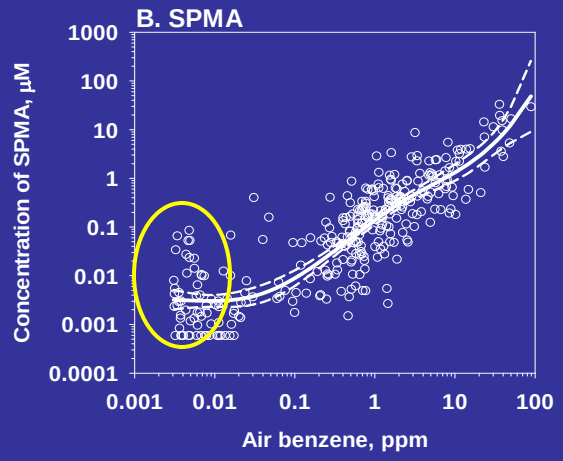


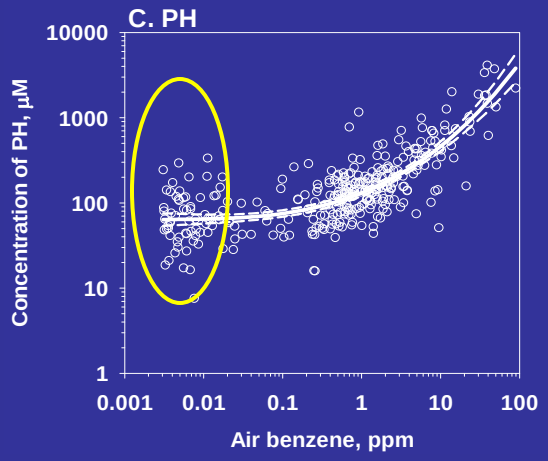


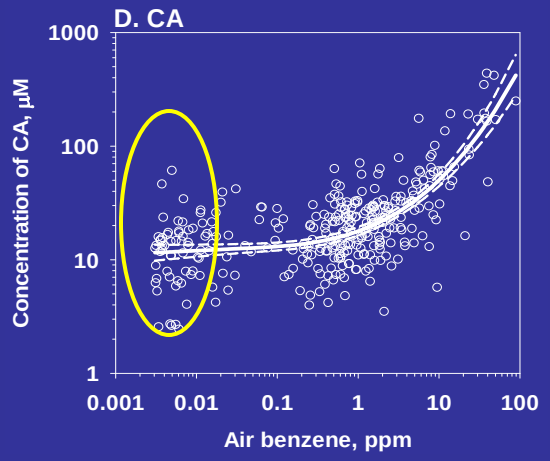
Kim et al., Carcinogenesis, 27:772 (2006)

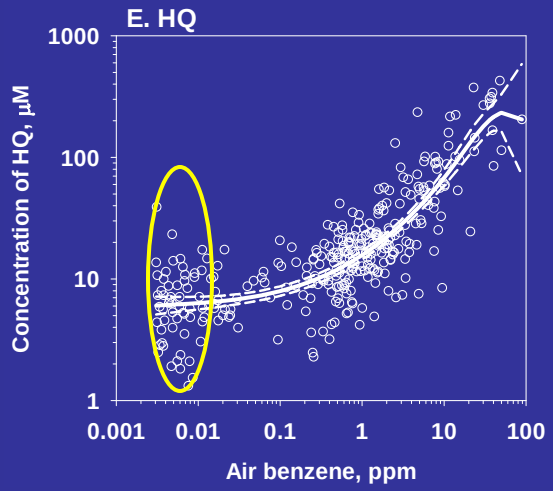
Modeling Metabolite Levels (Natural Splines)











Background levels

Kim et al., CEBP, 2006

Effects of Covariates [GLM+NS]

Metabolite	Adj.R^2	Parameter 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

Kim et al., CEBP, 2006

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

Sex: females produce more metabolites than males

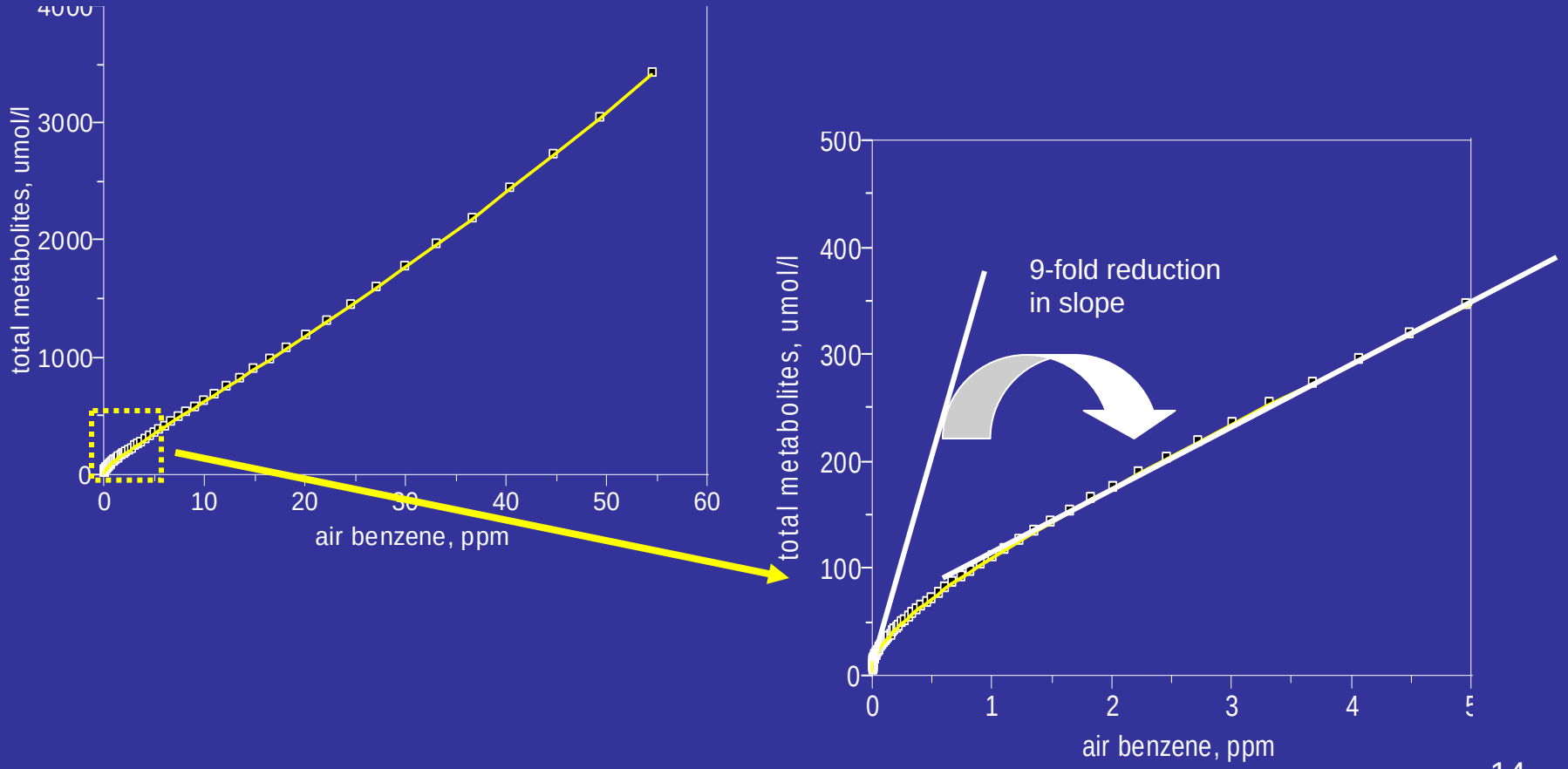
Smoking: Cigarette smoke contains HQ and CA

No effect of:

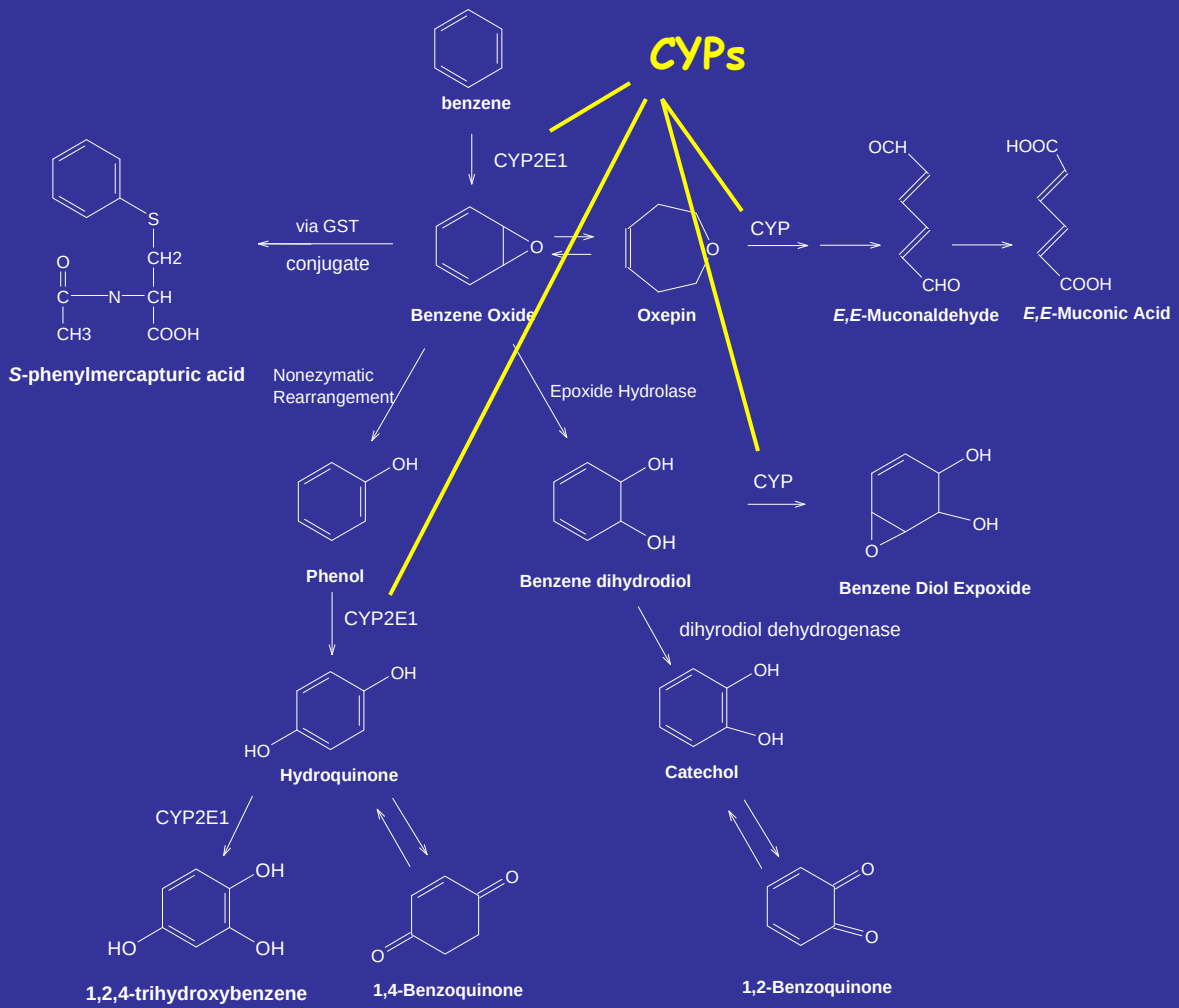
- Alcohol
- Toluene co-exposure

Dose-Related Metabolism of Benzene

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



Effects of Metabolism Genes



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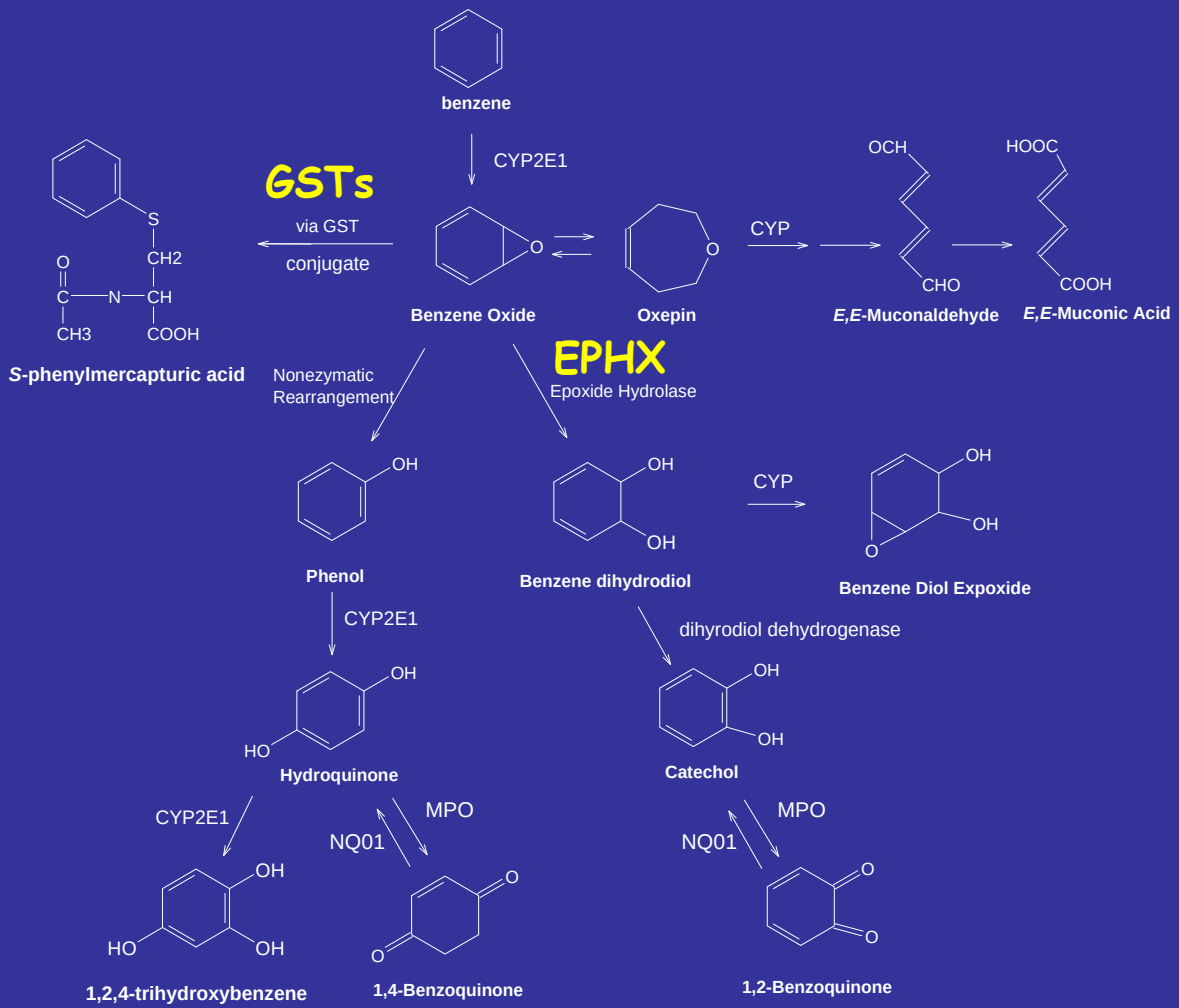

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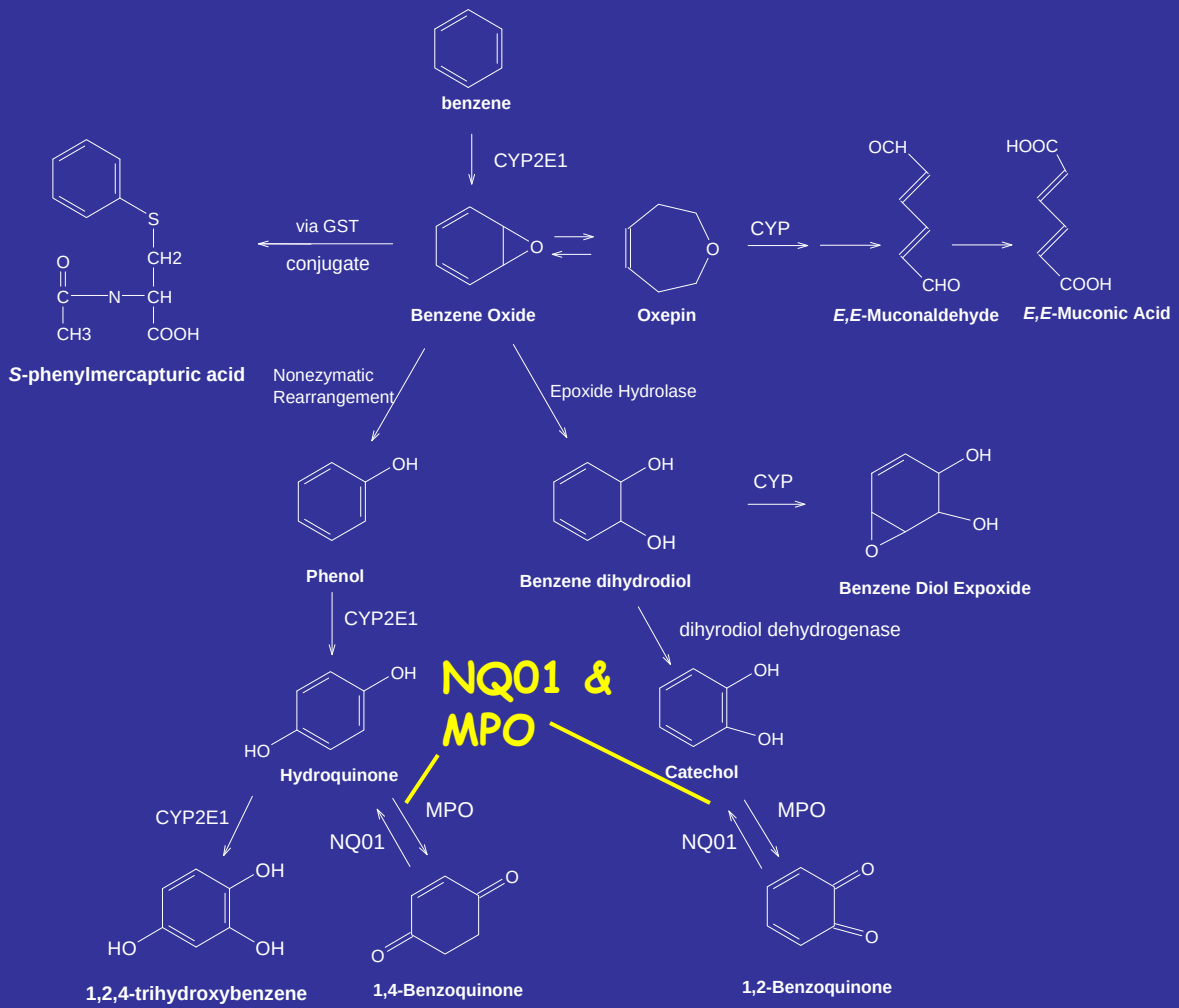

Magnitudes of Genetic Effects and Gene-Environment Interactions

SNP EFFECTS ON BENZENE METABOLITES
Ratio of (Var:Var)/(Wild:Wild)

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

- Several significant effects, most in expected directions
- Most effects are small (< 3-fold)

Kim et al., PGEN, in press

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

Kim et al., PGEN, in press

Magnitudes of Genetic Effects and Gene-Environment Interactions

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Evidence of gene-smoking interactions

Kim et al., PGEN, in press

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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