Metabolism and Peripheral Blood Effects in Shanghai Benzene Workers

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Background: benzene metabolism

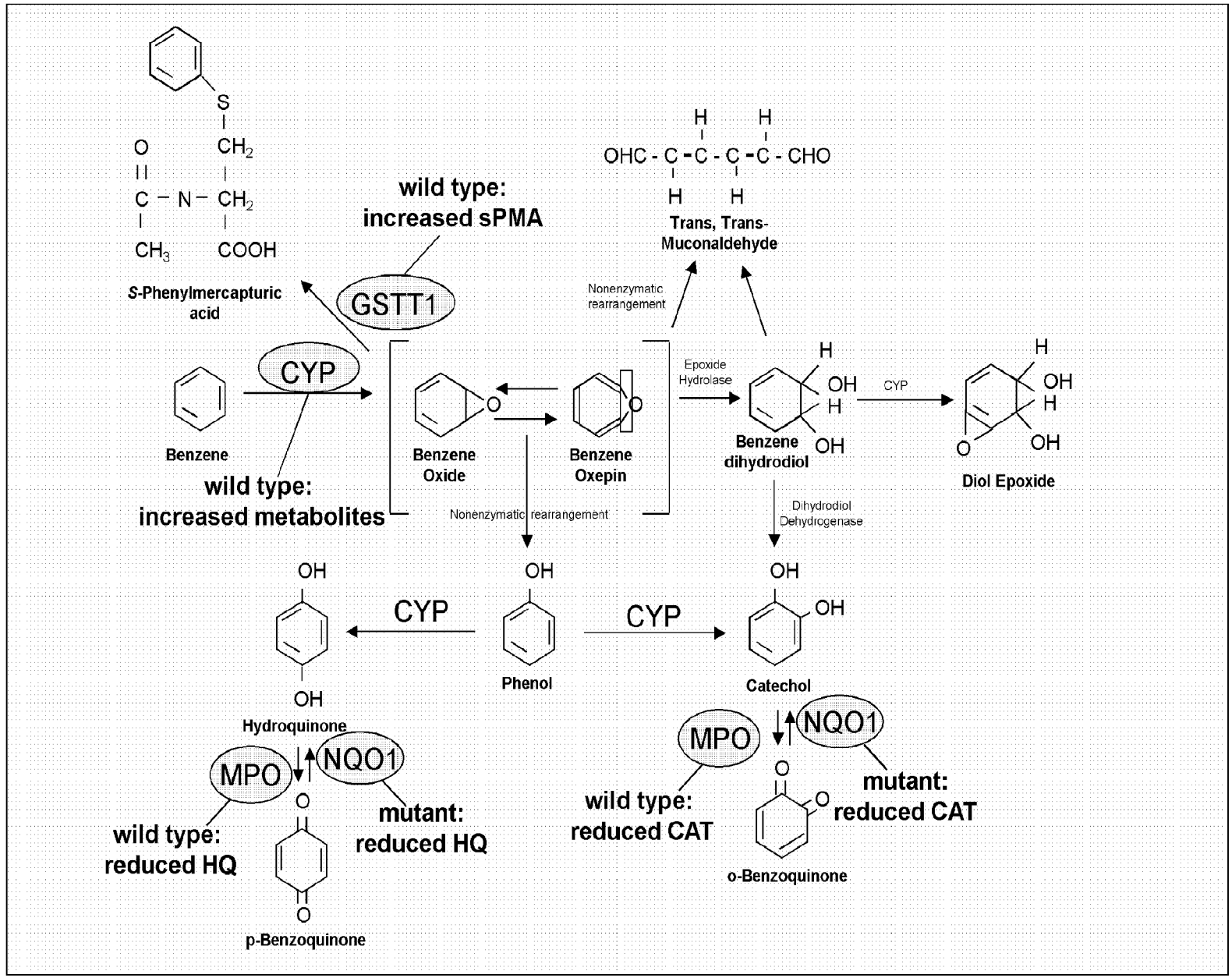
- sPMA, ttMA more sensitive, specific vs. phenolic metabolites
- Toluene may inhibit (Andrews 1977) or enhance (Wetmore 2008) metabolism, depending on dose
- Conflicting findings regarding single gene polymorphisms (SNP's)

Dougherty et al. 2008

	Positive	Negative
CYP2E1*5 CT or TT / ttMA	1	4
CYP2E1*5 CT or TT / Phenol	1	1
GSTT1 null / sPMA	4	4

- Different proportions / enhanced metabolism for lower exposures
  - ttMA favored at lower ambient exposures
  - 9x increase <1 ppm when ambient exposures predicted from urinary benzene (Kim 2006)

Benzene metabolism: expected influence of SNP's



Background: benzene and peripheral blood effects

- Well established effects for anemia (low RBC), leukopenia (low WBC), and thrombocytopenia (low PLT) . . . early data suggested more prevalent effects on RBC (Greenberg 1939), or WBC (Aksoy 1971; Kipen 1988)

- No consensus on most sensitive effect . . . candidates mentioned:
  - MCV (Yardley-Jones 1988; Collins 1991)
  - Lymphocytes (Goldstein 1988; Rothman 1996)
  - Neutrophils (Qu 2002)
  - Early progenitor cells (Lan 2004)

- Early studies showed clear effects >10 ppm . . . more recent studies show effects at lower levels

Study design / overview

- Cross-sectional study in five factories in and around Shanghai, China
- Approved by IRB's at University of Colorado and Fudan University
- Consent form, questionnaire, blood sample
- Post-shift urine sample in 2 of 5 factories for metabolite study
- Extensive exposure monitoring for benzene, toluene, xylene
  - Few other chemical exposures
- Five metabolites measured via GCMS (CAT, HQ, PH, ttMA, sPMA)
  - High LOD for sPMA prevented analyses at lower exposures
- Five SNP's determined via RFLP in four genes encoding for activating or detoxification enzymes
  - NQO1 465C >T, 609C >T
  - MPO 463G >A
  - CYP2E1 1019C >T
  - GSTT1 null
- Statistical analyses on 4 metabolites and 12 blood indices via correlations, general linear models, change-point regressions

Factories studied

	Type	Product / Process	Workers	Males	Females
A	Rubber	Rubber hoses & belts	352	250	102
B	Shoe	Glue soles to uppers	412	113	299
C	Sealant Products (rubber)	Diluent, adhesive	126	68	58
D	Pharmaceutical	Intermediates	128	116	12
E	Rubber	Rubber hoses	28	16	12

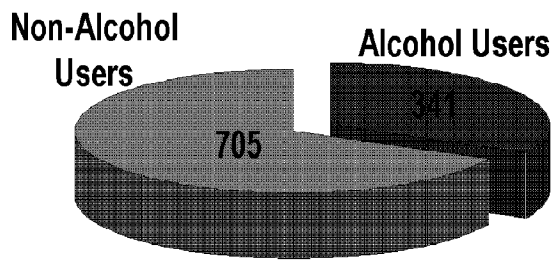
Metabolite Subpopulation

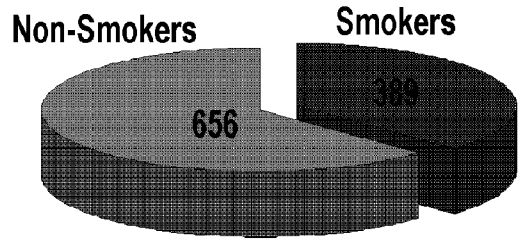
A	Rubber	Same	279	199	80
B	Shoe	Same	47	4	43

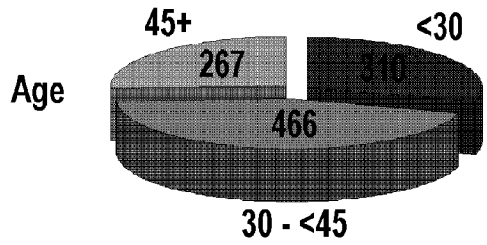
Population description

- Administered in-person questionnaire and consent form
  - 95-99% participation, similar across factories
  - Smoking validated with cotinine for metabolite workers, >98% agreement

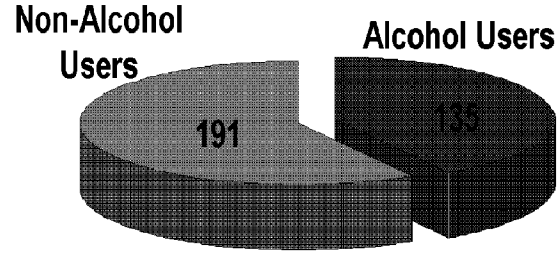
All Workers

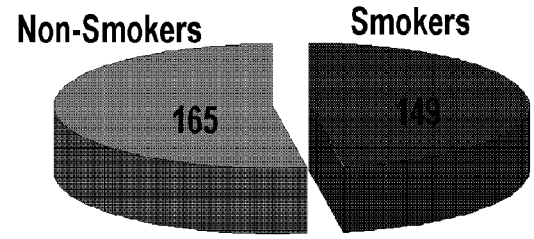


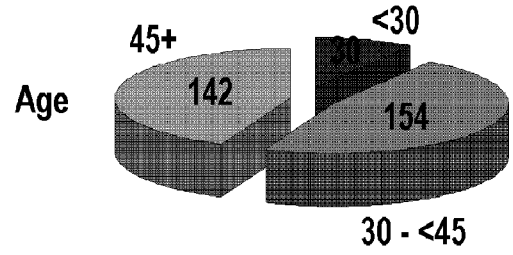




Metabolite Subpopulation







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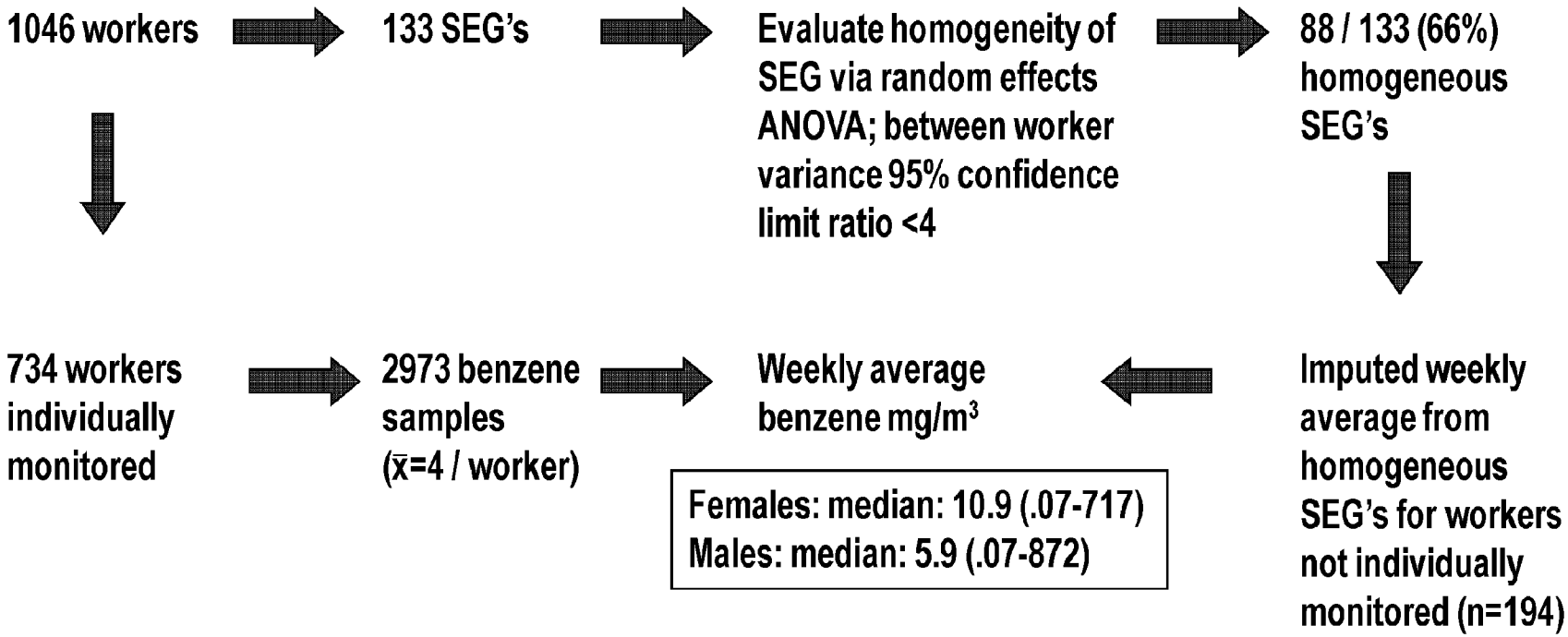
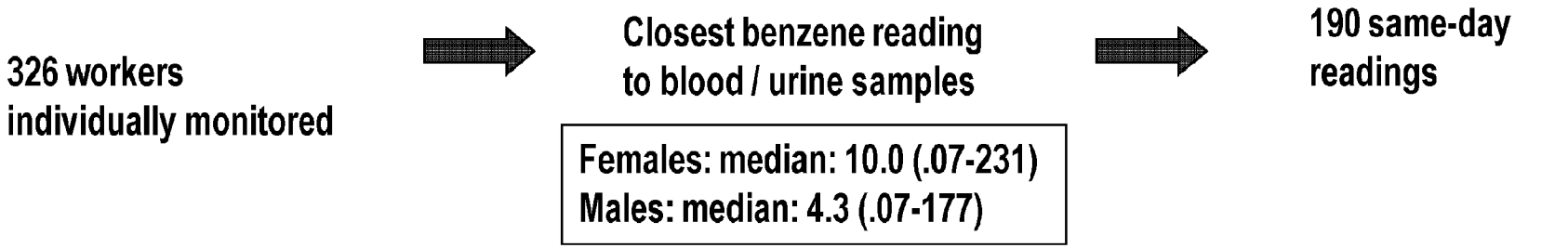
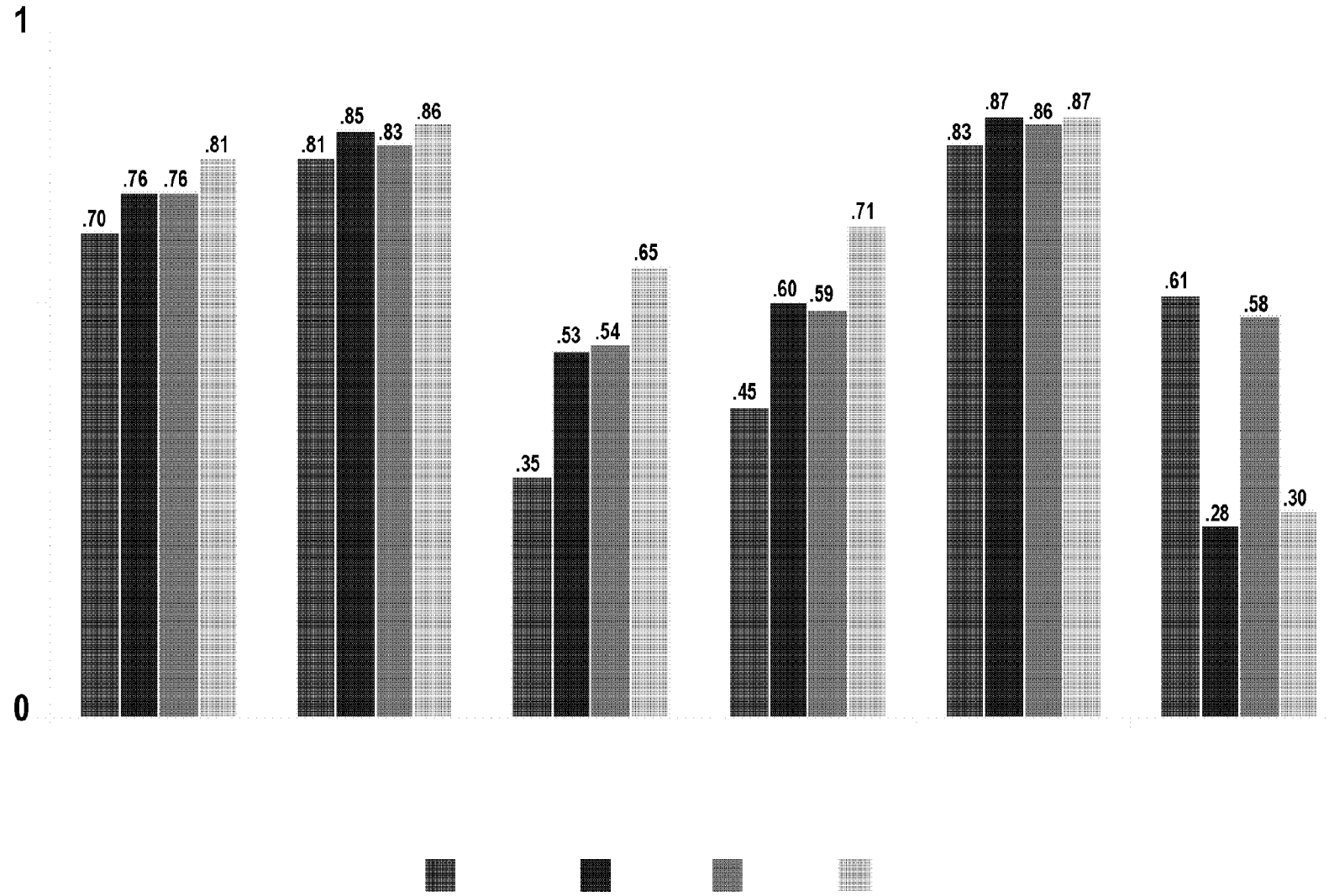
Exposure assessment



Metabolite Subpopulation



Benzene / metabolite correlations



HQ and ttMA particularly highly correlated with one another (r=0.94)

Prediction models for metabolites

	Baseline Model	Final Model (β coefficients)				
	Benzene β	Benzene	Benzene^2	Toluene	Smoking	BMI
CATECHOL	0.49***	-0.08	0.09**	0.28***	0.11	--
HYDROQUINONE	0.54***	0.25**	0.05*	0.10**	0.10	0.04*
PHENOL	0.58***	0.38***	--	0.27***	-0.21	--
ttMA	0.67***	0.55***	--	0.16**	-0.11	--

* p<.05; ** p<.01; *** p<.001

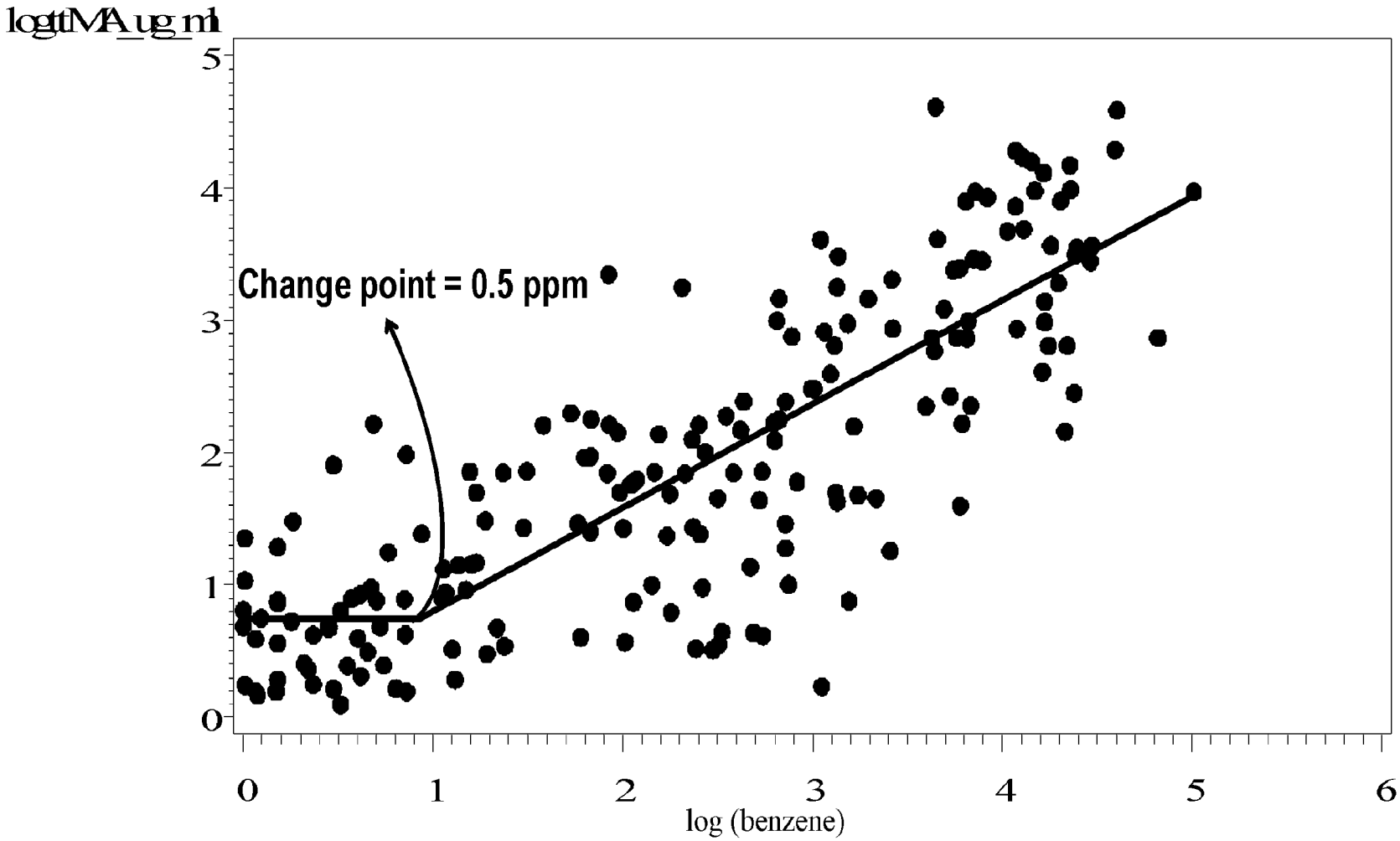
- BZ*TOL term did not enter final models (BZ, toluene correlation = 0.70)
- No SNP terms were important metabolite predictors

- Additional models run for BZ, TOL, BZ*TOL only:

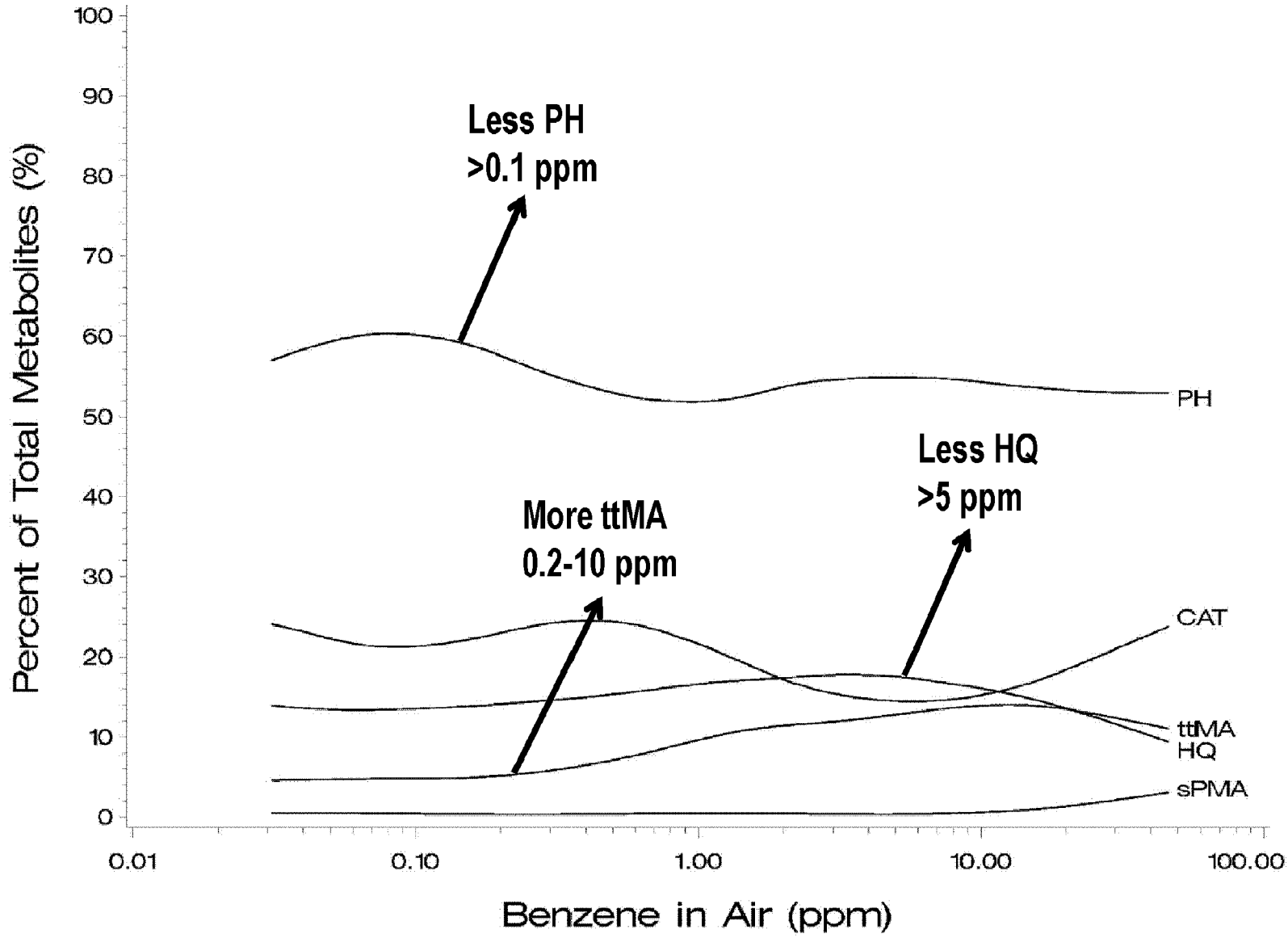
Metabolite	p-value for interaction term
CAT	<0.0001
HQ	0.37
PH	0.21
ttMA	0.72

Change-point regression results

- Change-point regressions determine response that is different than background response
- Lowest change point found for ttMA and HQ

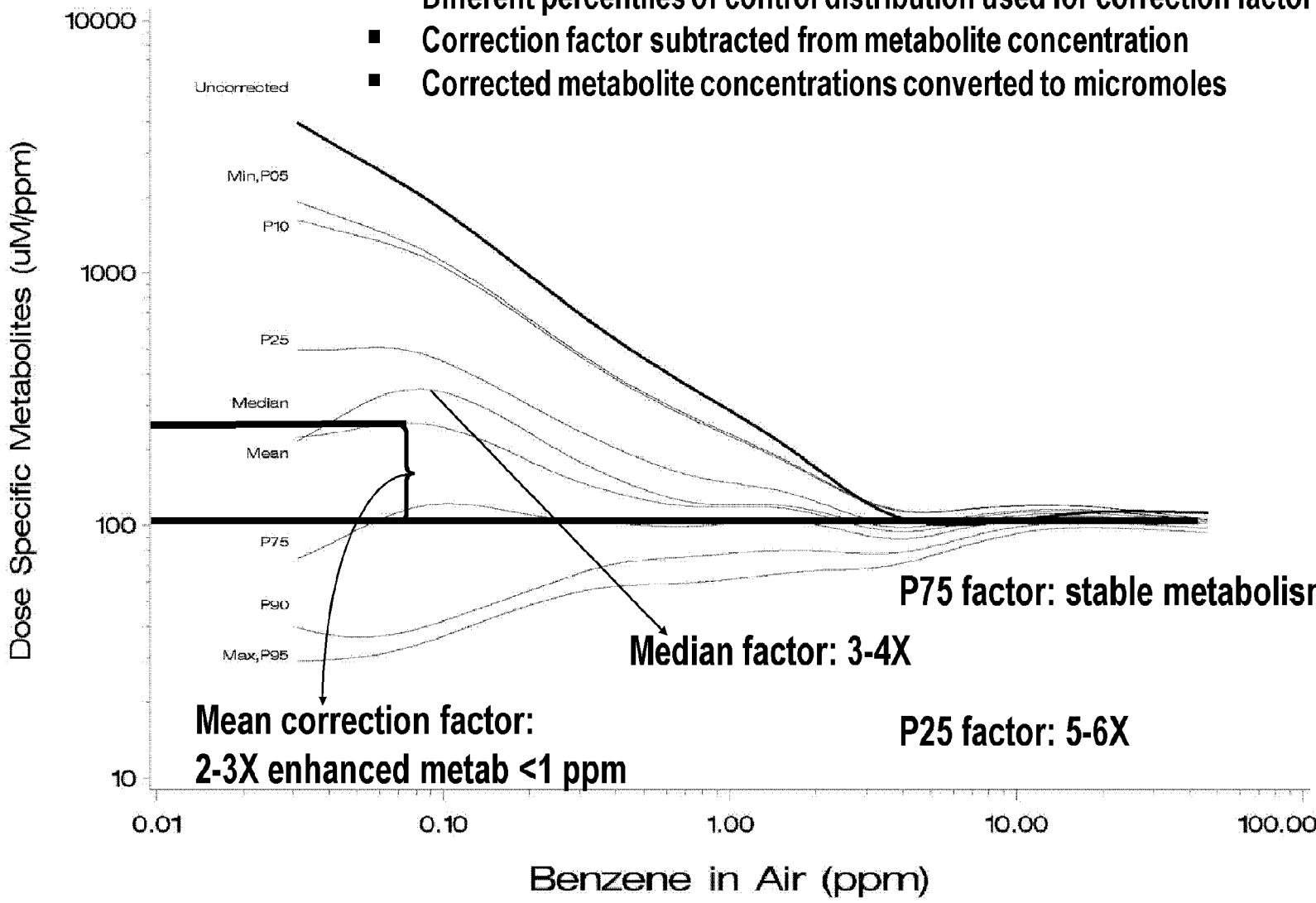


Benzene metabolite proportions across benzene concentrations



Metabolite production rate across benzene concentrations

- Background metabolite values identified in 27 controls
- Different percentiles of control distribution used for correction factor
- Correction factor subtracted from metabolite concentration
- Corrected metabolite concentrations converted to micromoles



Peripheral Blood Effects

n=928

Benzene effects on peripheral blood elements

- Twelve indices examined (6 WBC, 4 RBC, 2 PLT parameters)
- Continuous outcomes modeled via GLM, out-of-normal range defined using Chinese National norms and examined via logistic regression
- Covariates: smoking, age, BMI, alcohol, SNP's, gender

Parameter	Crude β (Benzene)	Adjusted β (Benzene)
WBC	-0.06*	-0.06**
Lymphocytes	-0.01	-0.02
Monocytes	-0.00	-0.00*
Neutrophils	-0.05**	-0.01*
ln (Eosinophils)	0.01	0.01
Basophils	-0.00*	0.00
RBC	-0.06***	-0.04***
HGB	-0.14*	-0.07***
MCV	0.26***	0.36***
RDW	0.03	0.02
Platelets	-1.64	-2.32**
MPV	-0.18***	-0.18***

* p<.05; ** p<.01; *** p<.001

Effect of toluene exposure

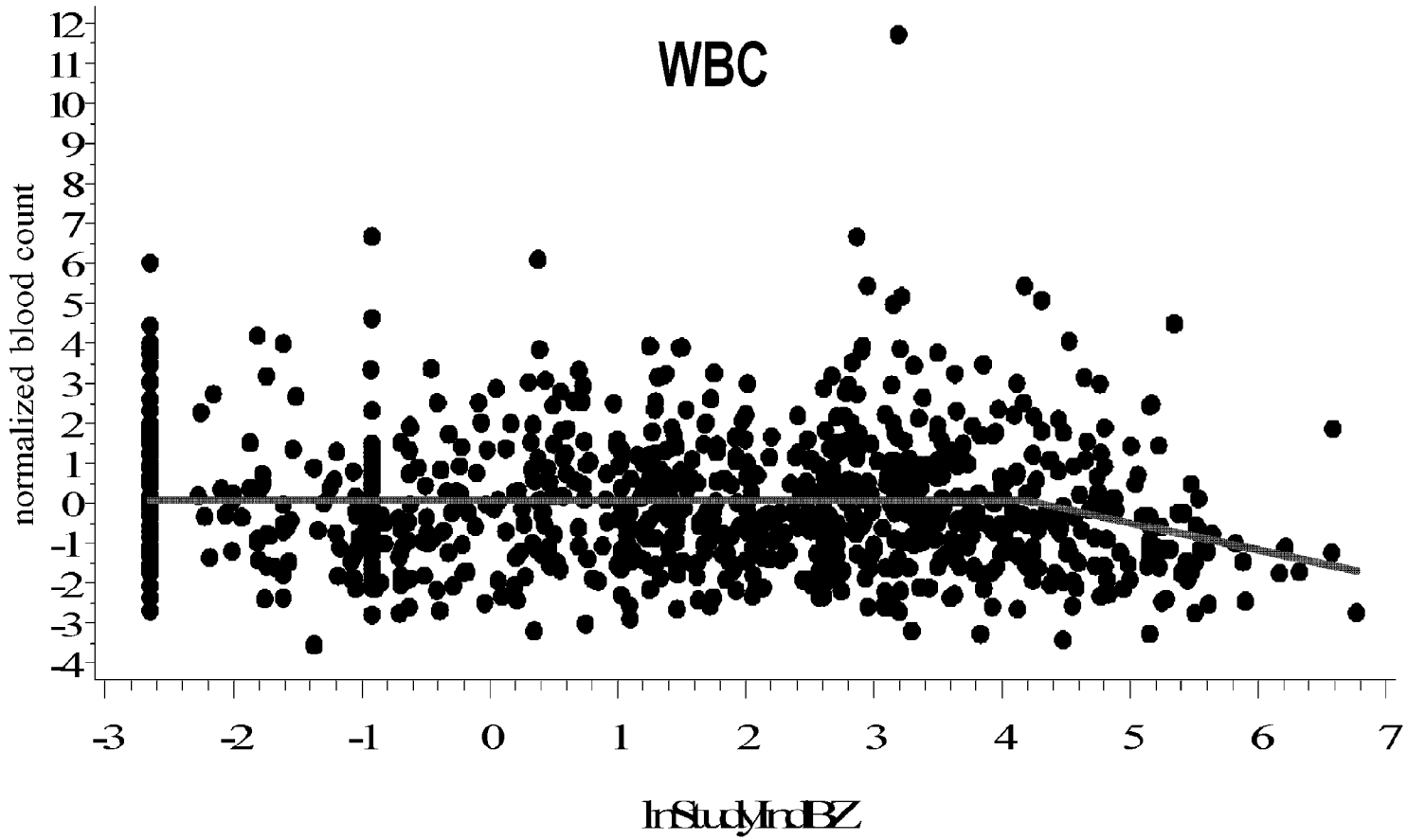
- Toluene confounded several blood index / benzene relationships
- Toluene / benzene exposure correlation = 0.66, thus collinearity may explain this observation

Toluene effect significant / benzene effect non-significant	Toluene increased / significant benzene effect	No toluene effect / significant benzene effect	No toluene or benzene effect
Neutrophils	Lymphocytes	WBC	Basophils
Monocytes	Eosinophils	MPV	RDW
RBC			
MCV			
Hemoglobin			
Platelets			

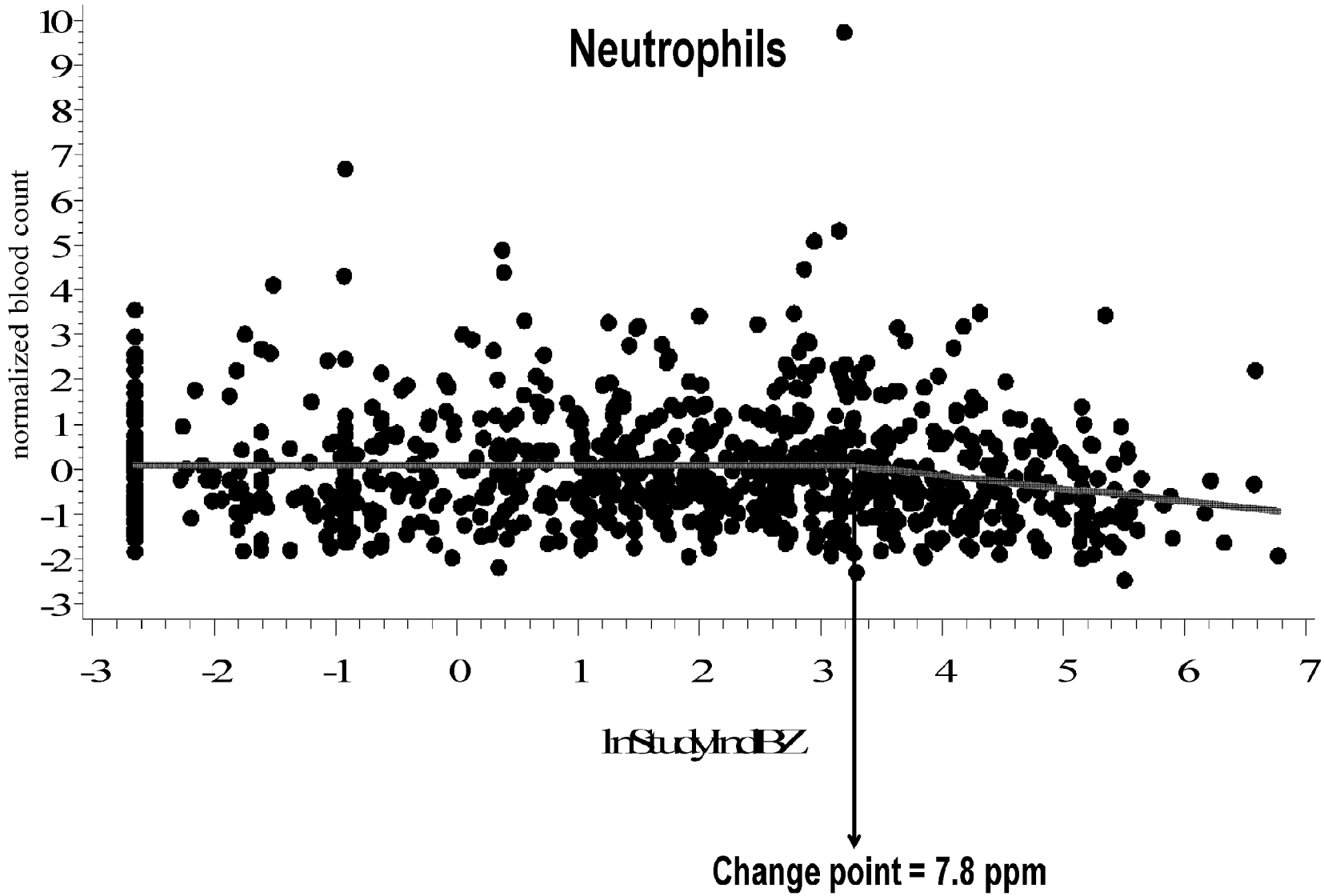
- Future analysis will assess toluene exposure and potential synergistic and antagonistic effects
- Present analyses is analogous to several studies in the literature where toluene co-exposure had occurred but was unaccounted for

Change-point regressions

- Examined whether background blood elements could be distinguished from benzene-induced effect
- Lower change points $\Rightarrow$ more sensitive effect
- Still may not represent clinically relevant effect

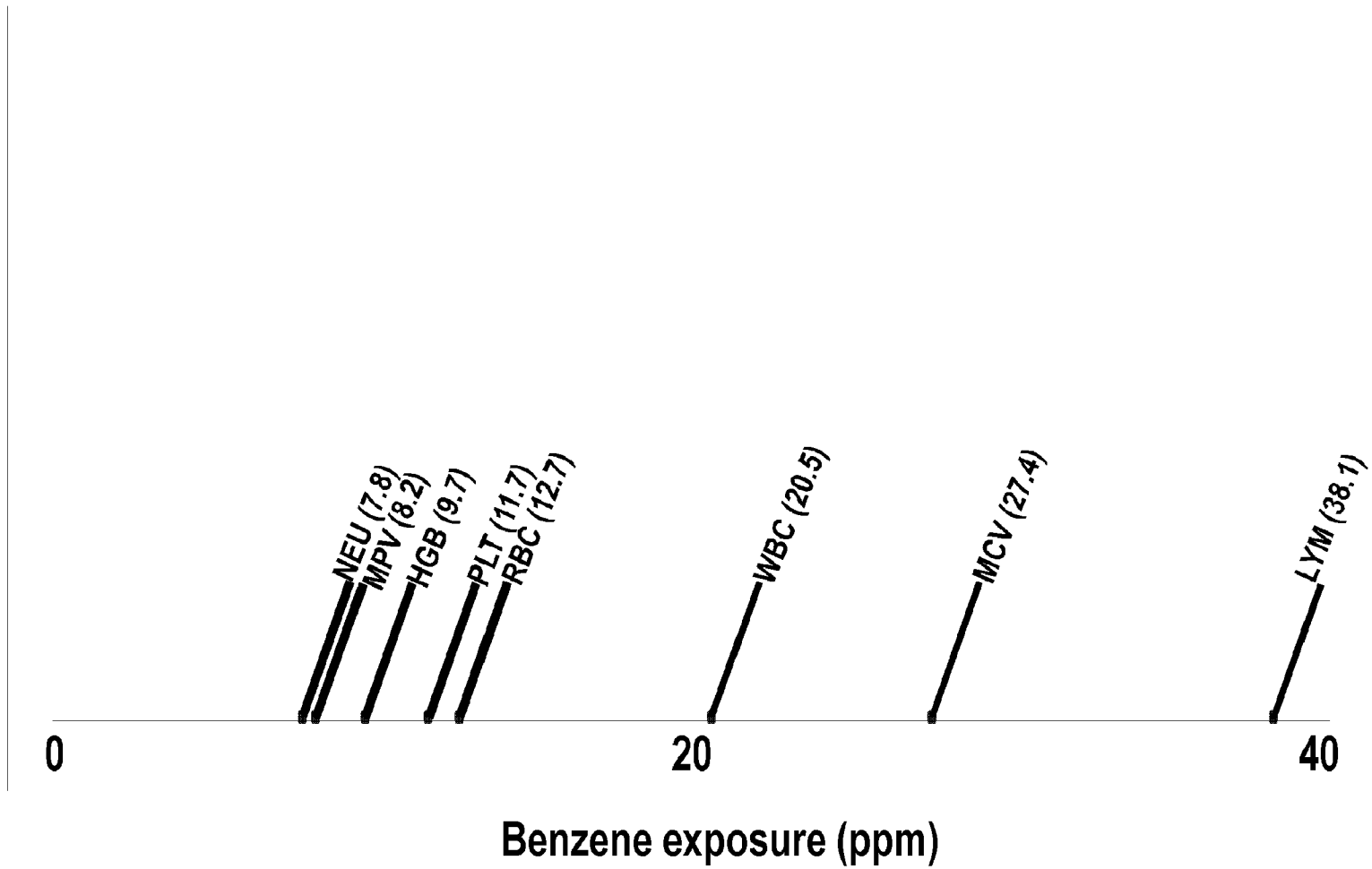


Neutrophil change-point regression results



Benzene concentrations affecting different blood elements

- Neutrophils and MPV showed lowest change points
- Lymphocytes showed highest change point

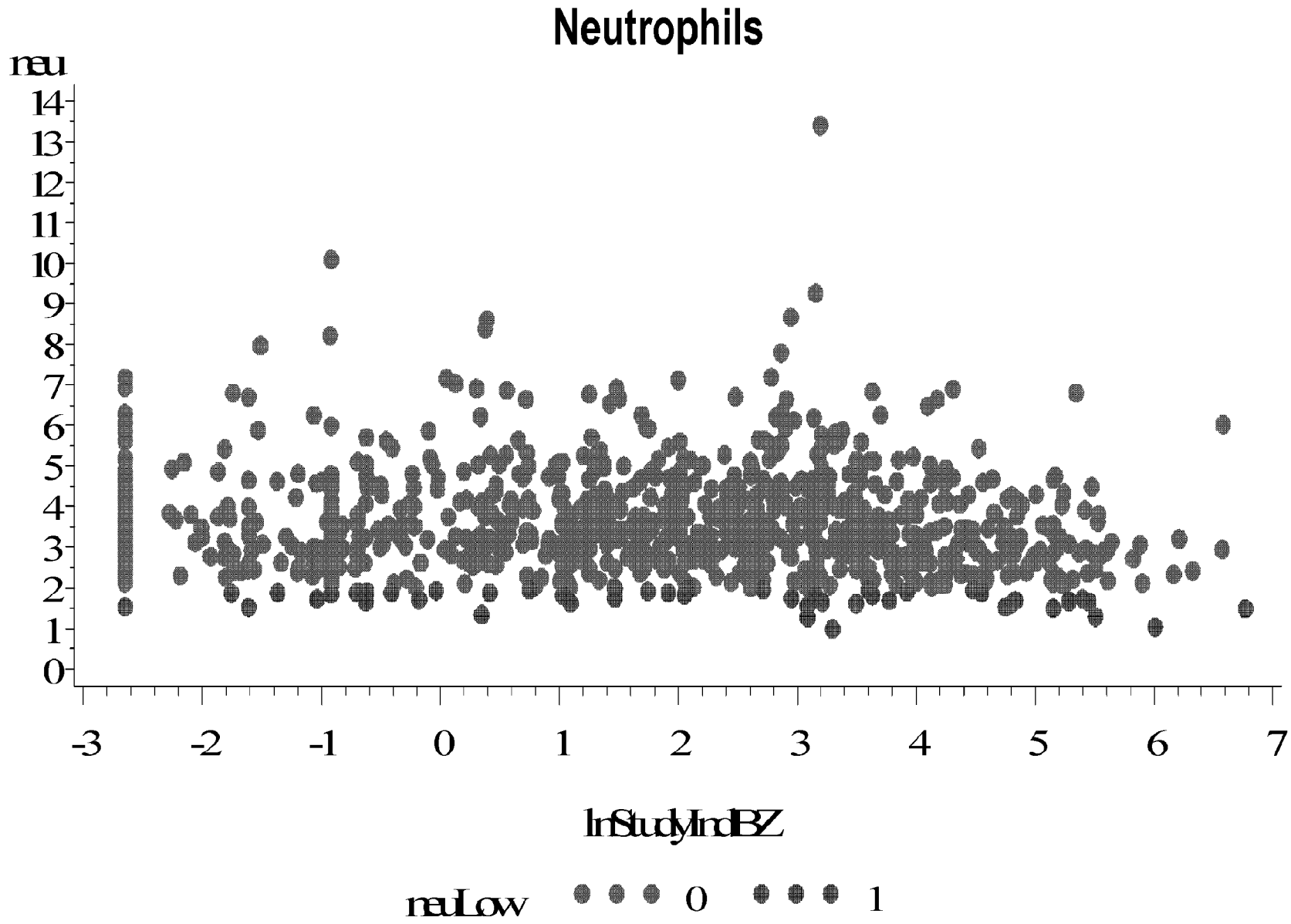


Logistic regression results for clinically relevant blood elements

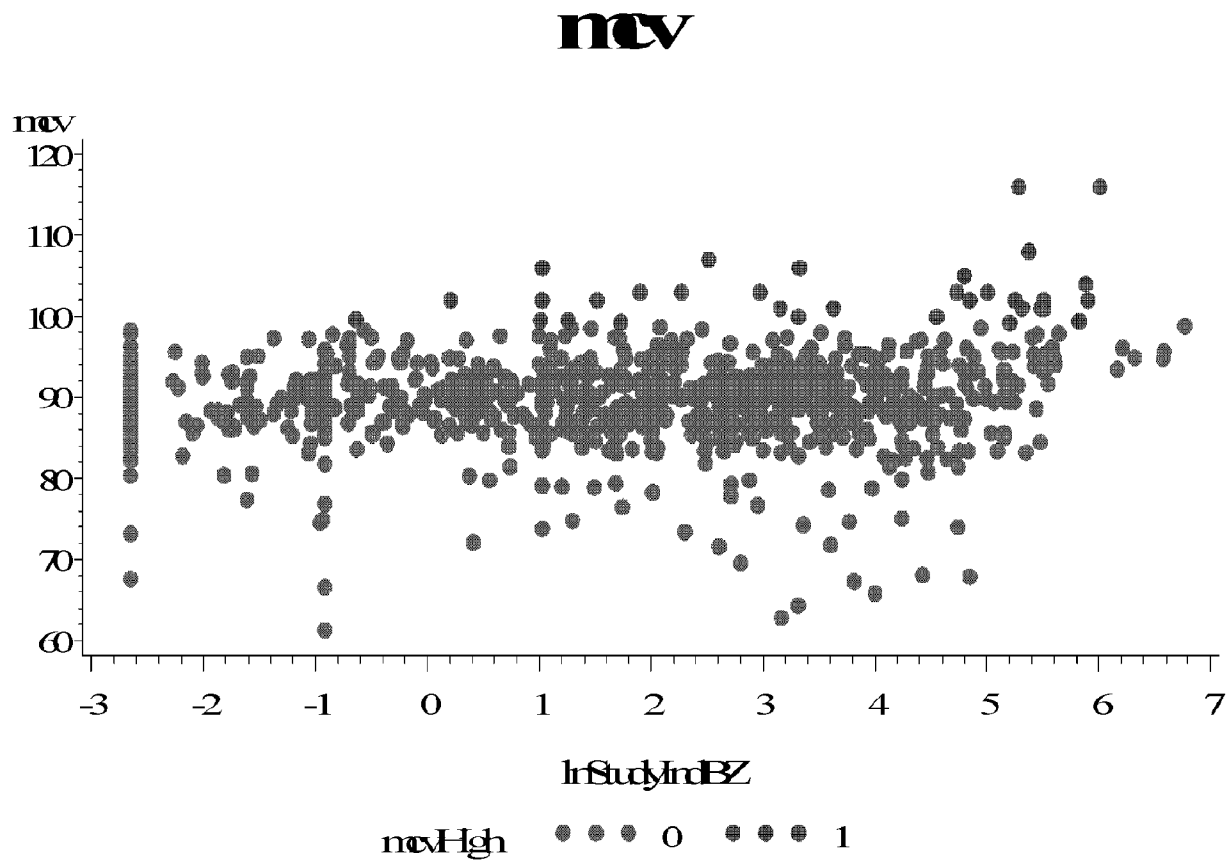
Parameter	OR	95% CI
WBC	1.18	1.00-1.39
Lymphocytes	1.04	0.91-1.19
Neutrophils	1.05	0.92-1.20
Eosinophils	1.07	0.88-1.30
RBC	1.28	1.12-1.47
MCV	1.68	1.35-2.10
HGB	1.11	0.99-1.24
Platelets	1.28	1.01-1.63

- Significant effects for: leukopenia, anemia, high MCV, and thrombocytopenia
- Strongest effects for MCV and RBC

Neutrophils: out-of-range values not related to benzene exposure



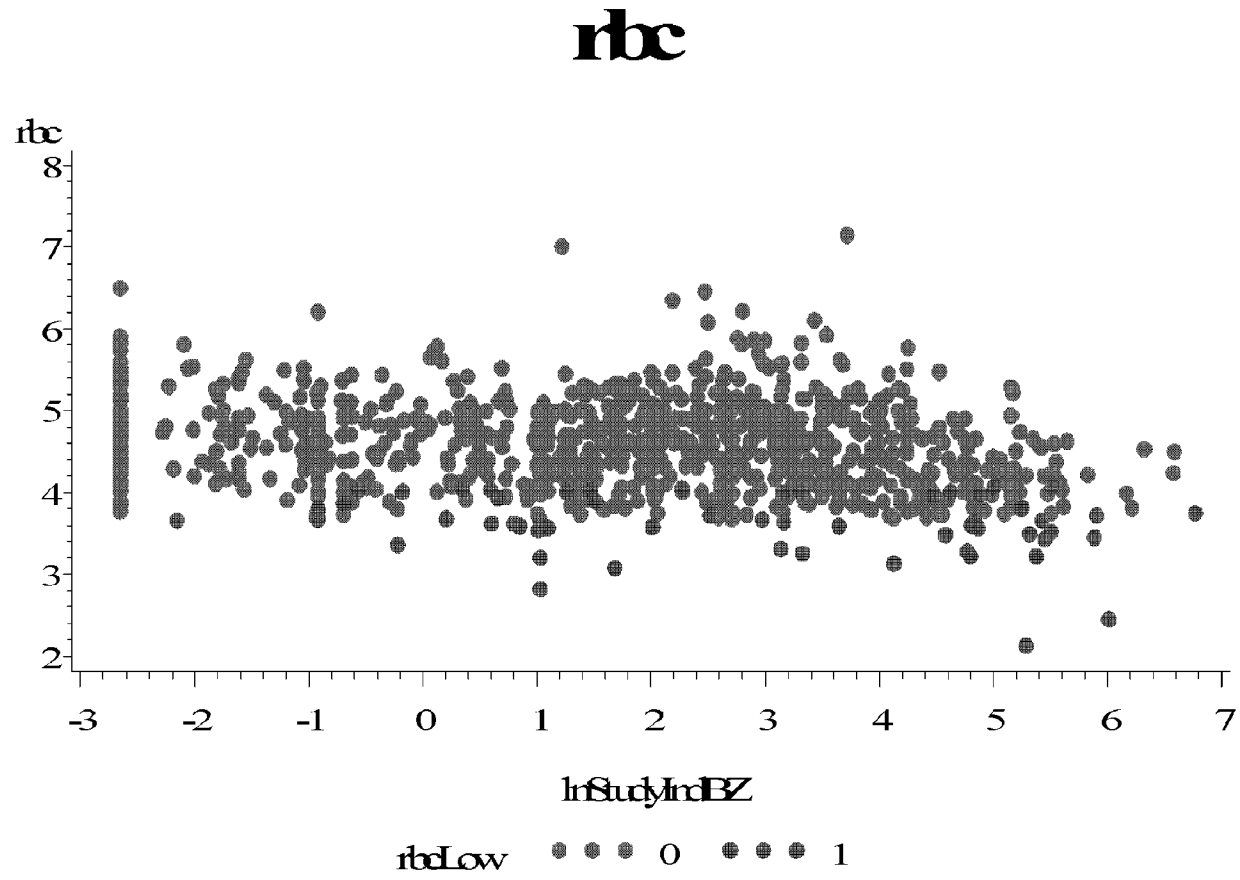
MCV: out-of-range values by benzene exposure



Logistic regression results by exposure category (OR, 95% CI):

< 1 ppm	1 - <10 ppm	10+ ppm
5.65 (0.62, 51.1)	5.91 (0.75, 46.5)	17.7 (2.35, 134.1)

RBC: out-of-range values by benzene exposure



Logistic regression results by exposure category (OR, 95% CI):

< 1 ppm	1 - <10 ppm	10+ ppm
10.5 (1.39, 79.7)	4.68 (0.61, 36.1)	14.7 (1.97, 110)

Summary / Conclusions

Metabolism

- Higher metabolic levels: males, smokers, alcohol use, toluene co-exposure
- No strong effects for: age, SNP's
- Benzene levels of 0.5 ppm can distinguish metabolites (HQ, ttMA) from background levels
- Metabolism rate slightly enhanced (2-3x) for lower (<1 ppm) exposures, although correction factor value is critical

Blood effects

- Benzene affects most blood indices, however, toluene co-exposure and collinearity under further investigation
- Most sensitive parameter for continuous indices is neutrophils (7.8 ppm) . . . appears to be a mild effect
- When clinically relevant effects are examined, benzene has an effect on WBC, RBC, PLT, and MCV
    - Stronger effects for RBC and MCV, albeit at higher concentrations

Collaborators

EMBSI and former EMBSI

Yimei Zhou, Min Chen, Mark Nicolich, Tom Armstrong

Cinpathogen / former Joint Molecular and Clinical Laboratory

Patrick Kerzic, Rich Irons

Fudan University

Fu Hua, Lu Lin

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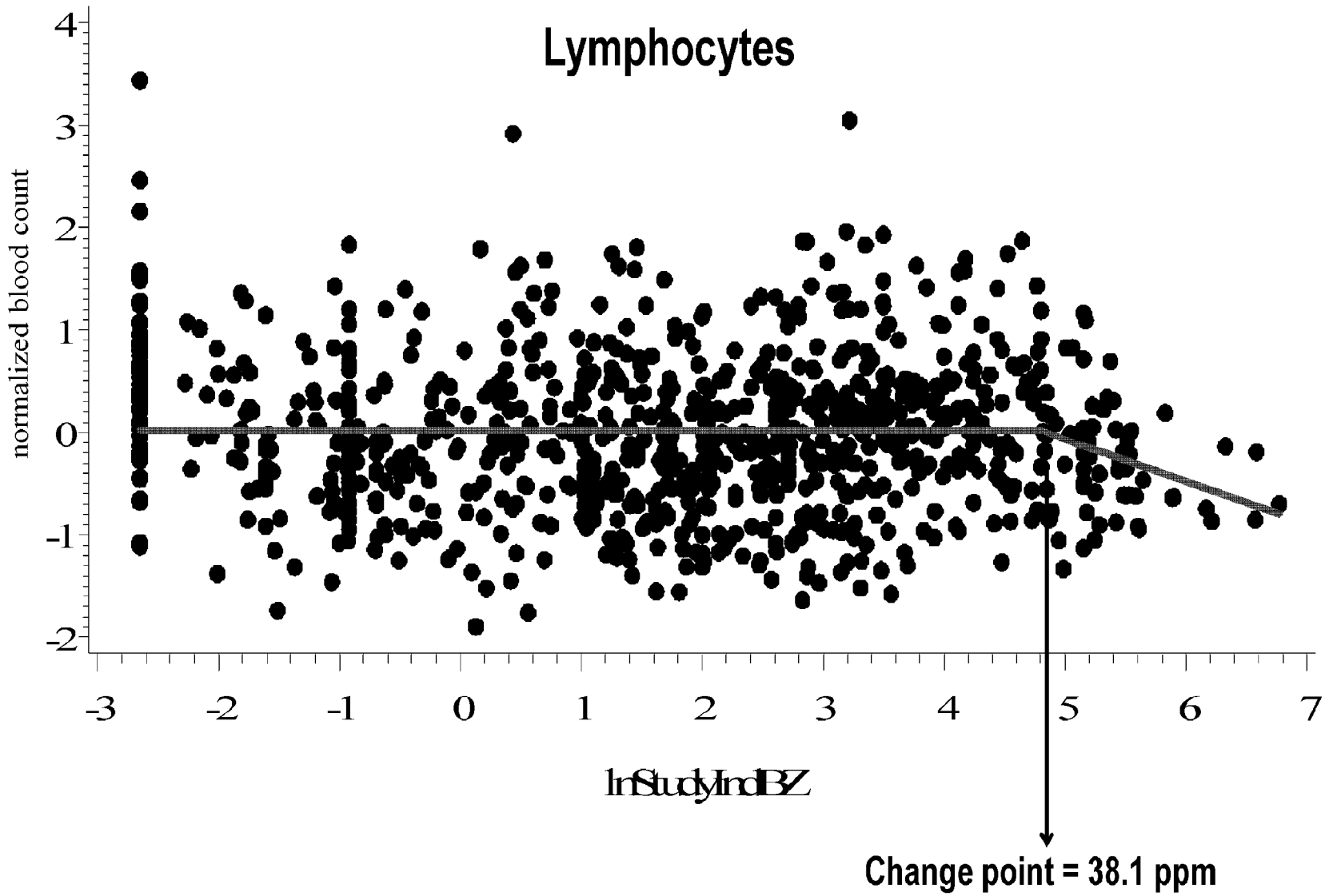
- SHS consortium (BP, Chevron, Conoco, ExxonMobil, Shell Chemical)
- Science Review Panel
- Ethics Review Panel
- Shanghai Municipal IPHS and CDC (Zhu Surong)
- Fudan University (and former) Miao Liuzhong
- Data Processing / QA – Susan Marcella (EMBSI), Gail Jorgensen (former EMBSI)
- University of Colorado IRB
- Fudan University Ethics Panel

Back-Up

Lymphocyte change-point regression results



Effect of NQO1*2 on hydroquinone levels