NexGen

Benzene

Example Approaches to Understanding Human Health Risks Associated with Environmental Exposures to Chemicals

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EPA United States Environmental Protection Agency

NIEHS National Institute of Environmental Health Sciences

NCGC NIH Chemical Genomics Center

CDC Centers for Disease Control and Prevention

Genes & Environment Laboratory

Benzene: A Prototype Environmental Leukemogen

Report from NexGen Risk Assessment working group
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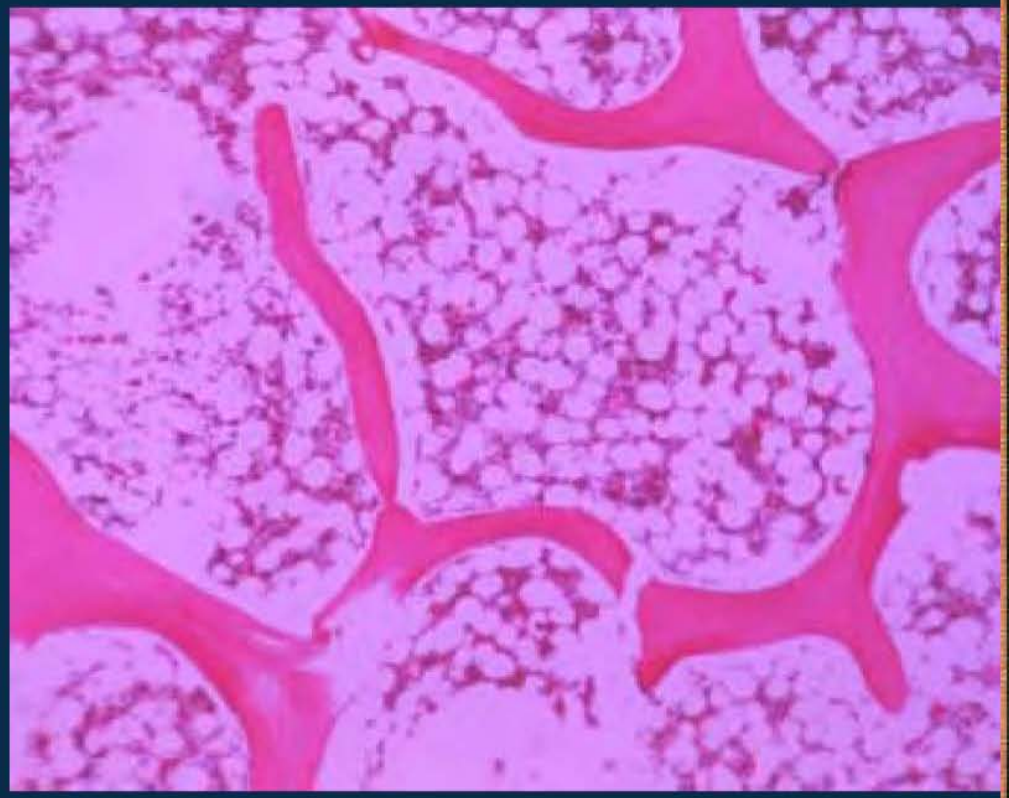
Benzene Carcinogenicity

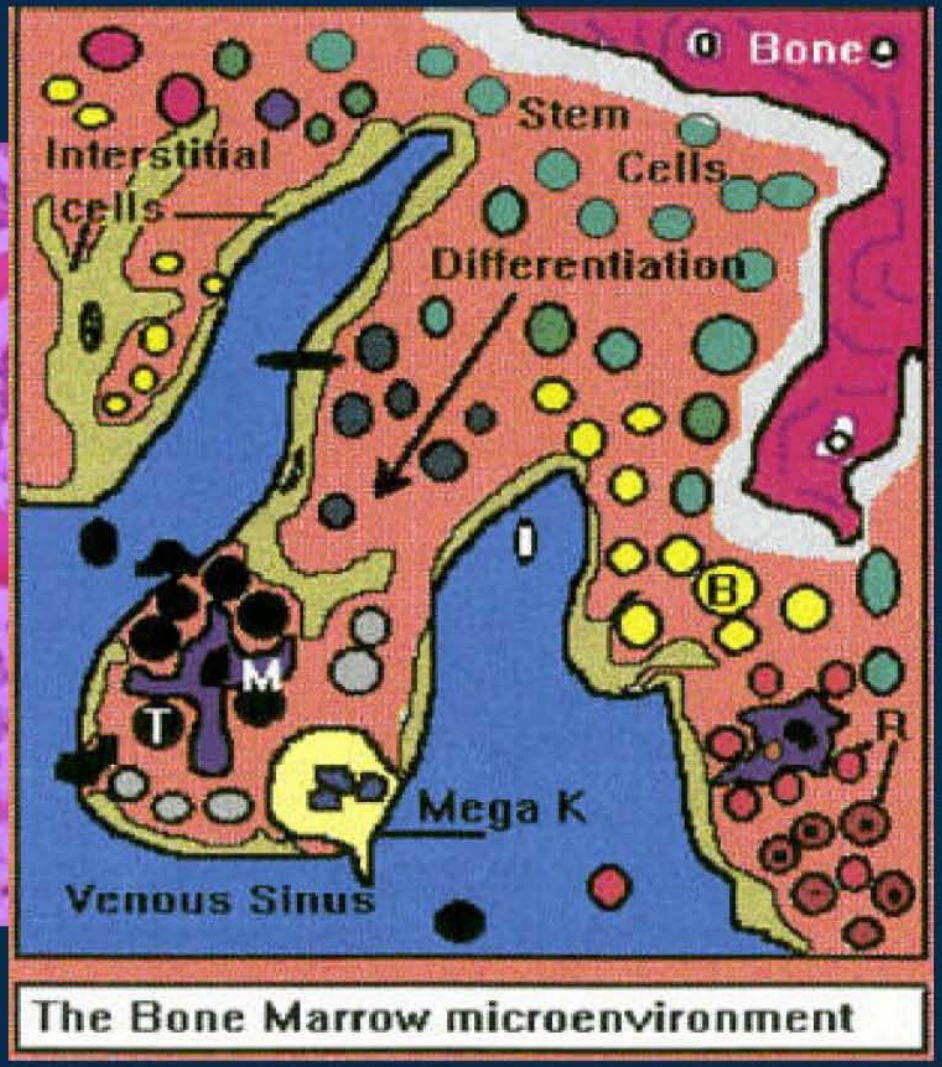
- Evidence of toxicity to the blood forming organs was first described in 1897
- Established cause of leukemia
- Probable cause of all forms of hematologic malignancy, including myelodyplastic synfromes, lymphoma and multiple myeloma
- No leukemia observed in standard rodent models – tumors of zymbal gland most sensitive site

Benzene would be negative in high-throughput screening

- Not cytotoxic
- Volatile, making it difficult to test in vitro
- Negative in Ames test – Does not generate mutagenic DNA adducts
- Does not bioaccumulate – is not a POP
- Requires metabolic activation at 2 sites in the body to produce bone marrow toxicity (CYP and peroxidase)
- Target cells are hematopoietic stem cells

Stem cells occupy an ordered microenvironment in marrow





The Bone Marrow microenvironment

Can new data and methods improve our understanding of risk in an important way?

YES, but in vitro methods to predict leukemogens need development

Human and animal biomarker data could be used to inform risk assessment

Outcome of Benzene Discussion

- In vitro testing of leukemogens requires a new model
  - Stem cells are target
  - $CD34^{+}lin^{-}$ from marrow/blood
  - Reside in a niche of support cells
  - Metabolic activation needed
  - Volatiles need to be testable

Animal tests are possibly predictive:

1) bone marrow toxicity showing pancytopenia;
2) positive micronucleus test

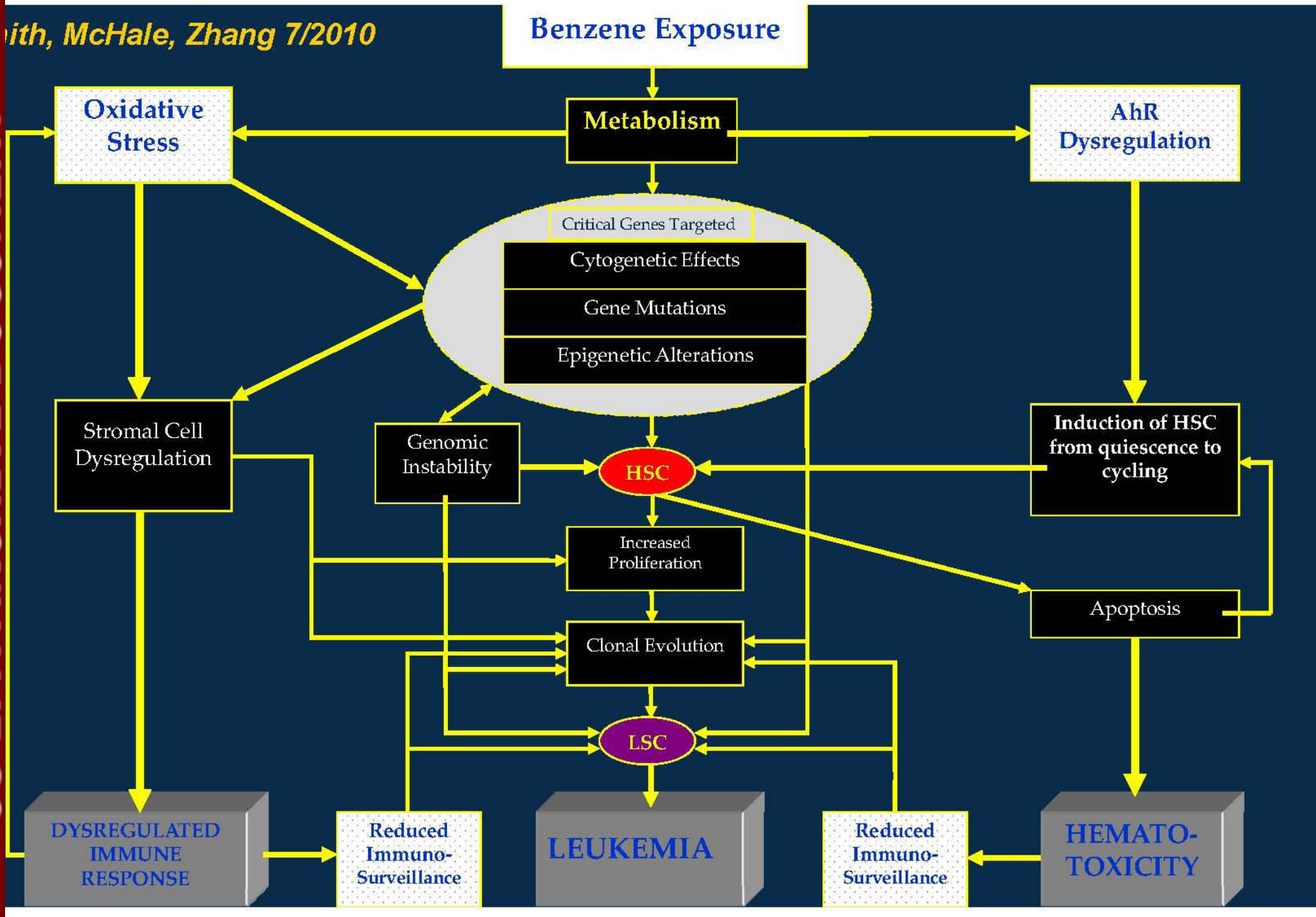
- Other models, e.g., zebrafish

Hypothesized mode of action with proposed key events for benzene-induced leukemia

Meek ME, Klaunig JE.
Chem Biol Interact. 2010; 184(1-2):279-85.

Key Events

1. Metabolism of benzene to a benzene oxide metabolite
2. Interaction of the benzene metabolite with target cells in the bone marrow
3. The formation of initiated, mutated bone marrow target cells
4. The selective clonal proliferation of these mutated cells
5. The formation of the neoplasm (leukaemia)



Conclusion on Mechanism of Action and Assessment of Risk at Low Doses

- Multiple key events and modifying factors involved in benzene-induced leukemia
- Will be challenging to produce a biological-based model for risk assessment
- No in vitro model exists – omics studies in CD34+ cells do not correlate with in vivo findings, but new 3D models of niche are being developed

Another approach:

- Use biomarkers to examine dose-response relationship in low-dose region (e.g. hematotoxicity, chromosome changes and altered gene expression)

What newly available data and/or knowledge are not included in current health assessments but potentially should be?

a. multiple epi studies since last assessment

b. 'omics' data incl. disease pathways

c. hematoxicity and chromosome damage data

e. genetic risk factors (SNPs, etc) - GWAS

f. toxicokinetics variability – two pathways

g. lifestage susceptibility (in utero, etc)

h. pre-existing conditions (obesity, blood disorders)

i. reproductive outcomes (sperm counts)

j. birth defect study

REPORTS

Hematotoxicity in Workers Exposed to Low Levels of Benzene

Qing Lan,[*] Luoping Zhang,[*] Guilan Li, Roel Vermeulen, Rona S. Weinberg, Mustafa Dosemeci, Stephen M. Rappaport, Min Shen, Blanche P. Alter, Yongji Wu, William Kopp, Suramya Waidyanatha, Charles Rabkin, Weihong Guo, Stephen Chanock, Richard B. Hayes, Martha Linet, Sungkyoon Kim, Songnian Yin, Nathaniel Rothman,[†] Martyn T. Smith[†‡]

Science
3 December 2004
Vol. 306 No. 5702
Pages 1633–1844 $10

Hematotoxicity: A Phenotypic Outcome of Benzene

Benzene Reduce Blood Cell Counts

There's no doubt that benzene, a widely used industrial chemical, can be harmful. Workers highly exposed to benzene fumes, for example, run an increased risk of leukemia and bone-marrow toxicity. But the risk from smaller exposures is unclear. Now a tightly controlled study in Chinese factories, reported on page 1774, provides reason for concern. Workers who inhaled less than 1 part per million (ppm) of benzene—an exposure considered safe under U.S. occupational guidelines—had fewer white blood cells than did unexposed workers.

Although the workers weren't sick, the results hint that low doses of benzene may alter the bone marrow and could lead to health problems, some experts say. The study also provides the first direct evidence in humans that benzene harms the progenitor cells that give rise to blood cells. "It

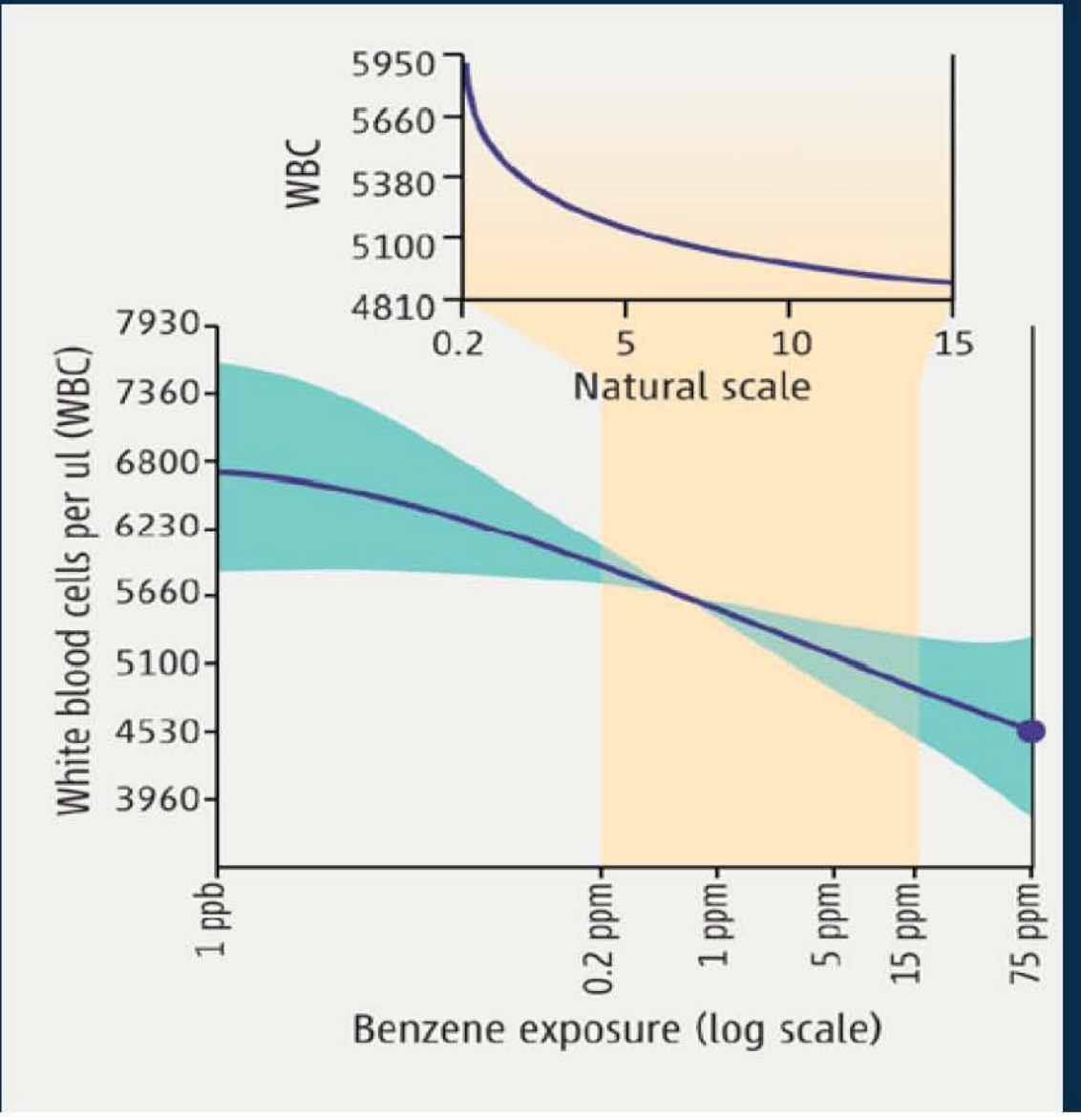
than did unexposed workers. But this also held true for the 109 workers exposed to less than 1 ppm benzene, even after controlling for smoking and other potential confounding factors. These workers had on average 15% to 18% fewer granulocytes and B cells than did unexposed workers—raising concerns about

Luoping Zhang of the University of California, Berkeley, and others in the research team also studied the effect of benzene on the

Spline regression analyses of WBC count and benzene exposure

- Modeling of data from 247 exposed and 139 control subjects
- No apparent threshold
- Evidence of supra-linear response in agreement with epi data

Lan Q et al. *Science* 312, 999, 2006



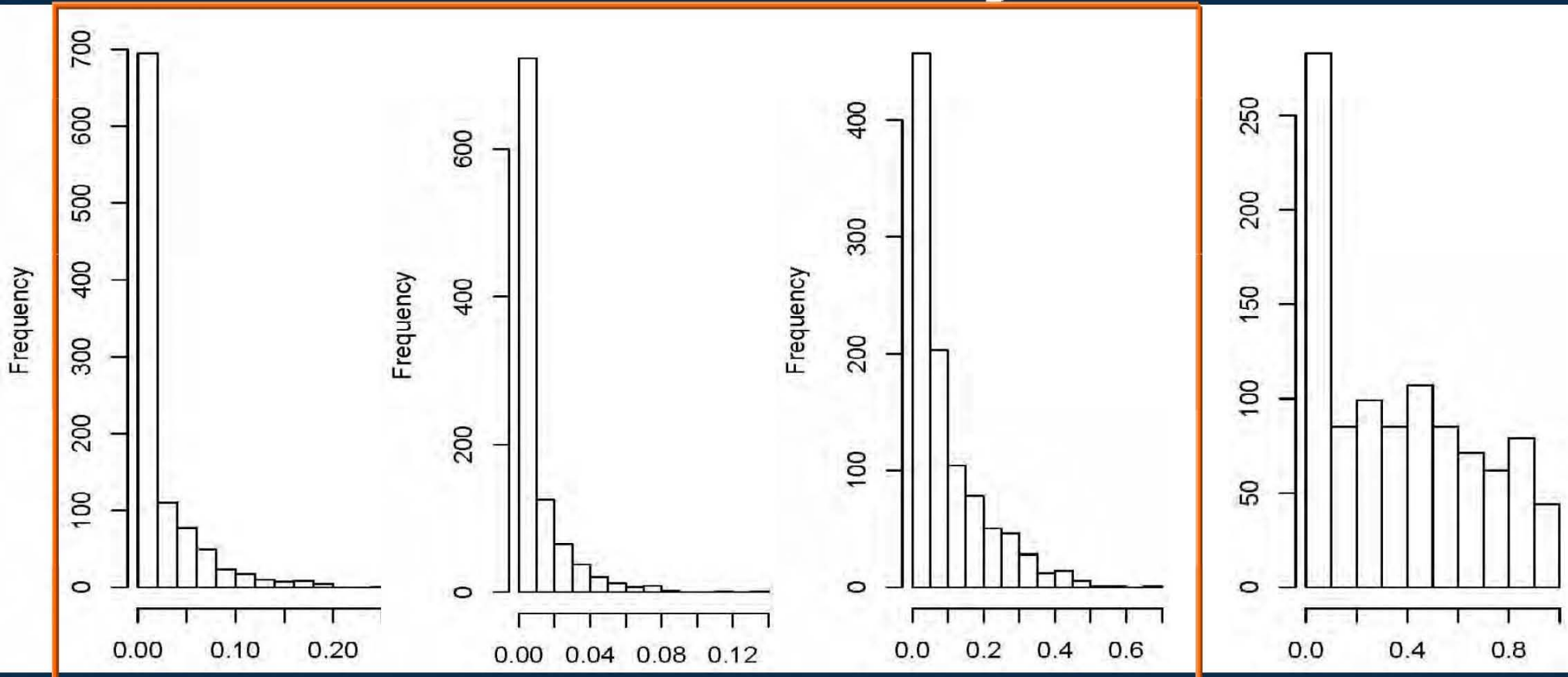
Collaborators on Benzene Study in Tianjin, China (2000-2001)

Roel Vermeulen & Nat Rothman

Stephen Rappaport with
Qing Lan and Songnian Yin

Cliona McHale & Alan Hubbard

Greatest Source of Variation lies between Subjects



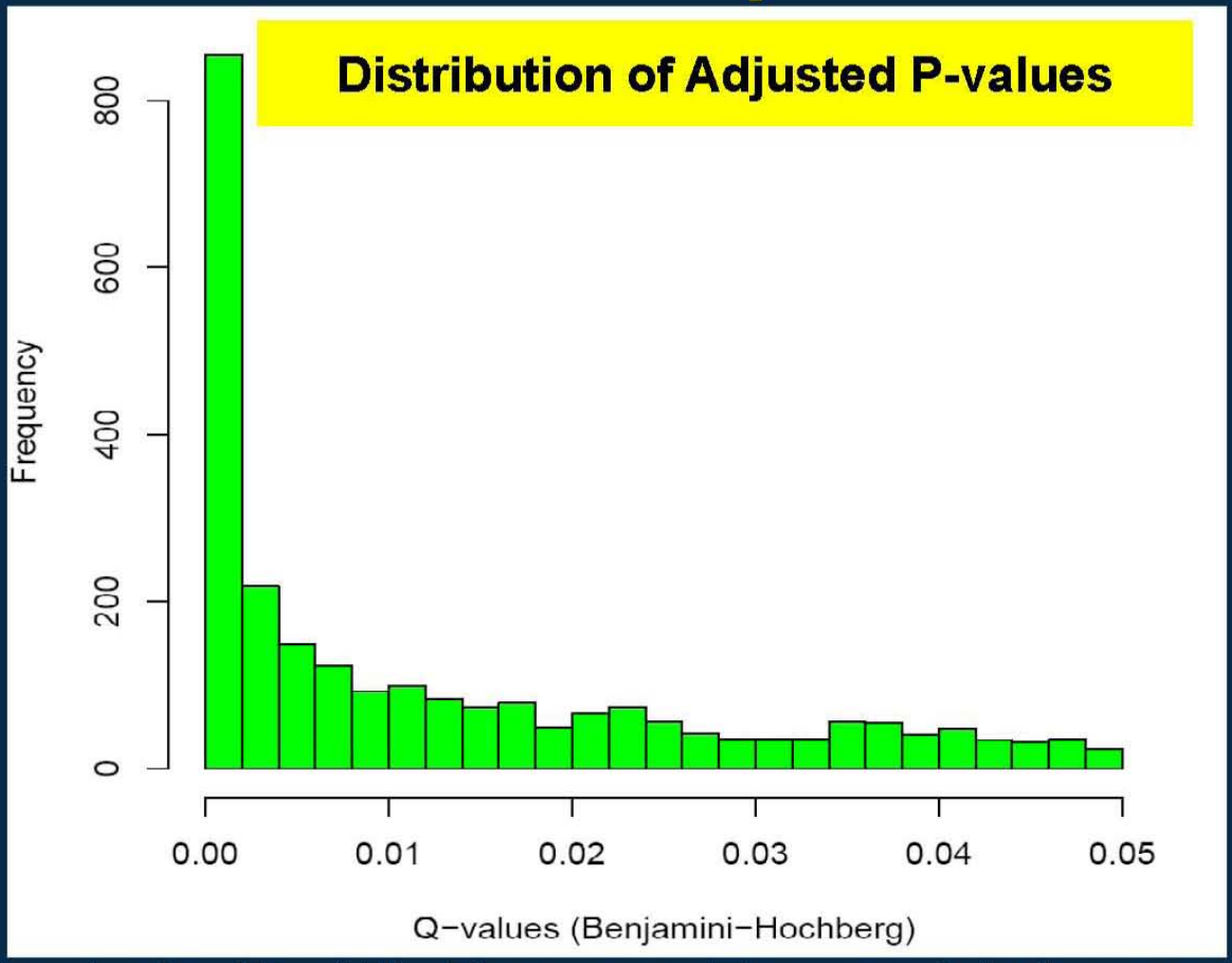
Chip **Hyb** **Label** **Subject**

Nuisance Variation

McHale C et al, EHP, 2010

Proportions of variance are shown for normalized data

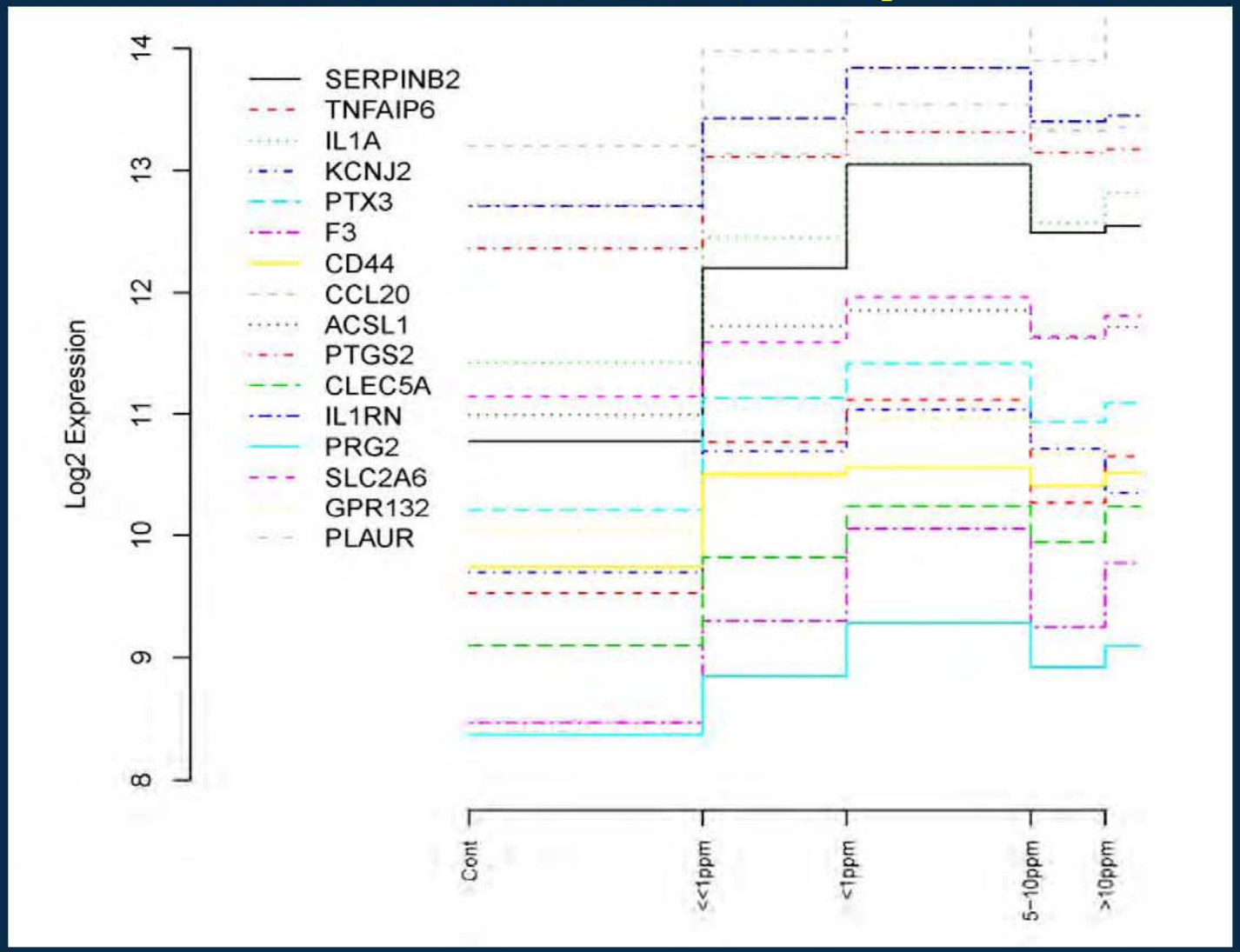
Highly Significant Changes in Gene Expression Associated with Low-Dose Benzene Exposure



McHale C et al, EHP, 2010

Controls (N=42); Benzene-Exposed (<1ppm; N=59)

Identified 16 genes that were up-regulated at all levels of benzene exposure



McHale C et al, EHP, 2010

A potential "signature" of benzene exposure?

Enriched Pathways Associated with Benzene

KEGGID	Pathway	*p* -value
path:hsa04620	Toll-like receptor signaling pathway	0.000
path:hsa04210	Apoptosis	0.000
path:hsa05221	Acute myeloid leukemia	0.000
path:hsa00190	Oxidative phosphorylation	0.000
path:hsa04662	B cell receptor signaling pathway	0.000
path:hsa04660	T cell receptor signaling pathway	0.001
path:hsa05120	Epithelial cell signaling in Helicobacter pylori infection	0.002
path:hsa04060	Cytokine-cytokine receptor interaction	0.003
path:hsa00563	Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	0.003
path:hsa05222	Small cell lung cancer	0.004

Disease Pathways Associated with Benzene

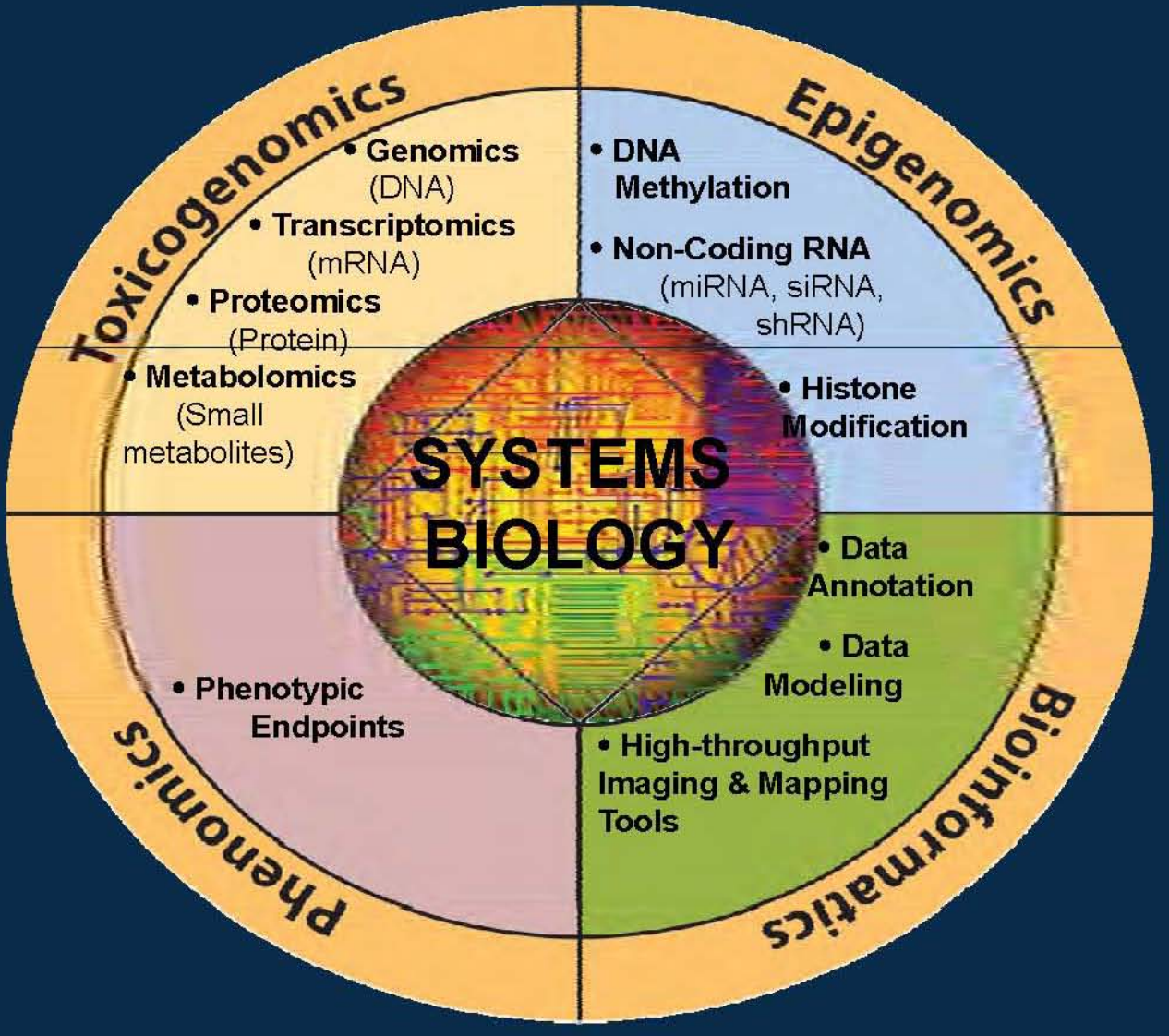
KEGG ID	Pathway	*p* -value	
		Overall	<1 ppm
path:hsa05221	Acute myeloid leukemia	0.000	0.002
path:hsa05222	Small cell lung cancer	0.004	0.002
path:hsa05212	Pancreatic cancer	0.039	0.007
path:hsa05220	Chronic myeloid leukemia	0.092	0.033
path:hsa05211	Renal cell carcinoma	0.109	0.024

Thomas R, Gohlke DM, Stopper GF, Parham FM and Portier CJ. (2009) *Genome Biology*, *10*:R44

Next step: Systems Biology Approach

Integration of transcriptomics, proteomics, and genomics with other data including epigenomics (methylomics and miRNomics) and phenomics, to yield a more complete picture of the individual and/or cellular response at the system levels.

Systems Biology: Wired Connections



Reverse engineering to predict risk

- Microarray (or sequencing) of RNAome to determine dose-response of AML pathway over larger range of exposures
- Phenotypically anchor with blood cell counts
- Add in more omics endpoints (genomics, epigenomics, proteomics, metabolomics, etc.) to produce systems biology approach

How can this new type of information best be incorporated into health assessments (cancer and noncancer) and utilized to inform risk managers and the public?

a. Support for epidemiological data conclusions (hazard ID)

b. Explore dose-response (shape, duration, timing – compare omic / systems data to epidemiology data)

c. Identify susceptible populations

d. Provide information on effect of co-exposures

What new policies and procedures are needed?

a. An in vitro test that uses stem cells in a 3D niche
b. Guidance on how to compare epi/exposure data with biomarker data ('omics, etc)
c. Develop training and procedures for use of 'omics data in risk assessment
d. Explore quantitative approaches for continuous health outcomes (eg., blood counts)

Next Steps

- Explore use of hematological parameter data to predict leukemia risk in a biomarker-based approach
- Explore systems biology-based risk model of benzene, integrating single and multiple datasets:
  - » Phenomic data
  - » Newly available data from multiple "omic" studies in humans at low exposures
  - » Disease-specific (AML) pathway data
- Examine predictability by comparison of "omic" and biomarker-based approaches with dose-response model based on leukemia epidemiology data
- Identify data gaps and opportunities for model refinement

Thanks to all participants in
Benzene group
and our scientific collaborators

Thanks to EPA, NCI and NIEHS for
supporting this work