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

Dr. Martyn Smith
University of California - Berkeley
Benzene: A Prototype Environmental Leukemogen

Report from NexGen Risk Assessment working group

Chairs: Martyn Smith, Kate Guyton, Bob Sonawane

School of Public Health
University of California, Berkeley
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 syndromes, 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
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
  - \( CD34^+\text{lin}^- \) 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


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
Hematotoxicity: A Phenotypic Outcome of Benzene

Hematotoxicity in Workers Exposed to Low Levels of Benzene

Qing Lan,* Luoping Zhang,* Guilan Li, Roel Vermeulen, Rona S. Weinberg, Mustafa Dosemecli, Stephen M. Rappaport, Min Shen, Blanche P. Alter, Yongji Wu, William Kopp, Surayya Waidyanatha, Charles Rabkin, Weihong Ge, Stephen Chanock, Richard B. Hayes, Martha Linet, Sungjoon Kim, Songnian Yin, Nathaniel Rothman, Martyn T. Smith

Benzene Reduce Blood Cell Counts

There’s no doubt that benzene, a widely used industrial chemical, can be harmful. Workers highly exposed to benzene become ill, for example, run an increased risk of leukemia and bone-marrow toxicity. But the risk from smaller exposure is unclear. Now a tightly controlled study in Chinese factories, reported on page 1798, provides some answers. Workers who breathed 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. The researchers say

Opportunity at Meridian Planum
Spline regression analyses of WBC count and benzene exposure

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

Lan Q et al. *Science* 312, 999, 2006
Collaborators on Benzene Study in Tianjin, China (2000-2001)

Stephen Rappaport with Qing Lan and Songnian Yin

Roel Vermeulen & Nat Rothman

Cliona McHale & Alan Hubbard
Greatest Source of Variation lies between Subjects

Proportions of variance are shown for normalized data

McHale C et al, EHP, 2010
Highly Significant Changes in Gene Expression Associated with Low-Dose Benzene Exposure

Distribution of Adjusted P-values

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

McHale C et al, EHP, 2010
Identified 16 genes that were up-regulated at all levels of benzene exposure

A potential “signature” of benzene exposure?

McHale C et al, EHP, 2010
## Enriched Pathways Associated with Benzene

<table>
<thead>
<tr>
<th>KEGG ID</th>
<th>Pathway</th>
<th>p -value</th>
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<tbody>
<tr>
<td>path:hsa04620</td>
<td>Toll-like receptor signaling pathway</td>
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<tr>
<td>path:hsa04210</td>
<td>Apoptosis</td>
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<tr>
<td>path:hsa05221</td>
<td>Acute myeloid leukemia</td>
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<tr>
<td>path:hsa00190</td>
<td>Oxidative phosphorylation</td>
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<td>path:hsa04662</td>
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<td>path:hsa04660</td>
<td>T cell receptor signaling pathway</td>
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<td>path:hsa05120</td>
<td>Epithelial cell signaling in Helicobacter pylori infection</td>
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<td>path:hsa04060</td>
<td>Cytokine-cytokine receptor interaction</td>
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<td>path:hsa00563</td>
<td>Glycosylphosphatidylinositol(GPI)-anchor biosynthesis</td>
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<tr>
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<td>Small cell lung cancer</td>
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## Disease Pathways Associated with Benzene

<table>
<thead>
<tr>
<th>KEGG ID</th>
<th>Pathway</th>
<th>Overall p -value</th>
<th>&lt;1 ppm p -value</th>
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<tbody>
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<td>path:hsa05221</td>
<td>Acute myeloid leukemia</td>
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<td>0.002</td>
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<tr>
<td>path:hsa05222</td>
<td>Small cell lung cancer</td>
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<td>path:hsa05212</td>
<td>Pancreatic cancer</td>
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<tr>
<td>path:hsa05211</td>
<td>Renal cell carcinoma</td>
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<td>0.024</td>
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</tbody>
</table>

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

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