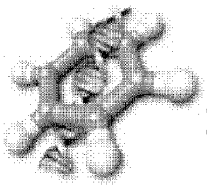
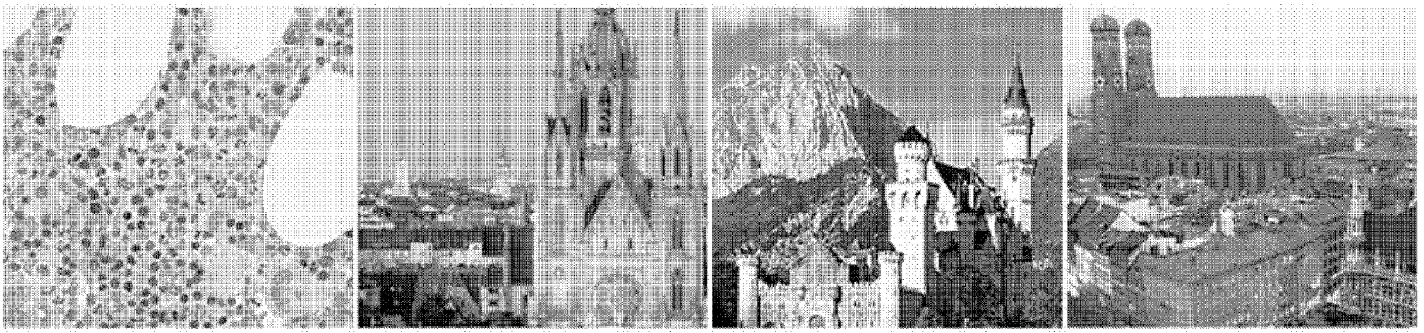


Benzene 2009:

Health Effects and Mechanisms of Bone Marrow Toxicity
Implications for t-AML and the Mode of Action Framework

September 7-11, 2009 • Technische Universität München • Munich, Germany



Benzene 2009

Program

Abstracts

Information for Attendees

Technical University of Munich
Munich, Germany
September 7-11, 2009

Organizing Committee

The members of the Organizing Committee took part in regular monthly conferences as the meeting developed.

Subcommittees, staffed by members of the Organizing Committee, worked between conference calls. The co-chairs of these committees were:

Organizing Committee: H. Greim, R. Snyder

Finance: H. Greim, B. Meek, J.M. Rice, R. Snyder

Program: M.G. Bird, H. Greim, R. Larson, R. Snyder

Publications: J. Bond, J.M. Rice

Communications: D. Kaden, D. Pyatt

Poster Committee: D. Kaden, S. Gross, D. Pyatt, C. Weisel

Travel Fellowship Committee: D. Kaden, R. Albertini, Y. Hirabayashi, G. Minsavage, T. Inoue

Local arrangements: H. Greim, I. Schaupp

List of members of the Organizing Committee:

Albertini, Richard	University of Vermont, Burlington, VT, USA
Bird, Michael	ExxonMobil Biomedical Sciences, Annandale, NJ, USA
Bolt, Hermann M.	Leibniz Research Centre (IfADo), Technical Univ. of Dortmund, Germany
Bond, James A.	Chemico-Biological Interactions, Santa Fe, NM, USA
Bus, James	Dow Chemical Company, Midland, MI, USA
Cagen, Stuart	Shell Chemical LP, Houston, TX, USA
Eastmond, David	University of California, Riverside, CA, USA
French, John	National Toxicology Program, NIEHS, NIH, Research Triangle Park, NC, USA
Gasiewicz, Thomas	University of Rochester, Rochester, NY, USA
Golding, Bernard T.	University of Newcastle upon Tyne, UK
Greim, Helmut	Technical University of Munich, Freising-Weihenstephan, Germany
Gross, Sherilyn	University of Colorado, Denver, Aurora, CO, USA
Hirabayashi, Yoko	National Institute of Health Sciences, Tokyo, Japan
Inoue, Tohru	National Institute of Health Sciences, Tokyo, Japan
Kaden, Debra	DakTox LLC, Arlington, MA, USA
Klaunig, James C.	Indiana University School of Medicine, Indianapolis, IN, USA
Larson, Richard	University of Chicago, Chicago, IL, USA
Levy, Len	Institute of Environment and Health, Cranfield, Bedford, UK
Meek, Bette	McLaughlin Centre for Population Health Risk Assessment, Ottawa, ON, Canada
Minsavage, Gary	Concawe, Brussels, Belgium
Monks, Terrence J.	University of Arizona, Tucson, AZ, USA
Pyatt, David	University of Colorado, School of Public Health, Superior, CO, USA
Rice, Jerry	Georgetown University Medical Center, Washington, DC, USA
Ross, David	University of Colorado, Denver, CO, USA
Schaupp, Isabel	Technical University of Munich, Freising-Weihenstephan, Germany
Schnatter, Robert A.	ExxonMobil Biomedical Sciences, Inc., Annandale, NJ, USA
Snyder, Robert	Ernest Mario School of Pharmacy of Rutgers University, Piscataway, NJ, USA
Sonawane, Bob	US Environmental Protection Agency, Washington, DC, USA
Trush, Michael	Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
White, Russell	American Petroleum Institute, Washington, DC, USA
Zhang, Luoping	University of California, School of Public Health, Berkeley, CA, USA

SPONSORS

Technical University of Munich
Klinikum rechts der Isar
Munich, Germany

American Petroleum Institute
Washington, DC, USA

Bayerisches Staatsministerium für Umwelt und Gesundheit
(Bavarian State Ministry of the Environment and Public Health)
Munich, Germany

CONCAWE
The Oil Companies' European Association for Environment,
Health and Safety in Refining and Distribution
Brussels, Belgium

Deutsches Museum
Munich, Germany

Environmental and Occupational Health Sciences Institute of
Rutgers, the State University of New Jersey and the
University of Medicine and Dentistry of New Jersey,
Robert Wood Johnson Medical School
Ernest Mario School of Pharmacy
Piscataway, NJ, USA

EUGT e.V.
Europäische Forschungsvereinigung für Umwelt und
Gesundheit im Transportsektor e.V.
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Munich Tourist Office
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National Institute of Health Sciences (NIHS)
Tokyo, Japan

Robert Bosch GmbH
Berlin, Germany

Society of Toxicology
Reston, VA, USA

United States Environmental Protection Agency
Washington, DC, USA

The Weinberg Group
Brussels, Belgium

Contribution from Health Canada, Ottawa, is also gratefully acknowledged

Symposium Schedule

Monday, September 7, 2009	***7:00 PM***	Reception & registration
Tuesday, September 8, 2009	***8:00 AM***	Greetings & welcome
	5:00 PM	Award Ceremony
	6:00 PM	End of Symposium Day 1
Wednesday, September 9, 2009	***8:00 AM***	Beginning of Symposium Day 2
	1:00 PM	End of Symposium Day 2; Snack & Beginning of Excursion
	1:30 PM	Departure to Starnberg at the latest
	3:00 PM	Lake Cruise on Starnberger See
	6:45 PM	Dinner at "UNDOSA" Restaurant
	anytime	Individual departure back to Munich
Thursday, September 10, 2009	***8:00 AM***	Beginning of Symposium Day 3
	5:00 PM	End of Symposium Day 3
	6:00 PM	Reception at Deutsches Museum with State Secretary Melanie Huml of the Bavarian State Ministry of the Environment and Public Health
	8:00 PM	End of reception
Friday, September 11, 2009	***8:30 AM***	Beginning of Symposium Day 4
	1:00 PM	End of Symposium

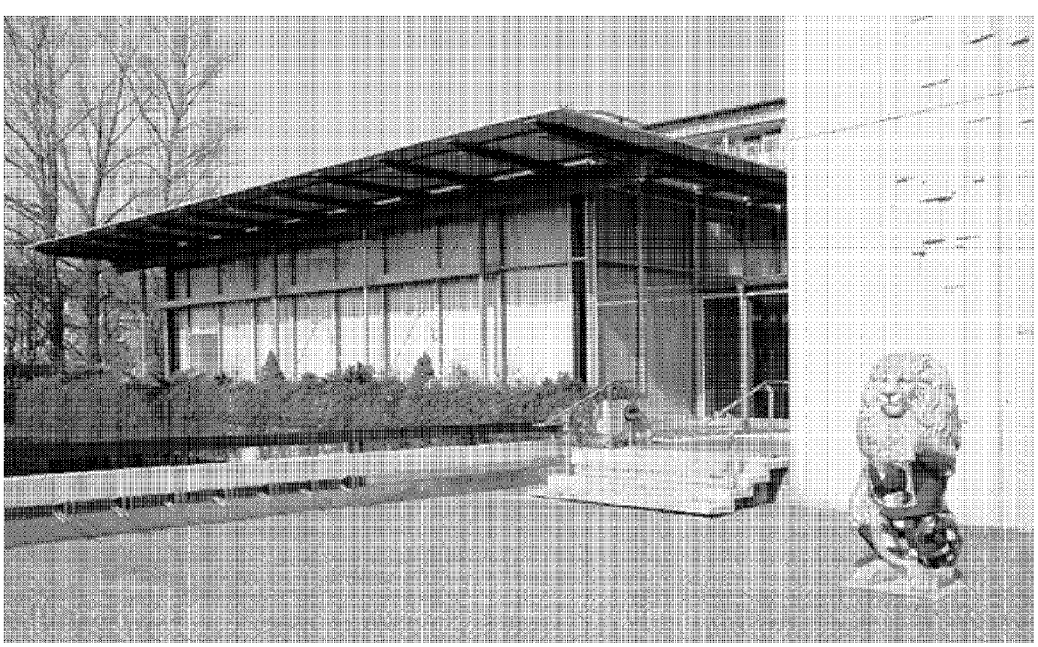
Location:

Hörsaal “Pavillon”

Klinikum rechts der Isar
der Technischen Universität München

Ismaninger Strasse 22
81675 München
Germany

(Entrance: Einsteinstrasse)



How to get there:

By train and subway:

a) Arrival "Hauptbahnhof" (central station):
Take subway (U-Bahn) U5, direction "Neuperlach Süd"
or take U4, direction "Arabellapark"
and get off at "Max-Weber-Platz".

b) Arrival "Ostbahnhof":
Take subway (U-Bahn) U5, direction "Laimer Platz"
and get off at "Max-Weber-Platz".

www.mvv-muenchen.de

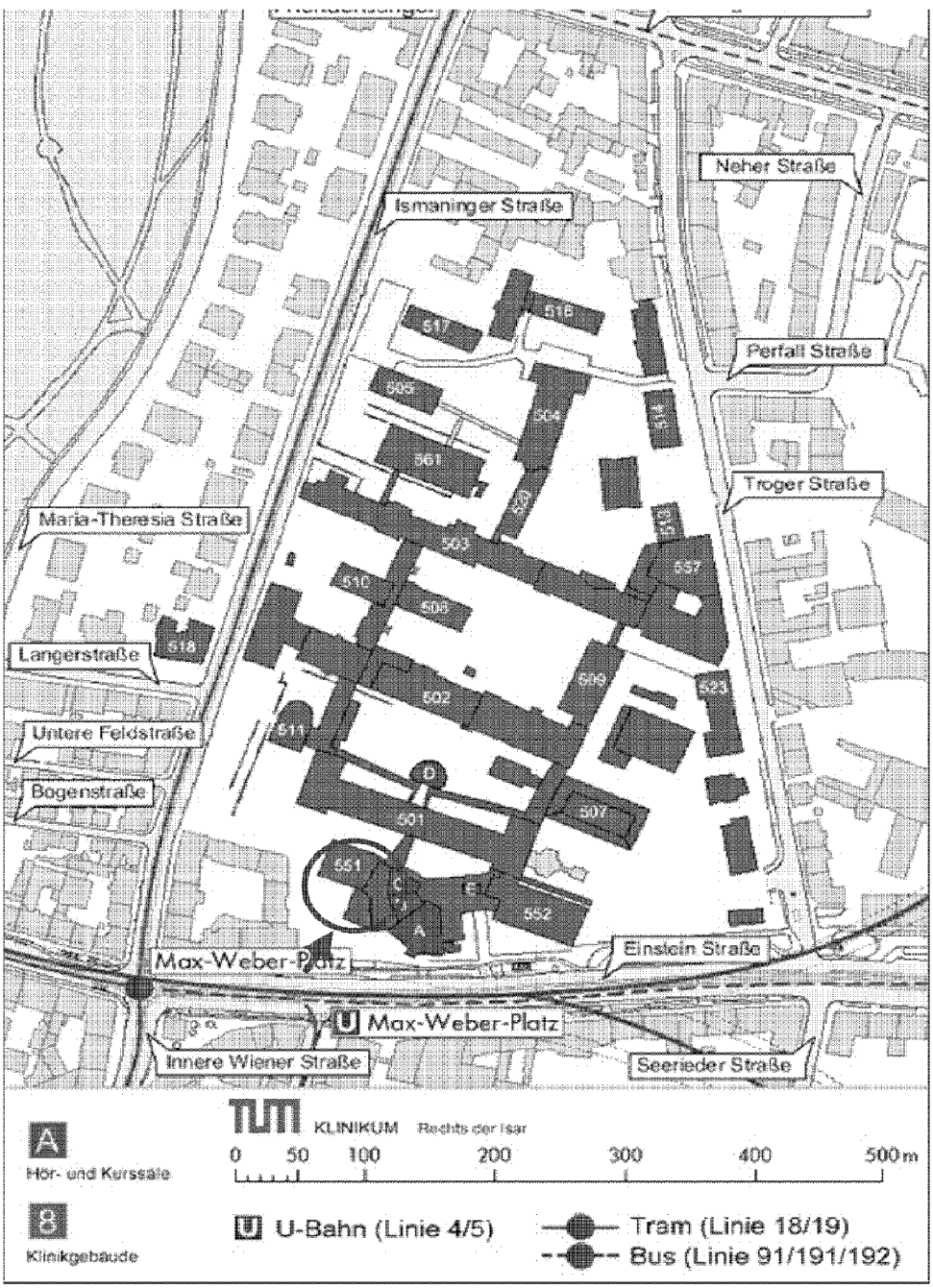
By air:

Arrival at Munich Airport (MUC):
Take a taxi or
take the "S-Bahn" S8 or S1 to "Hauptbahnhof"
or "Ostbahnhof".
Continue from there as explained in 'arrival by train'.

http://www.munich-airport.de/

Map:

Medical center „Klinikum rechts der Isar“ der Technischen Universität München (Hörsaal “Pavillon”: Building No. 551)



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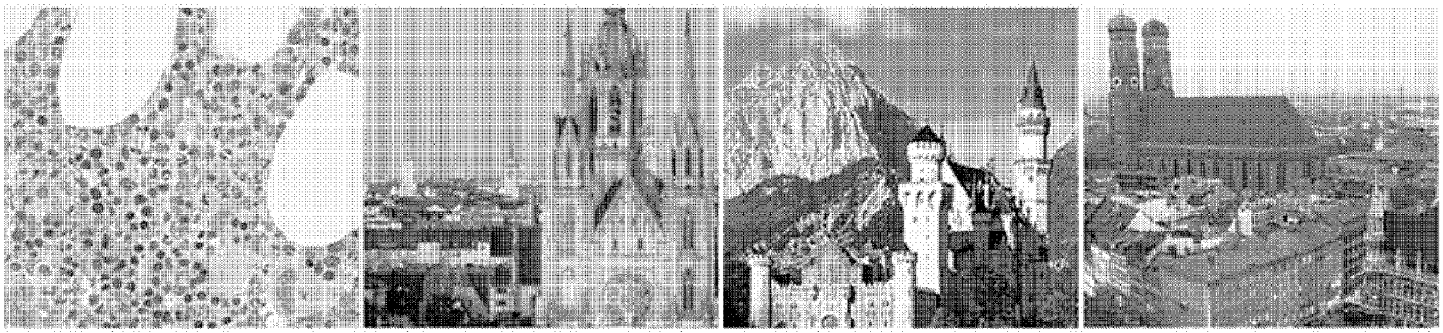


PROGRAM

Monday, September 7, 2009

7:00 PM Reception and registration

Tuesday, September 8, 2009

Platform Session 1: **Introduction to the Symposium.**
Co-chairs: **H. Greim, Technical University of Munich;**
H. M. Bolt, Leibniz Research Centre, Technical University of Dortmund

8:00 AM Greetings and welcome. H. Greim, Technical University of Munich

8:20 A century of research on the hematotoxic effects of benzene; aims of the symposium. R. Snyder, Rutgers, The State University of New Jersey, and EOHSI

8:40 Interpreting available data and identifying critical data gaps for benzene risk assessment. Contribution of the IPCS/ILSI framework for "Mode of Action/Human Relevance". B. Meek, University of Ottawa

Platform Session 2: **Bone marrow and leukemia.**
Co-chairs: **R. Larson, University of Chicago;**
D. Ross, University of Colorado.

9:00 Regulation of hematopoiesis by bone marrow stem cells. C. Peschel, Technical University of Munich

9:30 Regulation of hematopoiesis by the bone marrow environment. R. A. J. Oostendorp, Technical University of Munich

10:00 Break (Posters will be available for viewing during the break)

Poster Session Co-chairs: **D. Kaden, DakTox LLC, Massachusetts;**
S. Gross, University of Colorado, Denver

Platform Session 2 (continued)

10:30 The World Health Organization (WHO) classification of tumors of the hematopoietic and lymphoid tissues. J. Vardiman, University of Chicago

11:00 Myelodysplastic Syndrome (MDS): A case-case analysis of benzene exposure and clinical features using the WHO (2001)/(2008) criteria for diagnosis. R. Irons, Fudan University, Shanghai

11:30 Incidence and susceptibility to therapy-related leukemia. G. Leone, Catholic University of Rome

12:00 Implications of latency period between benzene exposure and risk of leukemia. G. Triebig, University of Heidelberg

12:30 Chromosome pathways, genetic changes, cooperating mutations, and candidate genes in primary and therapy related MDS and AML. M. Le Beau, University of Chicago

1:00 Lunch (Posters will be available for viewing during the lunch hour)

Platform Session 2 (continued)

2:00 MicroRNA, epigenetics, and copy number changes: New levels of gene regulation in acute myeloid leukemia (AML). R. Larson, University of Chicago

2:30 Epigenetic changes in therapy-related MDS/AML. M.T. Voso, Catholic University of Rome

Platform Session 3: **Recent studies and re-evaluation of data on the epidemiology of benzene-induced diseases.**

Co-chairs: **E. Taioli, SUNY, Downstate Medical Center, New York;**
P. Boogaard, Shell Health, The Netherlands

3:00 Ensuring comparability of benzene exposure estimates across three nested case-control studies in the petroleum industry in support of a pooled analysis. D. Glass, Monash University, Australia

3:30 Break (Posters will be available for viewing during the break)

4:00 Benzene as a cause of lymphoproliferative disorders. B.D. Goldstein, University of Pittsburgh

4:30-5:00 Shanghai Health Study: Hospital-based case-control studies of acute myeloid leukemia and non-Hodgkin lymphoid neoplasms in Shanghai. Analysis of environmental and occupational risk factors by WHO subtypes. Otto Wong, Applied Health Sciences, California

5:00 Award Ceremony

5:15-6:00 Poster session (posters will be attended by presenters)

Wednesday, September 9, 2009

Platform Session 3 (continued)

8:00 AM Metabolism and peripheral blood effects in moderately-exposed benzene workers in Shanghai. A. R. Schnatter, ExxonMobil Biomedical Sciences, New Jersey

8:30 Benzene and childhood leukemia. D. Pyatt, Summit Toxicology, Colorado

Platform Session 4: **Mechanistic studies: Reactive metabolites and reactive oxygen species.**
Co-chairs: **F. Oesch, University of Mainz;**
D. Schrenk, University of Kaiserslautern

9:00 Fate of benzene oxide. T. Monks, University of Arizona, Tucson

9:30 Human benzene metabolism following occupational and environmental exposures. S. Rappaport, University of California, Berkeley

10:00 Break (Posters will be available for viewing during the break)

10:30 Gene expression in benzene-exposed workers by microarray analysis of peripheral blood mononucleocytes: Induction and silencing of *CYP4F3A* and regulation of DNA-dependent protein kinase catalytic subunit in DNA double strand break repair. Yongyi Bi, Wuhan University, China

11:00 Modeling the formation and reactions of benzene metabolites. B.T. Golding, University of Newcastle Upon Tyne, UK

11:30 Influence of toluene co-exposure on benzene's metabolism and genotoxicity in mice using continuous and intermittent exposures. M. Bird, ExxonMobil Biomedical Sciences, New Jersey

12:00 Biological activity of hydroquinone thiol conjugates. S. Lau, University of Arizona, Tucson

12:30 Systems biology of human benzene exposure: Toxicogenomic studies. L. Zhang, University of California, Berkeley

1:00 Lunch and excursion

Thursday, September 10, 2009

Platform Session 5: Mechanistic studies: Transgenics and signal transduction.
Co-chairs: A. Boobis, Imperial College, London;
R. Irons, Fudan University, Shanghai

8:00 AM Benzene mode of action, hazard, and risk characterization in genetically-defined and modified mouse models. J. French, National Institute of Environmental Health Sciences, North Carolina

8:30 Hematopoietic neoplastic diseases in C3H/He and C57BL/6 mice after benzene exposure. Differences observed using microarrays. T. Inoue, National Institute of Health Sciences, Tokyo

9:00 The Ah receptor has an important role in the regulation of hematopoiesis. T. Gasiewicz, University of Rochester, New York

9:30 Benzene-induced toxicity is based on the AhR-mediated hematopoietic stem cells. Y. Hirabayashi, National Institute of Health Sciences, Tokyo

10:00 Break (Posters will be available for viewing during the break)
Poster Session Co-chairs: D. Pyatt, Summit Toxicology, Colorado;
C. Weisel, EOHSI and UMDNJ, New Jersey

Platform Session 5 (continued)

10:30 Benzene-initiated oxidative stress: Effects on embryonic signaling pathways. L. Winn, Queens University, Ontario, Canada

11:00 Metabolic factors in susceptibility to benzene toxicity. D. Ross, University of Colorado, Denver

Platform Session 6: **Mechanistic studies: DNA repair and chromosome damage.**
Co-chairs: **S. Kyrtopoulos, National Helenic Research Foundation, Athens;**
M. Ruchirawat, Chulabhorn Research Institute, Bangkok

11:30 The role of DNA repair in chemical carcinogenesis with special emphasis on benzene. A. Hartwig, Technical University of Berlin

12:00 DNA repair and t-AML. P. Karran, Cancer Research UK, London

12:30 Topoisomerase II, chromosome damage and leukemia. D. Eastmond, University of California, Riverside

1:00 Lunch (Posters will be available for viewing during the lunch hour)

Platform Session 7: **Exposure science and biomarkers.**
Co-chairs: **P. Farmer, University of Leicester;**
R. Albertini, University of Vermont

2:00 Measuring benzene exposure. C. Weisel, EOHSI and UMDNJ, New Jersey

2:30 Exposure to benzene in Thailand: Implications for carcinogenic risk. M. Ruchirawat, Chulabhorn Research Institute, Bangkok

3:00 Break (Posters will be available for viewing during the break)

3:30 Benzene metabolites block gap junction intercellular communication. E. Rivedal, Norwegian Radium Hospital, Oslo

4:00 Occupational benzene exposure and hematological parameters. G. Swaen, DOW Chemical, Terneuzen, The Netherlands

4:30 Biomarkers as mechanistic probes: Application to benzene toxicity. R. Albertini, University of Vermont

Friday, September 11, 2009

Session 8: **Selections from volunteered papers.**
Co-Chairs: **D. Kaden, DakTox LLC, Massachusetts;**
D. Pyatt, Summit Toxicology, Colorado

8:00 AM How much does benzene contribute to the overall burden of cancer due to occupation? L. Rushton, Imperial College London, UK

8:20 Benzene is associated with the development of severe but not moderate aplastic anemia in Shanghai, China. S. A. Gross, University of Colorado, Denver

8:40 Modulation of the benzene metabolite hydroquinone induced toxicity: Evidence for an important role of *fau*. S. H. Inayat-Hussain, Universiti Kebangsaan Malaysia, Kuala Lumpur

9:00 Methylation and expression analysis of tumor suppressor genes *p15* and *p16* in benzene poisoning. C. Xing, Chinese Center for Disease Control and Prevention, Beijing

Platform Session 9: **Mode of action and risk assessment.**
Co-chairs: **B. Sonawane, USEPA, Washington;**
L. Levy, Cranfield University, UK

9:20 Implication of recent data on benzene risk assessment. Weight of evidence for Mode of Action/ Human relevance and dose-response; critical data gaps. J. Klaunig, Indiana University, Indianapolis

9:50 The vanishing zero revisited: Thresholds in the age of genomics. Defining No Transcriptional Effect Levels (NOTEL) and the No Detectable Adduct Levels (NODAL) following carcinogen exposure *in vitro* and *in vivo*: Implications for risk assessment? H. Zarbl, EOHSI and UMDNJ, New Jersey

10:20 Break

10:50 **Panel Discussion**
Moderator: B. Meek, University of Ottawa

Questions to the panel:

a. What are the implications of recent data in relation to the weight of evidence for mode(s) of action of benzene-induced bone marrow damage and resulting risk?

b. What are the critical data gaps in consideration of mode of action/human relevance?

Panel: R. Albertini, H. M. Bolt, A. Boobis, J. Bus, D. Eastmond, S. Lau, M. Sandy

12:00 Summary of the panel discussion and comments on how the MOA approach can enhance future research on benzene. J. Klaunig, Indiana University

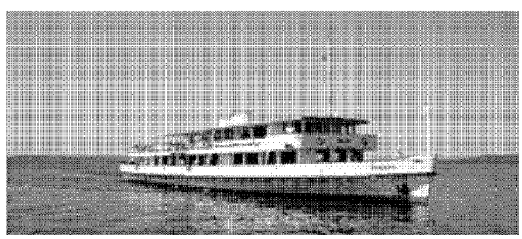
12:30 **Close of symposium, H. Greim**

Social Event:

Wednesday, 9th September 2009

Cruise on the Starnberger See

Dinner at the Restaurant UNDOSA, Starnberg



1:00 pm	**Lunch**
1:30 pm	**Departure by U-Bahn and S-Bahn to Starnberg***
3:00 pm	**Lake cruise with the "MS Bayern" from Starnberg landing stage**
6:30 pm	**Arrival back in Starnberg**
6:45 pm	**Dinner at the Restaurant UNDOSA**
→	**Individual departure back to Munich**

* The group will **take the U-Bahn U4/U5** (towards Laimer Platz or Westendstrasse) **to KARLSPLATZ** (Stachus)

From KARLSPLATZ we will **take the S-Bahn S6** (towards Tutzing / Starnberg) **to Starnberg**

Meeting point: Landing stage Starnberg
(within walking distance from S-Bahn station)
The ship will leave there at 3:00 pm
Please be on time!

RECEPTION

Thursday, 10th September 2009

6:00 PM – 8:00 PM

by invitation of

Melanie Huml

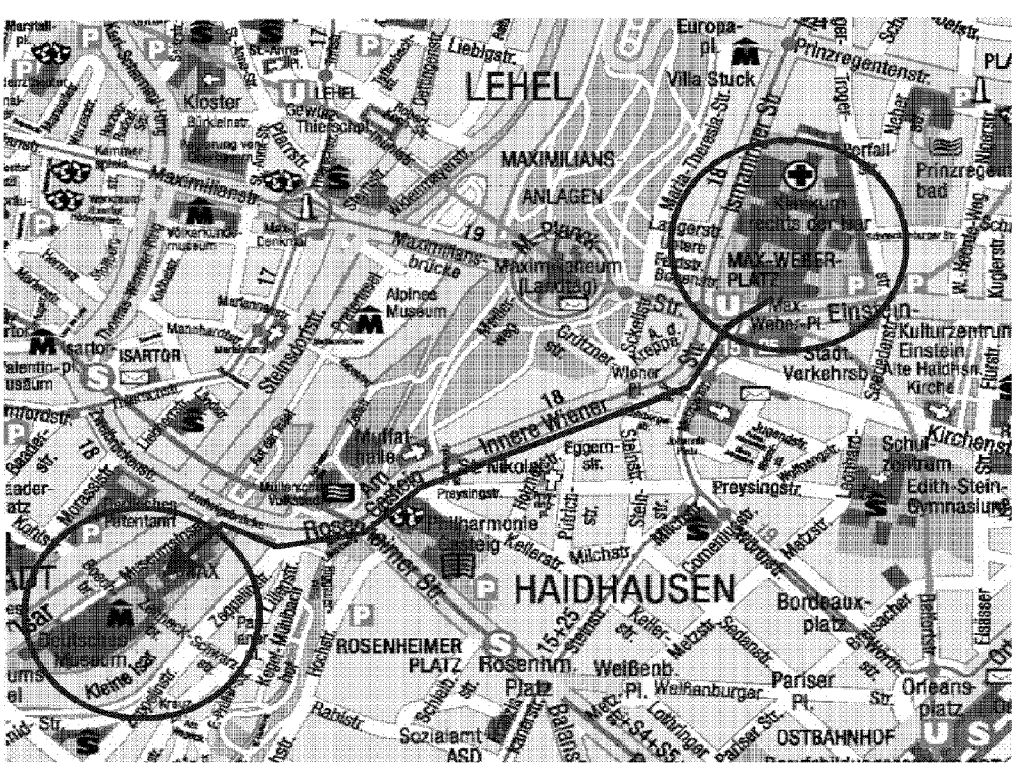
State Secretary of the Bavarian State Ministry of the Environment and Public Health

at

Deutsches Museum

(Masterpieces of Science and Technology)

Museumsinsel 1
80538 Munich
Germany



How to get to the Museum:

Walking distance from venue (15 Minutes)

or

take Tram No. 18 from Max-Weber-Platz station to Deutsches Museum station (3 stops)

List of Participants - Benzene Symposium 2009

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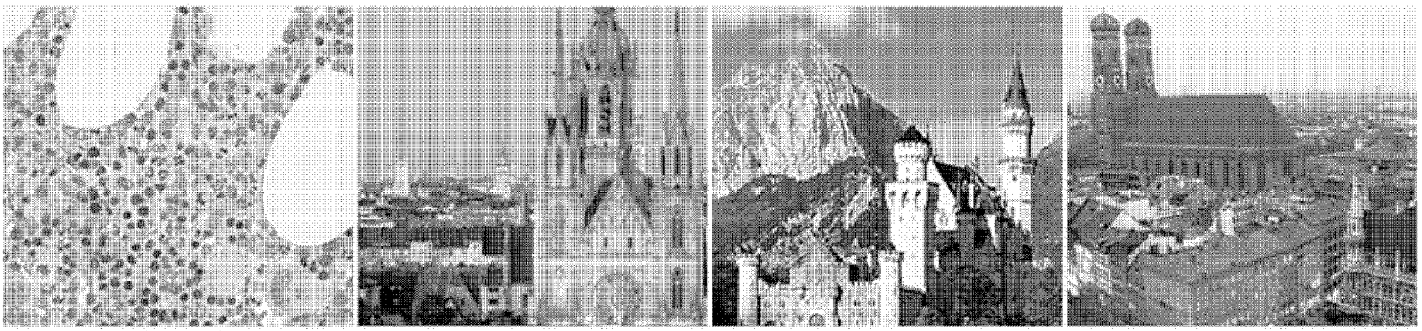
Benzene 2009:

Health Effects and Mechanisms of Bone Marrow Toxicity
Implications for t-AML and the Mode of Action Framework

September 7-11, 2009 • Technische Universität München • Munich, Germany



Abstracts:

Presentations

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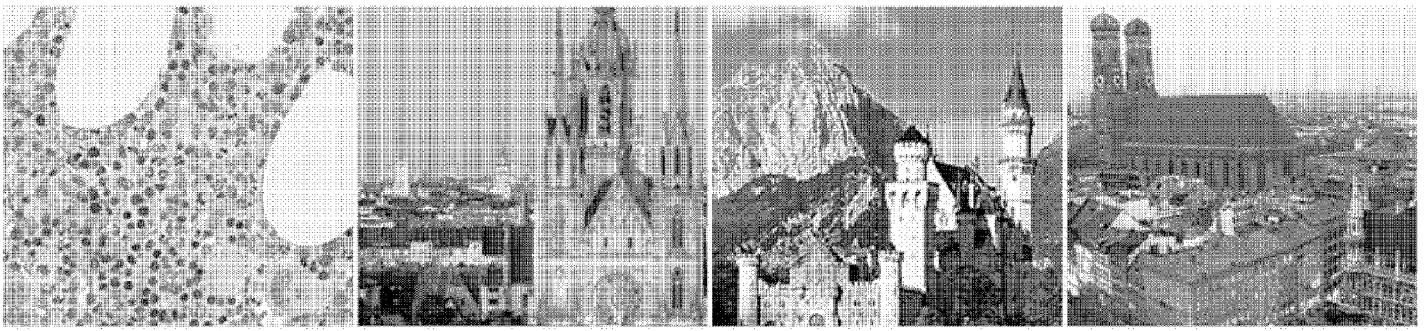

PLATFORM SESSION 1

Introduction to the Symposium

Co-chairs: **H. Greim**
Technical University of Munich,
Germany

H.M. Bolt
Leibniz Research Centre,
Technical University of Dortmund,
Germany

A century of research on the hematotoxic effects of benzene and aims of the Symposium. R Snyder, Rutgers, The State University of New Jersey School of Pharmacy and the Environmental and Occupational Health Sciences Institute, Piscataway, NJ, USA

More than a century has passed since Santesson (*Arch. Hyg. Berl.* **31**:336-376, 1897) described the hematotoxicity expressed in workers exposed to benzene in a Swedish tire factory. Over the next 50 years we learned that benzene exposure led to decreases in circulating blood cells resulting in aplastic anemia, impaired blood clotting, and immunotoxicity. Evidence that benzene exposure resulted in the development of one or more forms of leukemia was slower to develop. Efforts to understand the mechanisms by which benzene induced bone marrow damage were initiated by Parke and Williams (*Biochem. J.* **54**: 231-238, 1953), who studied benzene metabolism and argued that benzene metabolites might play critical roles in the etiology of bone marrow diseases. This is the fourth in a series of international symposia spanning approximately three decades which have been aimed at characterizing the effects of benzene exposure on people and examining the mechanisms of benzene-induced bone marrow damage in animal models *in vivo* and *in vitro*. During this year's symposium we will focus on the regulation of hematopoiesis by both bone marrow stem cells and the bone marrow microenvironment; a survey of the WHO classification of hematopoietic neoplasms and how the classification impacts on benzene epidemiology; treatment-related AML, its latency, related chromosome damage and similarity to benzene-induced AML; the range of potential benzene-induced hematopoietic cancers; analysis of the impact of benzene exposure on transgenic animals; effects on signal transduction; and biomarkers. The conference will terminate with a panel discussion which will make use of the Mode of Action/Human Relevance framework to analyze the current state of our understanding of benzene toxicity and related diseases, to attempt to integrate our current understanding of the benzene problem and point the direction towards more enlightening future studies.

Interpreting available data and identifying critical data gaps for benzene risk assessment. Contribution of the IPCS/ILSI framework for "Mode of Action/Human Relevance". ME (Bette) Meek, McLaughlin Centre for Population Health Risk Assessment, University of Ottawa, Canada

Mode of action is defined as a series of key biological events leading to an observed toxicological effect (for example, metabolism to a toxic entity, cell death, regenerative repair and tumors). While a hypothesized mode of action is supported by experimental observations and related mechanistic data, it contrasts with mechanism of action, which generally involves a detailed understanding of the molecular basis for an effect. An international framework to consider the weight of evidence for hypothesized modes of action in animals and their relevance to humans developed based on input from scientists worldwide, has been widely adopted and used by government agencies and international organizations. The framework, which was developed and refined through its application in case studies for principally non DNA reactive carcinogens, has more recently been extended to DNA reactive carcinogens, non-cancer endpoints and different life stages. In addition to increasing transparency, use of the framework promotes consistency in decision-making concerning adequacy of weight of evidence, facilitates peer input and review and identifies critical research needs. Iterative use of the framework also facilitates communication between assessors and researchers on key data gaps and encourages early assimilation of mechanistic data in hazard characterization and dose-response analysis. Available data on benzene are considered in the context of the framework as a basis for proposing key events in a hypothesized mode of action with the objective of focussing discussion during the Symposium on critical data gaps in a risk assessment context.

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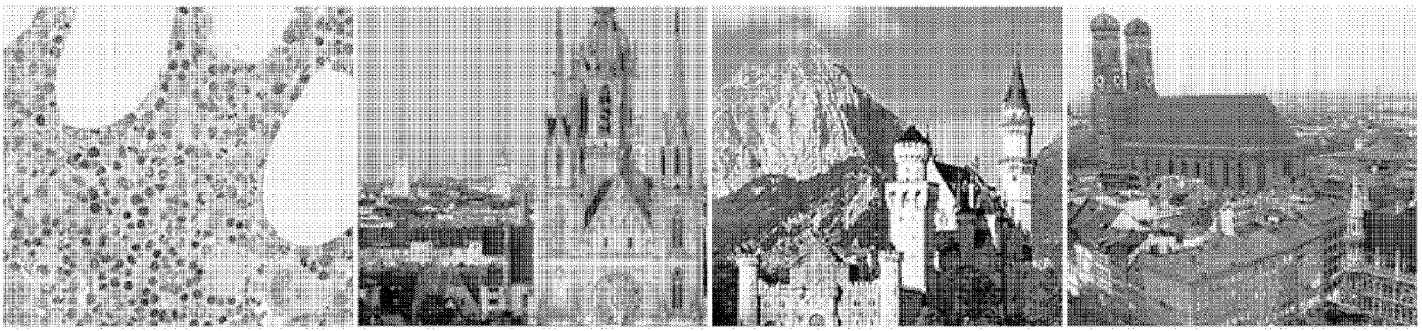

PLATFORM SESSION 2

Bone marrow and leukemia

Co-chairs: **R. Larson**
University of Chicago, USA

D. Ross
University of Colorado, USA

Regulation of hematopoiesis by bone marrow stem cells. C Peschel. Department of Internal Medicine III, Technical University of Munich, Germany

Self renewal und differentiation into different cell lineages, the key features of hematopoietic stem cells (HSC), are strictly regulated by cell autonomous and external, stromal cell derived signals. In myeloid leukemias HSCs are the initial target of the leukemogenic, transforming process which leads to unrestricted proliferation, disturbed cell cycle and apoptosis, and block of differentiation. At least in some leukemia subtypes, the capacity of self-renewal is acquired by the more mature progenitor cell pool. The discovery of disregulated molecular processes in leukemic stem cells will be essential for the development of truly targeted therapies aiming at eradication of the leukemic stem cell as prerequisite for definitive cure. Furthermore, the functional definition of leukemia-specific events will also improve our understanding of the regulation of normal early hematopoiesis. The receptor tyrosine kinase flt-3 is overexpressed in most AML cells. In appr. 30 % of AML, mutations of flt-3 are observed, leading to constitutive activation of this signalling pathway. Kinase inhibitors targeting flt-3 are available and have been tested in clinical trials, however, with moderate success. We investigated the effect of the flt-3 inhibitor SU5614 on various progenitor populations of primary AML cells (FLT-ITD mutation and FLT wildtype) in the presence of stromal cells with hematopoietic activity. In bone marrow cell samples derived from FLT3-ITD+ cells the number of more mature progenitor cells (CFU) was lower than in FLT-3-WT cells. However, the percentage of more primitive LTC is higher in CD34+FLT3 ITD+ samples. Committed CD34+ leukemic progenitors are inhibited in vitro by treatment with SU5614 both in FLT3-WT and FLT-ITD AML. However, direct contact with EL08-1D2 stroma protects committed progenitors as well as AML stem cells from inhibition by SU5614. In particular, primitive LTC from FLT3 ITD+ AML are protected from TK inhibition by stroma contact, resulting in net expansion of leukemic stem/ progenitor cells in these cultures. Alternative pathways mediated by stroma contact which activate AKT might allow independence of FLT3 signalling in FLT ITD+ leukemic stem cells thus allowing escape from TK inhibitor effects. This finding might explain the unsatisfactory clinical efficacy of FLT-3 inhibitors as single therapy and suggest the combination of FLT-3 inhibitors with compounds targeting primitive progenitors and/or the interaction of leukemic cells with the bone marrow microenvironment.

Regulation of hematopoiesis by the bone marrow environment. RAJ Oostendorp, Department of Internal Medicine III, Technical University of Munich, Germany

In adults, hematopoietic stem cells (HSC) reside in the bone marrow, surrounded by stromal cells. These stromal cells are thought to play an important role in the regulation of HSC proliferation and differentiation. We have identified several secreted factors, which are highly expressed by stromal cells maintaining HSC in culture. One of these factors is secreted Wnt signaling modulator frizzled-related protein 1 (Sfrp1). Loss of Sfrp1 in stromal cells increases production of hematopoietic progenitors, and, in knockout mice, dysregulates hemostasis. Loss of Sfrp1 also increases the HSC (Flk2- CD34- Lin- Sca1+ Kit+ (LSK)) cell numbers in bone marrow. Also, HSC and multipotent progenitors (MPP) show an increase in G0/G1 phase of cell cycle. On a molecular level, loss of Sfrp1 causes a decrease in the level of the intracellular wingless (Wnt)-signaling intermediate beta-catenin, suggesting that less canonical Wnt signaling occurs. Furthermore, gene expression studies showed a concomitant decrease of catenin-dependent transcriptional targets Ccnd1 and Dkk1 in Cd34- LSK cells, and increased expression of Pparg, Hes1 and Runx1 in MPP. To find out whether these effects were caused by intrinsic or extrinsic regulation, we performed transplantation experiments. Transplantation experiments showed no intrinsic effect of Sfrp1 loss on the number of HSC or the ability of $Sfrp1^{-/-}$ HSC to engraft irradiated recipients. In contrast, serial transplantations of wild-type HSC into $Sfrp1^{-/-}$ recipient mice show a progressive decrease of wild-type LSK and MPP numbers. These results demonstrate that Sfrp1 is required to maintain HSC homeostasis through extrinsic regulation of beta-catenin. In conclusion, our study shows the importance of analyzing stromal cells to define novel HSC regulators. In addition, we demonstrate that disruption of the microenvironment severely affects HSC maintenance.

The World Health Organization (WHO) classification of tumors of the hematopoietic and lymphoid tissues. J Vardiman, University of Chicago, Chicago, IL, USA

The WHO classification of myeloid and lymphoid neoplasms utilizes morphology, immunophenotype, genetics and clinical features to define disease entities of clinical significance. It is a "consensus" classification for which a number of experts have agreed on the classification and the diagnostic criteria. In general, the classification stratifies neoplasms according to their lineage (myeloid, lymphoid, histiocytic/dendritic cell) and distinguishes neoplasms of precursor cells from those comprised of functionally mature cells. Further subclassification is based on biologic, phenotypic, genetic, and clinical features. Five major subgroups of myeloid tumors are recognized: myeloproliferative neoplasms (MPN, comprised mainly of mature cells with effective proliferation), myeloid (and lymphoid) neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB* and *FGFR1* (defined largely by the genetic abnormalities and significant eosinophilia), myelodysplastic/ myeloproliferative neoplasms (MDS/MPN, comprised mainly of mature cells with both effective and ineffective proliferative components), myelodysplastic syndromes (MDS, immature and mature cells with ineffective proliferation), and acute myeloid leukemia (AML, comprised of blasts with impaired maturation). Genetic abnormalities play an important role as diagnostic criteria for further subclassification of some myeloid subgroups, particularly AML, in which a number of clinicopathologic entities are recognized that are associated with specific genetic rearrangements or mutations. Although therapy-related AML and MDS often have genetic defects identical to those found in de novo AML and MDS, therapy-related myeloid neoplasms are classified separately within the AML category to emphasize their unique clinical and biologic properties. Lymphoid neoplasms appear to recapitulate stages of normal B-, T-, and NK-cell differentiation and to some extent are classified according to the corresponding normal stage. Thus, neoplasms of B cells can be divided into those of precursor, pre-germinal center, germinal center or post-germinal center B-cells. Because NK cells are closely related to T cells, neoplasms of these two cell types are often considered together, and broadly categorized as precursor T cell neoplasms or as peripheral T/NK cell neoplasms. Some lymphoid tumors, however, are recognized by unique clinical or biologic features, such as lymphomas associated with immunodeficiency states associated with immunosuppressive therapy or with aging.

Myelodysplastic syndrome (MDS): A case-case analysis of benzene exposure and clinical features using the WHO(2001)/(2008) criteria for diagnosis. RD Irons [a], SA Gross, XQ Wang, A Le, C Yan, AR Schnatter, H Fu. [a] Fudan-Cinpathogen Clinical and Molecular Research Center, Institutes of Biomedical Sciences, Fudan University, Shanghai, China

We diagnosed and characterized the prevalence of hematopoietic and lymphoid disease for 2923 consecutive patients presenting at 29 hospitals from Aug 2003 to Jun 2007. Diagnoses were made by a single laboratory using WHO criteria based on morphologic, immunophenotypic, cytogenetic, FISH and molecular data. A total of 611 subjects (322 males/289 females) were prospectively diagnosed with MDS using WHO (2001) criteria. Update and re-evaluation of cases using MDS (2008) criteria resulted in 649 MDS cases. Refractory cytopenia with multilineage dysplasia (RCMD) accounted for 68% of total cases, refractory anemia with excess blasts (RAEB), 16.3%, refractory anemia (RA), 6.5% and MDS-unclassifiable (MDS-U), 4.5%, based on 2008 criteria. Subjects were administered questionnaires and information on previous disease, work histories and exposures to potential etiologic agents such as benzene (BZ) was obtained. A total of 80/649 (13.2%) were determined to have some BZ exposure. The frequency of clonal cytogenetic abnormalities in the total MDS series was 30%, the most common being +8 >del(20)q > -7 >-5 , while the analogous frequency in BZ-exposed cases was only 22.5%. To further investigate the clinical features of MDS that may be associated with BZ exposure, we identified a subset of cases with high BZ exposure. These signal cases were matched by age and gender to cases with no known BZ exposure. When contrasting high versus no BZ exposure cases, we found a high odds ratio (OR) for WHO subtypes MDSU (OR = 6), followed by RAEB (OR = 2) and RCMD (OR = 0.5). Bone marrow morphology consistent with multilineage dysplasia with abnormal eosinophils was strongly associated with BZ exposure (OR = 42). Surprisingly, the relative risk of clonal cytogenetic abnormalities was reduced for high BZ exposed cases (OR = 0.56), reflecting the pattern in the total case series. (This work was supported by the Benzene Health Research Consortium).

Incidence and susceptibility of therapy related leukemia. G Leone [a], MT Voso, L Fianchi, L Pagano. [a] Università Cattolica del Sacro Cuore, Policlinico Gemelli, Rome, Italy

Therapy-related myelodysplastic syndrome/acute myeloid leukemia (t-MDS/AML) is a treatment complication increasingly recognized in patients treated with radio- or chemotherapy for previous hematological malignancies or solid tumors. Distinct clinical entities have been described according to the primary treatment, corresponding to defined genetic lesions. Chromosome 7 and/or 5 losses or deletions are typical of alkylating agent-induced AML, while development of t-AML with balanced translocations involving chromosome bands 11q23 and 21q22 has been related to preceding therapy with drugs targeting DNA-topoisomerase II. In addition, anti-metabolites, and in particular the immunosuppressant azathioprine and fludarabine, have been shown to induce defective DNA-mismatch repair. This could promote survival of misrepaired cells giving rise to the leukemic clone. Leukemias developing after benzene exposure is similar to t-AML. Findings in the literature to date indicate that benzene may act like alkylating agents, causing alterations in chromosomes 5 and 7, and topoisomerase II inhibitors, thereby inducing t(21q22). Increased chromosomal aberrations are also observed among benzene-exposed but otherwise healthy workers. Individual predisposing factors, including polymorphisms in detoxification and DNA repair enzymes have been identified. Two genetic variants in key metabolizing enzymes, myeloperoxidase and NAD(P)H:quinone oxidoreductase, have been shown to influence susceptibility to benzene hematotoxicity. Combination of polymorphisms reducing detoxification with polymorphisms of DNA repair enzymes may significantly increase the t-MDS/AML risk. Among hematological malignancies, long-term survivors of Hodgkin's lymphoma are exposed to an increased t-MDS/AML risk, particularly when receiving MOPP-based, and escalated BEACOPP regimens, and when alkylators are combined to radiotherapy. Patients with Hodgkin's and non-Hodgkin's lymphoma are at highest risk when total body irradiation followed by autologous stem cell transplantation is used as rescue or consolidation. The addition of granulocyte-colony stimulating factor (G-CSF) and radiotherapy plays a significant role in t-AML following treatment of children with acute lymphoblastic leukemia. In non-hematological malignancies, treatment for breast cancer and germ-cell tumors has been associated with a 1-5% lifetime risk of both lymphoid as well as myeloid leukemia. In any case the risk of t-MDS/AML drops sharply after 10 years from treatment.

Implications of latency period between benzene exposure and risk of leukemia. G. Triebig. Institute and Outpatient Clinic of Occupational and Social Medicine, University of Heidelberg, Germany

From numerous epidemiologic studies is evidenced that risk after exposure to a carcinogen varies with time. Long latency period in the range of decades is regarded as a hallmark of cancer development. During the last years there has been an increasing discussion about the temporal variation between exposures to chemical and physical carcinogens such as asbestos, benzene, smoking or ionizing radiation and the risk of cancer. The results of different epidemiologic studies of occupational cohorts with benzene exposure and the development of leukemia and Non-Hodgkin-Lymphoma will be presented in order to find common aspects. In this context the data of 537 confirmed cases of leukemia as an occupational disease in Germany during the time period 1978 to 2007 will be analyzed for the temporal pattern. This includes first time of exposure, latency period and time since exposure.

Chromosome pathways, genetic changes, cooperating mutations, and candidate genes in primary and therapy-related MDS and AML. Z Qian, J Wong, AA Fernald, JM Joslin, TR Tennant, MM Le Beau [a]. [a] Section of Hematology/Oncology, University of Chicago, Chicago, IL, USA

Therapy-related MDS and AML (t-MDS/t-AML) are late complications of cytotoxic therapy used in the treatment of both malignant and non-malignant diseases. The most common subtype of t-AML (~75% of cases) develops after exposure to alkylating agents, is characterized by loss or deletion of chromosome 5 and/or 7 [-5/del(5q), -7/del(7q)], and arises in a hematopoietic stem/progenitor cell. In the University of Chicago's series of 306 patients with t-MDS/t-AML, 64 (21%) patients had abnormalities of chromosome 5, 85 (28%) patients had abnormalities of chromosome 7, and 65 (21%) patients had abnormalities of both chromosomes 5 and 7 (Smith et al. Blood 102:43, 2003). Survival times of t-MDS/t-AML patients are short (median 8 mos), and new therapeutic approaches are needed. Pedersen-Bjergaard (NEJM 152:1491, 2005) proposed that there are 8 different pathways involved in the pathogenesis of t-AML. Pathway I consists of patients who have abnormalities of chromosome 7, without chromosome 5 abnormalities. This subgroup typically has mutations of the RAS pathway (*KRAS1, NRAS, NF1, PTPN11*), methylation of the promoter of *CDKN2B* (*p15*), and a poor prognosis. Pathway II comprises patients with a -5/del(5q), mutations of *TP53*, genomic instability, and a poor prognosis. Haploinsufficiency of *RPS14*, *EGR1, NPM1*, and *CTNNA1* on 5q genes have been implicated in the pathogenesis of MDS/AML. In previous studies, we determined that *Egr1* acts by haploinsufficiency and cooperates with mutations induced by alkylating agents to induce myeloid leukemias in the mouse (Joslin et al., Blood 110:719, 2007). To identify mutations that cooperate with *Egr1* haploinsufficiency, we conducted a forward genetic screen using MOL4070LTR retroviral insertional mutagenesis, and determined that loss of one allele of *Egr1* shifts the disease spectrum to myeloid neoplasms. To date, we have identified two common integration sites (*Evi1* and *Gfi1b* loci). Of note is that the *EVI1* transcription factor gene is deregulated in human AMLs, particularly those with -7, and abnormalities of 3q. The GFI1/GFI1B transcription factors play a critical role in hematopoiesis. By cytogenetic analysis of 584 patients with t-MDS/t-AML, we determined that -5/del(5q) is significantly associated with a complex karyotype, characterized by trisomy 8 as well as loss of 12p, 13q, 16q22, 17p (*TP53* locus), chr. 18, and 20q. In addition, this subtype of t-AML is characterized by a unique expression profile (higher expression of genes involved in cell cycle control (*CCNA2, CCNE2, CDC2*), checkpoints (*BUB1*), or growth (*MYC*), loss of expression of *ICSBP*, and overexpression of *FHL2* (Qian et al., PNAS 99:14925, 2002).

MicroRNA, epigenetics, and copy number changes: new levels of gene regulation in acute myeloid leukemia (AML). RA Larson, University of Chicago, Chicago IL, USA

Alkylating agents, topoisomerase II inhibitors, ionizing radiation, and other hematotoxins induce DNA damage in hematopoietic stem cells that results in lesions such as -5/del(5q) and/or -7/del(7q) as well as other submicroscopic genetic lesions. Together with epigenetic alterations, these result in dysplasia and ultimately myeloid leukemia. Combinations of lesions are required to induce overt leukemia. Altering a small subset of signaling pathways leads to disruption of normal self-renewal, proliferation, differentiation, and apoptotic mechanisms that control the development of hematopoietic stem cells and their differentiation into mature effector cells. Recent studies have shown that cytogenetically normal (CN) AML is quite heterogeneous at the molecular level. Patients with CN-AML harboring mutations in *NPM1*, *FLT3*, *CEBPA*, *WT1* or expressing high levels of *BAALC*, *ERG*, or *MN1* have distinctly different clinical outcomes. *NPM1* mutations are independently associated with higher remission rates and longer disease-free and overall survival in AML. Copy number alterations (CNA) are deletions or amplifications of single genes. CNAs have been found at the breakpoints of known chromosomal translocations. High resolution genome-wide copy number analysis has identified genes that are the target of cryptic chromosomal translocations. Fewer CNAs have been detected in AML than in pediatric ALL. MicroRNAs (miRNAs) are non-coding small RNA molecules containing 19- to 25-nucleotides that arise by cleavage from 70-100 nucleotide precursors, typically encoded within introns. They hybridize to complementary mRNA targets and modulate protein expression by inhibiting translation and/or inducing degradation of target messenger RNAs, thereby repressing the expression of their coding genes. This new class of genes has recently been shown to play a pivotal role in malignant transformation. miRNAs are down-regulated in many tumors and thus appear to function as tumor suppressor genes. Genome-wide miRNA expression profiles have been associated with different subsets of AML. A microRNA signature that is associated with clinical outcome in patients with high-risk molecular features of AML (those who have *FLT3*-ITD, wild-type *NPM1*, or both) has been reported. This subgroup constitutes approximately 65% of patients with CN-AML and one-third of all patients with AML < 60 years old. Down-regulation of the microRNA-181 family contributes to an aggressive leukemia phenotype through mechanisms associated with the activation of pathways of innate immunity mediated by toll-like receptors and interleukin-1β.

Epigenetic changes in therapy-related MDS/AML. MT Voso, [a] F D'Alo,' M Greco, E Fabiani, M Criscuolo, F Guidi, S Hohaus, G Leone. [a] Istituto di Ematologia, Universita' Cattolica Sacro Cuore, Rome, Italy

Therapy-related Myelodyplastic Syndromes/Acute Myeloid Leukemias (t-MDS/AML) are one of the most compelling long term adverse events occurring in cancer survivors treated with chemo-radiotherapy regimes. Beside several well-described genetic lesions, a growing amount of data suggests that abnormalities in DNA methylation profile contribute to multistep secondary leukemogenesis. Two distinct alterations of normal DNA methylation patterns may occur in cancer: a global hypomethylation resulting in chromosomal instability and loss of genetic integrity, and promoter specific DNA hypermethylation which leads to silencing of tumor suppressor genes. Cytotoxic drugs and radiation have been shown to affect tissue DNA methylation profile. Radiation is able to induce a stable DNA hypomethylation in both target and bystander tissues (Koturbash, Biochem Biophys Res Commun 2005; Ilnytskyy, Environ Mol Mutagen 2009). Moreover benzene was shown to induce a reduction of LINE-1 and AluI methylation and an increase of p15 methylation in urban traffic officers (Bollati, Cancer Res 2007). Similarly, an increase in p15 methylation was observed in individuals who had been treated for lymphoma by intensive cytotoxic therapies, even if hematological parameters were normal (Preisler, Leukemia 2001). Studying methylation status of 13 genes in a small cohort of t-AML patients, Uehara et al (Int J Onc 2003) identified methylation of at least one gene in 55% of patients. The average time to the development of t-AML after treatment of the primary tumor was significantly shorter in methylated than unmethylated patients (49.3 months vs. 133.2 months, p=0.044). Moreover, p15 methylation correlates to monosomy/deletion of chromosome 7q, suggesting that it could be a relevant event in alkylating agent-induced leukemogenesis (Christiansen, Leukemia 2003). We have previously described hypermethylation of DAP-kinase and BRCA-1 in t-AML, when compared to de novo AML (48.3% vs 22.9%, p=0.01, for DAP-kinase; and 76% vs 31%, p=0.0002, for BRCA1) (Voso, Blood 2004; Scardocci, Br J Cancer 2006). Interestingly, methylation of the 2 promoters was frequently contemporary suggesting the existence of a methylator phenotype in t-MDS/AML. Furthermore, in 21 t-AML patients the time to develop t-AML after the primary tumor was significantly shorter in patients methylated for at least one gene than in unmethylated patients (43.5 months versus 111.1 months, p=0.01). These data suggest that promoter hypermethylation of genes involved in cell cycle control, apoptosis and DNA repair pathways is a frequent finding in t-MDS/AML and may contribute to secondary leukemogenesis. However, how the epigenetic machinery is disrupted after chemo/radiotherapy and during secondary carcinogenesis is still unknown, warranting further studies.

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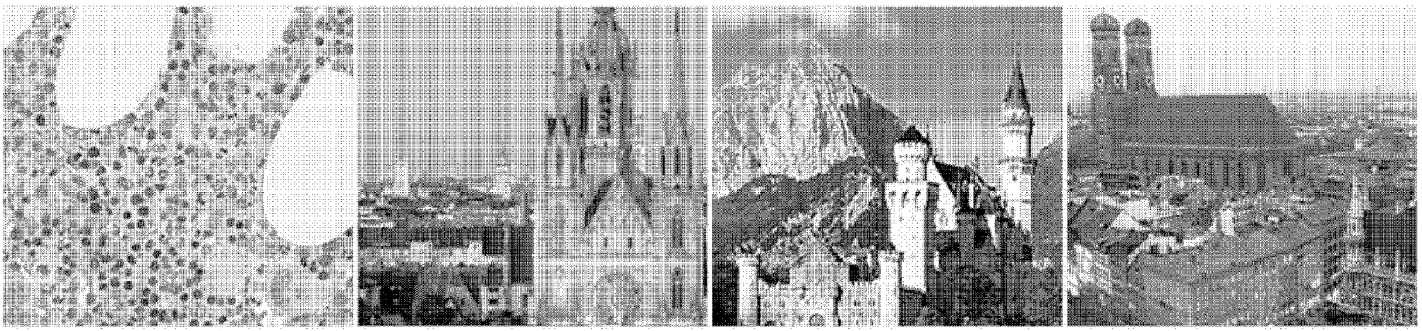

PLATFORM SESSION 3

Recent studies and re-evaluation of data on the epidemiology of benzene-induced diseases

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Ensuring comparability of benzene exposure estimates across three nested case-control studies in the petroleum industry in support of a pooled analysis. DC Glass [a], TW Armstrong, ED Pearlman, DK Verma, L Rushton, AR Schnatter. [a] Dept. of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia

Three case-control studies nested within large cohorts of petroleum workers in Canada, the UK and Australia, will investigate exposure to benzene in relation to haematopoietic cancers. The original studies were conducted at different points in time by different study teams, but the petroleum industry used similar technology in similar eras in each of these countries. The teams had communicated about methodology which resulted in a similarity in approach. However, potential differences in the exposure assessment methods needed to be investigated and resolved to optimize the compatibility of the data. Each study derived Base Estimate (BEs) in ppm, of average benzene exposure for jobs or tasks, based on measurements collected in the petroleum industry. Exposure estimates were then calculated for each line of work history for all individuals in each of the three studies. These were made by adjusting the BEs for site-and era-specific exposure-related variables such as loading technology, percentage benzene in the product etc. These estimates were termed workplace exposure estimates (WE). Each study was updated and the benzene exposure of new leukemia cases and controls was estimated in accordance with the respective study's original methods. To ensure that the exposure estimates were comparable among the studies, the WEs were allocated to one of several generic job groups, e.g., top loading tanker driver, mechanic. The AM, GM and range of the WEs were calculated, by study, for each generic job group. The WEs had been further stratified into eras (pre 1945, 1945-1960, 1960s & 1970s, 1980 onwards), reflecting technological changes in the industry. Although the studies covered some differing sectors of the industry and different time periods, there was sufficient overlap in eras in the downstream distribution sector to make comparisons possible. A difference of more than 10% between studies in the AMs for any generic job group-era WE was investigated to assess whether it was justified by differences in local exposure conditions, such as an enclosure versus open work area, or alternatively, the estimates were adjusted. Reconciliation of differences resulted in changes to a small number of underlying BEs, including the background values, to the BEs attributed to some individuals and in the allocation of jobs to the generic job groups.

Benzene as a cause of lymphoproliferative disorders. BD Goldstein. Graduate School of Public Health, Univ of Pittsburgh, Pittsburgh, PA, USA

Because benzene is a known cause of human leukemia there has been little reason for organizations such as IARC or the US National Toxicology Program to perform standard hazard identification of benzene as a cause of other cancers such as lymphocytic neoplasms. Applying the standard weight of evidence approaches used by these organizations to assess whether an environmental chemical causes human disease clearly shows that there is sufficient evidence to identify benzene as a cause of human lymphocytic tumors. Lymphomas were long ago reported in laboratory animals subjected to long-term benzene exposure. The epidemiological studies by themselves seem to this reviewer to reach the level of evidence required to be seen as sufficient. However, even if one were to discount the strength of the epidemiological evidence, there is sufficient mechanistic evidence to judge that benzene should be considered to be a cause of human lymphoproliferative neoplasms. There are two major reasons for this. 1) The criteria for assigning overall sufficiency of evidence for carcinogenicity has changed to allow greater scope for mechanistic evidence. IARC permits classification in Group 1 when there is less than sufficient evidence in humans but sufficient evidence in animals and "strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity". The presence in the lymphocytes of benzene-exposed workers of chromosomal abnormalities has been well documented, as has the role of chromosomal effects in carcinogenesis. Secondly, modern molecular biological approaches have demonstrated that the morphological distinctions that are made among hematological disease often have little to do with basic issues of causality. The early hematopoietic stem cell, which can be a target of benzene in causing myeloid cancers, can also become a lymphocytic cell type. Furthermore, the classification of lymphomas has evolved so that non-Hodgkins lymphoma now includes such formerly distinct disorders as chronic lymphocytic leukemia and multiple myeloma. The evidence appears conclusive that benzene is a cause of human lymphoid cancers.

A hospital-based case-control study of acute myeloid leukemia in Shanghai: Analysis of environmental and occupational risk factors by WHO subtypes. O Wong [a], F Harris, H Fu, et al. [a] Applied Health Sciences, San Mateo, California, USA

The objectives of the study were: (1) to investigate and identify potential environmental and occupational risk factors of acute myeloid leukemia (AML), and (2) to explore the relationships between risk factors and AML subtypes according to the World Health Organization (WHO) classification of myeloid neoplasms. The investigation was a hospital-based case-control study consisting of 722 confirmed AML cases and 1444 individually gender-age-matched patient controls at 29 hospitals in Shanghai. A 17-page questionnaire was used to obtain information on demographics, medical history, family history, lifestyle risk factors, employment history, residential history, and occupational and non-occupational exposures. Certain occupations of interest triggered a second questionnaire, which was occupation-specific and asked for more details about jobs, tasks, materials used and work environment. Exposure assessments were based on the questionnaires, on-site workplace investigations, data published in the Chinese literature, historical exposure measurements maintained by government health agencies, and expert opinions of a panel of local scientists who were familiar with workplaces in Shanghai. Risk estimates (odds ratios and 95% confidence intervals) of individual risk factors were calculated using conditional logistic regression models. A number of potential environmental and occupational risk factors were associated with an increased risk of AML (all subtypes combined) and/or individual subtypes; including home or workplace renovation, living on a farm, planting crops, raising livestock or animals, farm workers, metal workers, rubber and plastic workers, wood and furniture workers, printers, loading and unloading workers, automobile manufacturing, general construction, and food and drink industry (restaurants and other eateries). Exposures associated with an increased risk included benzene, metals, insecticides, herbicides, fertilizers, paints, glues and adhesives, and inks. Multivariate models were used to adjust for potential confounding exposures, and several potential risk factors were subsequently eliminated. The results of the investigation indicated that some risk factors applied to all or several subtypes (factors such as low-level education and living on a farm), while others were limited primarily to a single subtype (such as the association between raising livestock or animals and AML with multilineage dysplasia). Thus, some risk factors were subtype-specific. The difference in risk by subtype underscores the importance of the etiologic commonality and heterogeneity of AML.

A hospital-based case-control study of non-Hodgkin lymphoid neoplasms in Shanghai: Analysis of environmental and occupational risk factor by WHO subtypes O Wong [a], F Harris, H Fu, et al. [a] Applied Health Sciences, San Mateo, California, USA

The objectives of the study were: (1) to investigate and identify potential environmental and occupational risk factors of non-Hodgkin lymphoid neoplasms (NHLN), and (2) to explore the relationships between risk factors and NHLN subtypes according to the World Health Organization (WHO) classification of lymphoid neoplasms. The investigation was a hospital-based case-control study consisting of 649 confirmed NHLN cases and 1298 individually gender-age-matched patient controls at 25 hospitals in Shanghai. A 17-page questionnaire was used to obtain information on demographics, medical history, family history, lifestyle risk factors, employment history, residential history, and occupational and non-occupational exposures. Certain occupations of interest triggered a second questionnaire, which was occupation-specific and asked for more details about jobs, tasks, materials used and work environment. Exposure assessments were based on the questionnaires, on-site workplace investigations, data published in the Chinese literature, historical exposure measurements maintained by government health agencies, and expert opinions of a panel of local scientists who were familiar with workplaces in Shanghai. Risk estimates (odds ratios and 95% confidence intervals) of individual risk factors were calculated using conditional logistic regression models. A number of potential environmental and occupational risk factors were associated with an increased risk of NHLN (all subtypes combined) and/or individual subtypes; including home/workplace renovation, living on a farm, planting crops, raising livestock or animals, farm workers, fabric sewing and cutting workers, welders and sheet metal workers, masonry and plastering workers, product and chemical testing workers, toy manufacturing, agriculture industry, and beauty salon. Exposures associated with an increased risk included benzene, solvents, petroleum fuels, gasoline, metals, insecticides, herbicides, fertilizers, and glues and adhesives. Multivariate models were used to adjust for potential confounding exposures, and several potential risk factors were subsequently eliminated. The results of the investigation indicated that some risk factors applied to all or several subtypes (factors such as home/workplace renovation, and fabric sewing and cutting), while others were limited primarily to a single subtype (such as the association between solvents and precursor B-cell neoplasms). Thus, some risk factors were subtype-specific. The difference in risk by subtype underscores the importance of the etiologic commonality and heterogeneity of NHLN.

Influence of benzene exposure and other factors on metabolism and peripheral blood parameters in moderately exposed workers in Shanghai, China. AR Schnatter [a], P Kerzic, Y Zhou, M Chen, M Nicolich, K Lavelle, T Armstrong, M Bird, F Hua, R Irons. [a] ExxonMobil Biomedical Sciences, Inc., Annandale, NJ, USA

Benzene is an established leukemogen and bone marrow toxin yet the mechanism of these effects is still not known. The metabolism of benzene is thought to play a key role in these effects. Whether decrements in peripheral blood cells are also involved in marrow toxicity and leukemia is a matter of debate. Recent studies on benzene's metabolism have suggested that: (a) lifestyle habits such as smoking contribute to metabolite levels, (b) single nucleotide polymorphisms do not play a large role in levels of metabolites, (c) metabolism is more efficient at lower doses, (d) there may be important shifts in the proportion of metabolites over the range of exposures, and (e) toluene inhibits benzene metabolite formation. Recent findings on peripheral blood indices have variously suggested that lymphocytes, neutrophils or earlier, less differentiated cells may be more sensitive to benzene exposure. To test many of the findings which have resulted from recent studies, we studied 899 workers exposed to moderate levels of benzene in five factories near Shanghai China. Metabolite analyses focused on a subset of 208 workers in two factories for which five urinary benzene metabolites and same-day personal benzene exposure measurements were available. Benzene exposure was a strong predictor of metabolite levels. Segmented regression models suggest that benzene concentrations of 0.8 ppm are necessary to raise both hydroquinone and trans, trans muconic acid metabolites above background levels, while higher concentrations are necessary to affect background levels of phenol and catechol. Single nucleotide polymorphisms of key metabolizing and deactivating enzymes had little effect on metabolite levels. Despite previous data suggesting inhibition of benzene metabolism by toluene, in our data, toluene exposure showed a positive influence on metabolism, although the joint distribution of benzene and toluene exposure was compromised. When we examined metabolic efficiency across exposure, slightly increased metabolic efficiency was suggested for exposures below 1 ppm, although the magnitude of this effect was less than that observed in previous studies. In univariate analyses of peripheral blood counts, stronger effects for benzene exposure were noted for decreased erythrocyte and neutrophil counts and an increase in mean corpuscular volume. Further investigation of other blood parameters is ongoing.

Benzene and childhood leukemia. DW Pyatt [a,b], S Hays, L Alyward. [a] Summit Toxicology, LLP, [b] University of Colorado Health Sciences Center, Schools of Public Health and Pharmacy, USA

Chronic exposure to high concentrations of benzene is an established cause of AML in occupationally exposed workers. Based on this well documented association, it is not unreasonable to assume that children could also get AML if they were exposed to comparable levels of benzene. Fortunately, reports of such exposures and subsequent AML development in children are non-existent. However, the question of whether children can develop leukemia at far lower, environmental levels of benzene remains. The existing scientific evidence relevant to this question will be addressed. Leukemias are the most common malignancy in children under 15 years of age. The majority (~85%) of childhood leukemia cases are acute lymphoblastic leukemia (ALL), with the remainder being almost exclusively acute myeloid leukemia (AML). Chronic forms of leukemia, either myeloid or lymphoid are exceedingly rare in children. Acute infant leukemia occurs before the age of 1 and shares phenotypic features with both ALL and AML. At this point, the risk factors for childhood leukemia remain essentially unknown as established etiological factors such as genetic conditions, ionization radiation and certain chemotherapeutic agents can explain only a small portion of cases. As a result, efforts to identify other potential etiologies remain an active research area. From a biological, clinical and epidemiological point of view, childhood ALL and AML are distinctly different diseases. Nonetheless, many older epidemiology studies on childhood leukemia have combined both types into one etiological group which is strongly influenced by the predominant ALL cases. In contrast, newer studies have begun to segregate out these two main subtypes and are reporting that childhood AML and ALL do not typically share the same risk factors. Combining them into a single group complicates the interpretation of this literature and may even obscure potential etiological relationships. Most epidemiology studies specific to children have included one or more of the following exposure pathways for benzene: 1) direct exposure to the child (via a variety of sources); 2) maternal exposures during pregnancy or while breastfeeding; and 3) exposure to either parent before conception. These pathways are not mutually exclusive and frequently occur together. The timing of exposure (e.g. age of the child) has also been an important consideration. Unfortunately, very few studies contain quantified exposure information and many have no meaningful exposure estimates at all. The use of various surrogates for exposure such as proximity to gas stations or traffic density makes interpretation very difficult, particularly with regard to chemical exposures. Parental exposures to benzene or other chemicals in the workplace and its potential effect in the offspring have also been assessed (with varying degrees of scientific rigor) in multiple studies. While there are a few positive findings, the collective literature does not indicate that exposure to environmental levels of benzene is related to an increased risk of childhood leukemia. Our understanding would be strengthened by additional studies that accurately characterize exposures as well as differentiate between the various forms of leukemias observed in children.

Benzene 2009:

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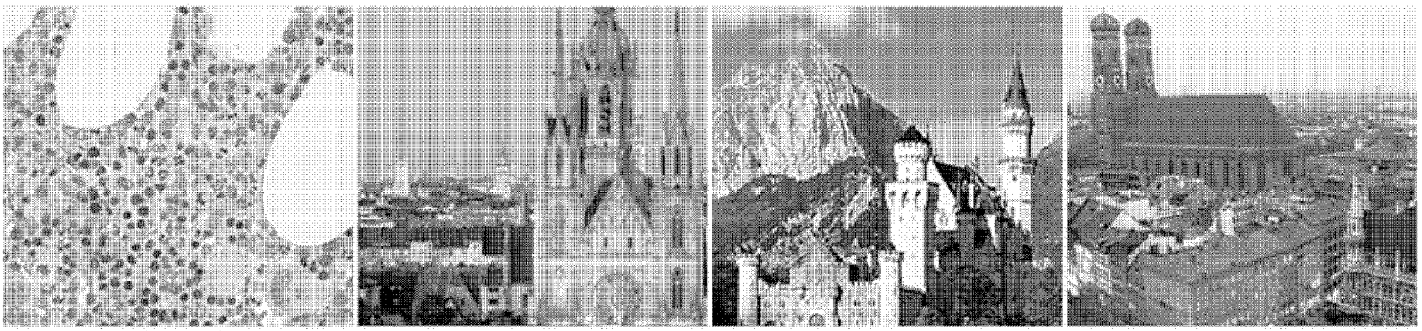

PLATFORM SESSION 4

Mechanistic studies: Reactive metabolites and reactive oxygen species

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The fate of benzene oxide. TJ Monks, Dept. Pharmacology & Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ, USA

Metabolism is a prerequisite for the development of benzene-mediated myelotoxicity. Benzene is initially metabolized via cytochromes P450 (primarily CYP2E1 in liver) to benzene oxide (BzO), which subsequently gives rise to a number of secondary products. Benzene oxides are assumed to equilibrate spontaneously with the corresponding oxepine valence tautomer, which can ring open to yield a reactive α-β-unsaturated aldehyde, *trans*, *trans*-muconaldehyde (MCA). Further reduction or oxidation of MCA gives rise to either 6-hydroxy-*trans*, *trans*-2,4-hexadienal or 6-hydroxy-*trans*,*trans*-2,4-hexadienoic acid. Both MCA and the hexadienal metabolite are myelotoxic in animal models. Alternatively, BzO can undergo conjugation with glutathione (GSH), resulting in the eventual formation and urinary excretion of *S*-phenylmercapturic acid. BzO is also a substrate for epoxide hydrolase, which catalyzes the formation of benzene dihydrodiol, itself a substrate for dihydrodiol dehydrogenase, producing catechol. Finally, BzO spontaneously rearranges to phenol, which subsequently undergoes either conjugation (glucuronic acid or sulfate) or oxidation. The latter reaction, catalyzed by cytochromes P450, gives rise to hydroquinone (HQ) and 1,2,4-benzene triol. Coadministration of phenol and HQ reproduces the myelotoxic effects of benzene in animal models. The two diphenolic metabolites of benzene, catechol and HQ undergo further oxidation to the corresponding *ortho*-(1,2-), or *para*-(1,4-)benzoquinones (BQ), respectively. Trapping of 1,4-BQ with GSH gives rise to a variety of HQ-GSH conjugates, several of which are hematotoxic when administered to rats. Thus, BzO gives rise to a cascade of metabolites that exhibit biological reactivity, and that provide a plausible metabolic basis for benzene-mediated myelotoxicity. BzO itself is relatively stable, and certainly capable of translocating from its primary site of formation in the liver to the bone marrow. However, therein lies the challenge, for although there exists a plethora of information on the metabolism of benzene, and the fate of BzO, there is a paucity of data on the presence, concentration, and persistence of benzene metabolites in bone marrow. The major metabolites in bone marrow of mice exposed to 50 ppm [^{3}H]benzene were muconic acid, and glucuronide and/or sulfate conjugates of phenol, HQ, and catechol. Studies with [^{14}C/^{13}C]benzene revealed the presence in bone marrow of protein adducts of benzene oxide, 1,2-BQ, and 1,4-BQ, the relative abundance of which was both dose and species dependent. Histones are bone marrow targets of [^{14}C]benzene, although the identity of the reactive metabolite(s) giving rise to these adducts remains unknown. Finally, hematotoxic HQ-GSH conjugates are present in the bone marrow of rats receiving the HQ/phenol combination. In summary, although the fate of BzO is known in remarkable detail, coupling this information to the site(s) and mechanism of action of benzene remains to be established.

Human benzene metabolism following occupational and environmental exposures. SM Rappaport [a], S Kim, G Li, Q Lan, R Vermeulen, S. Waidyanatha, L Zhang, S Yin, MT Smith, N Rothman. [a] School of Public Health, University of California, Berkeley, CA, USA

We previously presented evidence that human metabolism of benzene was likely governed by two saturable processes, including a hitherto unrecognized high-affinity enzyme which was responsible for an estimated 73 percent of total urinary metabolites [sum of phenol (PH), hydroquinone (HQ), catechol (CA), *t,t*-muconic acid (MU), and *S*-phenylmercapturic acid (SPMA)] in nonsmoking females exposed to benzene at sub-saturating (ppb) air concentrations. Here we extended the analysis to the major individual metabolites PH, HQ, CA and MU. For each metabolite, we modeled urinary levels as nonlinear functions of the benzene air concentrations, using data from 263 nonsmoking Chinese women (179 benzene-exposed workers and 84 control workers) with estimated benzene air levels ranging from less than 0.001 ppm to 299 ppm. The models were based upon Michaelis-Menten-like kinetics using the metabolite level as a surrogate for the enzyme velocity and the benzene air concentration as a surrogate for the substrate concentration. One model depicted benzene metabolism as a single saturable process (1-enzyme model) and the other as two saturable processes which competed for access to benzene (2-enzyme model). We evaluated model fits based upon the difference in values of Akaike's Information Criterion (ΔAIC) and we gauged the weights of evidence favoring the two models based upon the associated Akaike weights. For each metabolite, the 2-enzyme model provided a better fit than the 1-enzyme model with ΔAIC values decreasing in the order 9.511 for MU, 7.379 for PH, 1.417 for CA, and 0.193 for HQ. Based upon these data, the relative weights of evidence favoring the 2-enzyme model were: 116:1 for MU, 40:1 for PH, 2.0:1 for CA and 1.1:1 for HQ. Removal of one potential outlier from the HQ data (a control subject with very high urinary HQ) increased ΔAIC to 1.6 and the relative weight of evidence to 1.6:1 in favor of the 2-enzyme model. These results indicate that our earlier findings from models of total metabolites were driven largely by MU, representing the ring-opening pathway, and by PH, representing the ring-hydroxylation pathway. Using the fitted 2-enzyme models for these metabolites, we predicted ratios of MU/PF of 0.38 at 0.001 ppm, 0.32 at 0.01 ppm, 0.16 at 0.1 ppm, and 0.12 at 1 ppm of benzene. This indicates that the putative high-affinity enzyme (responsible for human benzene metabolism at ppb levels) favors the ring-opening pathway to a much greater extent than the low-affinity enzyme (probably CYP2E1 in liver) that metabolizes benzene at higher air concentrations. The predicted percentage of benzene that was metabolized by the putative high-affinity enzyme at an air concentration of 0.001 ppm was 88% based upon urinary MU and was 80% based upon urinary PH.

Gene expression in benzene-exposed workers by microarray analysis of peripheral blood mononucleocytes: Induction and silencing of *CYP4F3A* and regulation of DNA-dependent protein kinase catalytic subunit in DNA double strand break repair. Y Bi [a], Z Zhao, X Xiao, M Kong, HY Li, Y Xia, H Yan, X He, Q Ma. [a] School of Public Health, Wuhan University, Wuhan, Hubei, China

Benzene causes a spectrum of hematotoxicity ranging from reduction of peripheral blood cell counts to aplastic anemia and leukemia in humans. To investigate benzene-induced aberrant gene expression, we examined differential gene expression by microarray analysis of peripheral blood mononucleocytes from seven benzene-exposed workers and seven matched controls. Data revealed a number of aberrant gene expressions in benzene-exposed workers, among which 22 genes were up-regulated and 18 down-regulated. The genes are associated with several pathways involved in drug metabolism, DNA damage repair, immune function, and apoptosis. We characterized the induction and function of two genes, *CYP4F3* and DNA-dependent protein kinase catalytic subunit (DNA-PKcs). *CYP4F3A* encodes the leukotriene B4 (LTB4) ω-hydroxylase critical for the inactivation of LTB4 in polymorphonuclear leukocytes (PMN). *CYP4F3* mRNA was consistently elevated in all patients. Moreover, *CYP4F3A* was induced by benzene metabolite phenol in cultured promyelocytic leukemia cell line, HL-60, proerythroid cell line, K562, and ex vivo in human peripheral neutrophils at both mRNA and protein levels. Induction is concentration and time-dependent. *CYP4F3A*-specific siRNA was delivered into HL-60 cells using a lentiviral vector to probe the function of the gene. Silencing *CYP4F3A* reduced cell survival to 56, 44, 22, and 0% of controls at 3, 4, 5, and 6 days after silencing, respectively, revealing *CYP4F3A* as a critical positive regulator of HL-60 proliferation and growth. DNA-PKcs functions with DNA-PK regulatory subunits Ku70 and Ku80 to regulate non-homologous end joining (NHEJ) in DNA double strand break (DSB) repair. DNA-PKcs mRNA was consistently increased in the seven patients compared with matched controls in microarray analysis. Both DNA-PKcs mRNA and protein were induced by phenol in K562 cells in concentration and time-dependent manners. In a DSB model, phenol induced DSB in K562 cells as shown by increased expression of gamma H2AX, a marker of DSBs. The data demonstrated that phenol induces DSBs, which correlates with induction of DNA-PKcs and NHEJ. Since NHEJ is error-prone, induction of DNA-PKcs and NHEJ may contribute to mutations and leukemogenesis induced by benzene. In summary, we demonstrated for the first time that benzene and metabolite phenol induce *CYP4F3A* and DNA-PKcs in benzene poisoning patients and in vitro; induction of the genes may play a role in the pathogenesis of benzene hematotoxicity and serve as biomarkers of benzene exposure.

Modeling the formation and reactions of benzene metabolites. BT Golding [a], ML Barnes, C Bleasdale, A Henderson, D Jiang, X Li, E Mutlu, M Sadeghi. [a] School of Chemistry, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK

One or more of the muconaldehyde isomers is a putative product of benzene metabolism (R Snyder, *Human & Exp Toxicol* 2007, **26**, 687). As muconaldehydes are highly reactive dienals and potentially mutagenic they may be relevant to the carcinogenicity of benzene. Muconaldehydes may be derived through the oxidation of benzene oxide-oxepin, which are established metabolites of benzene, by cytochrome P450 mono-oxygenase. To extend our earlier investigations (see e.g. AP Henderson *et al*, *Chem Res Toxicol* 2005, **18**, 265) of the toxicology of benzenoid compounds and their metabolites, we have studied the oxidation of benzene oxide-oxepin and methyl-substituted derivatives by the one-electron oxidants cerium(IV) ammonium nitrate (CAN) and iron(III) *tris*(1,10-phenanthroline). Surprisingly, the muconaldehydes obtained were exclusively the (*E,Z*)-isomers. Oxidation of benzene oxide-oxepin with CAN or iron(III) *tris*(1,10-phenanthroline) hexafluorophosphate in acetone at –78 °C or in acetonitrile at –40 °C gave (*E,Z*)-muconaldehyde, which was a single diastereoisomer according to analysis by ^{1}H NMR spectroscopy. Reaction of toluene-1,2-oxide/2-methyloxepin with CAN gave (2*E*,4*Z*)-6-oxo-hepta-2,4-dienal. Similarly, the action of CAN on 1,6-dimethylbenzene oxide-2,7-dimethyloxepin gave (3*Z*,5*E*,)-octa-3,5-diene-2,7-dione. *In vivo*, benzene oxide-oxepin could suffer one-electron oxidation by cytochrome P450 mono-oxygenase, acting in a similar manner to that shown by H Sato and F P Guengerich (*J Am Chem Soc* 2000, **122**, 8099) for the oxidation of 1,2,4,5-tetramethoxybenzene. The observations presented may be relevant to the toxicology of benzene oxide-oxepin and other arene oxide-oxepins as we have previously shown that (*E,Z*)-muconaldehyde, analogously to (*Z,Z*)-muconaldehyde, affords pyrrole adducts with primary amines and the exocyclic amino groups of the DNA bases adenine and guanine. Independent of their possible toxicological significance, the experiments described provide preparatively useful routes to (*E,Z*)-muconaldehyde, (2*E*,4*Z*)-6-oxo-hepta-2,4-dienal and (3*Z*,5*E*,)-octa-3,5-diene-2,7-dione. This presentation will also describe methods for the trapping and analysis of reactive benzene metabolites, e.g. using the reactive diene 1,3-diphenylisobenzofuran to trap enals.

Influence of toluene co-exposure on benzene's metabolism and genotoxicity in mice using continuous and intermittent exposures. MG Bird [a], BA Wetmore, DJ Letinski, M Nicolich, M Chen, AR Schnatter, FT Whitman. [a] ExxonMobil Biomedical Sciences, Inc. Annandale, NJ, USA

Benzene exposure in occupational settings often occurs with concurrent exposure to toluene - an aromatic hydrocarbon which is also readily metabolized by CYP450 isozymes. While earlier mouse studies addressing co-exposure to benzene and toluene at high concentrations demonstrated a reduction in benzene-induced genotoxicity, we have previously found, using an intermittent exposure regimen with lower concentrations of benzene (50 ppm) and toluene (100 ppm), that toluene enhances benzene-induced clastogenic or aneugenic bone marrow injury in male CD-1 mice with significantly increased CYP 2E1, depleted GSH and GSSG levels, and increased the GSH/GSSG ratio. A follow up study also used the same daily and total co-exposures but over consecutive days and compared the effects of co-exposure on genotoxicity and metabolism in CD-1 mice both with and without buthionine sulfoximine (BSO) pretreatment. The toluene co-exposure doubled the genotoxic response (as determined by the erythrocyte micronucleus test) of benzene alone. Further, GSH depletion (BSO treatment) caused a reduction in this genotoxicity in both benzene exposed and benzene/toluene co-exposed mice. The results will be discussed in terms of the analyses of urinary metabolites from this and the intermittent exposure study. The discussion will also be based on the assessments of CYP2E1, epoxide hydrolase, quinone reductase, alcohol dehydrogenase, and aldehyde dehydrogenase activities. The results suggest that the presence of glutathione is necessary for benzene genotoxicity either as a metabolite conjugate or through an indirect mechanism such as TNF induced apoptosis.

Biological activity of hydroquinone thiol conjugates. SS Lau [a], C Kuhlman, A Fisher, TJ Monks. [a] Southwest Environmental Health Sciences Center, College of Pharmacy, University of Arizona, Tucson, Arizona, USA

Hydroquinone (HQ) is a metabolite of benzene, and in combination with phenol, reproduces benzene myelotoxicity. HQ readily oxidizes to 1,4-benzoquinone (1,4-BQ) followed by the reductive addition of glutathione (GSH). Subsequent cycles of oxidation and GSH addition gives rise to a variety of mono-, and multi-GSH substituted conjugates. Following administration of HQ/phenol (0.9 mmol/kg /1.1 mmol/kg, ip) to male Sprague-Dawley rats, 2-(GS)HQ, 2,5-(GS)HQ, 2,6-(GS)HQ and 2,3,5-(GS)HQ were all identified in bone marrow. 2-(CysGly)HQ, 2-(Cys)HQ, and 2-(NACys)HQ were also found in the bone marrow of HQ/phenol and benzene treated rats and mice, indicating the presence of an active mercapturic acid pathway within bone marrow. Moreover, 2,6-(GS)HQ and 2,3,5-(GS)HQ were hematotoxic when administered to rats. All of the (GS)HQ conjugates retain the ability to redox cycle and generate reactive oxygen species (ROS), and to alkylate target proteins. Recent *in vitro* and *in vivo* studies in our laboratory revealed lysine and arginine residues as primary targets of 1,4-BQ, 2-(GS)HQ and 2-(NACys)HQ adduction. In contrast 1,4-BQ-adduction of cysteine residues may be a transient interaction, where physiological conditions dictate adduct stability. The generation of ROS and alkylation of proteins may both contribute to benzene-mediated myelotoxicity, and the two processes may be inter-dependent. However, the precise molecular mechanism by which benzene and (GS)HQs induce hematotoxicity remains to be determined. Within 18 hrs of administration of HQ/phenol to Sprague-Dawley rats a significant decrease in blood lymphocyte count was observed. At this early time point, erythrocyte counts and hemoglobin concentrations remained within the normal range. Concomitant with the decrease in lymphocyte count, western analysis of bone marrow lysate, using (GS/NACys)HQ specific antibodies, revealed the presence of (GS)HQ-derived protein adducts. Specific identification of these *in vivo* (GS)HQ-protein adducts, and of ROS catalyzed protein modifications in bone marrow proteins is under investigation. The functional significance of these structurally altered proteins will also be assessed. (Supported by NIH grants GM070890 and ES006694).

Systems biology of human benzene exposure: Toxicogenomic studies. L Zhang [a], C McHale, Q Lan, G Li, Z Ji, R Vermeulen, A Hubbard, X Ren, M Shen, S Rappaport, S Yin, C Vulpe, S Chanock, MT Smith, N Rothman. [a]School of Public Health, University of California, Berkeley, California, USA

Toxicogenomic studies, including genome-wide analysis of susceptibility genes (genomics), gene expression (transcriptomics), protein expression (proteomics), and epigenomics (methylomics, miRomics), of human populations exposed to benzene are crucial to understanding gene-environment interactions, providing the ability to develop biomarkers of exposure, early effect and susceptibility. These toxicogenomic profiles, together with bioinformatics and phenotypic endpoints, comprise systems biology, which has the potential to comprehensively define the mechanisms by which benzene contributes to leukemia. We have recently applied a systems biology approach to a molecular epidemiology study of 390 subjects with varying levels of benzene exposure, with promising initial findings. We showed a significant decrease in almost all blood cell counts, in workers exposed to benzene (n=250), even at exposures below 1 ppm (n=109), compared to controls (n=140). This indicates that hematotoxicity, a phenotypic effect of benzene, is likely caused by an effect on hematopoietic progenitors. We further showed that the formation of progenitor colonies arising from bone marrow stem cells significantly declined with increasing benzene exposure, and that these progenitor cells were more sensitive to the effects of benzene than were mature blood cells. Analysis of transcriptomics, by microarray, in the peripheral blood mononuclear cells (PBMC) of exposed workers, identified genes and pathways (apoptosis, immune response, and inflammatory response) altered at high (>10ppm) and low (<1ppm) benzene levels. Serum proteomics by SELDI-TOF MS revealed proteins consistently down-regulated in exposed compared with control subjects. Preliminary epigenomics data showed effects of benzene and its metabolites on the DNA methylation of specific genes. In order to determine genes involved in susceptibility to benzene toxicity, genomic screens for candidate genes in yeast, and subsequent confirmation by RNAi in human cells, are being undertaken to expand upon the findings from earlier candidate gene analyses. Data on these and future biomarkers of exposure, early effect and susceptibility will be built into a large toxicogenomics database, to which we will apply bioinformatic approaches to understand the interactions among benzene toxicity, susceptibility genes, mRNA, and DNA methylation through a systems biology approach.

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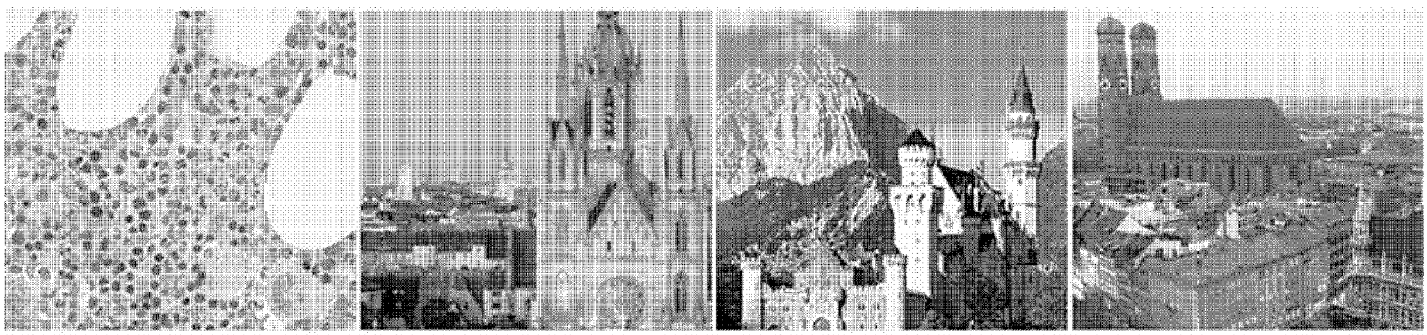
September 7-11, 2009 • Technische Universität München • Munich, Germany



PLATFORM SESSION 5

Mechanistic studies: Transgenics and signal transduction

Co-chairs: **A. Boobis**
Imperial College, London, UK

R. Irons
Fudan University, Shanghai, PR China

Benzene mode of action, hazard, and risk characterization in genetically-defined and modified mouse models. JE French [a], GA Knudsen, RK Kuester, IG Sipes, ML Cunningham, M Saulnier, L Pluta, D Abernethy, A James, S Boley, J Everitt, C Cole, P Schlosser[†], D Walter, SM Ward, JW Spalding, LA Hansen, J Seeley, RR Tice, J Mahler, RW Tennant, L Recio. [a] National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA

Benzene is a human carcinogen and a rodent multi-site carcinogen by multiple routes of exposure. Inherited and/or induced mutations in the *RAS* proto-oncogene related signaling pathways, the *TP53* tumor suppressor gene family, and other acquired or inherited mutations are highly associated with human and rodent cancers. Using multiple genetically-defined or modified mouse (GMM) models and routes of exposure we have shown that exposure to benzene by epicutaneous, inhalation, or oral contact can induce epidermal papillomas and carcinomas, and myeloproliferative disorder associated with leukemia in the bone marrow and peripheral blood. In addition, sarcomas, and splenic as well as thymic lymphomas that retained embryonic isoforms of T-cell receptors are observed. These tumors were characterized by activation of PKC mediated *Ras* signaling pathways and loss of *Trp53*, *Rad51*, and other tumor suppressor genes and aneuploidy, depending upon the mouse model employed. Interpretation of these mouse model studies may be confounded by the use of limited genetic backgrounds and tumor susceptibility. Using multiple haplotyped inbred strains, we have observed significant differences in absorption, distribution, metabolism, and excretion of benzene and metabolites following oral exposure. Studies are in progress to investigate inbred strain differences in hematotoxicity and genotoxicity in multiple strains to determine the variable range of response to inhaled benzene and identify causally related genes and their allelic variants. At present, safety assessment models use a limited set of genetically diverse models, which may be insufficient to evaluate observed differences in toxicity between human individuals under stringent protocols. We may better understand the outcome of the GMM models and future animal models by investigating the genetic basis for toxicity and disease and analyzing variable outcomes between models as complex quantitative traits. Determination of the genetic and epigenetic differences between benzene induced toxicity phenotypes across genetically diverse strains may improve hazard identification and provide reliable data for hazard and risk characterization and quantitative risk assessment relevant to exposed human populations when causally related genes in rodent models are anchored to human orthologs and a common mode or mechanism of action. (Sponsored by the Division of Intramural Research of the NIEHS and the NTP).

Hematopoietic neoplastic diseases in C3H/He and C57BL/6 mice after benzene exposure: Differences observed using microarrays. T Inoue, Center for Biological Safety and Research, National Institute of Health Sciences, Tokyo, Japan.

Benzene-induced hematotoxicity is mediated via aryl hydrocarbon receptor (AhR), which is specifically exhibited in the hematopoietic progenitor cells. Three major questions regarding benzene-induced hematopoietic neoplasms (HPNs) are, first, why is only a low incidence of HPNs induced at low-dose benzene exposure despite the significant genotoxicity of benzene even at low-doses? Second, why is there no linear plateau-like increase of HPNs in incidence at high-dose exposure despite a lower acute toxicity? Third, why are particular myeloid leukemias (AMLs) *not* commonly observed in mice even though AMLs are frequently observed in human cases after occupational benzene exposure? To determine whether *Trp*53-deficient C3H/He mice with AML-prone clarify the above questions, *Trp*53-deficient mice, both C57BL/6 and C3H/He mice, were exposed to benzene, 6 h/day, 5 days/week for 26 weeks, then observed for a life-time. As results, first, incidence of non-thymic lymphoma in C57BL/6 and AMLs in C3H/He mice showed non-threshold responses at the lower exposure level in *Trp*53-deficient mice; second, the incidence of thymic lymphoma in C57BL/6 and non-thymic lymphoma in C3H/He increased without plateau like ceiling; thus, the former threshold-like equivocal induction of HPNs at low-dose benzene exposure is assumed to be based on the DNA-repair potential in wild-type mice, and the latter limited increase in HPNs at a high-dose of benzene is due to excessive apoptosis in wild-type mice. Concerning the incidence of AMLs, although wild-type C3H/He mice, AML prone, induced 9% with 300 ppm, *Trp*53-deficient C3H induced 38% AMLs. Because AMLs were also observed in *Trp*53-deficient mice even in the C57BL/6 mice, benzene exposure may be a potent inducer of AMLs also in mice with some strain differences. Global gene expression profilings observed at 28 days after benzene exposure elucidated reciprocal strain differences for plausible hematopoietic tumorigenesis in expression gene profilings between C3H/He and C57BL/6 mice; such as dominant down modulation of Sltm and Cryl1 are supposed to result in suppression of apoptosis and genomic instability in C3H/He mice respectively, whereas dominant down modulation of Atrx / rad54 and Kdm2a are supposed to result in decrease of DNA repair and genomic instability respectively in C57BL/6 mice, implying these reciprocal gene expression changes induced by benzene exposure may lead each strain for different hematopoietic neoplastic pathways.

The Ah receptor has an important role in the regulation of hematopoiesis. TA Gasiewicz [a], KP Singh, FL Casado. [a] Department of Environmental Medicine, Univ of Rochester Medical Center, Rochester, NY, USA

The Ah receptor (AhR) belongs to the basic-helix-loop-helix (bHLH) Per-Arnt-Sim (PAS) family of transcription factors. In mammals, many of these proteins are involved in regulating responses to signals in the tissue environment such as hypoxia, oxidation-reduction status, and circadian rhythms. Although the AhR is well studied as a mediator of the toxicity of certain xenobiotics, the normal physiological function remains unknown. However, in the past several years, accumulating data from several laboratories support a hypothesis that the AhR has an important function in the regulation of hematopoietic stem cells (HSCs). Our laboratory demonstrated that persistent AhR activation by dioxin, a potent AhR agonist, results in altered numbers and function (reconstitution activity) of HSCs in mouse bone marrow. Data obtained by an analysis of HSCs from AhR null-allele (knock out; KO) mice also indicate that lack of AhR expression results in altered characteristics and function of these cells. HSCs from these animals are hyperproliferative and have altered cell cycle. In addition, aging AhR-KO mice show characteristics consistent with premature bone marrow senescence and are prone to hematopoietic disease characteristic of leukemia. Finally, preliminary data suggest that the expression of the *Ahr* gene is regulated under conditions that control HSC proliferation. Together, these findings from conditions of AhR dysregulation strongly suggest that the AhR has a normal role in the regulation of HSCs. More specifically, we believe that the presence of a normal functioning AhR provides an important advantage by regulating the balance between quiescence and proliferation and preventing the premature exhaustion of HSCs and sensitivity to genetic alterations. This function assists in the preservation of HSC function and long-term multi-lineage generation over the lifespan of the organism. This also implicates a role of the AhR in the aging process. Furthermore, these functions may affect the sensitivity of particular hematopoietic stem and progenitor populations to certain xenobiotics, including benzene. Defining the molecular mechanisms by which these events occur may lead to the identification of previously undefined roles of this transcription factor in particular human diseases, and could have important implications for the diagnosis and treatment of these diseases. (Supported by Grants ES01247, ES04862, and ES07026 from the National Institutes of Health.)

Benzene-induced toxicity is based on the AhR-mediated hematopoietic stem cells. Y Hirabayashi, Div. of Cellular and Molecular Toxicology, Center for Biological Safety and Research, National Institute of Health Sciences, Tokyo, Japan.

Benzene-induced hematotoxicity is an aryl hydrocarbon receptor (AhR)-mediated adverse effect that does not exhibit in AhR knockout (KO) mice. In the hematopoietic system, expression of AhRs is limited in the primitive progenitor cells; thus, a hierarchical hematopoietic impairment starts from primitive hematopoietic progenitor cells after benzene exposure. When one looks at wild-type recipient mice that had been lethally irradiated and repopulated with AhR KO bone marrow cells, owing to reconstruction of the marrow by AhR KO, no impairment is observed in the assay of granulo-macrophage colony forming units (GM-CFU) in the bone marrow after benzene exposure of the reconstructed mice. On the contrary, in mature white blood cells, benzene-induced hematopoietic cytotoxicity is observed in the same reconstructed mice; however, this benzene-induced hematopoietic cytotoxicity in mature white blood cells is not induced in the case of AhR KO mice repopulated with wild-type bone marrow cells after lethal dose of irradiation. Mechanism of benzene-induced hematopoietic toxicity in the matured blood cells without AhR expression is assumed to be based on the metabolites such as phenol and hydroquinone derived from hepatic AhR; thus, the former toxicity is from the metabolites of the wild-type hepatic AhR, whereas the latter is due to the lack of benzene-induced metabolism. Global gene expression of the bone marrow cells after benzene exposure showed that genes responsible to maintain hematopoiesis and hematopoietic niche were generally down regulated such as an up regulation of p38 MAP kinase resulted in apoptosis, and a down modulation of E-cadherin induced by coordination of Hif1- and, Zeb1 and Zeb2-down regulation after Zfhx1b down regulation. Among them, the expression of integrin alpha 4 is notably down regulated after benzene exposure, which is playing as a role of adhesion molecule between hematopoietic stem cells and niches for maintaining the stemness of hematopoietic stem cells and their dormancy of cell cycling. Direct observation of the hematopoietic progenitor cells, $Lin^{-}c\text{-}kit^{+}Sca\text{-}1^{+}$ (LKS) fraction, after benzene exposure showed an increased amount of intracytoplasmic reactive oxygen species (ROS) detected by ROS-reacting dye as compared with other blood cell fractions with lower amount of ROS. In conclusion, benzene-induced hematotoxicity is started from the induction of oxidative stress and the increased amount of the intracytoplasmic ROS in the LKS stem cells, followed by spreading out of whole blood cells, whereas toxicity of mature hematopoietic cells is induced by drug metabolism via hepatic AhR.

Benzene-initiated oxidative stress: Effects on embryonic signaling pathways.
HJ Badham [a], SJ Renaud, J Wan, LM Winn. [a] Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada

Approximately 90% of childhood cancers are of unknown etiology; however, it is hypothesized that *in utero* carcinogen exposure may contribute. Epidemiological studies have correlated parental exposure to benzene with an increased incidence of childhood leukemias. However, mechanisms of benzene-induced carcinogenesis following *in utero* exposure remain unknown. We hypothesize that *in utero* exposure to benzene causes alterations in the redox sensitive signaling pathways involving c-Myb, Pim-1, ERK, p38, and NF-kB via the production of reactive oxygen species (ROS) as a possible mechanism of *in utero* initiated carcinogenesis. Using a CD-1 mouse model we have shown increased oxidative stress in fetal tissue from embryos exposed *in utero* to benzene by measuring reduced to oxidized glutathione ratios, and increased levels of ROS in male fetuses using flow cytometry and the ROS sensitive fluorescent probe dichlorofluoroscein diacetate (DCFDA). In addition, using western blotting techniques we observed increased expression of fetal Pim-1, Pim-1 phosphorylation, c-Myb, and phosphorylated p38 (activated form) and lower protein levels of IKB-alpha, while phosphorylated ERK protein levels did not change. Interestingly, we found male fetuses more susceptible to benzene induced oxidative stress and alterations in embryonic signaling pathways, which is in agreement with the literature suggesting that males are more susceptible to benzene toxicity. Further studies evaluating the reason for this gender difference are ongoing.

Metabolic factors in susceptibility to benzene toxicity. D Ross, H Zhou, Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado, Denver, CO, USA.

Susceptibility to the toxic effects of benzene has been suggested to occur via variations in genes involved in benzene metabolism including cytochrome P450 2E1, epoxide hydrolases myeloperoxidase, glutathione-S-transferases and quinone reductases. However, genes involved in DNA repair, genomic stability and expression of cytokines and/or cell adhesion molecules have also been implicated in benzene susceptibility and an important role has also been suggested for p53 in influencing the response to benzene in animals. Consequently, individual susceptibility to benzene exposure in humans is likely to be multifactorial. Our work has focused on the quinone reductase NQO1 and the influence of the homozygous NQO1*2 polymorphism, which results in a null NQO1 phenotype, on benzene toxicity. The null NQO1 phenotype has been reported as a susceptibility factor for occupational benzene poisoning and NQO1 plays an important metabolic role in 1,4-benzoquinone detoxification. However, NQO1 may also impact other pathways in addition to metabolism of quinones due to protein-protein interactions or other mechanisms related to NQO1 activity. NQO1 has been implicated in stabilization of p53 and in maintaining microtubule integrity. NQO1 in bone marrow is primarily expressed in stromal cells and endothelial cells in stroma express high levels of NQO1. Since endothelial cells represent one of the major niches which influence bone marrow stem cell function, we have utilized transformed human bone marrow endothelial cells (HBMEC) as a model system to investigate the effects of benzene metabolites. HBMEC function was adversely affected by treatment with hydroquinone resulting in inhibition of endothelial cell tube formation, an effect modulated partly by upregulation of chondromodulin 1. In addition, inhibition of NQO1 activity in HBMEC using suicide inhibitors of NQO1 resulted in deficiencies of E-selectin, ICAM-1 and VCAM-1 adhesion molecule expression after TNFα stimulation. These results demonstrate how the metabolic susceptibility factor NQO1 may influence other potential susceptibility pathways for benzene toxicity such as p53 and adhesion molecule expression (Supported by NIH grant ES09554).

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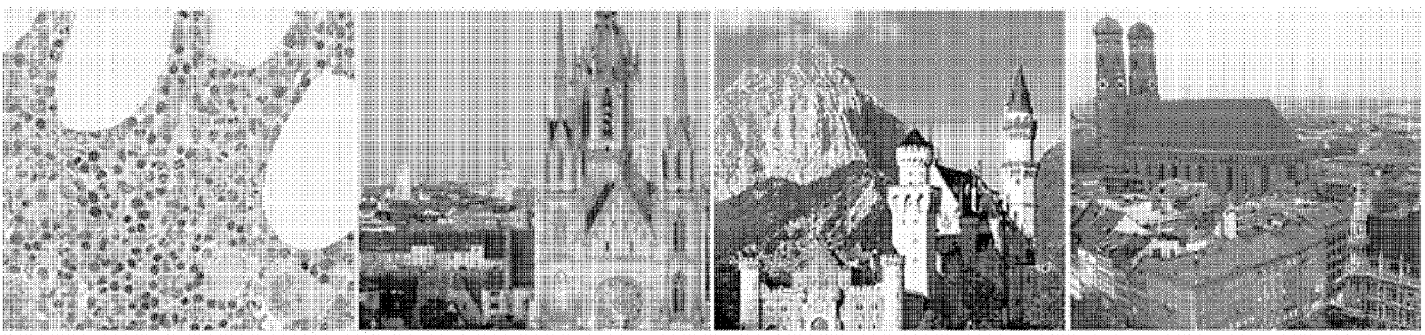

PLATFORM SESSION 6

Mechanistic studies: DNA repair and chromosome damage

Co-chairs: **S. Kyrtopoulos**
National Helenic Research Foundation,
Athens, Greece

M. Ruchirawat
Chulabhorn Research Institute,
Bangkok, Thailand

The role of DNA repair in chemical carcinogenesis with special emphasis on benzene. A Hartwig. Food Chemistry and Toxicology, Technical University of Berlin, Germany.

DNA damage is continuously induced by endogenous and exogenous agents. At the cellular level, DNA lesions interfere with DNA transcription and replication; potential consequences are cell cycle arrest, programmed cell death, mutagenesis, genomic instability and cancer. Perhaps most important, a complex network of different repair systems has evolved to maintain genomic integrity, such as base excision repair, nucleotide excision repair and mismatch repair. With respect to benzene, several active metabolites have been identified, including benzene oxide, phenol, hydroquinone and catechol, leading to different types of DNA lesions. Furthermore, the bioactivation of benzene may lead to the formation of ROS, which give rise to oxidative DNA lesions and altered signaling pathways. Even though the exact contribution of the respective metabolites to benzene-induced carcinogenicity is not yet resolved, different DNA pathways involved in the removal of benzene-induced DNA lesions have been identified and their impact on benzene-induced mutagenicity and carcinogenicity will be presented.

Secondary leukemia and the properties of therapeutic drugs. P Karran. Cancer Research UK, London Research Institute, Clare Hall Laboratories, UK

Therapy-induced acute myelogenous leukemia (tAML) is a relatively frequent complication of anti-cancer therapy. Traditionally, cancer treatments have employed combinations of cytotoxic drugs, most of which have the ability to damage DNA. The immediate consequence of this DNA damage, tumor cell death, is the desired outcome. The potential long-term hazards of this strategy are mutation and genetic instability that are likely to contribute to carcinogenesis. Interactions with the DNA repair pathways that normally protect cells against potentially hazardous genetic changes can be informative about possible etiologies of tAML. I will describe examples in which detailed knowledge of the DNA damaging properties of therapeutic drugs and how this damage causes cell death together with descriptions of the phenotype of tAML can help formulate hypotheses of tAML development.

Topoisomerase II, chromosome damage and leukemia. DA Eastmond, Dept. of Cell Biology & Neuroscience, University of California, Riverside, California, USA

While benzene is widely recognized as a human and animal carcinogen, the key mechanisms underlying its carcinogenic effects remain unknown. There is substantial evidence that chromosomal alterations play an important role in the development of chemically induced leukemias and are likely to contribute to benzene-induced leukemias. Inhibition of topoisomerase II (topo II) by benzene and its metabolites represents a potential mechanism by which benzene could induce its chromosome-altering effects. Previous work from our laboratory and others has demonstrated that bioactivated benzene metabolites are capable of inhibiting topo II in isolated enzyme and cell culture systems. Similarly, a decrease in topo II activity has been seen in the bone marrow of mice administered benzene *in vivo*. The focus of my presentation will be on integrating information about benzene genotoxicity, topo II inhibition, and leukemogenesis. In my presentation, I plan to: 1) Provide an overview of the genotoxic and chromosome-damaging effects of benzene; 2) Review the evidence that benzene and its metabolites can inhibit topo II *in vitro* and *in vivo*; and discuss its potential role in benzene toxicity; and, 3) Discuss this information in the context of what is known about leukemias induced by different classes of human leukemia-inducing agents and the mechanisms underlying chemical leukemogenesis. While the leukemias induced by benzene exhibit similarities with the other induced leukemias, they also exhibit a number of unique features. From current evidence it appears that multiple mechanisms are likely to contribute to benzene-induced leukemias, and that inhibition of topo II could potentially be involved in the development of some leukemia subtypes.

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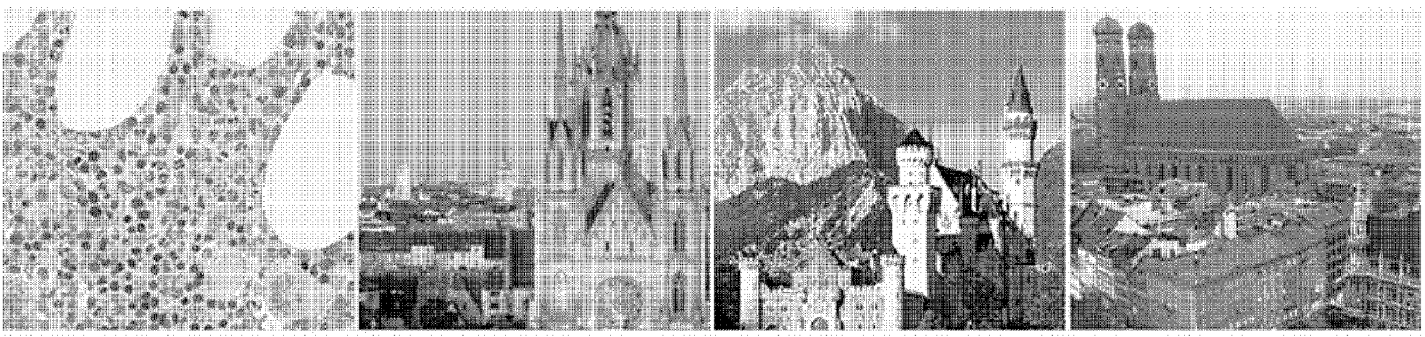

PLATFORM SESSION 7

Exposure science and biomarkers

Co-chairs: **P. Farmer**
University of Leicester, UK

R. Albertini
University of Vermont, USA

Measuring benzene exposure. CP Weisel, Environmental and Occupational Health Sciences Institute, RWJMS/UMDNJ, Rutgers University, Piscataway, NJ, USA

Benzene is ubiquitous in the environment and a common agent in industrial and transportation settings leading to widespread environmental and occupational exposures. Inhalation is the most common exposure route but benzene rapidly penetrates the skin and can contaminant water and food resulting in dermal and ingestion exposures. While less toxic solvents have been substituted for benzene, it is in petroleum products, including gasoline, and is a trace impurity in industrial products resulting in continued ppm occupational exposures. Emissions from gasoline/petrochemical industry are its main source to the ambient air, but a person's total inhalation exposure can be elevated from emissions from cigarettes, consumer products and gasoline powered engines/tools stored in garages attached to homes. Air samples are collected in canisters or on adsorbent with subsequent quantification by gas chromatography. Ambient air concentrations vary from sub-ppb range, low ppb, and tens of ppb in rural/suburban, urban, and source impacted areas, respectively. Short-term environmental exposures of ppm occur during vehicle fueling. Indoor air concentrations of tens of ppb occur in microenvironments containing indoor sources. Occupational and environmental exposures have declined where regulations limit benzene in gasoline (<1%) and cigarette smoking has been banned from public and work places. Similar controls should be implemented worldwide to reduce benzene exposure. Biomarkers of benzene used to estimate exposure and risk include benzene in breath, blood and urine; its urinary metabolites: phenol, *t,t*-muconic acid (*tt*MA) and S-phenylmercapturic acid (S-PMA); and blood protein adducts. The biomarker studies suggest sub to ppm benzene environmental exposures though non-benzene sources for urinary metabolites, differences in metabolic rates at occupational or animal doses, and the presence of polymorphisms may overestimate the projected exposures.

Exposure to benzene in Thailand: Implications for carcinogenic risk. M Ruchirawat, P Navasumrit, D Settachan, Chulabhorn Research Institute, Bangkok 10210, Thailand

Exposure to benzene in human populations can occur in occupational settings in which benzene is used or produced, or through the ambient air from traffic emission resulting from incomplete combustion of fossil fuel, or from other sources. Benzene exposure was studied in various population groups in Thailand. The first group consisted of gasoline service attendants and laboratory workers in quality control units of petrochemical factories who are occupationally exposed to benzene at levels ranging from 75 to 140 ppb. The second group consisted of traffic police and school children attending schools in the city center close to the traffic where ambient air was contaminated with benzene from traffic emission, as well as temple workers who were exposed to benzene from incense burning. Their exposure levels were approximately 15 ppb in traffic police, 5.5 ppb in school children and 13 ppb in temple workers. Co-exposure to 1,3-butadiene and PAH generated from the same sources occurred in the second group. Blood benzene and urinary muconic acid (MA) were used as biomarkers of exposure. 8-OHdG, DNA strand breaks and DNA repair capacity were measured as biomarkers of early effects of genotoxic compound exposure. In most population groups studied, the levels of benzene exposure correlated with blood benzene levels and urinary MA concentrations, as well as 8-OHdG levels in leukocytes but not with 8-OHdG levels in the urine as seen in the school children. In gasoline service attendants, laboratory workers, and temple workers, benzene exposure levels correlated with DNA strand breaks as well as DNA repair capacity. In traffic police and school children who were exposed to benzene from traffic emission, benzene exposure did not correlate with DNA repair capacity. DNA strand breaks and DNA repair capacity correlated with PAH levels in school children.

Benzene metabolites block gap junction intercellular communication. E Rivedal [a], G Witz, E Leithe. [a] Institute for Cancer Research, Norwegian Radium Hospital, Oslo, Norway

Chemicals contribute to carcinogenesis via multiple mechanisms and are associated with the induction of genetic changes. A large fraction of chemical carcinogens do however not induce direct DNA damage. The identification of such non-genotoxic carcinogens is a major challenge, due to their diverse modes of action. In order to accurately predict the potential carcinogenicity of chemicals and thereby their relevance in human diseases it is crucial to obtain mechanistic knowledge of their biological effects. The ability of chemicals to block intercellular communication via gap junctions has been associated with carcinogenesis as well as other pathological conditions. Thus, information concerning the effect of environmental contaminants on gap junctions is an important supplement to other data when assessing possible human health risk. Benzene is used in large quantities worldwide. Hematotoxicity and leukemia have been associated with benzene exposure, but the mechanisms involved are poorly understood. Aberrant gap junction intercellular communication (GJIC) has been linked to both cancer induction and interference with normal hematopoietic development. Our data indicate that inhibition of GJIC may play a role in benzene toxicity since benzene metabolites were found to block GJIC, with the ring-opened *Trans,trans*-muconaldehyde (MUC) as the most potent metabolite. We have studied the molecular mechanism underlying the inhibition of gap junction intercellular communication and showed that MUC induces cross-linking of the gap junction protein connexin43. The data suggest that cross-linking is responsible for the induced inhibition of GJIC, and that glutaraldehyde possesses similar effects as MUC. The reported association between glutaraldehyde exposure and enhanced risk of leukemia, as well as disturbance of hematopoiesis, strengthen the possible link between the effect of MUC on gap junctions, and the toxic effects of benzene.

Occupational benzene exposure and hematological parameters. GMH Swaen [a], L van Amelsvoort, JJ Twisk, E Verstraeten, R Slootweg, JJ Collins, C Burns. [a] Epidemiology, Health Services, The Dow Chemical Company, Terneuzen, The Netherlands

At high exposure levels, benzene exposure can cause hematological effects. There is conflicting evidence about potential hematological effects at low concentrations. Following a publication in *Science* that reported hematological effects at concentrations below 1 ppm, we have conducted an epidemiological study to further investigate this matter. Extensive exposure data and data from routine hematological examinations were available for Dow employees at the Terneuzen site in the Netherlands. We compared 8,532 blood samples of Dow employees with low benzene exposure to 12,173 samples of employees with no benzene exposure that were available for the period between 1981 and 2007. Based on 21,584 random benzene samples a Job Exposure Matrix (JEM) by job, department and year was made for all employees with benzene exposure. Next, the JEM was used to estimate benzene exposure in the year in which each blood sample was collected. Multiple logistic regression modeling was used to adjust for differences in age, cigarette smoking and month of blood sample collection. Next, the exposed group was stratified into three subgroups of exposure below 0.5 ppm, between 0.5 and 1 ppm and 1 ppm^{+}. The statistical analysis has not yet been fully completed and the final results will be presented at the conference.

Evaluation of the source to dose continuum for benzene is needed to augment biomarker measurements to adequately protect the public and eventually implement safeguards globally. Without such efforts new or revised control strategies may not address the most significant sources of human exposure to benzene.

Biomarkers as mechanistic probes: Application to benzene toxicity. RJ Albertini [a], SA Judice, MG Bird, L Recio, VE Walker. [a] University of Vermont, Burlington VT, USA

Most studies of benzene associated mutagenesis have focused on relationships between structural and numerical chromosome aberrations and leukemia/lymphoma. With the exception of reverse point-mutations in bacteria, benzene, through its metabolites, also induces gene level mutations although it is unclear if this is due to direct DNA reactivity or to indirect effects. Somatic mutations *in vivo* can assess the mutagenic potential of a treatment and can serve as mechanistic probes when mutations are characterized and clonal relationships established. Studies of *Hprt* mutations in splenic T-cells of C57BL/6 wild-type (*p53*+/+) and haplo-insufficient Trp53 (*p53*+/-) mice were undertaken to determine if gene mutations were induced at similar frequencies in the two strains under prolonged benzene exposure scenarios that produced thymic lymphomas preferentially in the latter and if these mutations in the *p53*+/- animals were enriched in T-cell clones that had lost the wild-type *p53* allele. Unexpectedly, benzene related *Hprt* mutation induction was fairly robust and equal in the two strains of mice for three exposure scenarios, each of which exposed the animals to 3000 ppm.h/week for 38 weeks. Analyses of *p53* in the *Hprt* mutant isolates from the *p53*+/- animals showed frequent loss of the *p53* mutant allele rather than the expected loss of the *p53* wild-type allele. Lineage analyses of clonally related *Hprt* mutant isolates showed that segregation of the *p53* alleles occurred after the *Hprt* mutations in proliferating T-cell clones. The high frequency segregation of the mutant disrupted *p53* transgenes in the *p53*+/- mice indicates that the tumor susceptibility of these animals is not due to genomic instability in clones of cells that have lost the wild-type *p53* allele. Although benzene is associated with *Hprt* mutations following prolonged exposures, shorter exposures at 1 to 200 ppm indicated that the mutagenic potency is low. T-cell *HPRT* mutations have been well characterized in humans but have not yet been studied in benzene exposed humans where questions of mutational spectra and genomic instability can be addressed. The recent development of a mutational assay for mutations of the phosphatidylinositol glycan class A gene (*PIGA*) will allow extension of these analyses employing high through-put systems capable of detecting mutations of several cell types including those of erythroid and myeloid origin.

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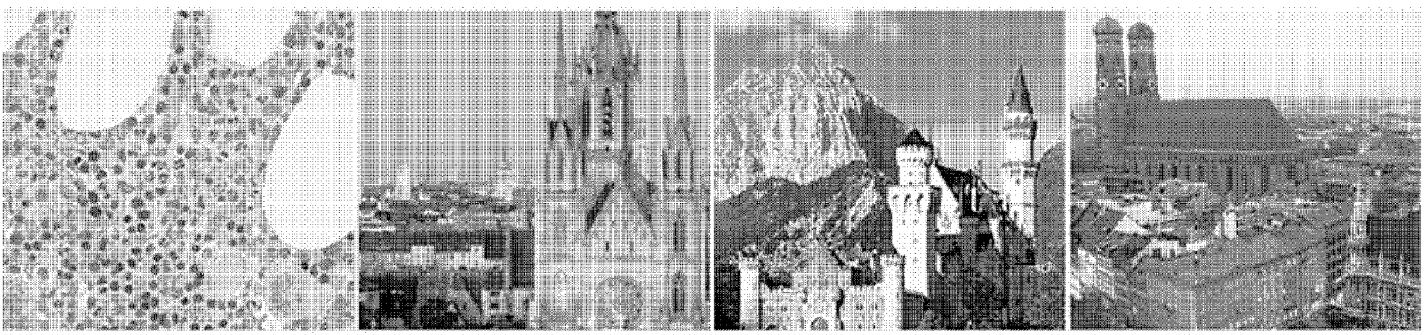


PLATFORM SESSION 8

Selections from volunteered papers

Co-chairs: **D. Kaden**
DakTox LLC, USA

D. Pyatt
Summit Toxicology, USA

How much does benzene contribute to the overall burden of cancer due to occupation?
L Rushton [a], L Fortunato, S Hutchings. [a] Imperial College London, UK

Work-related cancers are largely preventable. We are estimating the burden of occupationally-related cancer in Great Britain to identify agents, industries and occupations for targeting risk prevention. We estimate attributable fractions (AF) and numbers (AN) for cancer mortality and incidence for all cancers, agents and occupations classified as IARC group 1 and 2A carcinogens. Benzene is classified as a group 1 carcinogen i.e. there is strong human evidence. The AF requires knowledge of the risk of the exposure and the proportion exposed. Relative risks (RR) estimates for acute non-lymphocytic leukemia (ANLL) were obtained from published literature: (i) RR = 2.47, 95% CI 1.38 - 4.07 (Rinsky *et al.* 2002)) for industry sectors with high exposures (ii) RR = 1.32 (95%CI 0.49 - 2.88) (Lewis *et al.* 2000) for land transport (iii) RR = 2.17 (95%CI 0.9 - 5.2) (Collins *et al.* 2003) for workers in industrial chemical manufacturing (iv) RR = 1.11 (95%CI 0.3 - 2.83) (Bloemen *et al.* 2004) for industry groups with low levels of benzene exposure. A latency of 0 - 20 years was assumed for leukemia and we defined the risk exposure period (REP) for deaths occurring in 2005 as 1986 - 2005. National data sources were used for estimating proportions exposed over the REP in different industry sectors, taking account of changing employment levels and employment turnover. Monte Carlo methods were used to obtain random error confidence intervals (CI). We estimate the AF for ANLL due to exposure to benzene as 0.26% (95%CI 0 - 4.9%) giving 5 attributable deaths (95% CI 0, 103) and 8 attributable cancer registrations (95% CI 0,158). The wide confidence intervals reflect small RRs. For leukemia from all IARC 1 and 2A occupational carcinogens (benzene, ionising radiation, ethylene oxide, formaldehyde, 1,3,butadiene, non-arsenical pesticides) estimates are: AF 0.7% (95%CI 0 - 5.7), AN (deaths) 30 (95% CI 0 - 239), AN (registrations) 47 (95% CI 0 - 363). The estimated overall occupational burden in GB due to all cancers (24 different cancers) is 5.5% (8.5% men and 2.3% women) giving over 8,000 deaths and 14,000 registrations attributable to carcinogens at work. Of the 41 different carcinogens for which separate estimates were made, benzene is ranked 31. Our results might thus be interpreted as indicating that benzene should not be an immediate priority for health and safety strategic planning and that reasons other than burden are required for prioritising research effort in this area.

Benzene is associated with the development of severe but not moderate aplastic anemia in Shanghai, China. SA Gross [a,b], RD Irons, AR Schnatter, AT Le, J Ryder, WX Qin, TW Armstrong, GB Copely. [a] Molecular Toxicology and Environmental Health Sciences Program, School of Pharmacy, University of Colorado Denver, CO; [b] Fudan-Cinpathogen Clinical and Molecular Research Center, Institutes of Biomedical Sciences, Fudan University, Shanghai, China.

Benzene is historically thought to be a cause of aplastic anemia (AA) in individuals exposed to high concentrations, although quantitative epidemiology studies are rare. Herein, we report the findings of a case control study of 137 consecutive patients diagnosed with AA in Shanghai, China over a 4 year period. Diagnoses were made by a single laboratory and subjects were age- and gender- matched to two hospital-based controls. Questionnaires were administered to all cases and controls, and information obtained on previous disease, work histories and exposures to potential etiologic agents such as benzene. Subjects and their controls were further categorized into AA subtypes (moderate (MAA) and severe (SAA)) and odds ratios (OR) were calculated for individual risk factors. The strongest associations were observed for exposure to benzene and SAA (OR 3.12, 95% CI 1.12-8.65) and life on a farm and MAA (OR 3.08, 95% CI 1.44-6.56). Using a Conditional Logistic Regression model for analysis of multiple risk factors, consistent associations between the risk factors (i.e. benzene and life on a farm) and variation in subtypes of AA (i.e. SAA and MAA) emerged as the best explanations for the development of the disease (R^2 = 10.1% and 9.9%, respectively). These findings suggest that individual subtypes of AA may have distinct etiologies, and while exposure to benzene poses a significant risk of developing AA, the majority of cases of this disease remain unexplained. (This work was funded by the Benzene Health Consortium as part of the Shanghai Health Study.)

Modulation of the benzene metabolite hydroquinone induced toxicity: Evidence for an important role of *fau*. SH Inayat-Hussain [a], HA Ibrahim, NF Mahbub, EL Siew, KM Chan, NF Rajab, GT Williams, D Ross. [a]Faculty of Allied Health Sciences, Univ Kebangsaan Malaysia, Kuala Lumpur, Malaysia

Hydroquinone (HQ) being a major metabolite of benzene has been shown to induce reactive oxygen species and DNA damage leading to inhibition of human T-lymphocytes proliferation and induction of apoptosis in hematopoietic cells. Using functional expression cloning, several genes have been isolated which inhibit apoptosis induced by several genotoxic agents including etoposide, cisplatin and uv irradiation. In this study, we examined the potential role of these genes, which were stably transfected into mouse W7.2 T-cells and which regulate cell death in different ways, in HQ induced cytotoxicity. These stable transfectants were overexpressed with *rFau* (antisense sequence to *F*inkel-Biskis-Reilly murine sarcoma virus- *a*ssociated *u*biquitously expressed gene), 4n10 (a transdominant fragment of protein phosphatase 4 which down-regulates endogenous protein phosphatase 4), full-length RACK 1 (Receptor for Activated protein C-Kinase 1) and pc3n3, which up-regulates endogenous RACK1. Assessment of HQ induced cytotoxicity using MTT assay revealed that only *rFau* inhibited cytotoxicity. Further investigation confirmed that HQ also attenuated apoptosis as assessed by annexin V/PI after 14 hr treatment. Since *fau* overexpression enhances apoptosis in genotoxin induced T-cell apoptosis, our data demonstrates that down regulation of *fau* by overexpression of its reverse sequence (*rfau*) may play an important role in the pathophysiology of HQ induced myelotoxicity. The potential mechanisms of *rfau* inhibition of HQ induced apoptosis are currently being investigated. (Supported by UKM-GUP-TKP-08-022-075)

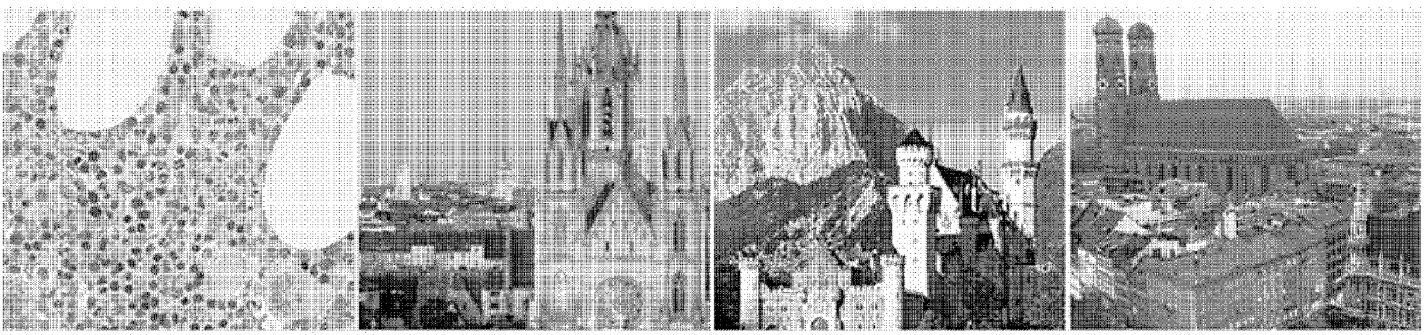
Methylation and expression analysis of tumor suppressor gene p15 and p16 in benzene poisoning. CH Xing [a], QF Wang, HY Tian, SN Yin, GL Li. [a] National Institute of Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention, Beijing, China.

Benzene is an important industrial chemical and component of cigarette smoke, gasoline, and automobile emissions. Benzene causes hematotoxicity and leukemia, particularly acute myeloid leukemia (AML). The underlying mechanism of benzene poisoning (BP), however, is poorly understood. In AML, inactivation of tumor-suppressor gene p15 and p16 is one of the most common genetic events, and promoter DNA hypermethylation has shown to be the primary mechanism that leads to the loss of expression of these two genes. To investigate whether benzene negatively affects the expression of p15 and p16 through DNA methylation, we carried out a case-control study in Chinese occupational benzene poisoning patients. Eleven cases of BP and 8 controls who were matched for age ($\pm$ 5 yr), sex, working duration and job title with BP were recruited. Expression levels of p15 and p16 were examined by quantitative real-time polymerase chain reaction (PCR). Bisulfite-PCR pyrosequencing was used to quantitate the level of DNA methylation in the promoter regions of p15 and p16. p15 presented downregulation in 63% (7/11) of benzene poisoning compared to 38% (3/8) of the controls. p16 was downregulated in 80% (8/10) of BP compared to 20% (1/5) of the controls. The methylation levels of third CpG site within p15 promoter region were higher among BP group and controls compared with the genome DNA which was used as built-in control (12.6%,10.8% and 9%, respectively).The average percentage of methylated cytosines of p16 was higher in BP group than in controls (12.4%, 11.3%, respectively; $p>0.05$). A weak negative correlation was presented between p15 mRNA level and methylation level in third CpG site within the promoter region of p15 (Pearson $r = -0.2$, $p > 0.05$). p16 mRNA level decreased with increasing methylation (Pearson $r = -0.64$, $p > 0.05$). The fourth CpG site in p16 promoter is located within the consensus binding sequence for olfactory neuron-specific transcription factor. A significant negative correlation between mRNA level and the fourth CpG site was exhibited (Pearson $r = -0.88$, $p < 0.05$). Our report demonstrated that mRNA expression of p15 and p16 is significantly downregulated in BP patients. Hypermethylation in promoter CpG islands is likely to contribute to the downregulation of these two genes. Further in-depth studies, utilizing large number of samples, are needed to fully understand the molecular mechanism involved in the tumor-suppressor gene inactivation in benzene-related diseases.

Benzene 2009:

Health Effects and Mechanisms of Bone Marrow Toxicity
Implications for t-AML and the Mode of Action Framework

September 7-11, 2009 • Technische Universität München • Munich, Germany



PLATFORM SESSION 9

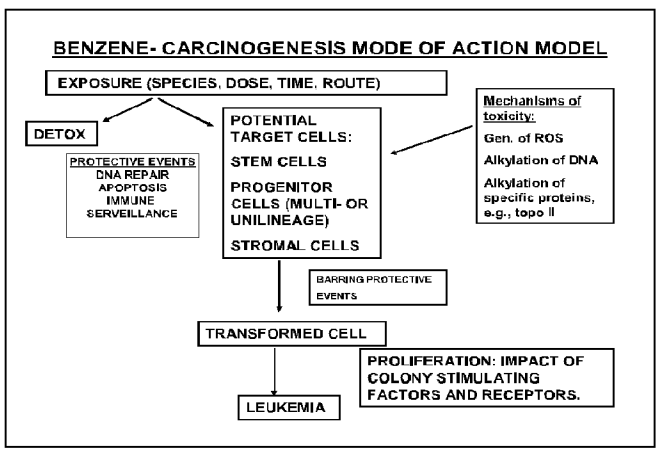
Mode of action and risk assessment

Co-chairs: **B. Sonawane**
USEPA, Washington DC, USA

L. Levy
Cranfield University, UK

Implication of recent data on benzene risk assessment. Weight of evidence for mode of action/ human relevance and dose-response; Critical data gaps. JE Klaunig [a], R Snyder.
[a] Center for Environmental Health, School of Medicine, Indiana University, Indianapolis, IN, USA

Utilizing new information provided during this symposium in addition to previously published reports, a draft proposal for the mode of action for benzene induced carcinogenesis will be presented. In the case of benzene induced leukemia, several key steps are provided for consideration including; 1) the activation of benzene in the liver to phenolic metabolites, 2) the delivery of these metabolites to the bone marrow with the targeting stem cells, progenitor and/or stromal cells, 3) additional conversion of the benzene metabolites to semiquinones and quinones or the generation of reactive oxygen species,
4) induction of damage to tubulin, histones topoisomerase II, and/or DNA in the target cells; 5) resulting in the induction of a transformed cell 6) with subsequent selective cell proliferation of the transformed cell (via CSF and cytokines) to form the leukemia. Discussion of the proposed mode of action and the identification of critical data and data gaps will be addressed.



The vanishing zero revisited: Thresholds in the age of genomics. Defining No transcriptional effect levels (NOTEL) and the No detectable adduct levels (NODAL) following carcinogen exposure *in vitro* and *in vivo*: Implications for risk assessment?
H Zarbl [a], J Glick, P Vouros. [a] Environmental and Occupational Health Sciences Institute, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ, USA, 08854.

The continued increase in the sensitivity of analytical techniques has generated much debate on the role of low level exposures in risk assessment. In the present study we explored dose dependent transcriptional responses to carcinogens *in vitro* and *in vivo,* and examined how transcriptional responses were related to the formation of genotoxic DNA adducts. For *in vitro* studies, logarithmically growing human BEAS-2B bronchial epithelial cells were dosed for 24 hours with the active metabolite of PhIP (N-hydroxy-PhIP) at concentrations ranging from 10^{-5} to 10^{-11} M. Total RNA and DNA were simultaneously isolated from each of the exposed cultures. Changes in gene expression as a function of carcinogen dose were measured using Affymetrix GeneChip Human Genome U133 Plus™ microarrays. For measurements of adduct formation, genomic DNA was digested to mononucleosides and the adducted bases enriched by solid phase extraction. DNA adduct levels were measured using an Agilent ion trap MS with HPLC Chip Cube interface. Using this system, adducts were detected in the dosing range of 10^{-5} to 10^{-9} M PhIP concentrations. Significantly, doses that failed to yield detectable levels of adducts (NODAL) corresponded closely to the doses that had no observable effect on the cell's gene expression profile (NOTEL). Similar *in vivo* experiments using rats exposed to decreasing, non-tumorigenic doses are in progress. These finding and similar finding by other researchers could have significant implications for risk assessment when dealing with genotoxic compounds and carcinogens.

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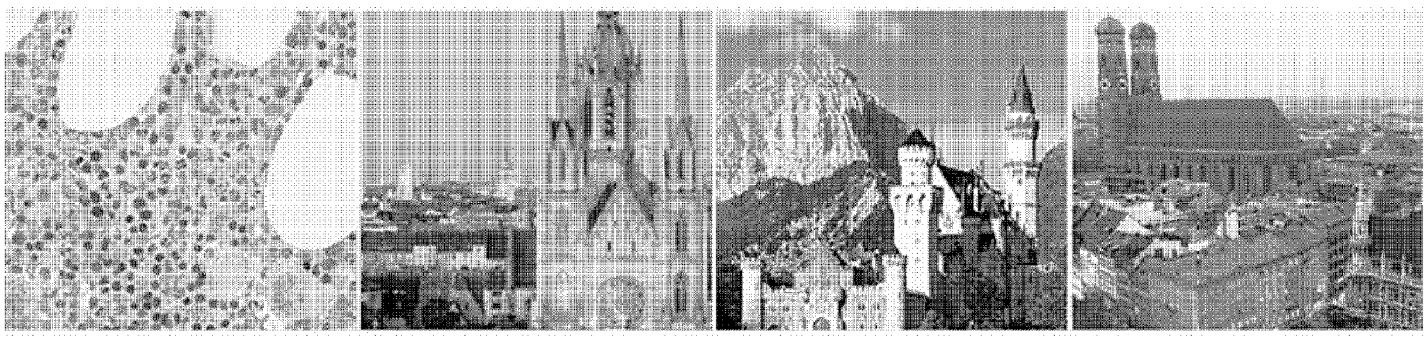

Abstracts:

Posters

Exposure assessment for case-control (CC) epidemiology studies based in Shanghai, China: Summary of methods and results. T Armstrong[a], Y Zhou, C Zhang, S Bowes, Y Liang, O Wong, F Hua, A Schnatter. [a] TWA8HR Occupational Hygiene Consulting, NJ, USA

As part of a suite of epidemiology studies of leukemia, lymphoma and aplastic anemia conducted in Shanghai from 2001 to 2008, retrospective job and task focused exposure assessments (EA) of cases and matched controls were completed for benzene and selected other hazards of concern. Trained interviewers administered structured questionnaires (in Chinese) to study subjects recruited from 31 hospitals. Initial classifications (exposed, likely unexposed, or uncertain), done by team members not informed of case or control status, were made to focus subsequent investigations. Benzene EA resources beyond questionnaires included industry-specific extracts from the Shanghai Municipal Institute for Public Health Supervision database, Chinese literature on benzene exposures, on-site workplace investigations, summaries of key regulatory and technology changes, and task simulations. A Shanghai-based expert panel (EP) made preliminary exposure assignments, which were further refined via logic and consistency checks with source data, resulting in range estimates 0 to 4 (none, <1, 1 to 10, >10 to 100 and >100 mg/m^3) for benzene. For other hazards, sources included the EP's knowledge of relevant industries, and the Chinese and Western literature. A random 20% of the benzene EAs were checked by two independent approaches using job titles and key task data extracted from questionnaires. The CC database included over 21,000 work history events (WHE, or jobs). For study subjects, 754 WHE underwent further review for benzene exposure. The 754 WHE arose from diverse industries and trades including: shoe manufacturing, rubber production, printing, painters and machine repair. Nearly 40% of these WHs rated ≥ category 2 benzene exposure, with rank counts and percents of: 0 (150, 20%), 1 (317, 42%), 2 (144, 19%), 3 (101, 13%) and 4 (42, 6%). Review of initial differences between study ratings and the 20% sample ratings suggested that the more detailed data (beyond questionnaire extracts) available to the study team improved rating specificity. For other exposures, the EA covered 19,157 WH events with 15,231 in the final CC database. The most prevalent general category exposures included agricultural chemicals (2077), petroleum products (1339) and metals (908). The number, eras and diversity of WH events presented a large, challenging EA project, but the broad information base assembled supported the extensive, multiple substance EA. Information about the nature, extent and range of regulatory and technology changes in China, and the available Chinese exposure literature, were key sources on chemical uses that enhanced the EA approach.

Do we agree? : Comparison of semi-quantitative exposure assessments by three independent approaches. TW Armstrong[a], JW Cherrie, RF Herrick, M Chen, SM Bowes, AR Schnatter. [a] TWA8HR Occupational Hygiene Consulting, Branchburg NJ, USA (current affiliation)

As part of a review of exposure assessments (EA) for a population case-control study of acute myeloid leukemia and lymphoid neoplasm, we undertook a comparative evaluation of the study's semi-quantitative retrospective EA for benzene. The overall study goal was to rate the benzene exposure for each subject's described tasks. The study approach (TWA's) drew on broad information available, including historical monitoring data, Chinese medical/exposure literature, on-site workplace investigations, reports on technology changes for key industrial sectors, prior discussions with and written review notes from the Chinese experts. A stratified random process was used to select a 20% sample from the study database. Two raters (JWC, RFH) were provided partial data (from questionnaires only): start and end date for the work history, job title, type of industry, and a one to two sentence description of job duties. JWC's assessments were based on a structured subjective judgment approach developed for use in industry case-control studies, with results of then mapped to the study categories. RFH rated the exposures via the professional judgment, considering information on industry, job title and activities, materials used, processes, duration of work, and year. Following the ratings, TWA provided additional information from the study database that provided the rationale for study ratings that differed from the ratings of either JWC or RFH. Two of the raters (JWC, RFH) had somewhat less information than that drawn upon for the study EA. Due to these differences, a strict comparison of inter-rater comparability was not conducted. Weighted Kappas (generally in the range between 0.4 and 0.69) indicated reasonable agreement. Two categories represent no and very low (<1 mg/m^3) exposure, so comparisons were completed on "exposed" versus the two lowest categories as "unexposed". Those simple Kappas indicated moderate agreement. Another comparison showed scores were within plus/minus one category for approximately 85% of the subjects. JWC and RFH reviewed the additional information TWA provided and indicated this additional information would generally have altered their ratings in the direction of the study ratings. The three methods gave good agreement. The study ratings (TWA's) drew on additional information not available in the data extracts provided to RFH and JWC. Subsequent consideration of this additional information further improved the agreement of the raters. The comparison study suggests the additional information improved the EAs (TWA's) used in study epidemiologic analyses.

Interpretation of urinary and blood benzene biomarkers of exposure for non-occupationally exposed individuals. SM Arnold [a], PS Price, SH Robison, MF Hughes, PJ Boogaard, MD Dryzga. [a] The Dow Chemical Company, Midland, MI, USA

This study relates published measurements of benzene biomarkers to air exposure concentrations. Benzene has three reliable biomarkers of exposure for non-occupationally exposed individuals: urinary benzene, urinary S-phenylmercapturic acid (SPMA), and blood benzene. Published linear regression equations for benzene and SPMA in urine were used to relate urinary measurements to average air concentrations, and for comparison to the USEPA reference concentration (RfC) of 0.030 mg/m^3 (9 ppb). The urinary benzene concentration relating to the USEPA RfC was 0.24 ng/L (range 0.04 to 0.46 ng/L), and the urinary SPMA concentration was 3.0 ug/g creatinine (cr.) (range = 0.1 to 6.8 ug/g cr.). These values compare to urinary concentrations (central tendency values for non-smokers) reported in the literature ranging from 0.11 to 0.25 ng/L for benzene and 0.3 to 6.5 ug/g cr. for SPMA. As expected, the urinary concentrations for smokers were generally higher. The results of this analysis indicate that for non-occupational exposure, urinary benzene and SPMA levels correlate well with reported ambient air benzene levels and non-smokers are generally at or below the urinary biomarker level corresponding to the USEPA RfC. For benzene urinary metabolites with significant background sources, t,t-muconic acid (MA), phenol (PH), catechol (CA), and hydroquinone (HQ), we determined the upper bound urinary concentration of benzene metabolites that could occur as a result of benzene exposure; therefore, permitting a determination of the levels of each metabolite from the background sources (e.g., smoking, diet, pharmaceuticals, etc.). Measured metabolite concentrations were 11 (MA), 57 (PH), 405 (CA), and 310 (HQ) times greater than the maximum theoretical amount predicted from the benzene exposure concentration. Assuming that the contribution of benzene will need to be twice the background levels of a metabolite in order to be properly detected, the detection limits of benzene for metabolites known to have other sources are 0.08 ppm for MA, 0.5 ppm for PH, 2.3 ppm for CA, and 2.7 ppm for HQ. The most recent CDC-NHANES data for blood benzene indicate levels of 0.017 to 1.4 ng/ml, which are consistent with ranges previously reported in the literature. An evaluation of the blood benzene data and documented activity patterns, such as recent refueling of vehicles and close proximity to cigarette smoke, is underway. (This abstract does not represent US EPA policy)

The production of hydroquinone in fetal liver tissue is associated with an increased incidence of liver tumors in mice after *in utero* exposure to benzene. H Badham [a], L Winn. [a]Department of Pharmacology and Toxicology, Queen's University, Kingston, ON, Canada

Approximately 90% of childhood cancers are of unknown etiology; however it is hypothesized that *in utero* carcinogen exposure may contribute. Benzene is a known adult carcinogen in humans and mice. The cancer risks and mechanisms of toxicity associated with *in utero* benzene exposure are unknown, however bioactivation of benzene into reactive metabolites such as hydroquinone (HQ) is thought to play a crucial role. We propose that *in utero* exposure to benzene causes the production of hydroquinone in fetal tissue that can cause altered cell signaling and/or damage cellular constituents such as proteins, lipids and DNA ultimately leading to an increased risk of developing cancer later in life. **Objectives**: This study aimed to measure HQ concentration in fetal liver tissue shortly after *in utero* exposure to benzene as well as determine the cancer incidence in offspring up to 1 year after birth. **Methods**: Pregnant CD-1 mice were injected i.p on gestational days (GD) 8, 10, 12, and 14 with corn oil, 200 mg/kg, or 400 mg/kg benzene. One, 2, and 4 hrs after the last injection on GD14, fetal livers were collected, homogenized, and extracted with ethyl acetate. The organic layer of the samples was brought to dryness under N_2, then derivitized with Tri-Sil Reagent. HQ concentration was measured by gas chromatography-mass spectrometry. In additional studies, offspring of dams exposed to corn oil, 200, or 400 mg/kg benzene were followed to 1 year after birth. At 1 year of age, offspring were euthanized and necropsied. **Results**: HQ production in male fetuses peaked 2 hrs after *in utero* exposure to benzene and interestingly was highest in the 200 mg/kg benzene exposure group (40 pg/mg tissue) compared to 400 mg/kg benzene (25 pg/mg tissue). HQ production in female fetuses peaked at 1hr after *in utero* exposure to benzene, however, only reached 25 pg/mg tissue in both 200 and 400 mg/kg benzene exposure groups. Interestingly, liver tumor incidence in 1 year old male offspring was 17 %, 45%, and 22% in control, 200 mg/kg and 400 mg/kg benzene exposure groups respectively. Liver tumor incidence in 1 year old female offspring was 0%, 4%, and 0% in control, 200 mg/kg and 400 mg/kg benzene exposure groups respectively. This is the first demonstration that *in utero* exposure to the environmental pollutant benzene can induce tumor formation in mice and raises the possibility that benzene exposure to pregnant women could contribute to similar cancers in children. These results also indicate that the production of the reactive benzene metabolite, HQ after *in utero* benzene exposure may be responsible for the increased tumor incidence in offspring.

FRY gene expression is a biomarker of leukemia type and phase. J Graham [a], H Zarbl.
[a] Dept. of Environmental and Occupational Medicine, RWJMS, EOHSI, University of Medicine and Dentistry of New Jersey, Piscataway, NJ, USA

Rat strains differ dramatically in their susceptibility to mammary carcinogens. Using a genetic backcross between the resistant Copenhagen (Cop) and sensitive Fischer 344 (F344) strain, we mapped a Mammary Carcinoma Susceptibility (*Mcs*) locus (LOD score ~8.6) to the long arm of rat chromosome 12. The *Mcs* locus comprises a 5.6 megabase region whose synteny is conserved on the long arm of human chromosome 13. Our genetic linkage studies indicated that the rat *Fry* gene is a mammary carcinoma susceptibility locus. Interestingly, studies have indicated a B-cell chronic lymphocytic leukemia tumor suppressor gene on the long arm of chromosome 13. We recently analyzed published microarray data from over 280 leukemia samples available on the Oncomine 3.0 Cancer Profiling Database. Normalized microarray data was analyzed via the modified t-test for unequal variances and gene covariance was determined using the Pearson correlation calculation. Our analysis showed that the *FRY* gene is under-expressed in acute lymphoblastic leukemias relative to acute myeloid leukemias ($p<0.0001$). We also observed that *FRY* expression was increased in blast crisis remission, when compared to *FRY* expression in blast crisis ($p<0.02$). Additionally, in a majority of the tumors examined *FRY* expression was positively correlated with the expression of *NDR1* ($n=199$, $p<0.03$) and with the alpha subunit of the L-type calcium channel, *CACNA1D* ($n=245$, $p<0.0001$), implicating its involvement in calcium signaling. Our results indicate that *FRY* expression is lost or significantly reduced in B-cell and T-cell lymphoblastic leukemias and increased in patients who do not experience relapse, raising the possibility that *FRY* may serve as a biomarker of leukemia type and prognosis. Our previous analysis of *FRY* expression in human breast cancers indicated that *FRY* is under-expressed in Estrogen Receptor negative (ER-) tumors and in Elston Grade 3 tumors, suggesting that *FRY* may serve as a biomarker of breast carcinoma hormonal status and differentiation. Prior to our studies in rat mammary cells, *Fry* was a predicted gene whose sequence was highly conserved throughout eukaryotic evolution. In *Drosophila* the fry (*furry*) protein is involved in epithelial cell proliferation, separation, morphogenesis and polarization. Fry regulates the activity of the Tricornered family of kinases, the human homologs of which are NDR kinases, several of which are classified as tumor suppressor genes (LATS1/2) or as likely proto-oncogenes (NDR1/2). These findings suggest that the *FRY* gene modulates susceptibility to carcinogenesis by its effects on NDR kinases. *We are currently investigating the effects of FRY in carcinogenesis by transfecting wild-type FRY into breast tumor and lymphoblastic leukemia cells which have low FRY expression.*

Interpreting benzene biomonitoring data in a public health risk context using biomonitoring equivalents. S Hays [a], L Aylward, D Pyatt. [a] Summit Toxicology LLP, Lafayette, Colorado, USA

A Biomonitoring Equivalent (BE) is the concentration or range of concentrations of a biomarker of exposure for a chemical consistent with existing exposure guidance values. Substantial efforts have been developed over the past two decades to biomonitor for benzene exposures in a range of occupational (primarily) and environmental (more recently) exposure scenarios. Biomonitoring for occupational exposures poses fewer problems for interpretation because exposures are usually elevated over environmental (background) exposures and the timing of exposures are known and the timing of sample collection can be controlled. Biomonitoring for environmental exposures poses more challenges because exposures are often extremely low and the timing of discrete exposure events are often unknown and sample collections are randomly timed with respect to exposures. When developing BEs, critical factors that go into selection of preferred biomarkers include: sensitivity, specificity, half-life, and ease of interpretation in a risk assessment context (which factors in issues associated with mode of action for the toxicities of concern). In this presentation, the strengths and weaknesses of the various biomarkers for benzene are discussed and BEs are presented for the preferred biomarkers. These BEs should provide a means of interpreting population-based biomonitoring studies for environmental exposures in a public health risk context.

In vivo hydroquinone (HQ) exposure impairs functional activity of peritoneal cells.

CB Hebeda [a], SMD Macedo, DMH Cavalcanti, JMC Ferreira Jr, MGT Sousa, SR Almeida, S Farsky.
[a] University of Sao Paulo; Department of Clinical and Toxicological Analyses, Sao Paulo, Brazil.

HQ is a phenolic compound found in cigarette smoking, medicines and foods. Also, it is obtained from endogenous metabolism of benzene. We have shown that rats exposed to HQ (5 or 10 mg/kg; ip) present impaired leukocyte migration to the lung during allergic inflammation. Here we investigated the role of HQ exposure on functional activity of peritoneal cells related to acquired inflammatory response. **Methods:** Male Wistar rats were exposed of HQ (5 or 10 mg/kg), ip; once a day, 8 doses with an interval of 2 days every 5 doses. Control animals received vehicle (saline:ethanol; 1:10). Twenty four hours after later exposures, resident peritoneal cells were collected and ovalbumin (OVA) *in vitro* stimulated to assess CD40, CD80, CD86, CD18 and ICAM-1 membrane expression by flow cytometry and to investigate concentrations of IFN-gamma, IL-10 and IL-4 in the supernatant by ELISA; and to quantify killing activity to *Candida albicans* using optical microscopy. The experiments were conducted according to Ethics Committee in Animal Experiments n.53/2008 – Number Protocol – 65. **Results**: HQ did not modify expressions of CD80, CD40, CD86, CD18 and ICAM-1 by peritoneal cells; did not alter concentrations of IL-4 and IL-10, but reduced concentrations of IFN-gamma (37% vs. control); reduced killing activity (331% vs. control). Incubation of cells with recombinant IFN-gamma reversed the decreased killing activity. **Discussion:** Together, results obtained suggest that *in vivo* exposure to HQ reduces peritoneal cell functions related to acquired inflammatory response, by a mechanism dependent on reduced IFN-gamma secretion. [Financial Support: CAPES; FAPESP 03/04013-8].

Gasoline: A complex chemical mixture or a dangerous vehicle for benzene exposure.

JJ Keenan [a], SH Gaffney, D. Galbraith, P. Beatty, and D.J. Paustenbach. [a] ChemRisk, Inc., San Francisco, CA, USA

Gasoline broadly describes fuels used in internal combustion engines. It is a complex and highly variable volatile mixture of over 500 saturated and unsaturated hydrocarbons. In terms of examining the human health implications following exposure to the many components of gasoline, benzene is the most studied. The current benzene content of gasoline in the United States (< 1%) is lower than that found in European forms of gasoline (1-4%) but is higher than any other consumer product. However, benzene is not the only known or suspected carcinogen in gasoline. Others include 1, 3 butadiene, ethylbenzene, and methyl-tertiary butyl ether (MTBE). The purpose of this analysis is to summarize and evaluate the available data on gasoline exposed cohorts in the published literature. Over 70 studies were initially obtained for review, with many subsequently excluded, primarily due to significant additional exposures to other chemicals or mixtures. Thirty-two studies were identified that met our inclusion criteria. These studies were evaluated for workplace description, cohort demographics, time periods of exposure and follow-up, diagnostic methods, control population source, and methods of evaluating exposure. Case control studies reported occasional weak associations between gasoline exposed populations and laryngeal cancer, hematopoietic malignancies, kidney cancer, male breast cancer, pancreatic cancer, stomach cancer, and bladder cancer. Cohort studies focused primarily on the risk of kidney cancer and hematopoietic malignancies in gasoline station attendants and gasoline distribution workers. Overall, although gasoline is known to contain benzene, the majority of cohort studies did not report a significantly increased risk of hematopoietic malignancy or solid cancers following acute or chronic gasoline exposures. While the debate about whether gasoline should be considered a carcinogen will likely continue, it is important to remember that gasoline usage involves complex exposures to many chemical agents, and studies should take this into consideration when providing estimates of health risk in human populations.

Analysis of hydroquinone and catechol in peripheral blood of benzene-exposed workers. PJ Kerzic [a], P Liu, P Pan, Y Zhou, AR Schnatter, RD Irons. [a] Fudan-Cinpathogen Clinical and Molecular Research Center, Institutes for Biomedical Sciences, Fudan University, Shanghai, China

We have developed a gas chromatography-mass spectrometry method for analysis of benzene (BZ) metabolites in human urine and blood. Here we describe peripheral blood concentrations of hydroquinone (HQ) and catechol (CAT) in total, protein bound, and unbound (free) forms obtained from BZ-exposed factory workers and controls. Total and unbound metabolites were directly measured in independent experiments, while bound forms were calculated as [total]-[unbound]. In this subset of a larger study, breathing zone benzene, toluene, and xylene were measured for the duration of a workshift, and end-shift blood samples taken from 143 individuals. Potential lifestyle and environmental influences were assessed by questionnaire and bioassay, and single nucleotide polymorphisms in xenobiotic metabolizing enzymes NQO1, MPO, Cyp2E1, and GSTT1 were also analyzed for potential contribution to differences in blood metabolite concentration. Positive correlations were observed between same-day BZ exposure and total CAT, bound CAT, total HQ, and bound HQ ($r>0.634$, $p<0.05$ for all), when compared to controls, while unbound CAT and HQ did not positively correlate with BZ exposure. Nearly all of the metabolites found in blood were bound to protein (CAT 96-99+%, HQ 78-92+%), and when the ratio of bound to unbound metabolites were compared in subsets of exposed workers, the increase in blood metabolite concentration was nearly all due to an increase in the protein-bound molecule. These findings suggest that a threshold for conjugation does not exist within the exposure spectrum studied. Correlations between exposure and blood metabolite concentration among subsets defined by genetic and lifestyle differences are also presented. This method demonstrates the feasibility of analyzing benzene metabolites in human blood, and should allow for further investigation of the health effects of benzene and its metabolites. [This work was supported by the Benzene Health Research Consortium].

Effect of hydroquinone on DNA double strand break and DNA-PKcs expression in HL-60 cells. MM Kong [a], WT Song, H You, JH Sun, Q Ma, YY Bi. [a] School of Public Health, Wuhan University, Wuhan, Hubei, China

Hydroquinone (HQ), a reactive metabolite of benzene, is myelotoxic and leukemogenic in humans. Carcinogenicity of HQ is dependent upon its conversion to reactive free radical species, such as semiquinone radical, in the bone marrow. Redox cycling of these radicals produces reactive oxygen species that damage DNA and induce DNA double strand breaks (DSBs). In mammalian cells, DSBs induce substantial phosphorylation of histone H2AX (γ-H2AX) at sites of DSBs, a marker of DSB formation. DSBs are mainly repaired through the error-prone, non-homologous end-joining pathway (NHEJ). NHEJ requires the DNA-dependent protein kinase (DNA-PK) that consists of two regulatory subunits (Ku70 and Ku80) and a catalytic subunit (DNA-PKcs). In this study, we investigated DSB formation in human promyelocytic leukemic HL-60 cells treated with HQ. Cells were treated with HQ of 10, 25, 50, or 100 μM for 6, 18, or 24 h. γ-H2AX foci formation was observed with fluorescent microscopy. Significantly higher amounts of γ-H2AX were found in cells treated with 50 and 100 μM HQ for 18 h and 24 h, respectively, indicating HQ induces DNA double strand breaks in concentration and time-dependent manners. To examine the DSB repair activity after HQ treatment, we analyzed the expression of DNA-PKcs in HL-60 cells at both transcription and translation levels. Cells were treated with HQ as above. The results showed a dose and time dependent induction of DNA-PKcs, indicating increased DSB repair activities after HQ treatment. Our findings reveal a role of HQ in the induction of DSB and DSB repair, both of which are relevant to HQ-induced myelotoxicity and leukemogenesis.

The effect of CYP4F3 gene silencing on HL-60 cell proliferation. YH Li [a], MM Kong, H You, H Wang, H Yan, Q Ma, YY Bi. [a] School of Public Health, Wuhan University, Wuhan, China

CYP4F3 is expressed in human polymorphonucleocytes (PMN), monocytes, and cells that differentiate into monocyte-like cells. Expression of CYP4F3 is altered during the differentiation of leukocytes. The mechanism that regulates the expression of CYP4F3 remains unknown. CYP4F3 has the ability to metabolize both leukotoxins and leukotriene B4 (LTB4) and thereby, potentially plays important roles in modulating inflammatory response. CYP4F3-catalyzed reactions generate various octanoid products that may have potent, but as-yet-undefined, biological effects. We have previously reported that phenol, a major metabolite of benzene, significantly induces the expression of CYP4F3 at both mRNA and protein levels in cultured promyelocytic leukemia cells (HL-60) and ex vivo in human neutrophils. Additionally, benzene metabolite hydroquinone induces apoptosis of HL-60 cells. In this study, we analyzed the function of CYP4F3 in relation to benzene hematotoxicity by silencing CYP4F3 in HL-60 cells. A lentiviral vector system was constructed and used to deliver a small interfering RNA (siRNA) specific for the human CYP4F3 gene into HL-60 cells. Scrambled siRNA was used as a negative control. Expression of siRNA was monitored by detection of GFP fluorescence. The results revealed that silencing of CYP4F3 reduced the cell numbers to 56.25, 43.75, and 21.88% of control at 3, 4, and 5 days after CYP4F3 silencing. On the sixth day, no viable fluorescent cells were observed. Cell survival and growth were also examined using the MTT assay. Expression of CYP4F3 siRNA effectively inhibited the growth of HL-60 cells, which was dose-dependent of the recombinant lentivirus. The findings indicate that CYP4F3 may act as an important positive regulator of HL-60 proliferation. We are currently investigating the relation between induction of CYP4F3 in HL-60 cells after phenol exposure and HL-60 cell growth.

Utilizing functional genomics in yeast to discover novel biomarkers of benzene toxicity in humans. M North [a], J Shuga, A Loguinov, V Tandon, L Zhang, MT Smith, CD Vulpe. [a] Department of Nutritional Science and Toxicology, University of California, Berkeley, CA, USA.

The carcinogenic organic compound benzene is used extensively in industrial processes such as plastic production, and is also present in cigarette smoke and gasoline. In addition, there are thousands of benzene-contaminated sites in the USA alone. With a large workforce exposed to the compound, and the ever-increasing identification of benzene exposure-associated diseases, there is a mounting effort to identify biomarkers for susceptibility. *Saccharomyces cerevisiae* is the best-characterized simple eukaryotic organism, and has hundreds of tools available for genetic study. This includes a complete set of non-essential gene deletions, genetically tagged so that individual strains can be identified in competitive growth experiments. Using this deletion set, parallel systematic analysis can be completed to identify mutant strains that are susceptible to toxicant treatments. We have used this technique to establish the cellular processes targeted by three of the most well studied benzene metabolites: hydroquinone, catechol, and 1,2,4-benzenetriol. Comparing the global deletome profiles of the metabolites revealed that deletion of certain genes renders sensitivity to all three compounds, for example the epimerase *RPE1*. This particular result indicates a requirement for NADPH in metabolite detoxification; unsurprising since NADPH is required for the function of oxidoreductases, which reduce the more toxic quinone forms of these metabolites (produced through autoxidation). Conversely, we also found that some mutations cause sensitivity to only one or two of the metabolites, for example *ira2*, a negative regulator of Ras and homolog of the mammalian tumor suppressor gene *NF1*. The role of *NF1* in mammalian benzene toxicity has also been investigated, in both mice and human cell lines. Several of the genes identified have direct human homologs with conserved biological function, and many more have functional orthologs, supporting the notion that the mechanisms of toxicity identified are relevant to human disease. This study highlights *S. cerevisiae* as a simple but valuable model for identifying biomarkers for genetic susceptibility to toxicant-related disease.

Job and task based analysis of benzene air concentrations associated with refinery operations. JM Panko [a], SH Gaffney, ML Kreider, KM Unice, AM Burns, DJ Paustenbach, LE Booher, RH Gelatt. [a] ChemRisk, Inc., Pittsburgh, PA, USA

In this study, historical exposures to benzene were quantified for workers at four U.S. refineries from 1976 to 2006. The results of more than 12,000 long term (> 180 minutes) personal industrial hygiene air samples which were associated with 50 job titles and the results of more than 4,000 short term (<180 minutes) industrial hygiene air samples which were associated with 24 task bins were evaluated for inclusion in this analysis. The air sampling results for both datasets were found to be approximately lognormal based on probability plots and the benzene detection frequency was approximately 43% and 35% for the long term and short term samples, respectively. For the long term dataset, there was a statistically significant difference in benzene air concentration based on operational status (routine, turnaround, startup), and thus all subsequent analyses were conducted on datasets divided accordingly. The job titles and tasks most frequently sampled included those with potential direct contact with refinery product streams including job titles such as the process technician, machinist, pipefitter/welder, and laboratory technician and tasks such as blinding/breaking lines, product sample collection, sample analysis, equipment cleaning and repair, and gauging. The ANOVA indicated that for most jobs, the associated air concentrations were not dependent on refinery area. However, for some job titles, benzene air concentrations depended upon the area of refinery in which the worker was stationed. Certain areas, such as waste treatment, reformer, the tank farm, and the lube extraction unit, were statistically different than other areas of the refinery for several different job titles. Where a statistical difference in benzene air concentration by area occurred, new job categories (a specific combination of operational status, job title and area) were formed for all further analyses. With regard to the short term samples, the ANOVA indicated that air concentrations associated with each task were not dependent on job title, except for blinding and breaking and equipment cleaning and repair tasks. Similarly, the ANOVA indicated that the air concentrations associated with each task were not dependent upon area of the plant in which the task was conducted, except for blinding/breaking. The tasks with the highest air concentrations of benzene were associated with tank cleaning in the tank farm and blinding/breaking lines at the reformer and tank farm. Changes over time in benzene air concentrations associated with job titles and tasks were also evaluated. For several job titles and tasks, there was a statistically significant decrease in benzene air concentration between the 1976-1989 period and 1990-2006 periods. This study provides a job and task-focused analysis of occupational exposure to benzene during refinery operations, and it should be useful for reconstructing refinery workers' exposures to benzene over the past 30 years.

Comparison of modeled and measured concentrations of airborne benzene from the use of petroleum-based solvents spiked with low levels of benzene. DJ Paustenbach [a], JS Knutsen, DM Murbach, J Sahmel, AK Madl. [a] ChemRisk, San Francisco, CA, USA

In the absence of quality monitoring data, to estimate airborne concentrations of evaporating volatile organic compounds (VOCs), exposure assessors typically rely upon their professional judgment to appropriately select and use one of many consumer and industrial hygiene exposure models. A study was undertaken to assess the accuracy of several models to predict indoor air concentrations of benzene resulting from the manual application of products containing low or trace amounts of benzene. First, a simulation study was designed and conducted to assess the effects of six factors on air concentrations of benzene. These factors included: (1) the concentration of benzene in the solvent, from 0.001% to 1% by volume, (2) the applied solvent volume, from 50 ml to150 ml, (3) the applied surface area, from 1 ft^2 to 9 ft^2, (4) the application technique, using either a rag or a spatula, (5) the solvent formulation, using either paint thinner or a consumer product containing mainly naphthenic and aliphatic petroleum distillate, and (6) the air exchange rate, from 4 – 10 air changes per hour (ACH). A total of 24 personal and 71 area air samples were collected to characterize both near- and far-field exposures to airborne benzene in each of these scenarios. In each sampling event, two personal and six area samples were collected over durations of 15 – 25 minutes. Personal and area concentrations of benzene ranged from 0.009 to 0.6 ppm and 0.004 to 0.2 ppm, respectively. There was a significant difference between near-field (personal) and far-field (area) concentrations, but significant differences were not observed between area samples collected at 1, 3, or 5 ft from the solvent handling activity. Following the simulation study, air concentrations were estimated for the same scenarios using two models: a Monte Carlo simulation using a two-zone model and a turbulent diffusion model with steady, exponentially decaying, and instantaneous generation rates of benzene vapor. Model estimates compared favorably with measured air concentrations at low concentrations, although they did not adequately capture the marked non-linear correlation between benzene concentrations in the bulk and those measured in air. This research provides an additional platform for professional judgment and a basis for considering reasonable extrapolations from the twelve scenarios examined in the simulation study.

Alternative explanation of reported increases in low dose metabolism of benzene.
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In 2006, Kim et al. reported the dose-related production of urinary benzene metabolites in workers from a Chinese shoe-making factory and concluded that there was a dramatic change in benzene metabolism over an air exposure range of 0.027 ppm and 90 ppm (*Carcinogenesis* 27:772-781; *Cancer Epidemiol. Biomarkers Prev.* 15:2246-2252). This change resulted in a ninefold increase in the amount of urinary phenol excreted per unit of benzene exposure at the lower doses. A review of the data from the study's publications indicates that the finding of changes in the production of urinary metabolites with air concentration of benzene is very sensitive to the correction for the background levels of urinary metabolites. In addition, one step in the process of correcting for background (subtraction of the median rather than the mean of the background urinary levels of the benzene metabolites) may have resulted in an overestimate of metabolite production at low exposures. In order to investigate this issue, we developed a simulation model based upon the published findings from the study to investigate the impact of alternative approaches of correcting for background levels of phenol in urine. This modeling confirmed that the use of the median rather than the mean is a possible alternative explanation of the elevated production rates of phenol. The results of this modeling are presented in this poster. The next step in our investigation will be to verify this finding by reanalyzing the original raw data. To this end, we have requested access to the raw data under the US Freedom of Information Act and plan to complete the assessment in the near future.

Headspace solid-phase micro-extraction for trace analysis of ethylbenzene in human exposure monitoring.
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Conventional analytical methods for organic pollutants in water require extraction of these pollutants, using hazardous and toxic solvents. Solid phase microextraction (SPME) is a solvent-free equilibrium extraction method, in which proper calibration can allow quantitative determinations of organic pollutants at a very good sensitivity without the use of any toxic organic solvent. Because individual VOCs are generally present in urine only at trace levels, a sensitive and accurate determination technique is essential. Therefore, this study describes the optimization of headspace solid phase microextraction (HS-SPME) followed by GC-FID for ethylbenzene in spiked urine. Through this investigation, the parameters affecting the extraction procedure and gas chromatographic determination of ethylbenzene, including extraction time, temperature, desorption temperature, desorption time, salt addition, sample pH, sample volume and sample agitation were studied. An optimized headspace extraction was carried out at 30°C for 6 min in the presence of 0.2 gml^{-1} of NaCl in the sample solution. Also, the sample volume and sample pH were optimized at 5 ml and 7 (neutral pH), respectively. Desorption of the analytes was carried out for 60 sec. at 250°C. The optimized procedure was also validated with three different pools of spiked urine samples and showed a good reproducibility over six consecutive days as well as six within-day experiments. Through this study, the accuracy, linearity, detection limits of the method were also determined. The HS-SPME-GC-FID technique provided a relatively simple, convenient, practical procedure, and successfully applied to determine ethylbenzene in spiked urine. This optimized technique promised to be an appropriate procedure for evaluating the other aromatic hydrocarbon analogues when both environmental and occupational exposure monitoring are of interest.

Meta-Analysis and causal inference: A case study of benzene and non-Hodgkin lymphoma. DL Weed. DLW Consulting Services LLC, Kensington, MD, USA

Meta-analysis is an important method in the practice of occupational epidemiology, with a legitimate but limited role to play in causal inference. Meta-analysis provides an assessment of consistency—one of several classic causal criteria—through tests of heterogeneity and an assessment of differences across studies. It may also provide an increase in the precision of effect estimates. Causal inference, however, involves much more: strength of association, dose-response, and biological plausibility, to name only the most commonly used criteria. Causal claims, therefore, should not emerge from meta-analyses as such. In a recent meta-analysis of epidemiological studies of benzene exposure and non-Hodgkin lymphoma (NHL), however, the authors, Steinmaus et al. (2007), do exactly that. Using studies from a previous narrative review in which no causal claim was made, Steinmaus et al. performed a meta-analysis and concluded that their results represented new evidence that benzene causes NHL. Despite a lack of consistency (i.e significant heterogeneity), weak associations, no evidence of dose-response, no effort to provide an assessment of biological plausibility, and no new epidemiological evidence, the authors, nevertheless, changed their conclusion from association to causation. Using the Steinmaus et al. (2007) meta-analysis as a case study, this paper provides cautionary and critical comments about the use of meta-analysis in causal inference in occupational epidemiology.

The role of DNA-PKCs in the biological effect of a benzene metabolite: Phenol toxicity to human K562 cells in vitro. X Xiao [a], WT Song, H You, H Wang, YA Lu, Q Ma, YY Bi. [a] School of Public Health, Wuhan University, Wuhan ,China

Chronic benzene toxicity is of major concern, however the molecular mechanisms remain unclear. We report that the PRKDC-coding DNA-dependent protein kinase catalytic subunit (DNA-PKcs) was found to be up-regulated in PBMCs among patients with varying degrees of chronic benzene toxicity, using microarray analysis. The potential importance of DNA-PKcs in the spectrum of hematotoxicity elicited by benzene, ranging from the reduction of peripheral blood cell counts to aplastic anemia and leukemia, was inferred due to the fact that the DNA-PKcs take part in non-homologous end joining (NHEJ) to repair DNA double strand breaks (DSBs), which may be error-prone and lead to aberrant chromosomes. A DSB model of DNA damage was first established using the CML cell line K562 exposed to phenol, a benzene metabolite, in a concentration gradient. The expression of gamma H2AX was utilized to test the quantity of DSB, by means of immunofluorescence. The results showed that the extent of DSBs in K562 is dependent on phenol concentration ($p<0.01$). DNA-PKcs mRNA was then tested by real-time PCR, indicating that phenol can induce DNA-PKcs mRNA expression ($p<0.01$). Similarly, Westernblot analysis showed that the expression of DNA-PKcs is dependent on phenol concentration, as well as time-dependent ($p<0.05$). We also investigated the expression of Ku70/80, the dimeric DNA-binding regulatory subunits of DNA-PK, which are also key factors in NHEJ. Results were similar to that obtained for the DNA-PKcs, showing that Ku80 expression is dependent on phenol concentration, as well as time-dependent ($p<0.05$). Together, these results suggest that phenol is able to induce DSB formation in K562 cells, as well as elicit DNA-PKcs expression at both the gene and protein level. The current work is focused on testing the misrepair frequency of NHEJ in K562, which enrolls the DNA-PKcs and Ku70/80.