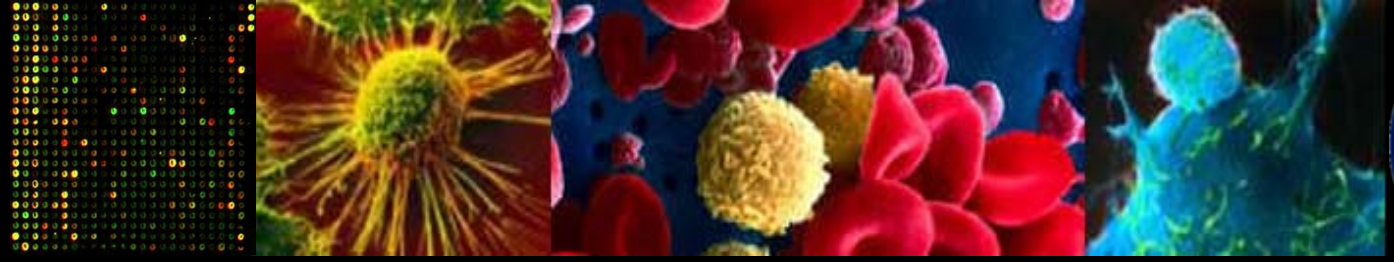


Human Health Hazard Indicator Workshop

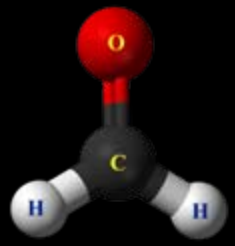
Sacramento, Cal EPA | March 15-16, 2010

# *Towards Toxicity Testing in the 21st Century*: A new global and systematic paradigm to identify potential human carcinogens

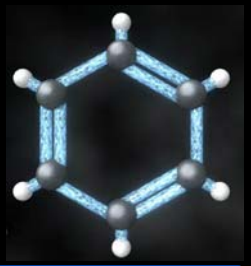

## Luoping Zhang

School of Public Health
University of California, Berkeley

http://sph.berkeley.edu/faculty/zhang.php




# Commercially Available *vs.* Tested Chemicals



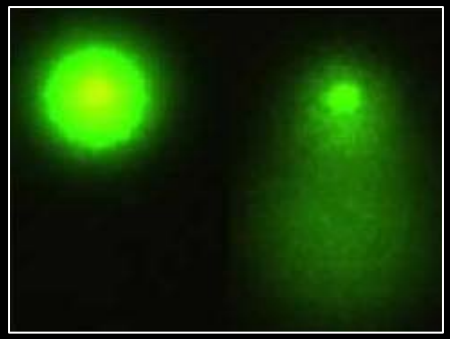
| | |
|---|---|
| # Inventory of Chemicals in *Toxic Substances Control Act* | ~ 75,000 |
| # Commercially Significant Chemicals (produce > 25,000 lbs/yr) | 6,500–7,000 |
| # Commercially Significant Chemicals (produce >1,000,000 lbs/yr) | > 2,200 |
| # Chemicals evaluated by **IARC** | > 900 |
| # Chemicals listed on **IRIS** (EPA) | 545 |
| # Chemicals reviewed by **CERHR / RoC** (NTP) | 23 / 246 |
| # Chemicals listed as carcinogens/toxicants | ~ 750 |
| # Chemicals listed under ***Proposition 65*** by **OEHHA** (CalEPA) | 829 |

## Current System – Problem

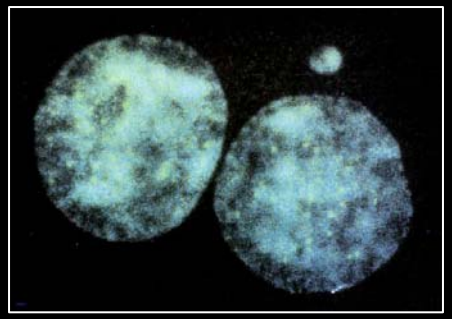
- Time consuming
- Resource intensive
- Assay limitations
- Difficult in meeting today's challenges

## New System – Solution

- Broader coverage of chemicals
- Reduce cost & time
- Use fewer animals, minimize suffering
- More robust scientific basis for assessing health effects



# Current Genotoxicity Testing Methods



**DNA Damage & Repair Assays**

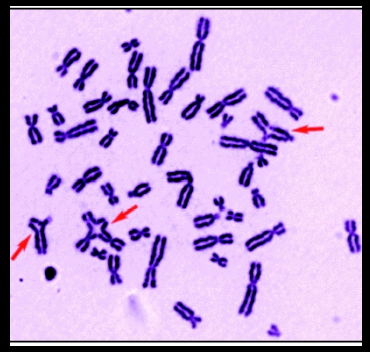
- Comet
- Comet FISH

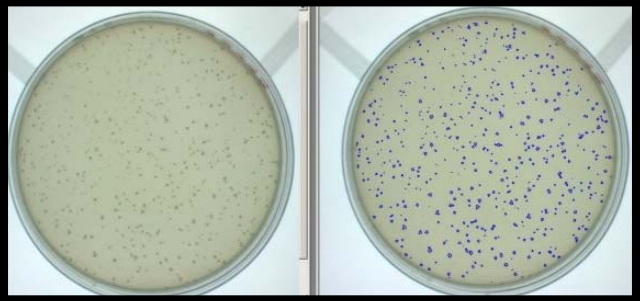
**Gene Mutation Assays**

- Ames Test
- *HPRT/TK* Genes

**Cytogenetic Methods**

- Chromosome Aberration (CA)
- Sister Chromatid Exchange (SCE)
- Micronuclei (MN)





# Chromosome aberrations correlate with cancer risk

| Study | Subjects | Person -years | Follow-up period | Cancer cases | OR-High CA (95% CI) |
|---|---|---|---|---|---|
| Nordic Study, 1990 | 1,787 | 7,547 | 1970–1985 | 26 | 1.80 (0.98–3.01)[a] |
| Hagmar, 1994 | 1,979 | 17,666 | 1970–1988 | 66 | 2.10 (1.50–2.80)[a] |
| Bonassi, 1995 | 1,455 | 16,190 | 1969–1994 | 44 | 1.82 (1.11–2.81)[b] |
| Hagmar, 1998 | 3,541 | 43,827 | 1970–1995 | 91 | 2.01 (1.35–2.89)[b] |
| Smerhovsky, 2001 | 3,973 | 37,775 | 1975–1999 | 144 | 1.60 (1.01–2.37)[c] |
| Hagmar, 2004 | 3,554 | 58,798 | 1965–1993 | 150 | 1.87 (1.28–2.74)[d] |
| Rossner, 2005 | 11,862 | 113,967 | 1975-2000 | 485 | 1.13 (0.89-1.43)[d] |
| Boffetta, 2007 | 6,340 | 54,655 | 1978-2002 | 200 | 1.81 (1.20-2.73)[d] |

[a]SIR: Standardized Incidence Ratio; [b]SMR: Standardized Mortality Ratio; [c]HR: Hazard Ratio; [d]RR: Relative Risk.

Bonassi S et al. (2004) *Cytogenet Genome Res.* 104:376–382

Fucic A et al. (2007) *Toxicology Letters.* 172:4–11

# Chemical exposure induces the highest CA frequency in human populations

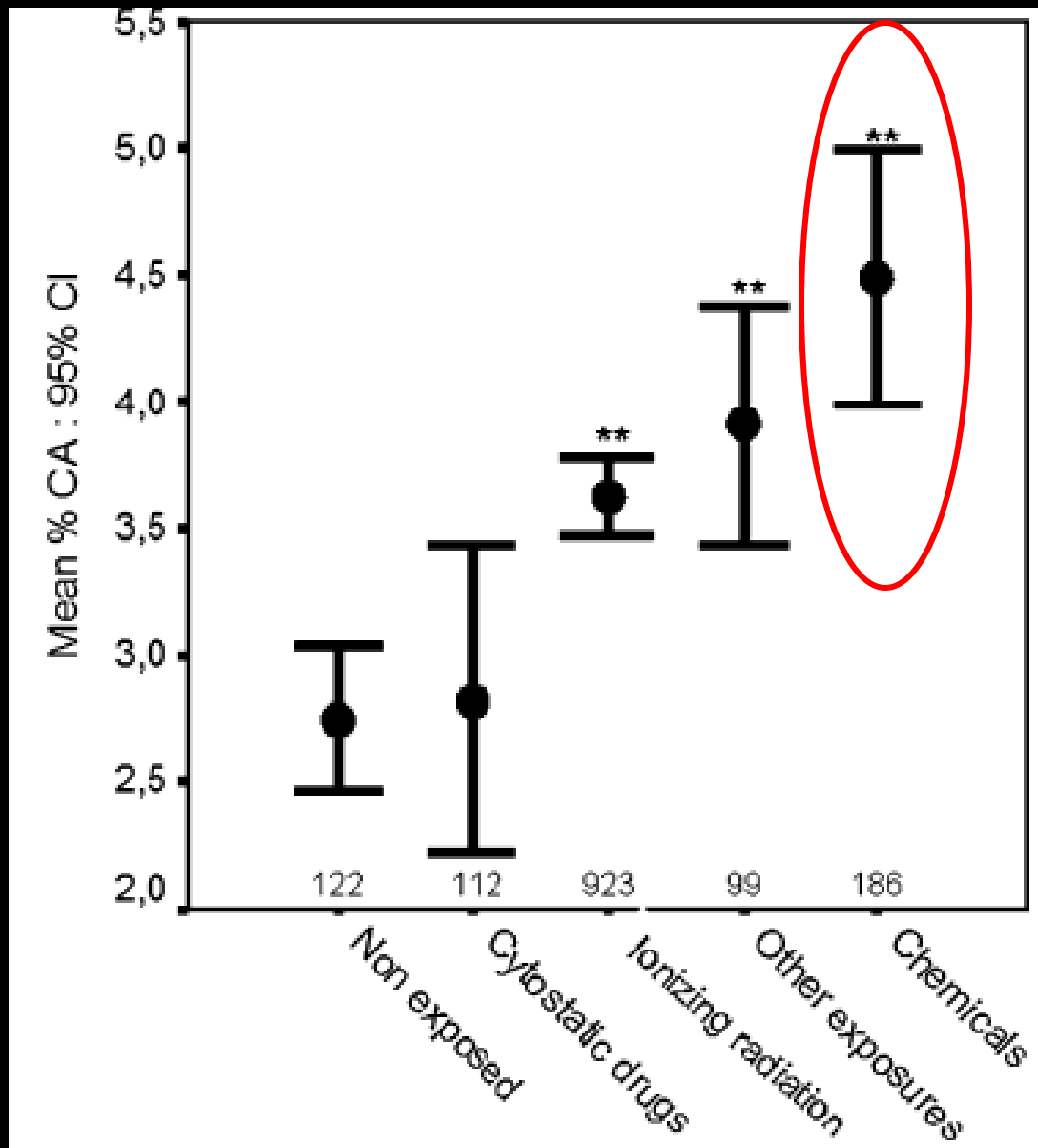



Fig. 1. Mean frequency of cells with chromosomal aberrations (CA) in groups occupationally exposed to physical and chemical genotoxic agents and in unexposed referents (**$p < 0.01$). The number of subjects in each exposure category is shown below the symbols.

Fucic A et al. (2007) *Toxicology Letters.* 172:4–11.

Chemicals → CAs → Cancer Risk

→ Potential Carcinogens?

**Should we consider all chemicals that significantly increase chromosome aberrations in human population studies as possible human carcinogens (Group 2B)?**

# Human Cytogenetics of Chemical Exposure

| Chemicals | CAs | IARC Group |
|---|---|---|
| Benzene | + | 1 |
| Vinyl chloride | + | 1 |
| Ethylene oxide | + | 1 (mechanistic data) |
| Epichlorohydrin | + | 2A |
| Styrene | + | 2B |
| Welding fumes | + | 2B |
| 6-Mercaptopurine | + | 3 |
| Methotrexate | + | 3 |
| Pyrimethamine | + | 3 |

Sorsa M, et al. (1992) *Mechanisms of Carcinogenesis in Risk Identification*, IARC.

**Group 3 and other chemicals that significantly increase chromosomal aberrations in human studies would be classified as possible human carcinogens (Group 2B)**

# Benzene Study in Tianjin, China

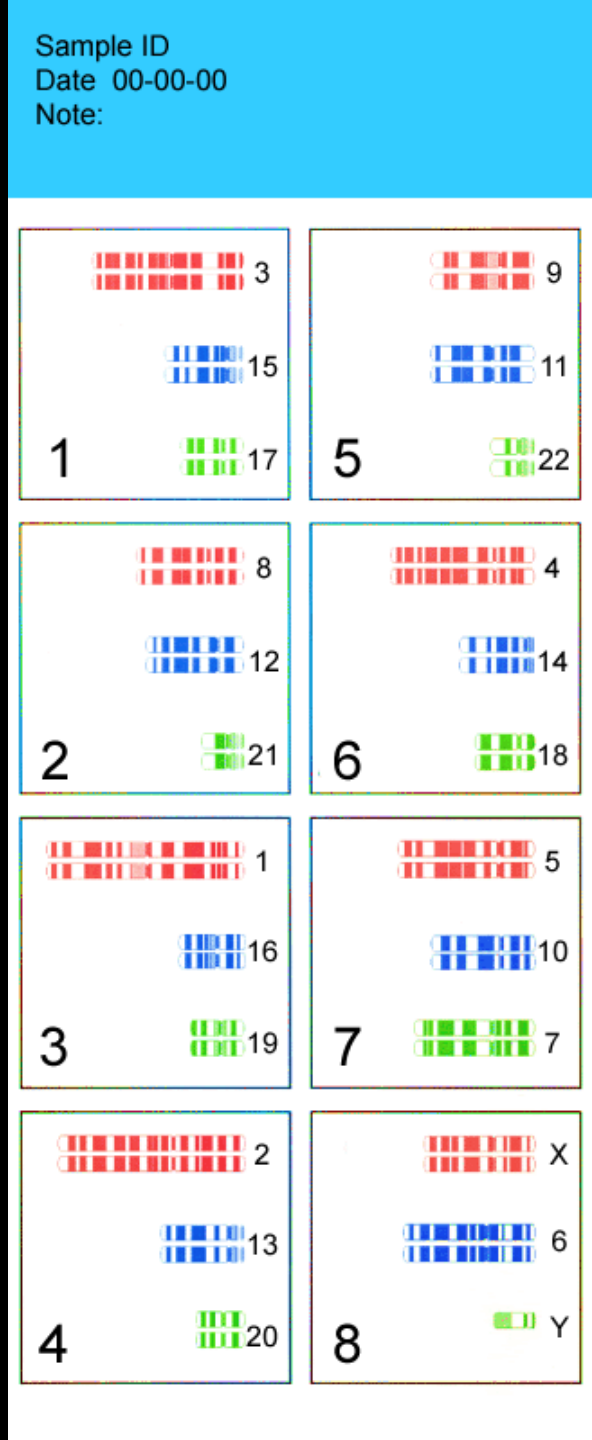
Lan Q, Zhang L et al. *Science*, 306:1774-6, 2004

- 250 healthy shoe manufacturing workers from two factories with **benzene exposure***
- 109 of the 250 workers had exposure **< 1 ppm** (U.S. standard)
- 140 healthy age sex matched **unexposed controls** from clothing factories

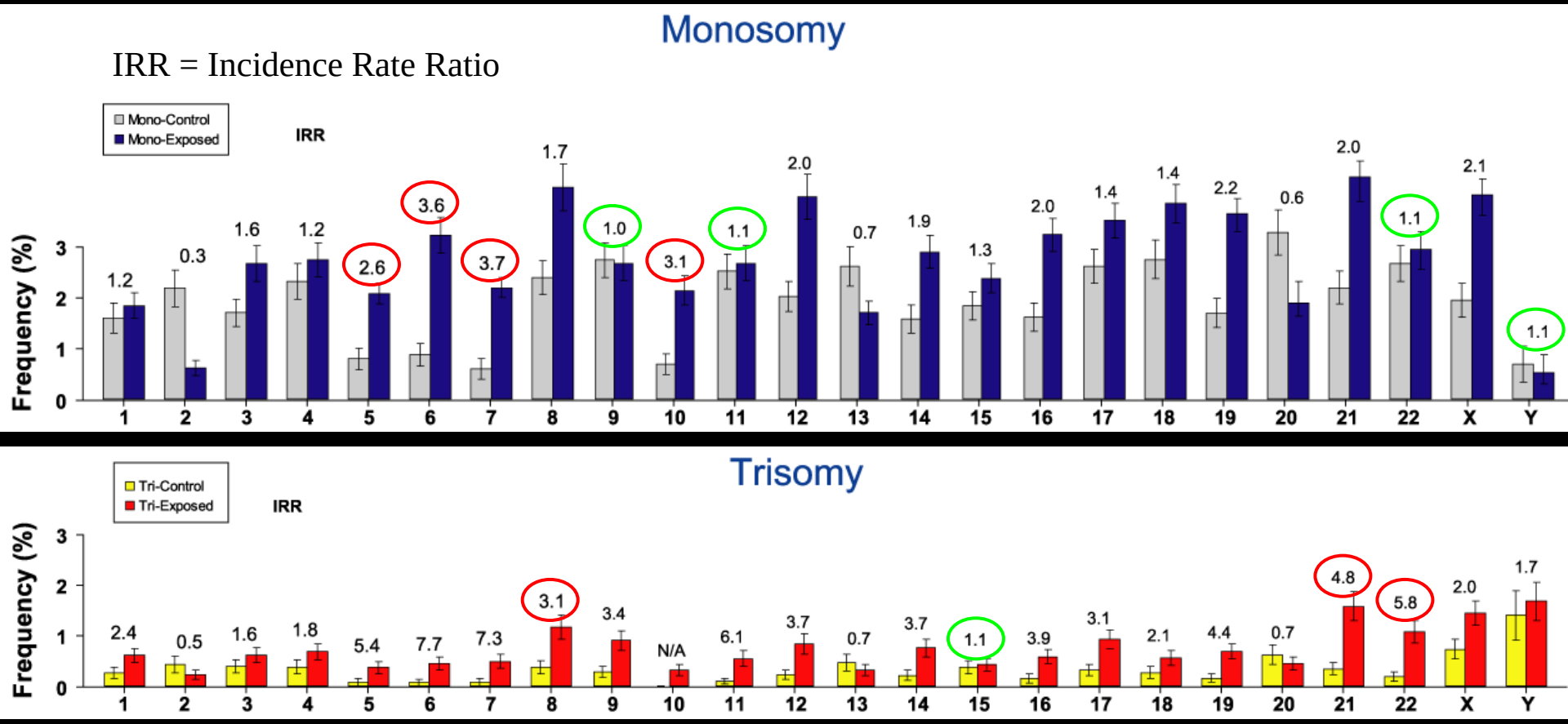
*Group means: 0.57 – 28.73 ppm

# OctoChrome FISH

1. Simultaneous hybridization and examination of all 24 chromosomes on the same slide;
2. Detection of specific chromosomal aneuploidy and rearrangements related with human leukemia and lymphoma;
3. Allows examination of many more metaphase spreads than other assays such as SKY and/or G-banding; and
4. More cost-effective and efficient than SKY and/or G-banding.



# Effect of Benzene Exposure on Frequency of Aneuploidy of Chromosomes 1-22, X & Y



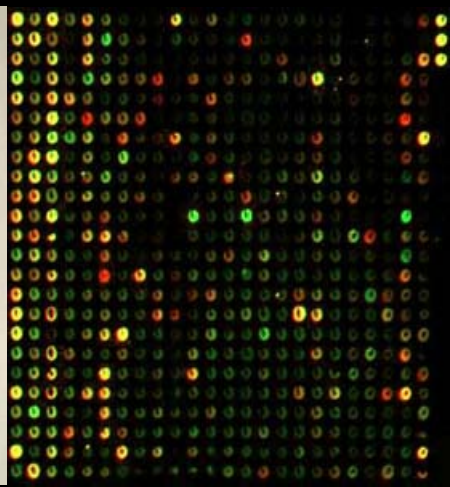

Zhang et al. *Chem.-Biol. Interact.*, 2005.

# Modes of Action for Well Characterized Compounds

1. DNA reactivity / covalent binding
2. Gene mutations
3. Chromosomal breakage
4. Aneuploidy
5. Enzyme-mediated DNA damage/repair
6. Epigenetic
7. Cell signaling: Nuclear receptor-mediated
8. Cell signaling: Other
9. Immune response modulation
10. Inflammation
11. Cytotoxicity/compensatory cell proliferation
12. Mitogenic
13. Chronic metabolic or physiologic overload
14. Nutrient deficiency related
15. Interference with GJIC
16. Other

**New predictive markers are needed for other MOAs:**

***Array-based omic technologies can potentially examine all MOAs***



# The 21st Century Toxicity Testing

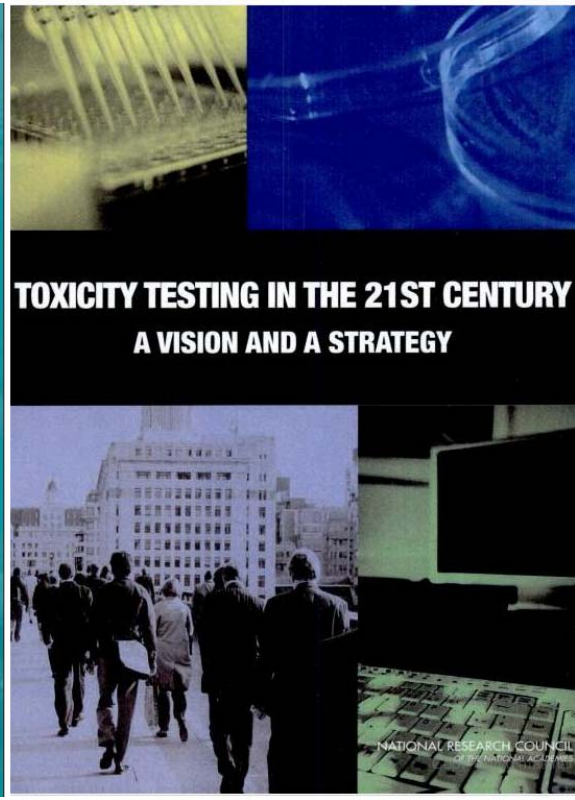
THE NATIONAL ACAD

REPORT IN BRIEF

October 2007

## Applications of Toxicogenomic Technologies to Predictive Toxicology and Risk Assessment

The new field of toxicogenomics presents a potentially powerful set of tools to better understand health effects from exposures to toxicants in the environment. However, realizing the potential of this nascent field to improve public health decisions will require a concerted effort to generate data, to make use of existing data, and to study data in new ways—an effort requiring funding, interagency coordination, and data management strategies.



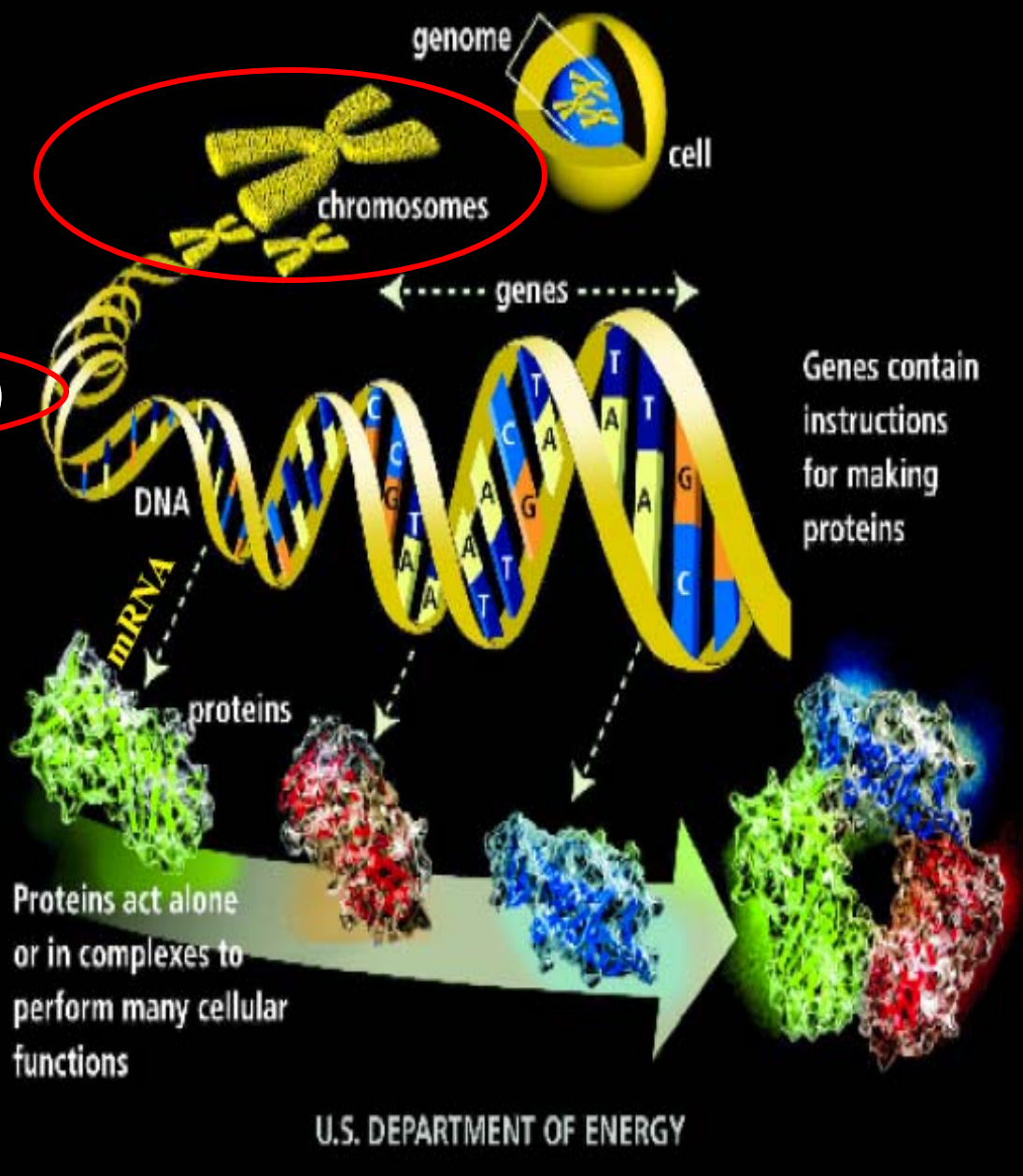

**New emerging technologies such as array-based *Toxicogenomics* are indeed needed to better predict chemical toxicity.**

# Toxicogenomics is an array-based technology:

- Genomics (DNA)
- Transcriptomics (mRNA)
- Proteomics (Protein)
- Metabolomics (Bio-product)

**Transcriptomics** (or **gene expression profiling**) is the study of mRNA—the intermediary step between genes and proteins that indicates genes that are active (as opposed to dormant or silent).

http://dels.nas.edu/dels/rpt_briefs/toxicogenomic_technologies_final.pdf



# Transcriptome Studies of Exposed Humans

| Study | Chemical | Subj. # | Sample | Platform | Dif. Exp. Gene* |
|---|---|---|---|---|---|
| Forrest, 2005 | Benzene | 12 | PBMC | Affymetrix | 2129 (6.5%) |
| McHale 2009 | Benzene | 16 | PBMC | Affymetrix | 2692 (8.2%) |
| | | | | Illumina | 1828 (7.9%) |
| McHale, 2007 | TCDD | 26 | PBMC | Affymetrix | 135 (0.4%) |
| Argos, 2006 | Arsenic | 40 | PBL | Affymetrix | 468 (2%) |
| Wu, 2003 | Arsenic | 24 | PBL | cDNA Array | 62 (8.8%) |
| Lu, 2001 | Arsenic | 12 | Liver | Clontech Atlas | 60 (10%) |
| Fry, 2007 | Arsenic | 32 | Cord BL | Affymetrix | 447 (1-2%) |
| Andrew, 2008 | Arsenic | 21 | PBL | Affymetrix | 259 (<1%) |
| van Leeuwen, 2007 | Cig. smoke | 18 | PB | Phase-1 | 34-76 (5.7-12.7%) |
| Beane, 2007 | Cig. smoke | 107 | Bronch. E. | Affymetrix | 175 (0.95%) |
| Rim, 2007 | Welding fumes | 40 | PB/WBC | House Spot Array | 103 (1.2%) |
| Wang, 2005 | Metal fumes | 28 | PB | Affymetrix | 546 (2.5%) |

PBMC: peripheral blood mononuclear cells; PBL: peripheral blood leukocytes; Cord BL: cord blood; PB: peripheral blood; and WBC: white blood cells.

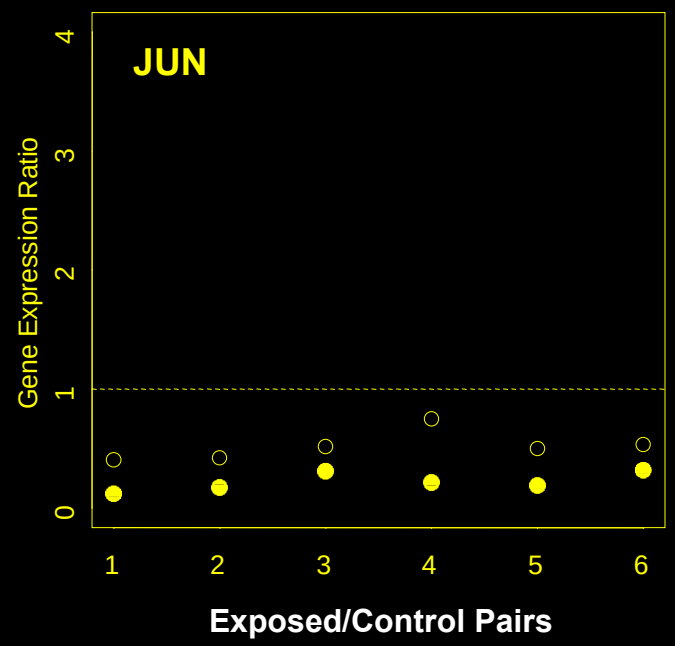
# Discovery of Novel Biomarkers by Microarray Analysis of Peripheral Blood Mononuclear Cell Gene Expression in Benzene-Exposed Workers

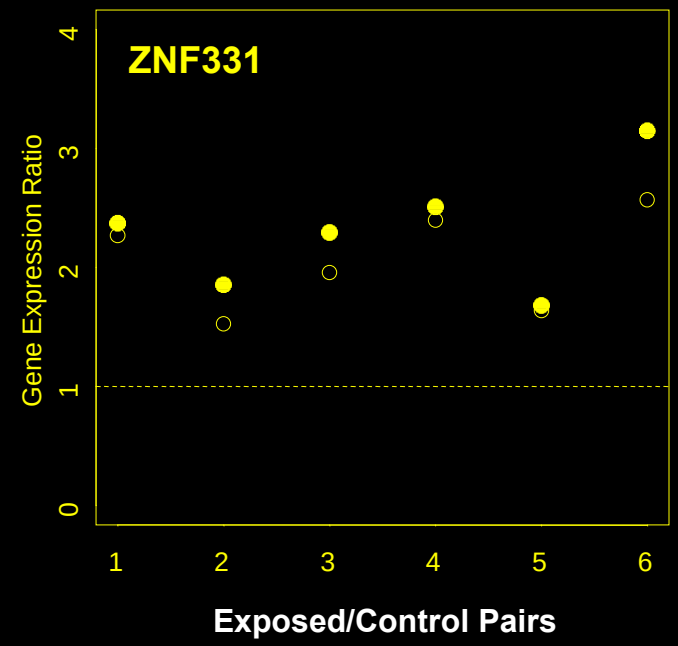
*Matthew S. Forrest,[1] Qing Lan,[2] Alan E. Hubbard,[1] Luoping Zhang,[1] Roel Vermeulen,[2] Xin Zhao,[1] Guilan Li,[3] Yen-Ying Wu,[1] Min Shen,[2] Songnian Yin,[3] Stephen J. Chanock,[2] Nathaniel Rothman,[2] and Martyn T. Smith[1]*

Environmental Health Perspectives • VOLUME 113 | NUMBER 6 | June 2005

- **6 exposed* vs 6 controls by Affymetrix Chips**
- **Differentially expressed genes identified**
- **6 genes confirmed by real time PCR in 28 subjects**

● Real Time
○ Microarray




*47.3 ± 24.3 ppm

# Changes in the peripheral blood transcriptome associated with occupational benzene exposure identified by cross-comparison on two microarray platforms

Cliona M. McHale [a,*], Luoping Zhang [a], Qing Lan [b], Guilan Li [c], Alan E. Hubbard [a], Matthew S. Forrest [a], Roel Vermeulen [d], Jinsong Chen [a], Min Shen [b], Stephen M. Rappaport [e], Songnian Yin [c], Martyn T. Smith [a], Nathaniel Rothman [b]

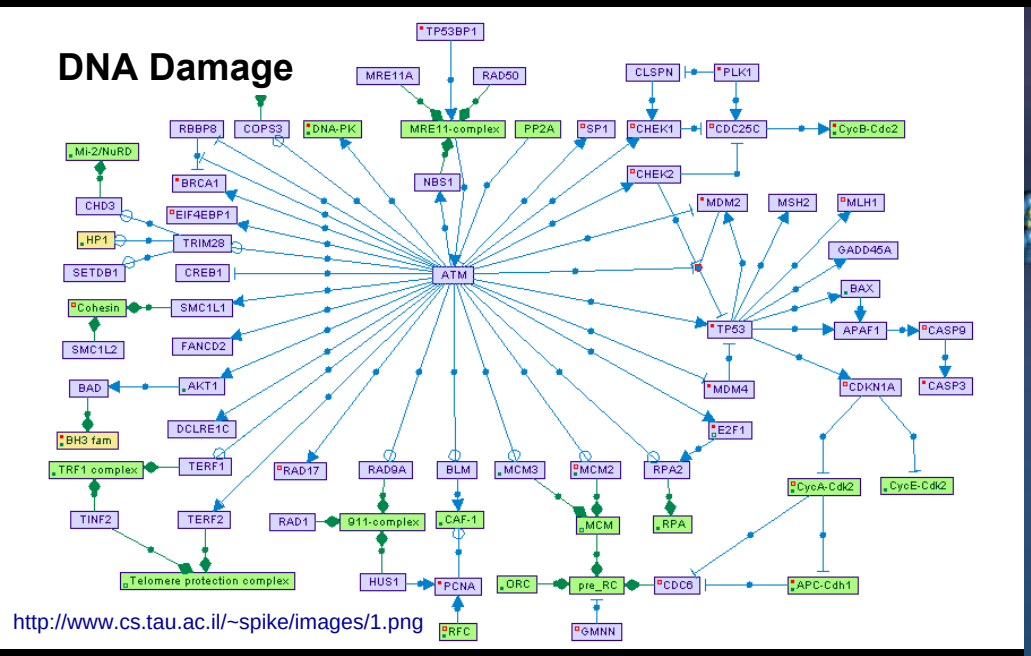
McHale C et al. (2009) *Genomics*, Apr;93(4):343-9.

| AFFY | | | ILLUMINA | |
|---|---|---|---|---|
| **P-value** | **Ratio** | **Symbol** | **P-value** | **Ratio** |
| **Down** | | | | |
| 3.98E-05 | **0.179** | **HSPA1B** | 3.74E-05 | **0.190** |
| 6.57E-05 | **0.339** | **HSPA1A** | 1.48E-04 | **0.320** |
| 1.47E-05 | **0.393** | **JUN** | 2.43E-05 | **0.334** |
| 1.45E-02 | **0.546** | **KLF6** | 1.10E-04 | **0.411** |
| 4.37E-07 | **0.603** | **FLJ39779** | 1.92E-04 | **0.514** |
| 1.33E-03 | **0.617** | **PPP1R15A** | 4.12E-05 | **0.546** |
| 4.35E-02 | **0.741** | **MPL** | 4.52E-04 | **0.420** |
| **Up** | | | | |
| 1.05E-03 | **1.821** | **PLK2** | 1.45E-04 | **1.621** |
| 8.78E-03 | **1.817** | **ZNF331** | 6.38E-06 | **1.623** |
| 5.09E-04 | **1.809** | **MCEMP1** | 9.11E-05 | **1.950** |
| 5.84E-04 | **1.756** | **SLC16A3** | 3.99E-04 | **1.691** |

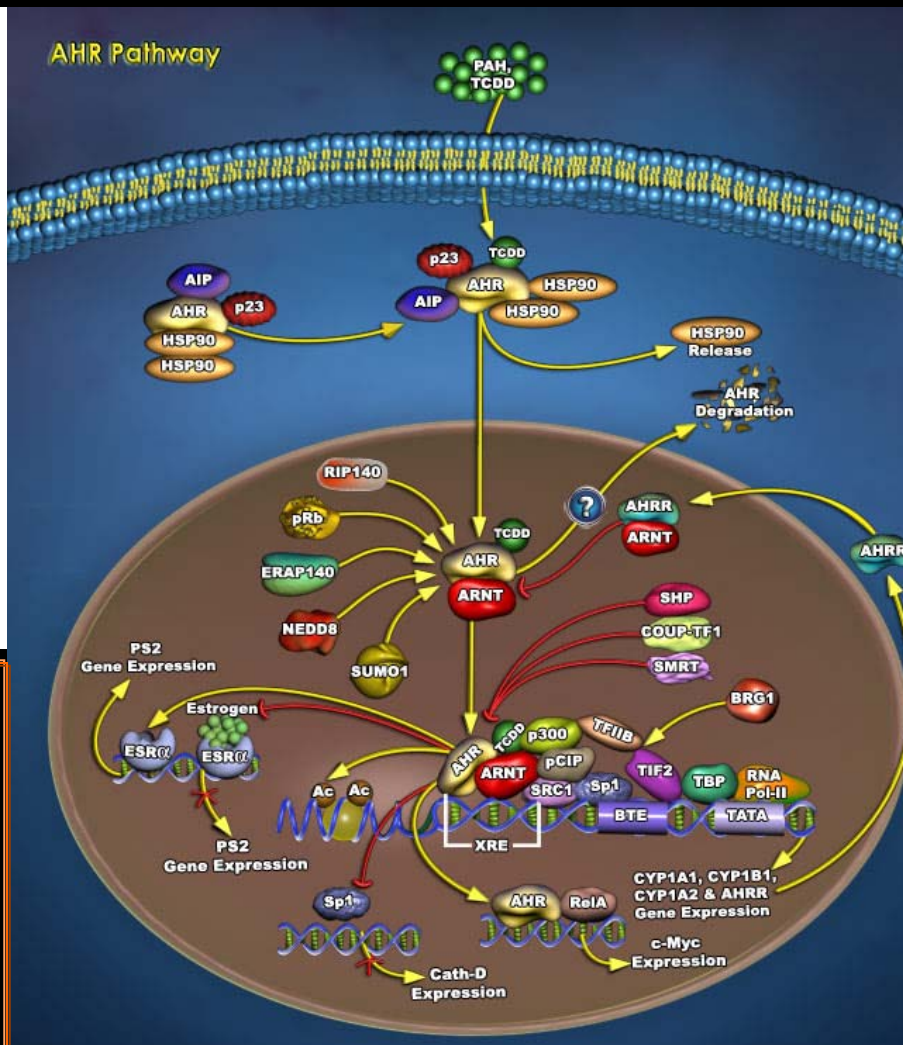
- **8 BZ exposed and 8 control workers**
- **Good concordance among genes with average fold-change ≥ 1.3**
- **Ratio similarity among common genes on both MicroArray platforms**

# Gene Expression: Approach #1

## Examine specific pathways or gene sets known to be involved in cancer




http://www.cs.tau.ac.il/~spike/images/1.png



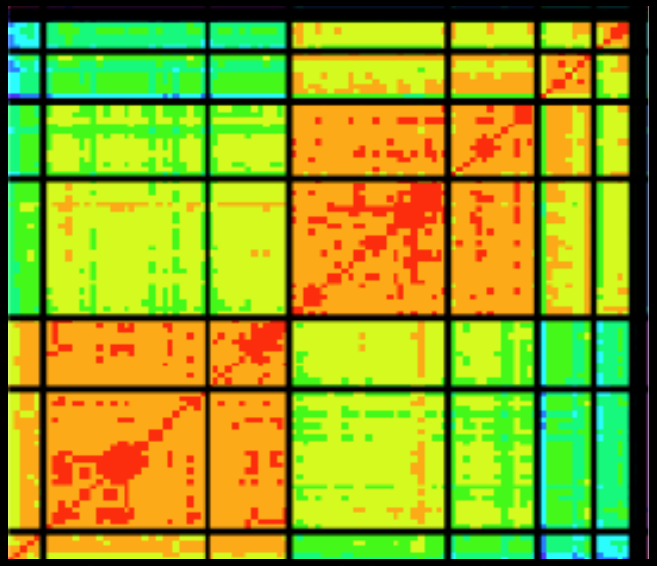

www.ambion.com/images/AHR%20Pathway.jpg

How should we apply these pathway analyses to hazard identification?

# Gene Expression: Approach #2

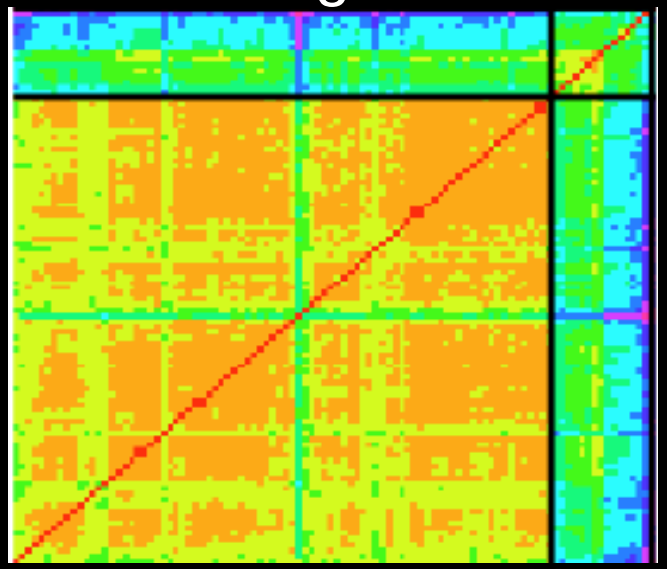
**Examine gene expression profiles of known carcinogens (Group 1) and compare to that of novel chemical, X. If same as Group 1 then consider X to be possible human carcinogen (Group 2B)?**

Benzene vs controls



(7 clusters identified)

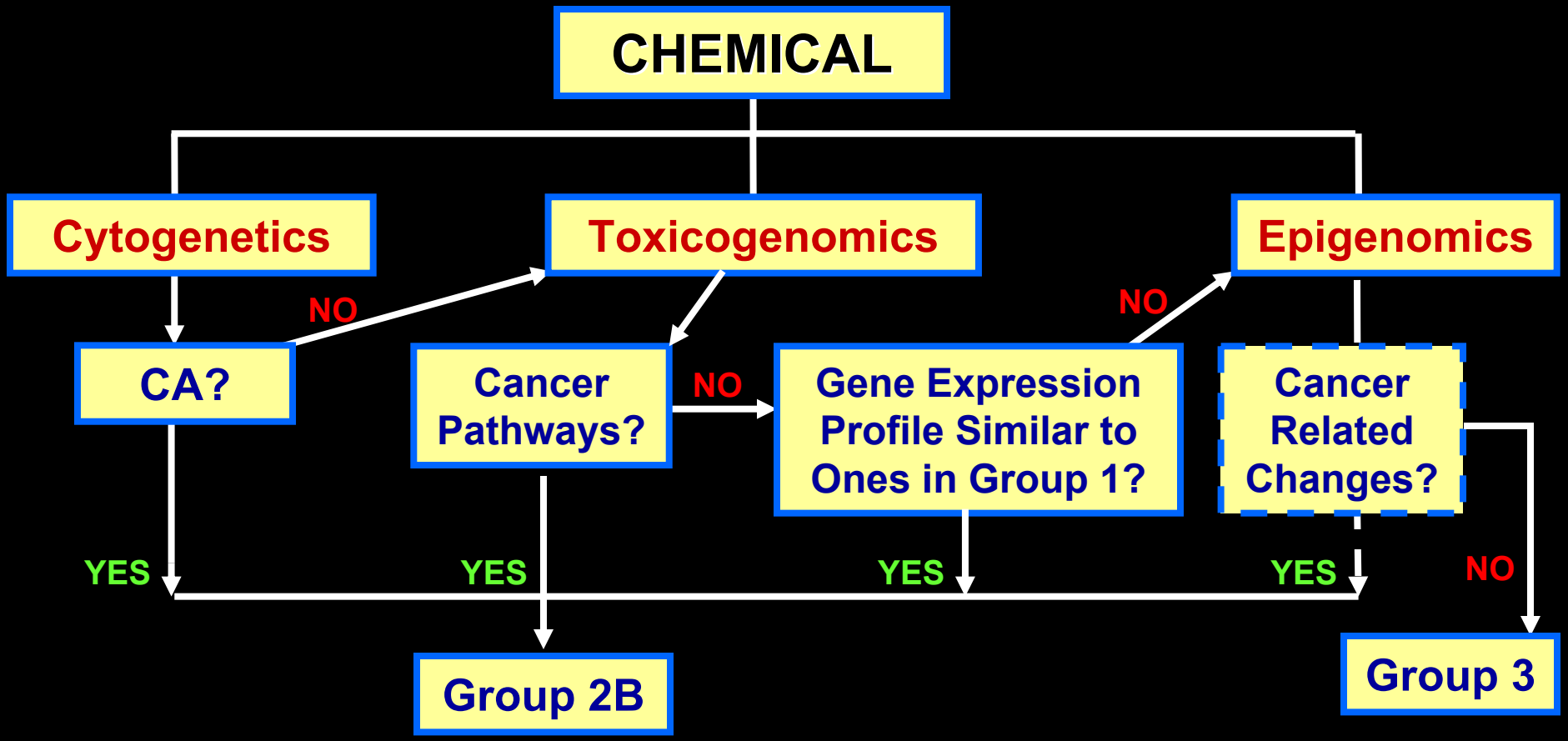
Dioxin high vs low



(only 2 clusters identified)

Clustering analysis using HOPACH

# Chemical Evaluation in Human Studies



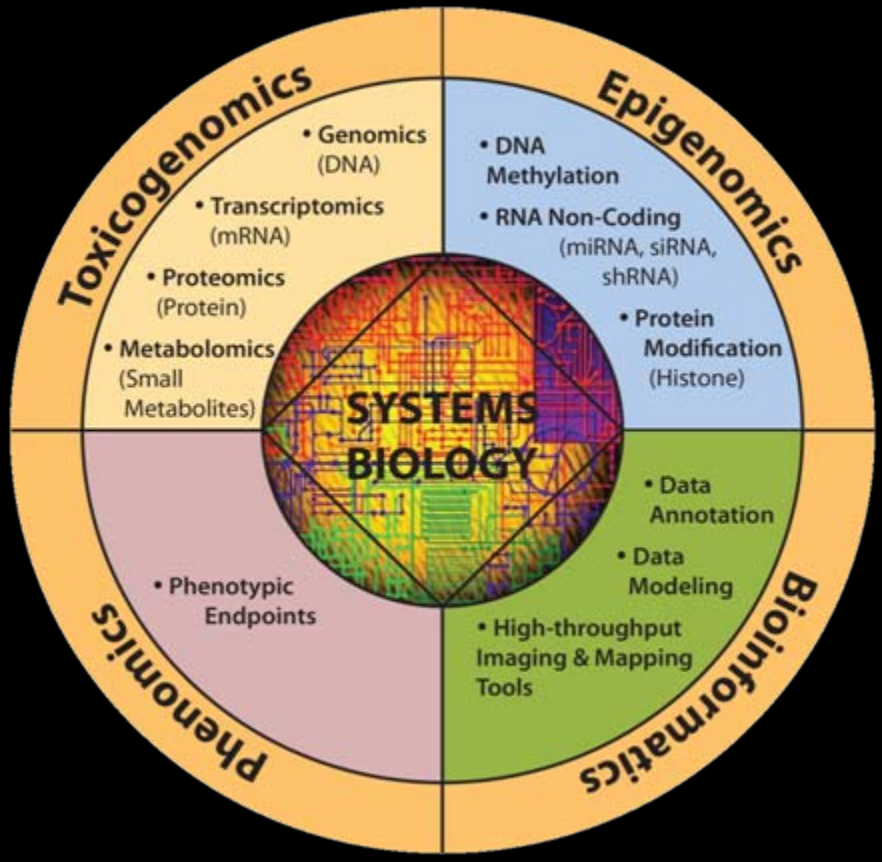
Review

Improving prediction of chemical carcinogenicity by considering multiple mechanisms and applying toxicogenomic approaches☆

Kathryn Z. Guyton [a,*], Amy D. Kyle [b], Jiri Aubrecht [c], Vincent J. Cogliano [d], David A. Eastmond [e], Marc Jackson [f], Nagalakshmi Keshava [a], Martha S. Sandy [g], Babasaheb Sonawane [a], Luoping Zhang [b], Michael D. Waters [f], Martyn T. Smith [b]

# Systems Biology Approach

- Is an interdisciplinary field that focuses on the systematic study of complex interactions in biological systems
- Employs a holistic approach to study components and interactions in the network of DNA, RNA, proteins & biochemical reactions
- Utilizes powerful tools including toxicogenomics, epigenomics, bioinformatics, and phenomics

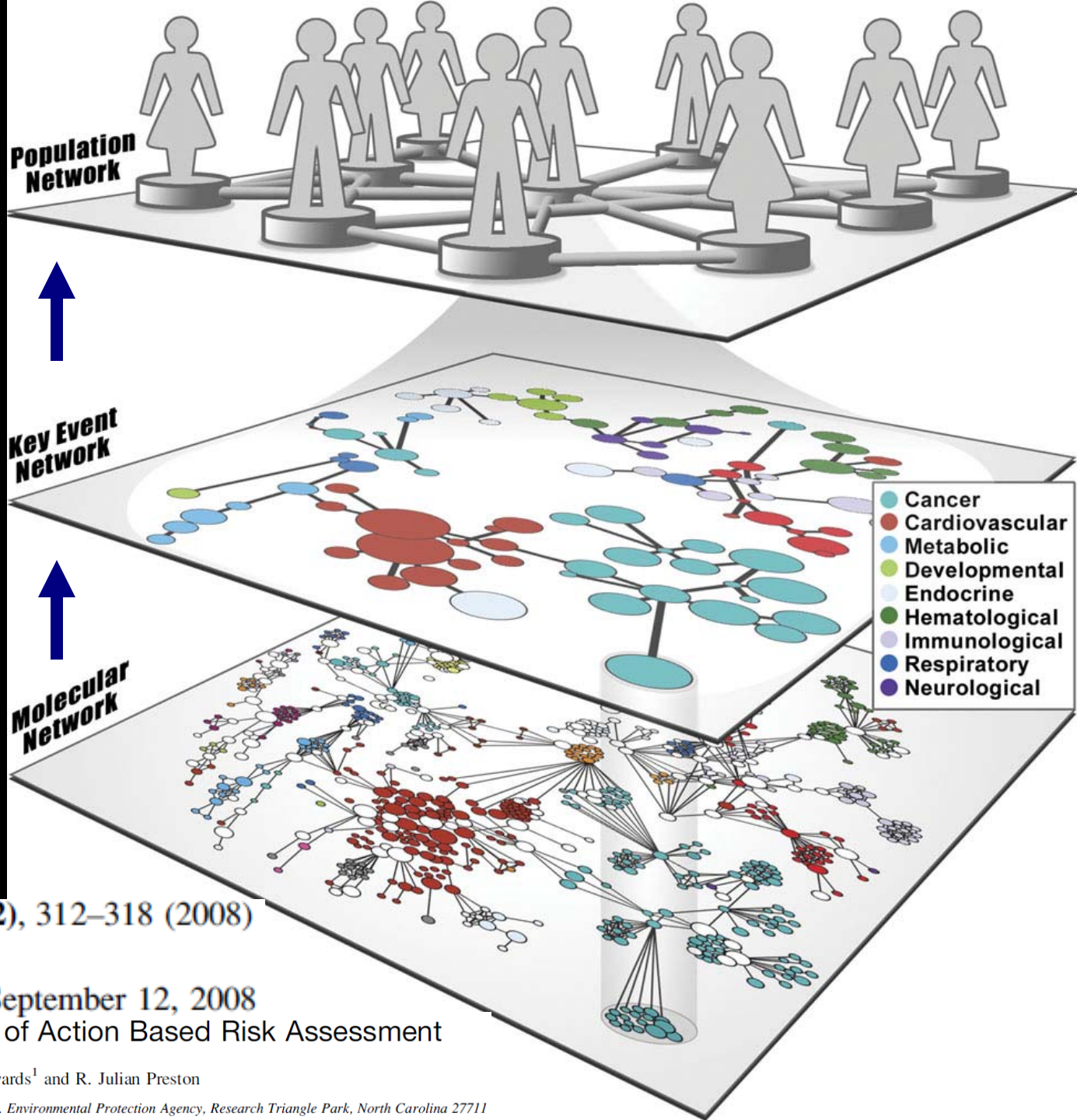


Review

Systems biology of human benzene exposure

Luoping Zhang[a,*], Cliona M. McHale[a], Nathaniel Rothman[b], Guilan Li[c], Zhiying Ji[a], Roel Vermeulen[d], Alan E. Hubbard[a], Xuefeng Ren[a], Min Shen[b], Stephen M. Rappaport[a], Matthew North[e], Christine F. Skibola[a], Songnian Yin[c], Christopher Vulpe[e], Stephen J. Chanock[b], Martyn T. Smith[a], Qing Lan[b]

# Systems Biology & MOA-Based Risk Assessment




TOXICOLOGICAL SCIENCES **106(2)**, 312–318 (2008)
doi:10.1093/toxsci/kfn190
Advance Access publication September 12, 2008

Systems Biology and Mode of Action Based Risk Assessment

Stephen W. Edwards[1] and R. Julian Preston

*National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711*

# Conclusions

1. Human biomarker studies should be a priority to identify potential carcinogens;
2. Chromosome aberrations are a validated biomarker of cancer risk in humans, and can be employed for new hazard identification;
3. Array based technologies could examine essentially all MOAs, and can be applied in human biomarker studies;
4. New predictive biomarkers and/or pathways related to cancer and Group 1 chemicals are proposed to be applied for testing possible carcinogens; and
5. Systems biology is a powerful and recent approach to MOA discovery and risk assessment.

# Thanks

Martyn T. Smith
Cliona McHale
Zhiying Ji
Alan Hubbard
Steve Rappaport
Christine Skibola
Nat Rothman
Qing Lan
G Li and S Yin
X Tang and L Li

UCB, NCI, China CDC and GDPCC

NIEHS National Institute of Environmental Health Sciences
~ Your Environment is Your Health ~