Genomics and Pathways
Prediction and Dose-Response

Christopher J. Portier, Ph.D.
Director
National Center for Environmental Health
Agency for Toxic Substances and Disease Registry
Three Issues to Address

- **Using pathway-based approaches**
  - Strengthen findings seen in animals and humans
  - Predict disease

- **Cumulative risk assessment**
  - Pathways as a common paradigm for linkage

- **Mixtures**
  - Pathways for identifying synergies
  - We still need a method for addressing mixtures and risk
Major Themes

- Measurement of multiplexed signals
  - Environmental
  - Ecological
  - Biological
- Greater acquisition and use of data from humans
- Systems thinking (ecological frame of mind)
- Complex analyses of complex systems
  - Ability to predict
Systems Biology for the Individual

Gohlke and Portier (2006)
Research and the Human Model

Gohlke and Portier (2006)

Epidemiology

Human Clinical Laboratory

Animal Models

Tissue Cultures

Molecular Biology

Cell Cultures

Boverhof and Zacharewski (Tox. Sci., 2006)

Gene Space → Protein Space → Metabolite Space

Gohlke and Portier (2006)
Boverhof and Zacharewski (Tox. Sci., 2006)
Interaction Network: Our Environment and Our Health
Predicting human health risk using pathways and their canonical structure

- Animal-Based Mechanistic Data \((in vivo or in vitro)\)
- Animal-Based Carcinogenicity Data \((in vivo)\)
- Human-Based Mechanistic Data \((in vivo or in vitro)\)
- Human-Based Carcinogenicity Data \((epidemiology)\)

A: Animal-Based Mechanistic Data \((in vivo or in vitro)\) -> Pathway-Based Analysis
B: Animal-Based Carcinogenicity Data \((in vivo)\) -> Pathway-Based Analysis
C: Human-Based Mechanistic Data \((in vivo or in vitro)\) -> Pathway-Based Analysis
D: Human-Based Carcinogenicity Data \((epidemiology)\) -> Pathway-Based Analysis
E: Pathway-Based Analysis -> Human-Based Mechanistic Data \((in vivo or in vitro)\)
Identifying Important Disease Pathways

Human Genetics + Human Disease

Sets of Pathways

Identify Pathways Related to Human Disease

High Throughput High Content Chemical-Specific Data

Predict Chemical-Gene-Disease Interactome

New ‘omics & HTS Data

Predict Risks

Develop Screens

Set Priorities

Fingerprint Toxicants

Develop Hypotheses
The Genetic Association Database is a gene-centered archive of published scientific papers on human genetic association studies.

- **Database Contents**
  - 28347 records on human gene-phenotype (mostly complex disease) relationships

- **Data used in our analysis**
  - Manual phenotype grouping and better annotation
  - 8,825 unique associations between 2088 genes and 208 disease phenotypes

Comparative Toxicogenomics Database

http://ctd.mdibl.org/

• Interactions between environmental factors and genes/proteins in diverse organisms are curated from the published literature using both algorithm based methods as well as manual curation.
• Environmental factor identifiers used in the literature are annotated using MeSH chemical terms.

Unsupervised Clustering of Pathways with Phenotypes

Cardiovascular
Metabolic

Cancer

Immune

Endocrine

Neuropsychiatric

Gohlke et al. (BMC Systems Biology, 2009)

Renin-angiotensin
PPAR
Insulin

Cell cycle
Erbb

Toll like receptor
Cytokine
Jak-Stat

Steroid hormone
Calcium
VEGF
Tyrosine metabolism
Structurally-Enhanced Pathway Enrichment Analysis (SEPEA)

Thomas et al. (Genome Biology 2009)

Heavy Ends Rule
- Specificity of the pathway at the receptor end
- Ultimate response by downstream components
- Intermediate components typically shared between pathways

Sequential Best Rule
- Pathways with multiple affected components close together are more reliably linked.
Relating Across Pathways

Signal transduction
Avg. of 16 diseases associated with each signaling pathway

Metabolism
Avg. of 4 diseases associated with each metabolic pathway
Critical Toxicity Pathways:

- Ranked highly for both phenotype and environmental factor
  - Metabolism by cytochrome P450
  - Retinol metabolism
  - Jak-STAT signaling
  - Adipocytokine signaling
  - Toll-like receptor signaling

- Other important pathways
  - Apoptosis
  - p53 signaling pathway
  - Cell-cycle pathway
  - PPAR signaling pathway
### Microarray data from B6C3F₁ mice following 90 day exposure

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Short Name</th>
<th>NTP No.</th>
<th>Route[^a]</th>
<th>Dose</th>
<th>Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Amino-2,4-dibromoantraquinone</td>
<td>ADBQ</td>
<td>383</td>
<td>Food</td>
<td>20,000 ppm</td>
<td>Liver</td>
</tr>
<tr>
<td>Methyleneg Chloride</td>
<td>MECL</td>
<td>306</td>
<td>Inhalation</td>
<td>4,000 ppm</td>
<td>Liver</td>
</tr>
<tr>
<td>N-Methylolacrylamide</td>
<td>MACR</td>
<td>352</td>
<td>Gavage (Water)</td>
<td>50 mg/kg</td>
<td>Liver</td>
</tr>
<tr>
<td>Tris(2,3-dibromopropyl)phosphate</td>
<td>TDPP</td>
<td>76</td>
<td>Food</td>
<td>1,000 ppm</td>
<td>Liver</td>
</tr>
<tr>
<td>2,2-Bis(bromomethyl)-1,3-propanediol</td>
<td>BBMP</td>
<td>452</td>
<td>Food</td>
<td>1,250 ppm</td>
<td>Other</td>
</tr>
<tr>
<td>1,2-Dibromoethane</td>
<td>DBET</td>
<td>86</td>
<td>Gavage (CO)</td>
<td>62 mg/kg</td>
<td>Other</td>
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<tr>
<td>Ethylene Oxide</td>
<td>ETOX</td>
<td>326</td>
<td>Inhalation</td>
<td>100 ppm</td>
<td>Other</td>
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<tr>
<td>Naphthalene</td>
<td>NPTH</td>
<td>410</td>
<td>Inhalation</td>
<td>30 ppm</td>
<td>Other</td>
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<tr>
<td>Vanadium Pentoxide</td>
<td>VANP</td>
<td>507</td>
<td>Inhalation</td>
<td>2.0 mg/m³</td>
<td>Other</td>
</tr>
<tr>
<td>1,4-Dichlorobenzene</td>
<td>DCBZ</td>
<td>319</td>
<td>Gavage (CO)</td>
<td>600 mg/kg</td>
<td>Liver</td>
</tr>
<tr>
<td>Propylene glycol mono-t-butyl ether</td>
<td>PGBE</td>
<td>515</td>
<td>Inhalation</td>
<td>1,200 ppm</td>
<td>Liver</td>
</tr>
<tr>
<td>Tetrafluoroethylene</td>
<td>TFEL</td>
<td>450</td>
<td>Inhalation</td>
<td>1,250 ppm</td>
<td>Liver</td>
</tr>
<tr>
<td>1,2,3-Trichloropropane</td>
<td>TCPN</td>
<td>384</td>
<td>Gavage (CO)</td>
<td>60 mg/kg</td>
<td>Liver</td>
</tr>
<tr>
<td>2-Chloromethylpyridine hydrochloride</td>
<td>CMPH</td>
<td>178</td>
<td>Gavage (Water)</td>
<td>250 mg/kg</td>
<td>No</td>
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<tr>
<td>Diazinon</td>
<td>DIAZ</td>
<td>137</td>
<td>Food</td>
<td>200 ppm</td>
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<tr>
<td>Iodoform</td>
<td>IODO</td>
<td>110</td>
<td>Gavage (CO)</td>
<td>93 mg/kg</td>
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<tr>
<td>Malathion</td>
<td>MALA</td>
<td>24</td>
<td>Food</td>
<td>14,800 ppm</td>
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<tr>
<td>4-Nitroanthranilic acid</td>
<td>NAAC</td>
<td>109</td>
<td>Food</td>
<td>10,000 ppm</td>
<td>No</td>
</tr>
<tr>
<td>Tetrafluoroethane</td>
<td>TFEA</td>
<td>...[^d]</td>
<td>Inhalation</td>
<td>50,000 ppm</td>
<td>No</td>
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<tr>
<td>Trichlorofluoromethane</td>
<td>TCFM</td>
<td>106</td>
<td>Gavage (CO)</td>
<td>3,925 mg/kg</td>
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</tr>
<tr>
<td>Air</td>
<td>ACON</td>
<td></td>
<td>Inhalation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td>CCON</td>
<td></td>
<td>Gavage (CO)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed</td>
<td>FCON</td>
<td></td>
<td>Food</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>WCON</td>
<td></td>
<td>Gavage (Water)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[^a]: Route of administration.
[^d]: Not specified.
Predicted Liver Cancer from Microarray Data

Liver Carcinogens

Above red line - clearly positive
Below blue line - clearly negative
X - actual data prediction
Green range - x-validation range
Predicted Liver Cancer from Microarray Data

Liver Carcinogens

Above red line - clearly positive
Below blue line - clearly negative
X - actual data prediction
Green range - x-validation range
Predicted Liver Cancer from Microarray Data
Colorectal Cancer
Liver Cancer
Prostate Cancer
Evaluating Dose-Response Patterns

- Methylene Chloride
- Naphthalene
- 1,4-Dichlorobenzene
- 1,2,3-Trichloropropane
- Propylene glycol mono-t-butyl ether
Pathways can be used to identify compounds with common activation patterns. These can be linked with specific diseases. Dose additivity can be used if pathways seem to be altered in similar directions. Up- and down-regulations are identical. More complicated if patterns of alteration are different.
Mixtures with Different Mechanisms

- Pathways can help to distinguish mechanisms
- Need a new paradigm for aggregating risks
  - Dose additivity won’t work
  - Aggregation needs to be done in the risk range
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