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Emerging Technologies in Toxicity Testing:How will this Impact Risk Assessment?

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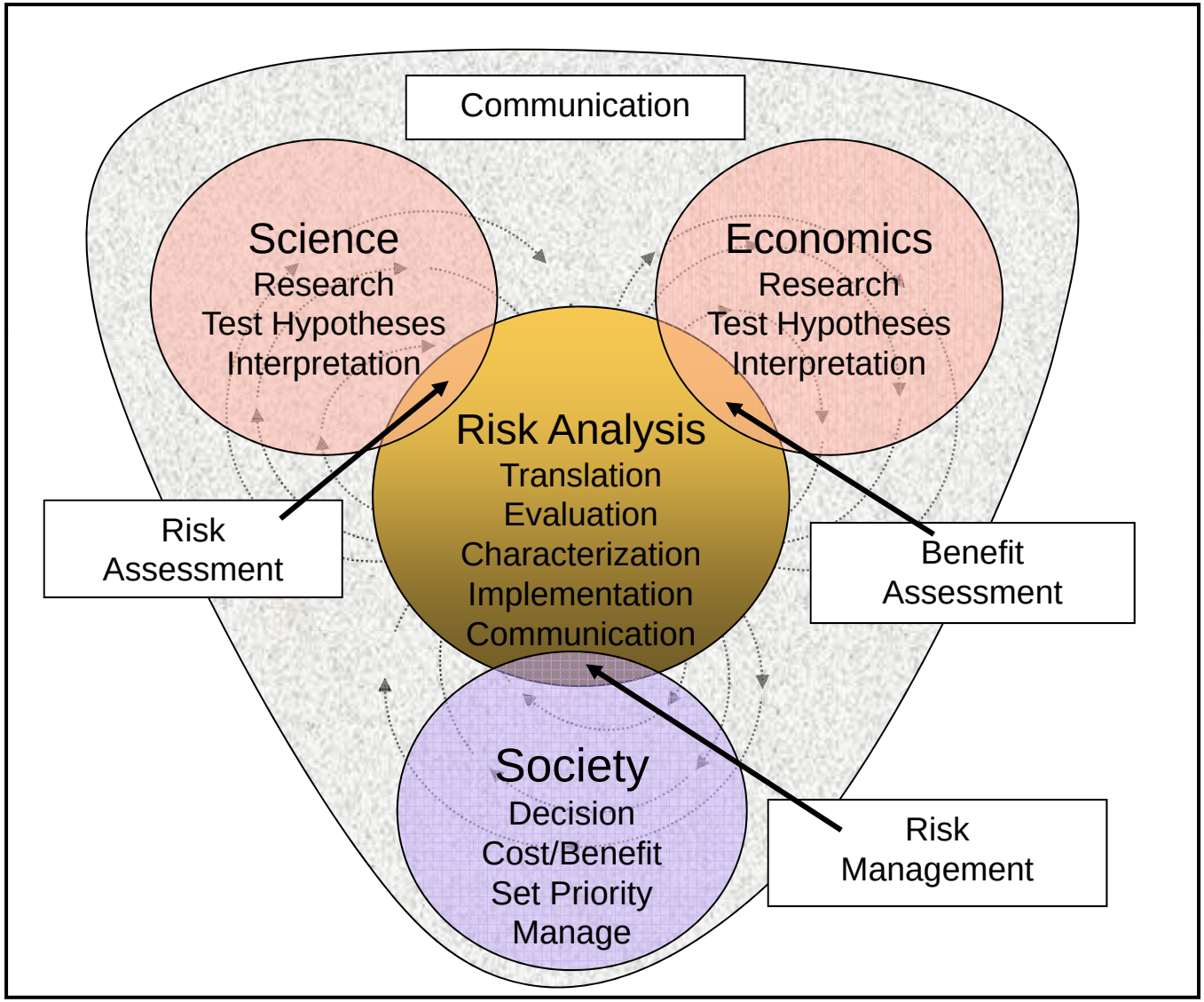
National Institute of Environmental Health Sciences

NTP National Toxicology Program

U.S. Department of Health and Human Services
National Institutes of Health
National Institute of Environmental Health Sciences


Communication
Science
Research
Test Hypotheses
Interpretation
Economics
Research
Test Hypotheses
Interpretation
Risk Analysis
Translation
Evaluation
Characterization
Implementation
Communication
Risk
Assessment
Benefit
Assessment
Society
Decision
Cost/Benefit
Set Priority
Manage
Risk
Management

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Evaluation of the weight of the evidence - IARC

Cancer in humans

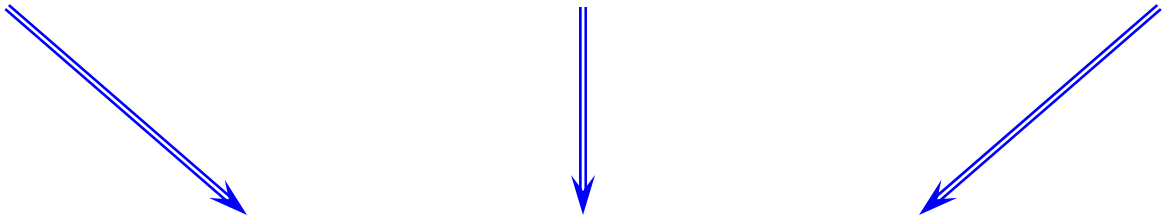
- *Sufficient evidence*
- *Limited evidence*
- *Inadequate evidence*
- *Evidence suggesting lack of carcinogenicity*

Cancer in experimental animals

- *Sufficient evidence*
- *Limited evidence*
- *Inadequate evidence*
- *Evidence suggesting lack of carcinogenicity*

Mechanistic and other relevant data

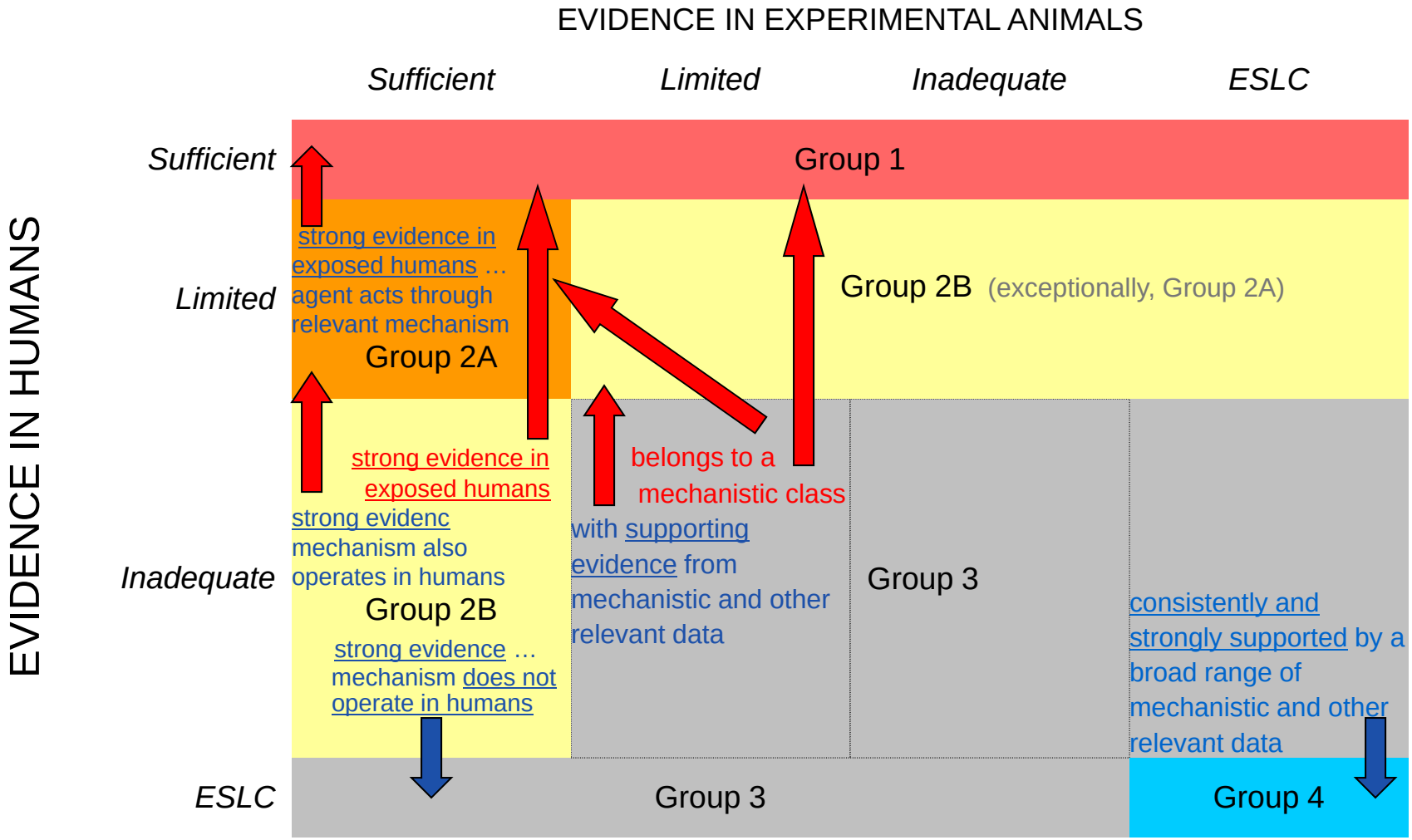
- Mechanistic data “weak,” “moderate,” or “strong”?
- Mechanism likely to be operative in humans?



Overall evaluation

- Group 1 *Carcinogenic to humans*
- Group 2A *Probably carcinogenic to humans*
- Group 2B *Possibly carcinogenic to humans*
- Group 3 *Not classifiable as to its carcinogenicity to humans*
- Group 4 *Probably not carcinogenic to humans*

Human data predominates, but all data is used



Modified from Vincent Cogliano, IARC

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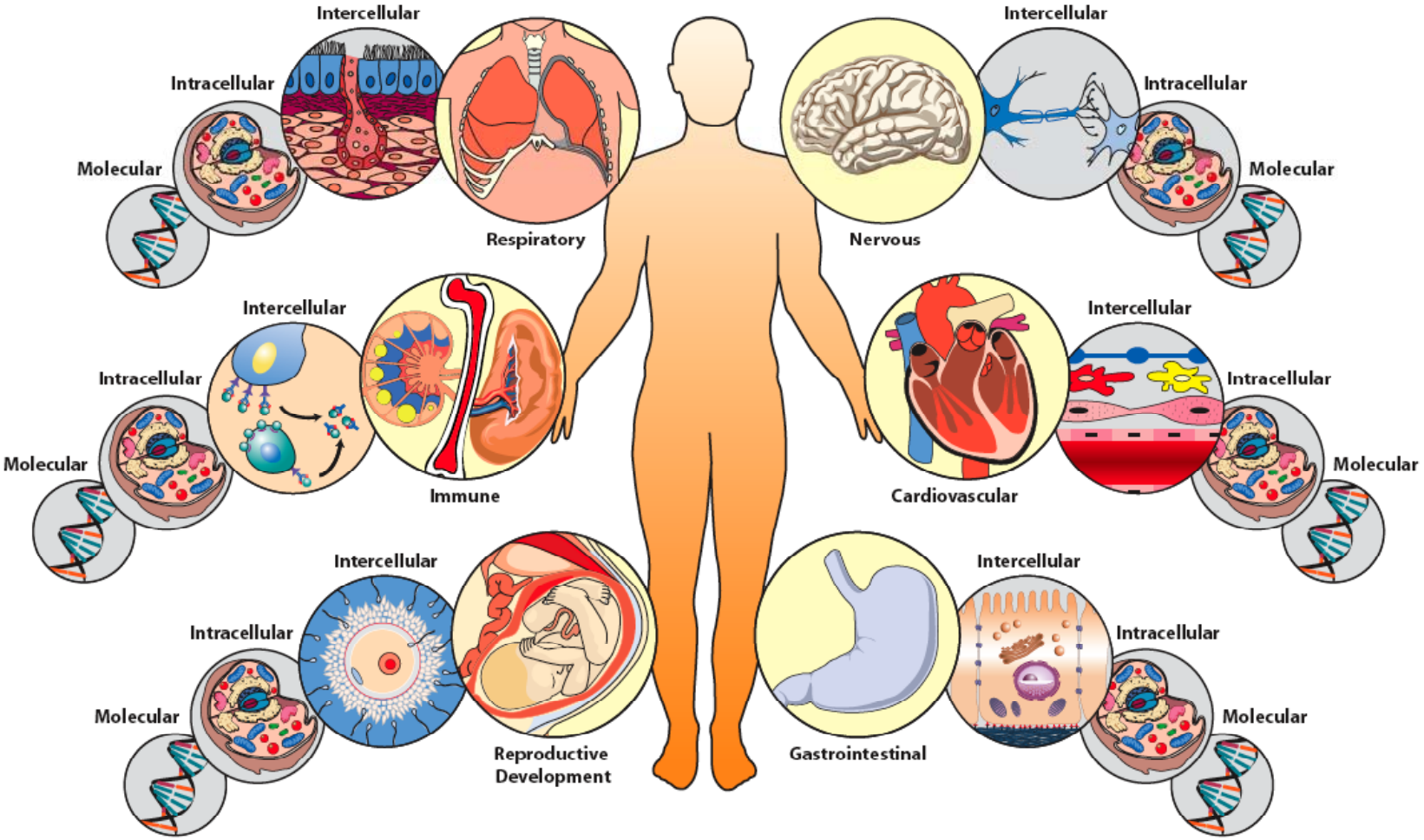
Systems Biology for the Individual



Gohlke and Portier (2006)

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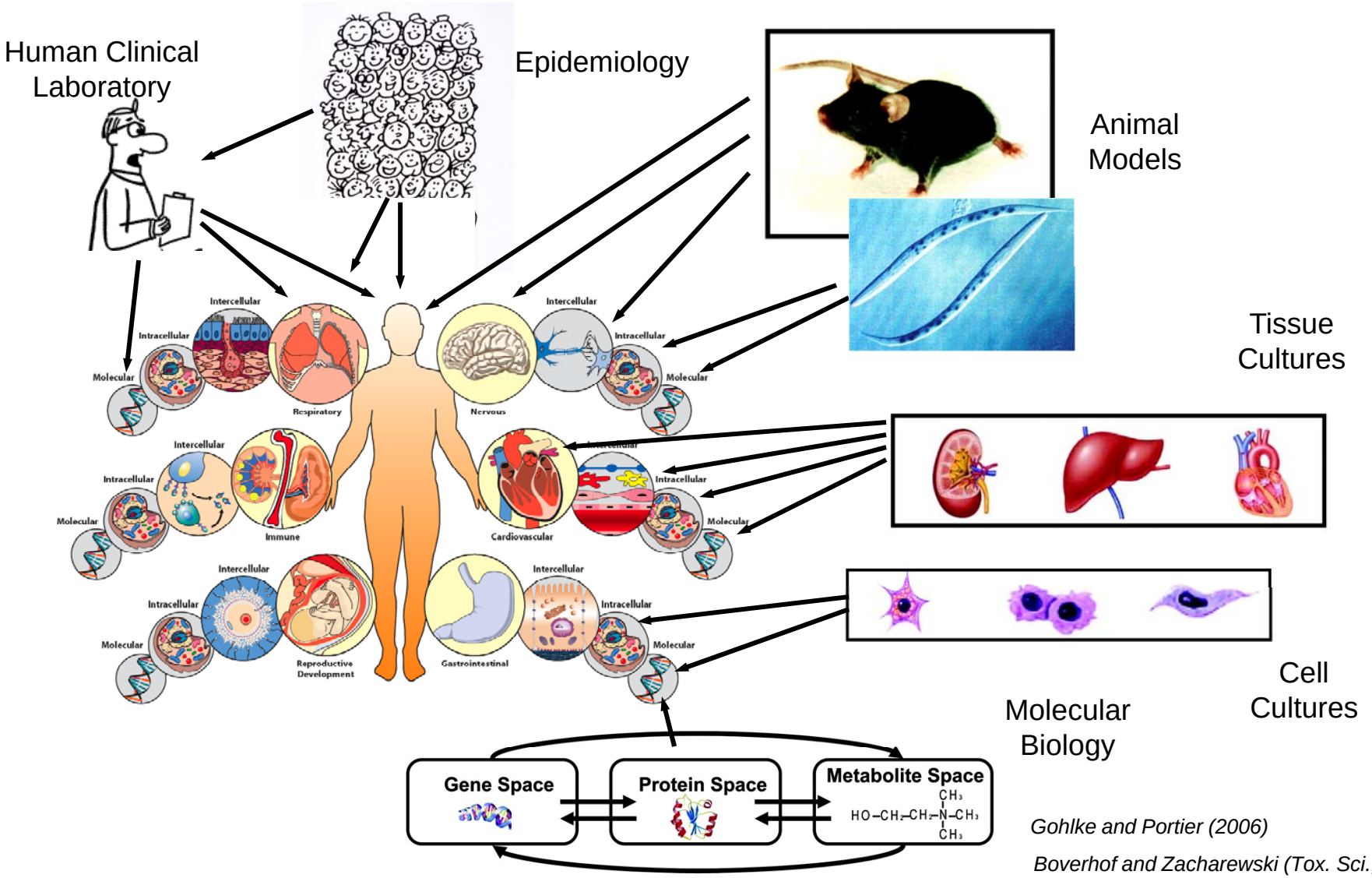
Research and the Human Model



Gohlke and Portier (2006)

Boverhof and Zacharewski (Tox. Sci., 2006)

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Research and the Human Model

Human Clinical Laboratory

Epidemiology

Animal Models

Tissue Cultures

Cell Cultures

Molecular Biology

Gene Space

Protein Space

Metabolite Space

Gohlke and Portier (2006)

Boverhof and Zacharewski (Tox. Sci., 2006)

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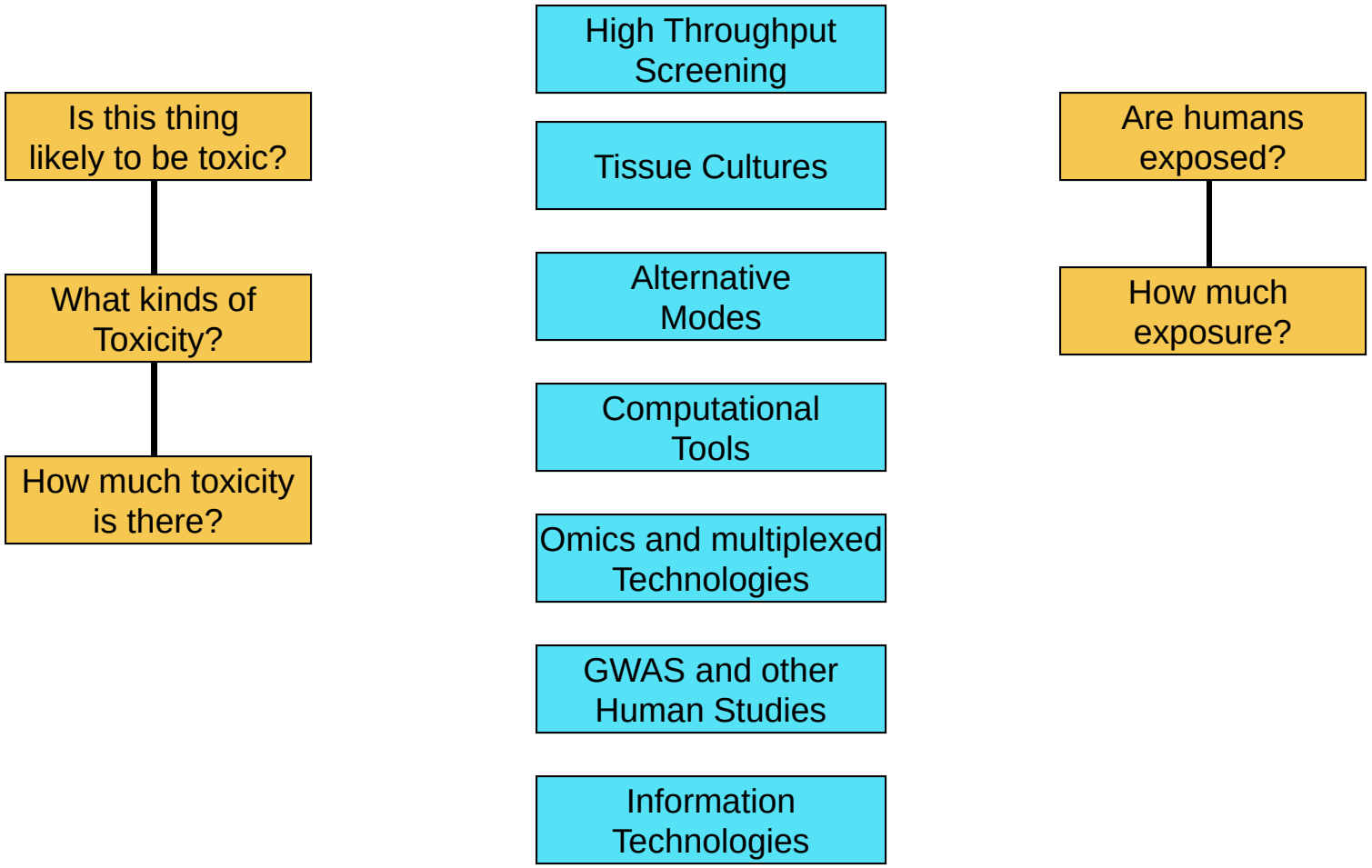
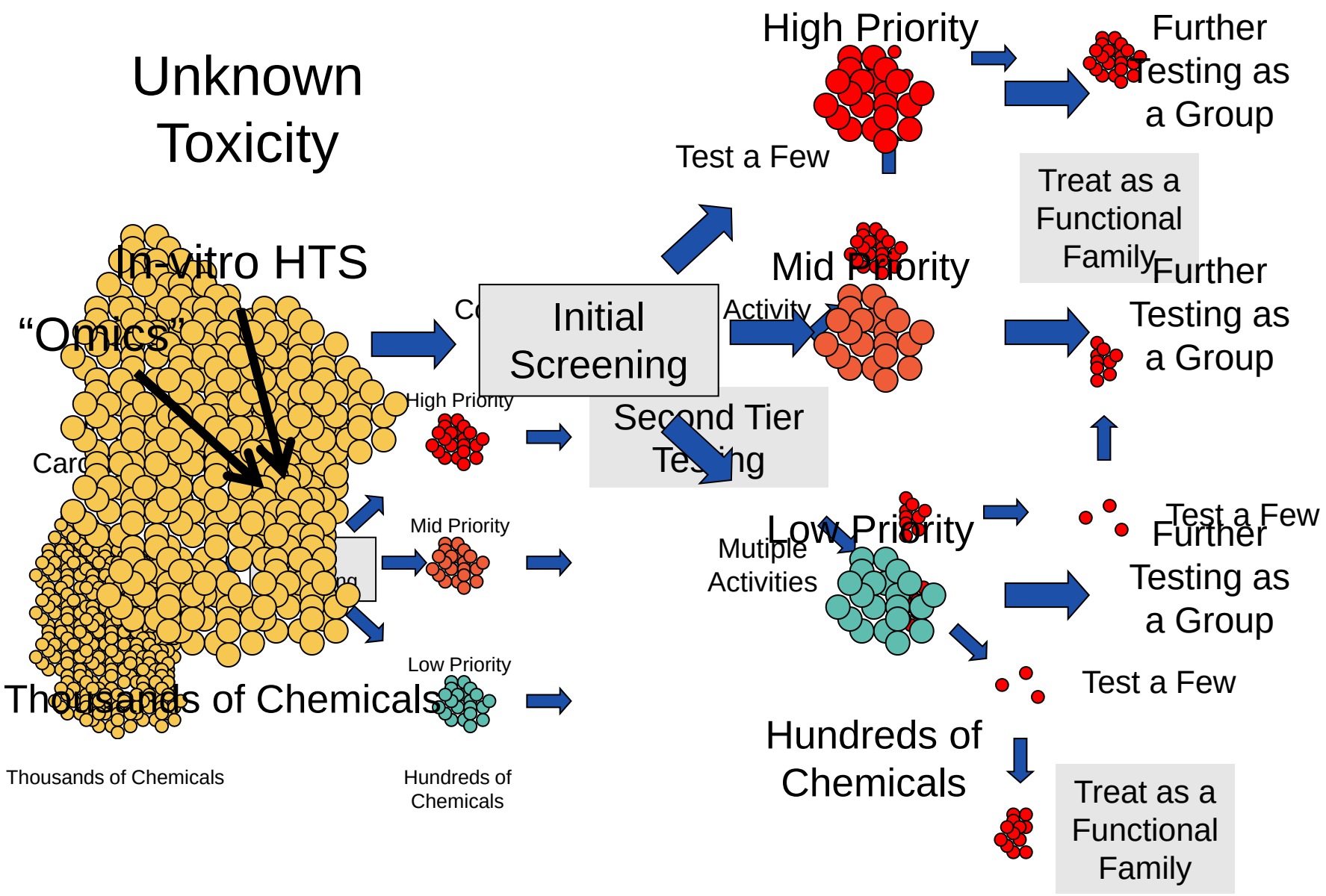
Emerging Screening Tools and the Risk Assessment Process




Unknown Toxicity
In-vitro HTS "Omics"
Thousands of Chemicals
Initial Screening
Activity
High Priority
Mid Priority
Low Priority
Second Tier Testing
Test a Few
High Priority
Further Testing as a Group
Treat as a Functional Family
Mid Priority
Further Testing as a Group
Low Priority
Mutiple Activities
Test a Few
Further Testing as a Group
Test a Few
Hundreds of Chemicals
Treat as a Functional Family
Thousands of Chemicals
Hundreds of Chemicals

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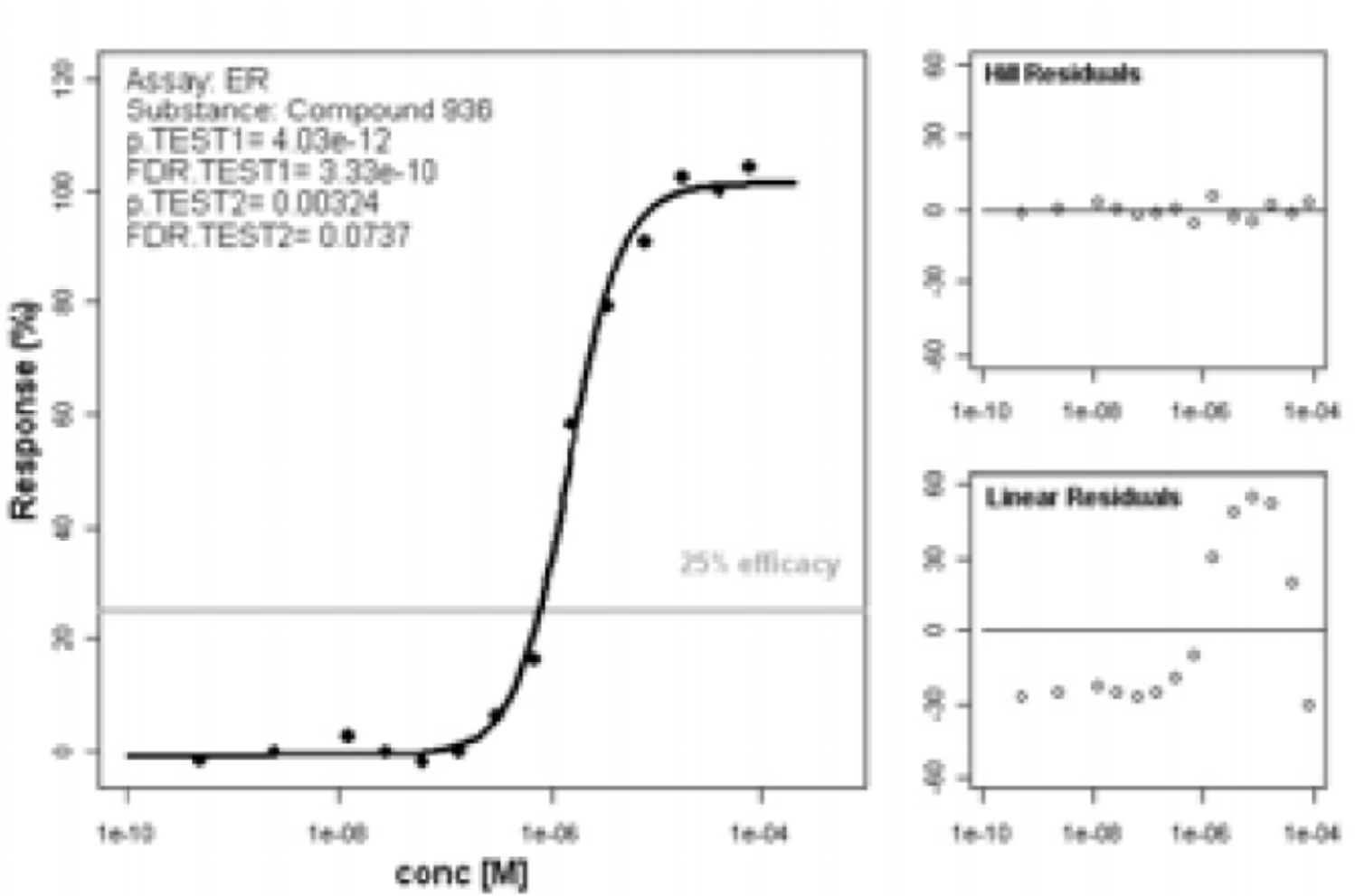
What constitutes a positive? Should it be a single number?



Shockley et. al.. SOT 2010

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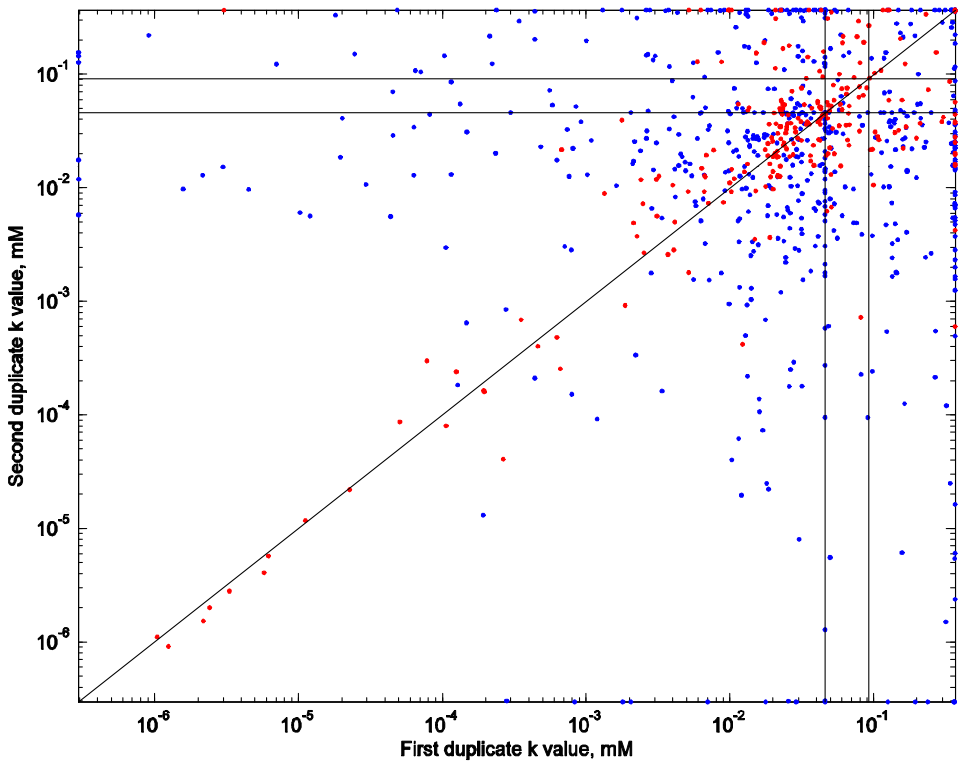
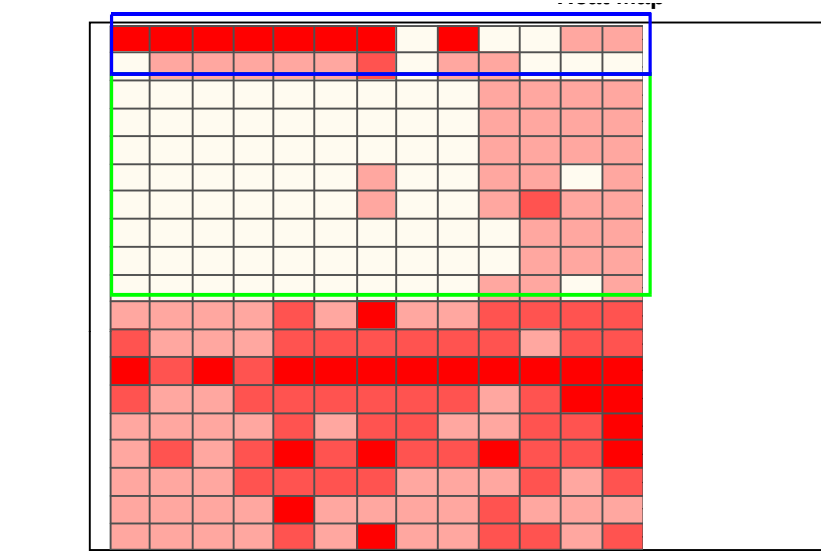
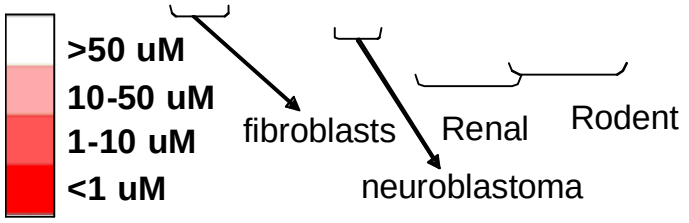
HTS Analyses



Reproducibility



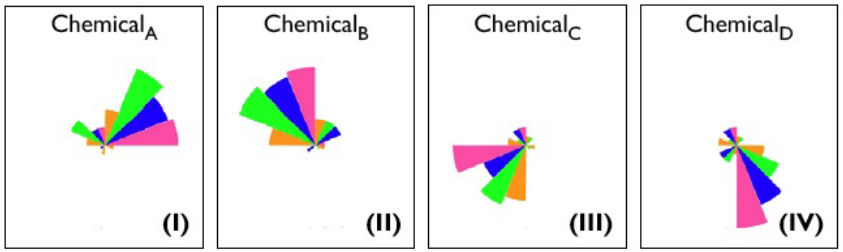


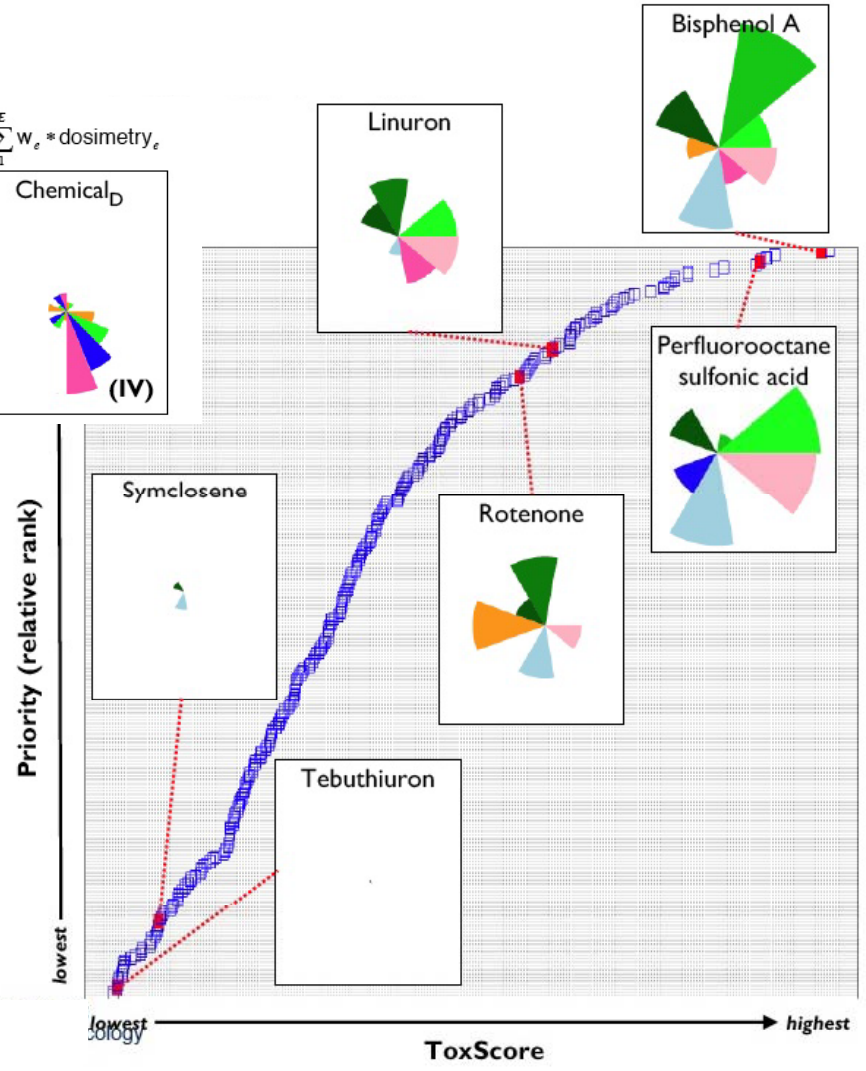
Species Concordance

ToxScores: combining information to set priorities

$$\text{ToxScore} = \sum_{1}^{C} w_c * \text{chemProp}_c + \sum_{1}^{I} w_i * \text{assay}_i + \sum_{1}^{P} w_p * \text{pathway}_p + \sum_{1}^{E} w_e * \text{dosimetry}_e$$

Example ToxScores prioritizing chemicals for Cancer, Systemic, Reproductive or Developmental testing, respectively.





Kavlock, 2009

Commercial Tools: GeneGo (an example)

- Chemical Reports
  - Major Metabolites
  - 76 QSAR predictions
    - Protein binding
    - Cancer
    - Alzheimer's
    - Etc
  - Compounds predicted to have similar activity
    - For both parent and metabolites
  - Possible cellular (receptors, proteins, etc.) targets
    - Both parent and metabolites
  - Functional pathways likely to be targeted

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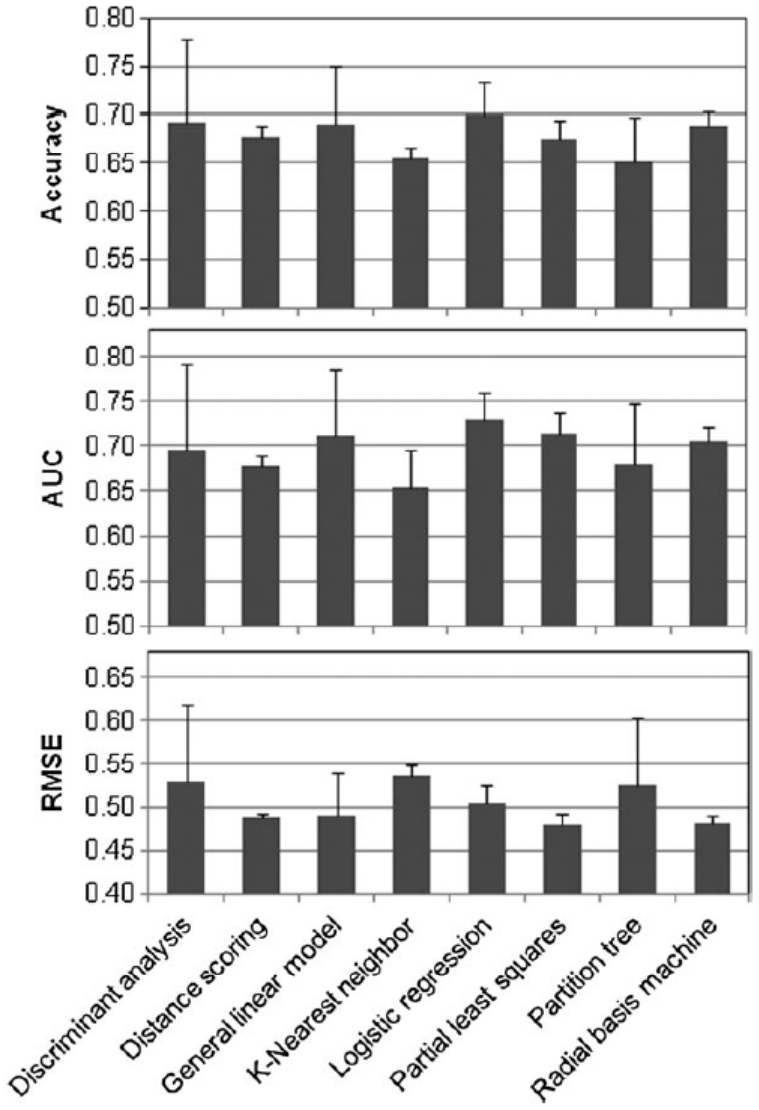
Comparison of the average performance of eight different statistical classification methods



Thomas et. Al. Toxicological Sciences 112(2), 311–321 (2009)

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How good is good enough?

The predictive capacity of the 3T3 Neutral Red Uptake assay to identify substances with acute oral LD50 > 2000 mg/kg

	TP	FP	FN	TN	Sens	Spec	Acc	PPV	NPV
JRC	23	14	2	12	92	46	69	62	86
HSL	18	14	1	11	95	44	66	56	92
IIVS	24	17	1	12	96	41	67	59	92

TP: True Positive
FP: False Positive
FN: False Negative
TN: True Negative

Prieto et. Al., JRC, SOT 2010

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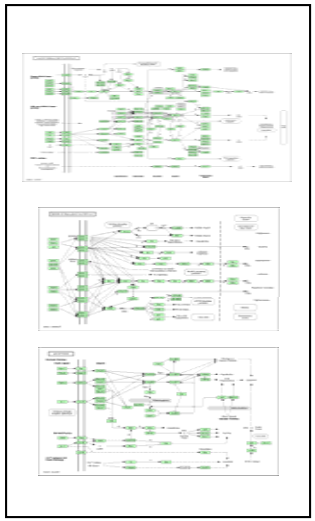
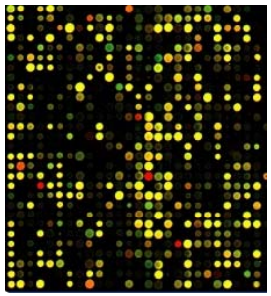
Identifying Important Disease Pathways

Human Genetics
+
Human Disease



Sets of Pathways

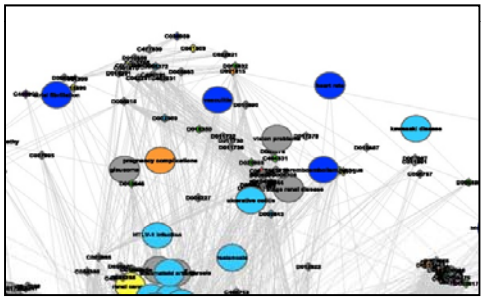
Identify Pathways Related to Human Disease



High Throughput High Content Chemical-Specific Data

New 'omics Data

Predict Chemical-Gene-Disease Interactome



Predict Risks

Develop Screens

Set Priorities

Fingerprint Toxicants

Develop Hypotheses

Genetics, Pathways and Human Disease

- Observations
  - Genetic polymorphisms associated with human diseases
    - Example: ALDH2 isoenzyme polymorphism (mutated form) ALDH2*2 is unable to complete phase 2 metabolism of alcohol leading to increased acetaldehyde levels in blood resulting in increased blood pressure and flushed appearance
  - Part of a pathway related to glycolysis and gluconoegenesis
  - Other aspects of this pathway can lead to increases in acetaldehyde
    - So can biofuels?
  - All of these should be associated with the same symptoms (or phenotype or disease)
  - If chemicals block the activity of ALDH2, they should also increase risk of the same phenotype

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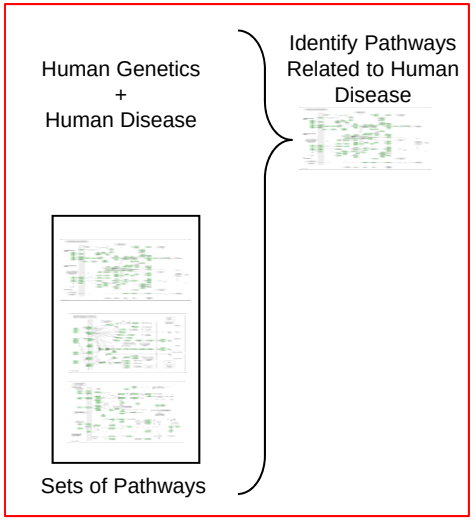
Genetic Association Database
geneticassociationdatabase.nih.gov



- 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

Becker et al. (2004) *Nature Genetics* 36(5)

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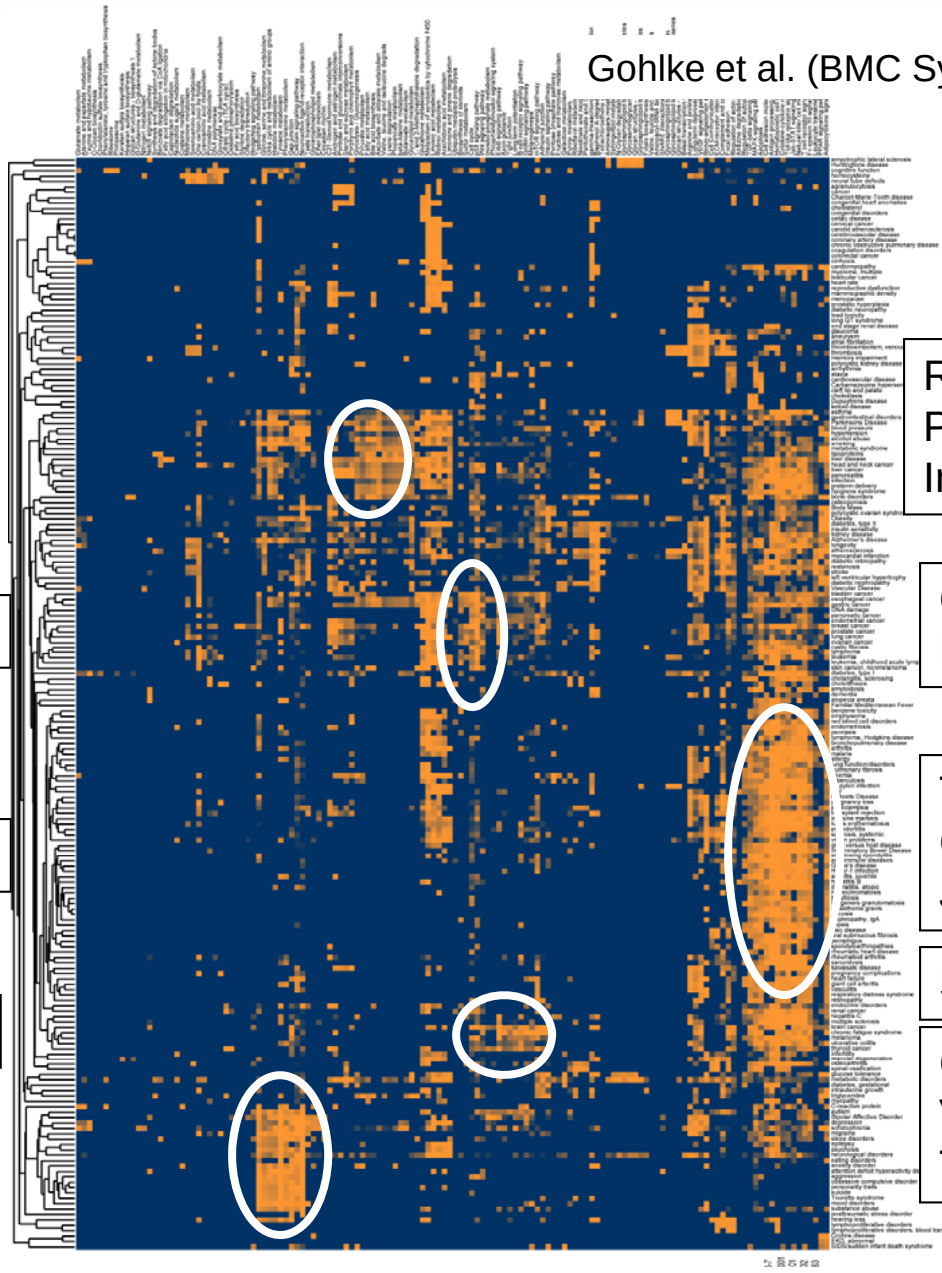
Unsupervised Clustering of Pathways with Phenotypes

Gohlke et al. (BMC Systems Biology, 2009)



Cardiovascular
Metabolic

Cancer

Immune

Endocrine

Neuropsychiatric

Renin-angiotenson
PPAR
Insulin

Cell cycle
Erbb

Toll like receptor
Cytokine
Jak-Stat

Steroid hormone

Calcium
VEGF
Tyrosine metabolism

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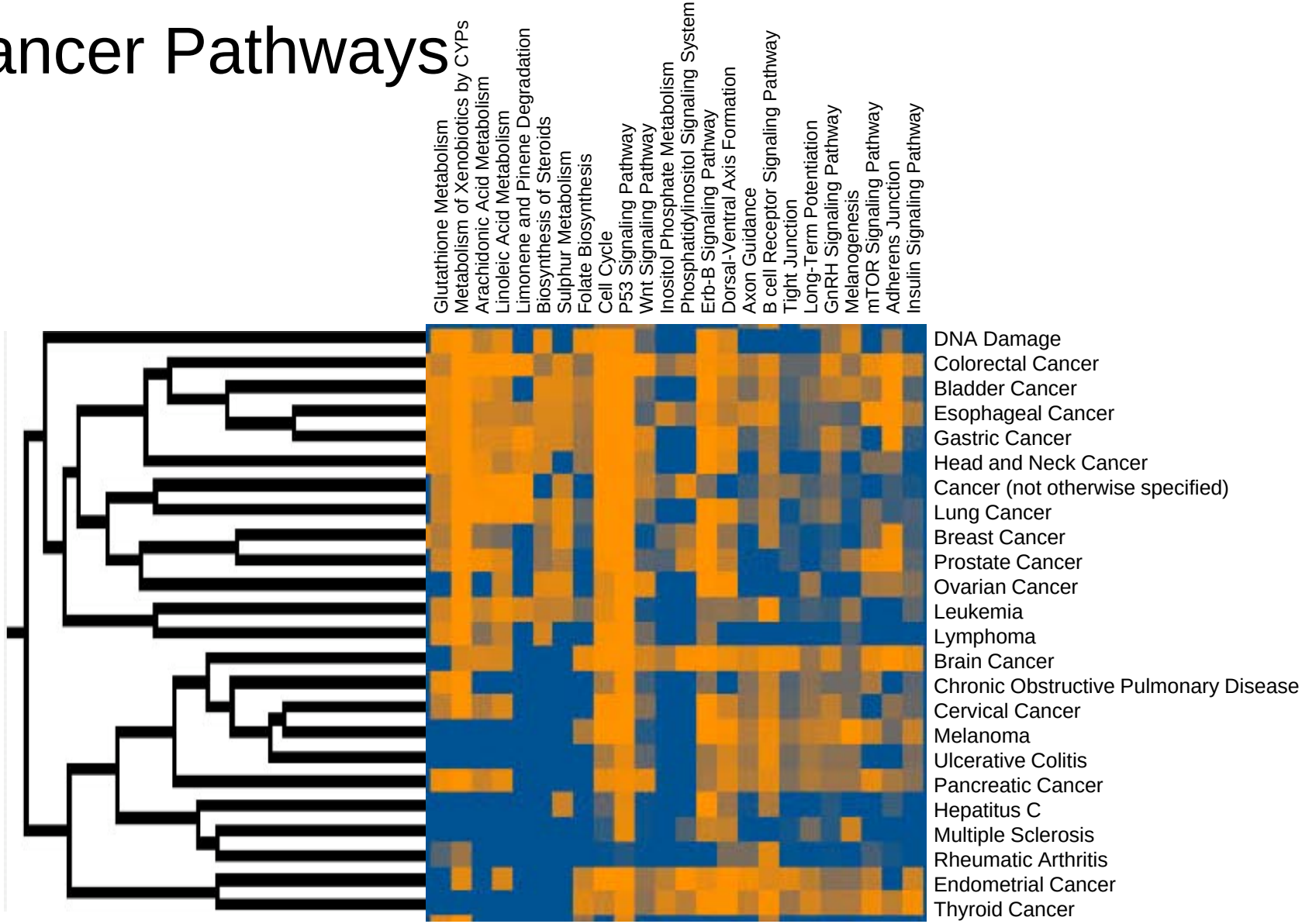
Cancer Pathways



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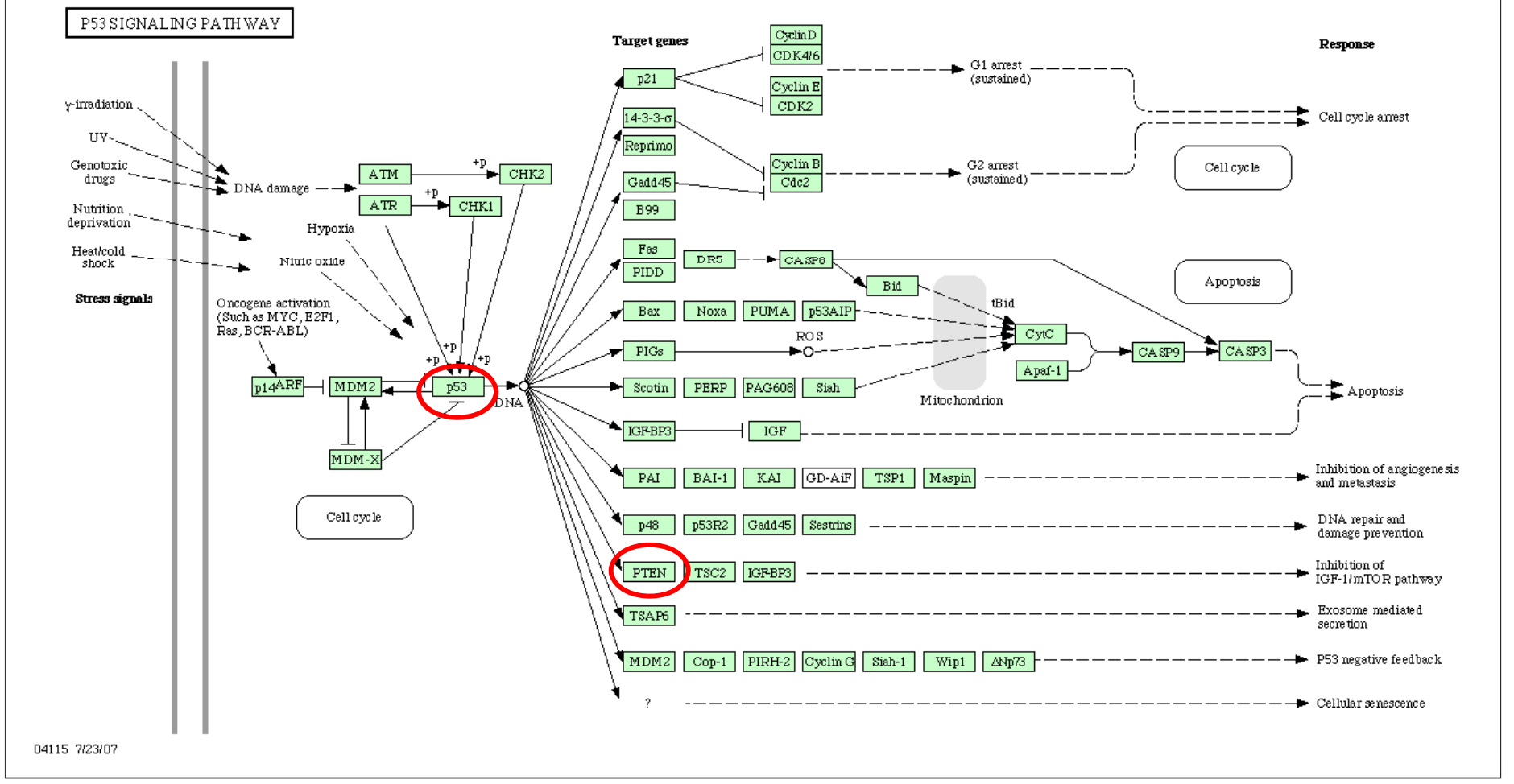
A Subway Map of Cancer Pathways Hahn and Weinberg, Nature Reviews, 2002



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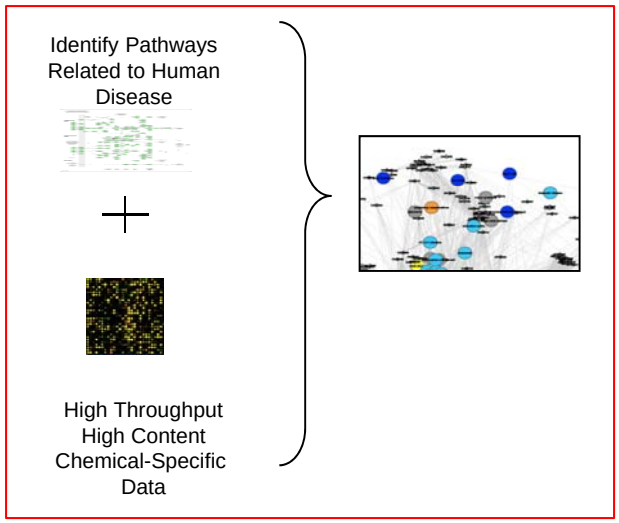
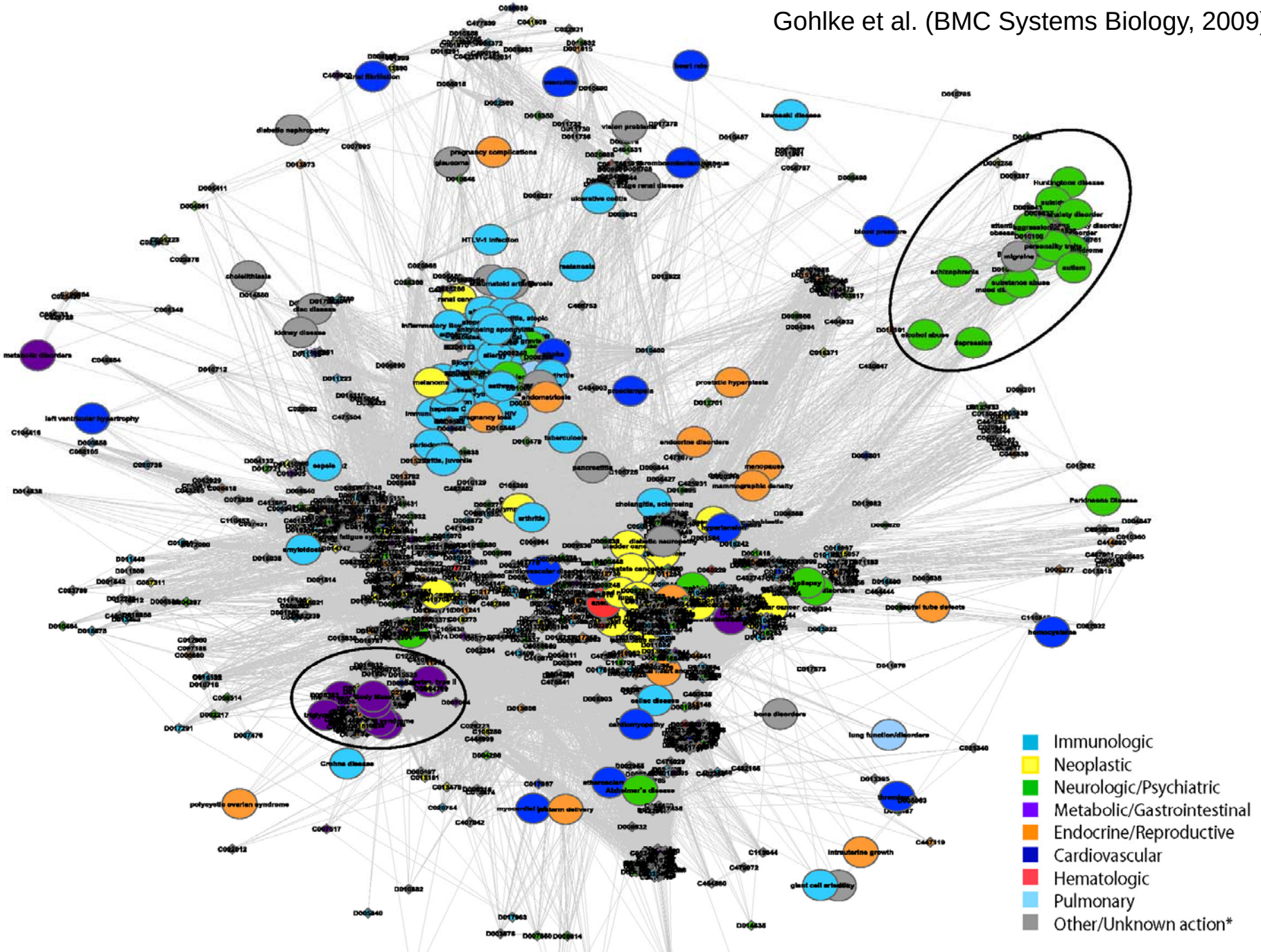
Comparative Toxicogenomics Database
http://ctd.mdibl.org/

- 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.



Mattingly et al. 2006. Toxicol Sci. 92(2):587-95.

Gohlke et al. (BMC Systems Biology, 2009)



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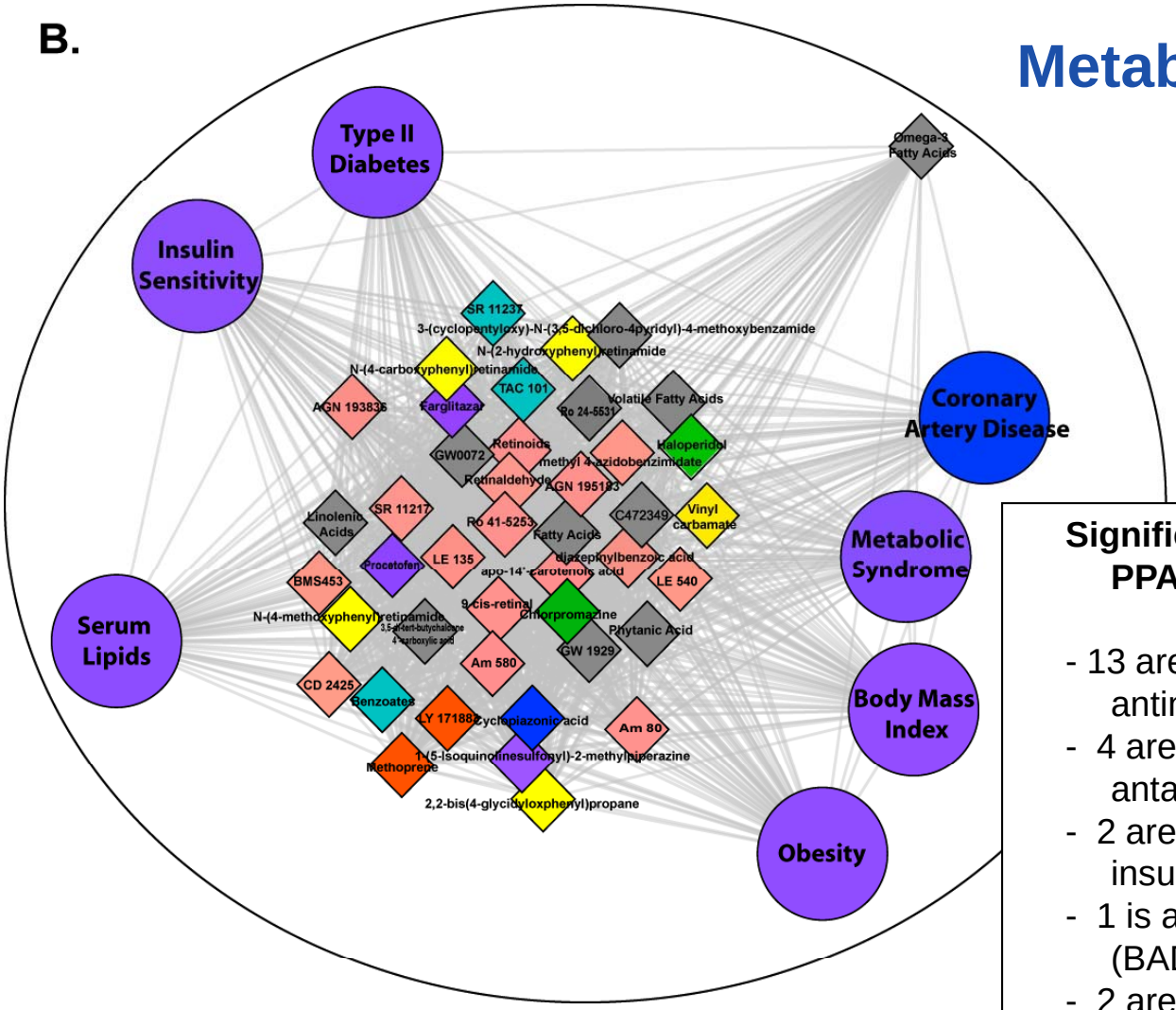
Metabolic Syndrome Cluster



Significance in Adipocytokine and PPAR signaling define this cluster:

- 13 are retinoids (including 4 antineoplastic agents).
- 4 are known PPAR alpha/gamma antagonist/agonists:
- 2 are used to treat hyperlipedemia and insulin resistence
- 1 is a Bisphenol A derived epoxy (BADGE)
- 2 are anti-psychotics known to cause weight gain
- Essential omega 3 fatty acids

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Neurologic Disease Cluster

- Phenotypes
  - Aggression
  - Alcohol Abuse
  - Anxiety Disorder
  - Attention Deficit Disorder
  - Autism
  - Bipolar Affective Disorder
  - Depression
  - Huntington's Disease
  - Migraine
  - Mood Disorders
  - Obsessive Compulsive Disease
  - Personality Traits
  - Schizophrenia
  - Substance Abuse
  - Suicide
  - Tourette Syndrome
- Exposures
  - D 21266 (amine)
  - Plicamycin
  - Nafenopin
  - Naphthyridines
  - Nordihydroguaiaretic Acid
  - Nystatin
  - Organophosphorus Compounds
  - Oxygen
  - Pentazocine
  - Pentobarbital
  - Pilocarpine

Gohlke et al. (BMC Systems Biology, 2009)

Screening new chemicals for activity

- Pure prediction from a computational model
- Screening using HTS, geneomics and a variety of other tools
  - Look to see what it predicts for activity from a model like ours
  - Look to see what chemicals have the same activity and use a toxic equivalency factors approach

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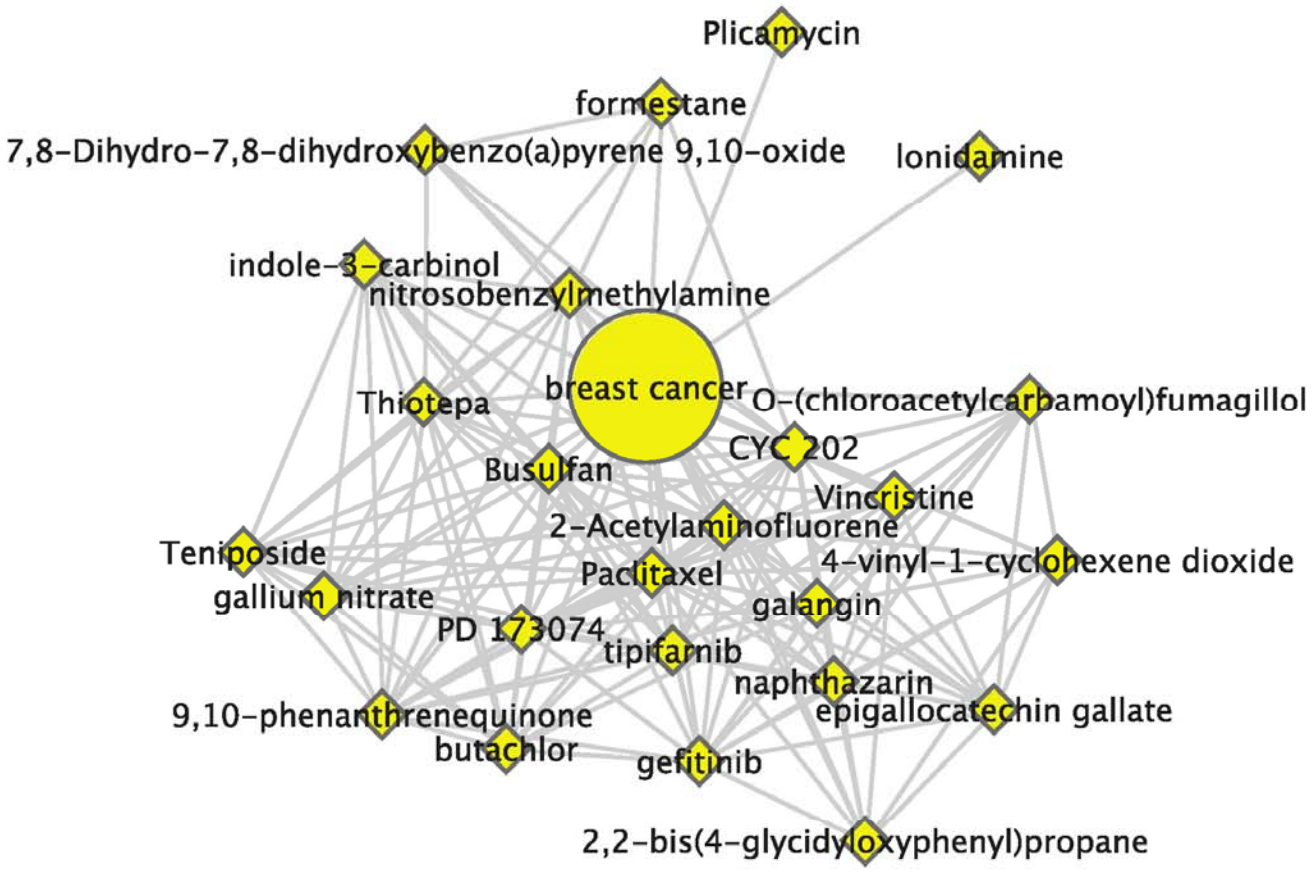
Chemicals linked to breast cancer



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Enriched Human Susceptibility Pathways
Breast Cancer

KEGG Pathway ID	*Pathway description*	*SEPEA_NT3*	*SEPEA_NT3**
path:hsa04370	vegF signalling pathway	1.69E-04	5.14E-04
path:hsa04662	B-cell receptor signalling	3.32E-04	3.51E-04
path:hsa04630	Jak Stat signalling	8.91E-04	0.0417
path:hsa04520	Adherens junction	0.0014	0.1438
path:hsa04810	Regulation of actin cytoskeleton	0.0027	0.0717
path:hsa04150	mTOR signalling	0.0047	0.0052
path:hsa04664	Fc epsilon RI signalling	0.0081	5.99E-04
path:hsa04510	Focal adhesion	0.0103	0.0648
path:hsa04012	ErbB signalling	0.0103	8.51E-04
path:hsa04210	Apoptosis	0.0108	7.97E-04
path:hsa03440	Homologous recombination	0.0147	0.0016
path:hsa04660	T cell receptor signalling	0.0182	0.001
path:hsa04010	MAPK signalling	0.0183	0.0183
path:hsa04910	insulin signalling	0.0191	0.0032
path:hsa04514	Cell adhesion molecules	0.0274	0.2407
path:hsa04115	P53 signalling	0.0306	0.0093
path:hsa04620	Toll-like receptor signalling pathway	0.0391	0.0193

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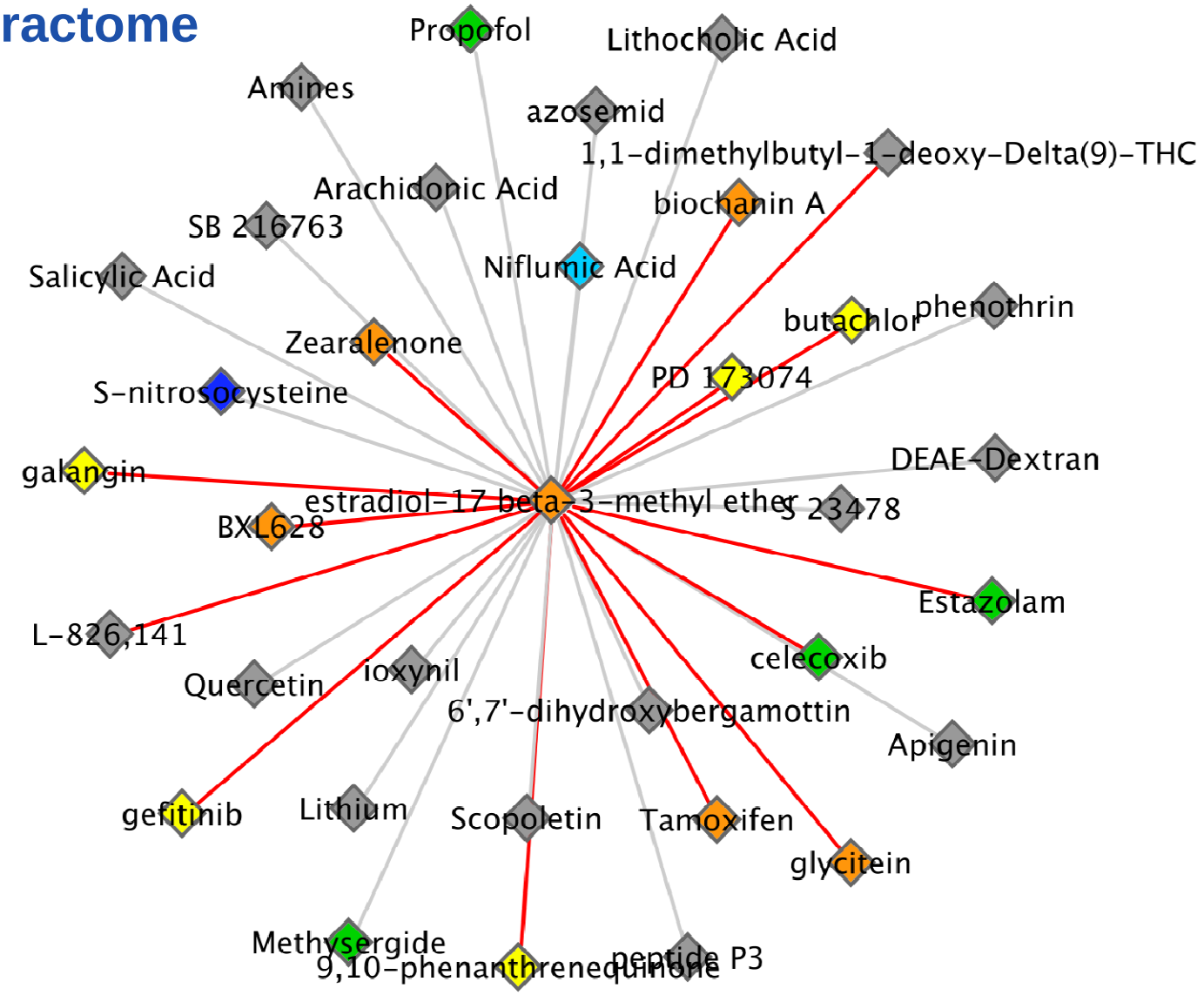
Estradiol Interactome



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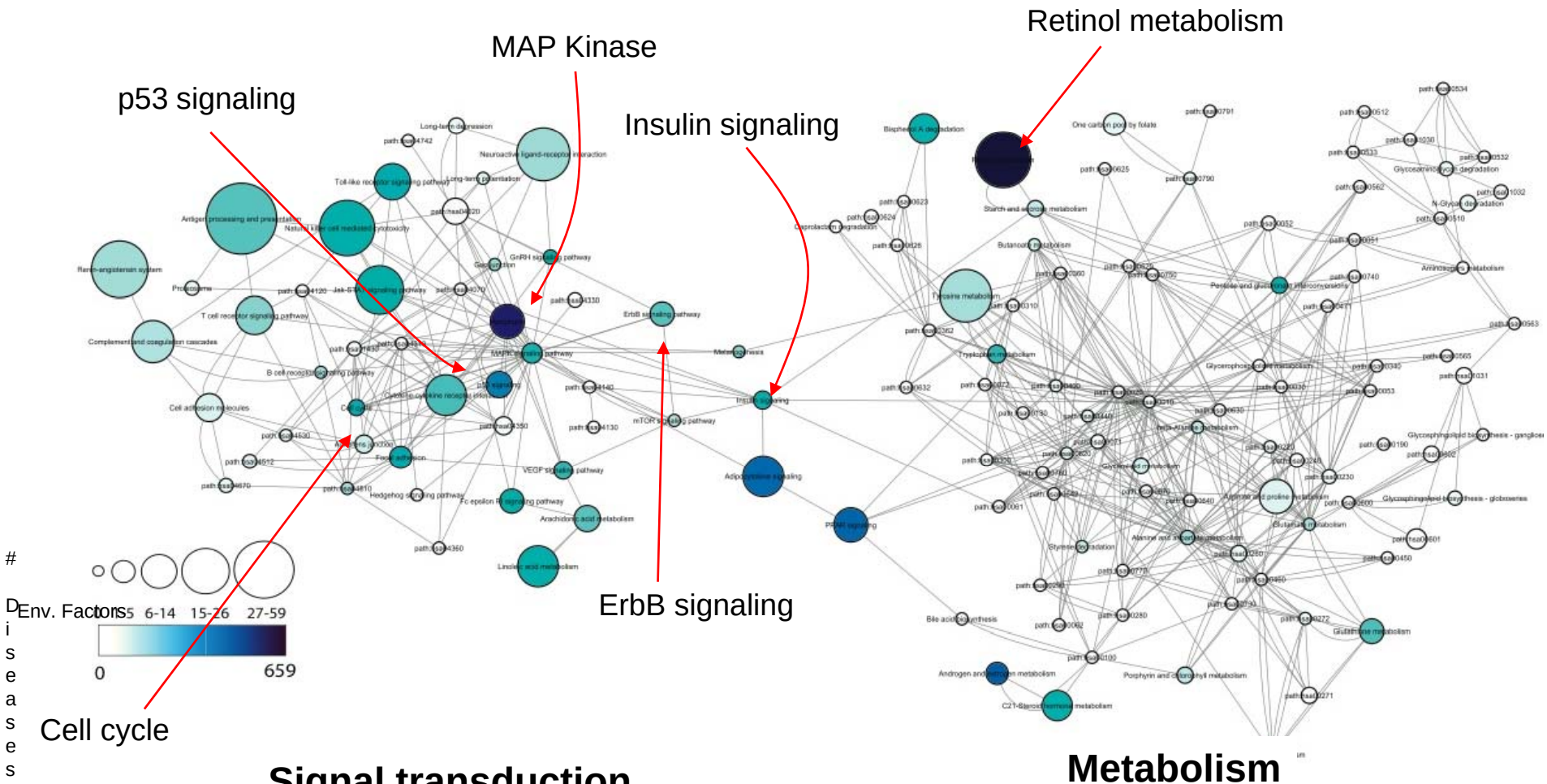
Identifying and Screening Pathways



Signal transduction

Avg. of 16 diseases associated with each signaling pathway

Metabolism

Avg. of 4 diseases associated with each metabolic pathway

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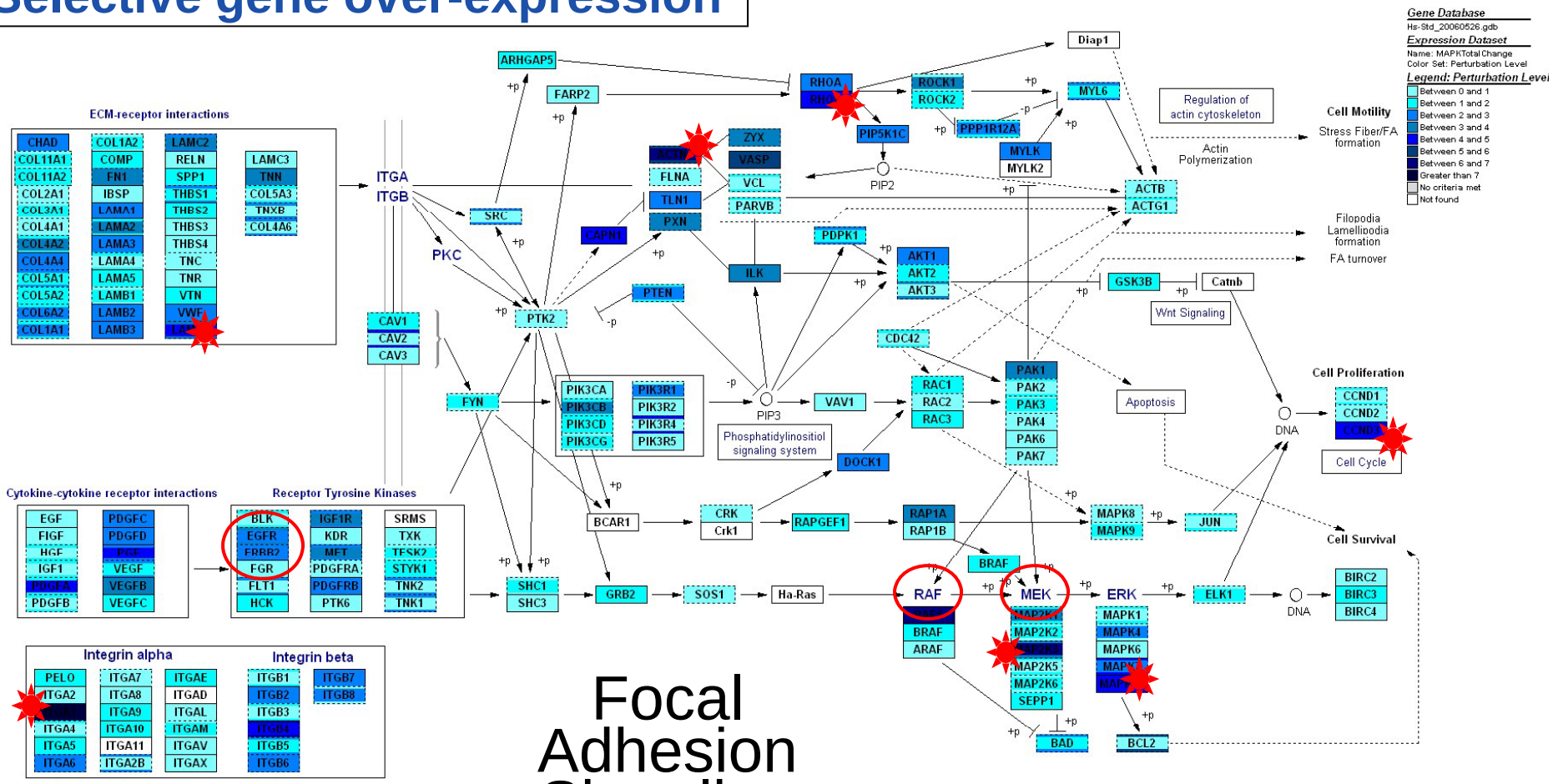
Finding targets for screening
Selective gene over-expression



Focal Adhesion Signaling

Creighton et al., Cancer Research, 2006

Commercial Tools: GeneGo (an example)

- Chemical Reports
  - Major Metabolites
  - 76 QSAR predictions
    - Protein binding
    - Cancer
    - Alzheimer's
    - Etc
  - Compounds predicted to have similar activity
    - For both parent and metabolites
  - Possible cellulr (receptors, proteins, etc.) targets
    - Both parent and metabolites
  - Functional pathways likely to be targeted

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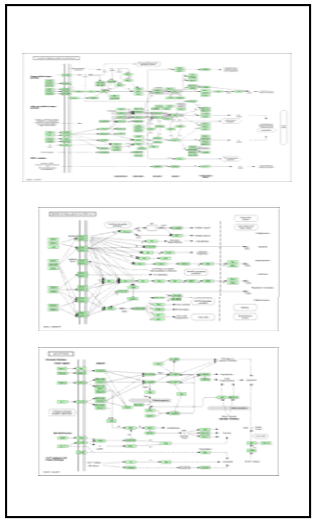
Identifying Important Disease Pathways

Short Term Assay in Rodents + 'omics

+



Sets of Pathways

Identify Pathways Related to Rodent Short-term Response

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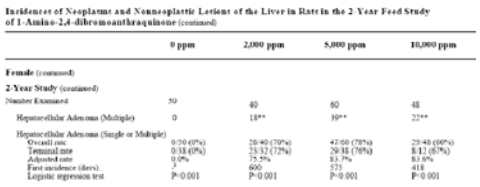
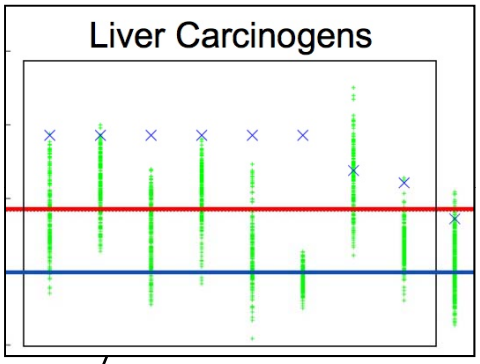


Incidence of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the 2-Year Feed Study of 1-Amino-2,4-dibromoanthraquinone (continued)

	0 ppm	2,000 ppm	5,000 ppm	10,000 ppm
Female (continued)				
2-Year Study (continued)				
Number Examined	50	40	50	48
Hepatocellular Adenoma (Multiple)	0	18**	39**	22**
Hepatocellular Adenoma (Single or Multiple)	0/50 (0%)	28/40 (70%)	47/50 (70%)	29/48 (60%)
Overall rate	0/33 (0%)	23/32 (72%)	29/38 (76%)	8/13 (67%)
Terminal rate	0.0%	75.7%	83.7%	83.6%
Adjusted rate	1	600	575	418
First incidence (days)	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression test				

Cancer Response in Chronic Bioassay

Short-Term to Long-Term Prediction Model



Predict Chronic Exposure Response

Statistic for Testing

Dose-Response

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Microarray data from B6C3F$_1$ mice following 90 day exposure

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

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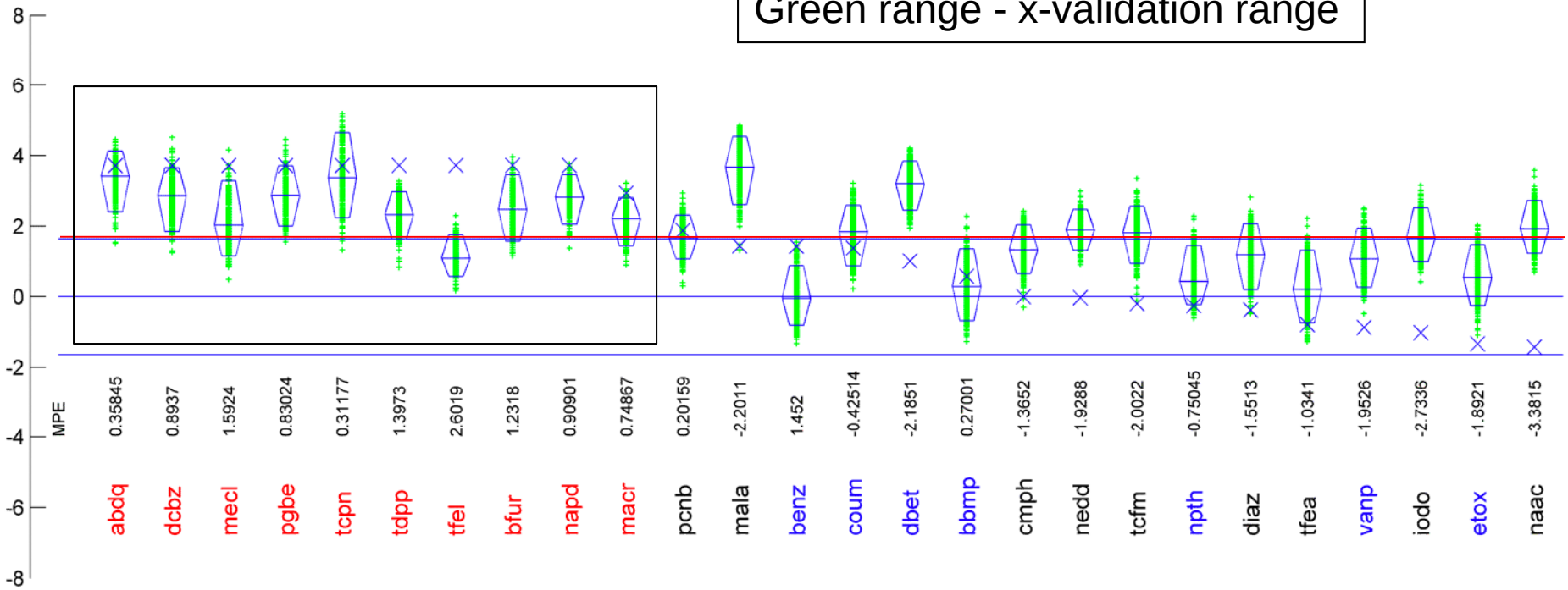
Predicted Liver Cancer from Microarray Data

Liver Carcinogens

Above red line - clearly positive
Below blue line - clearly negative
X - actual data prediction
Green range - x-validation range



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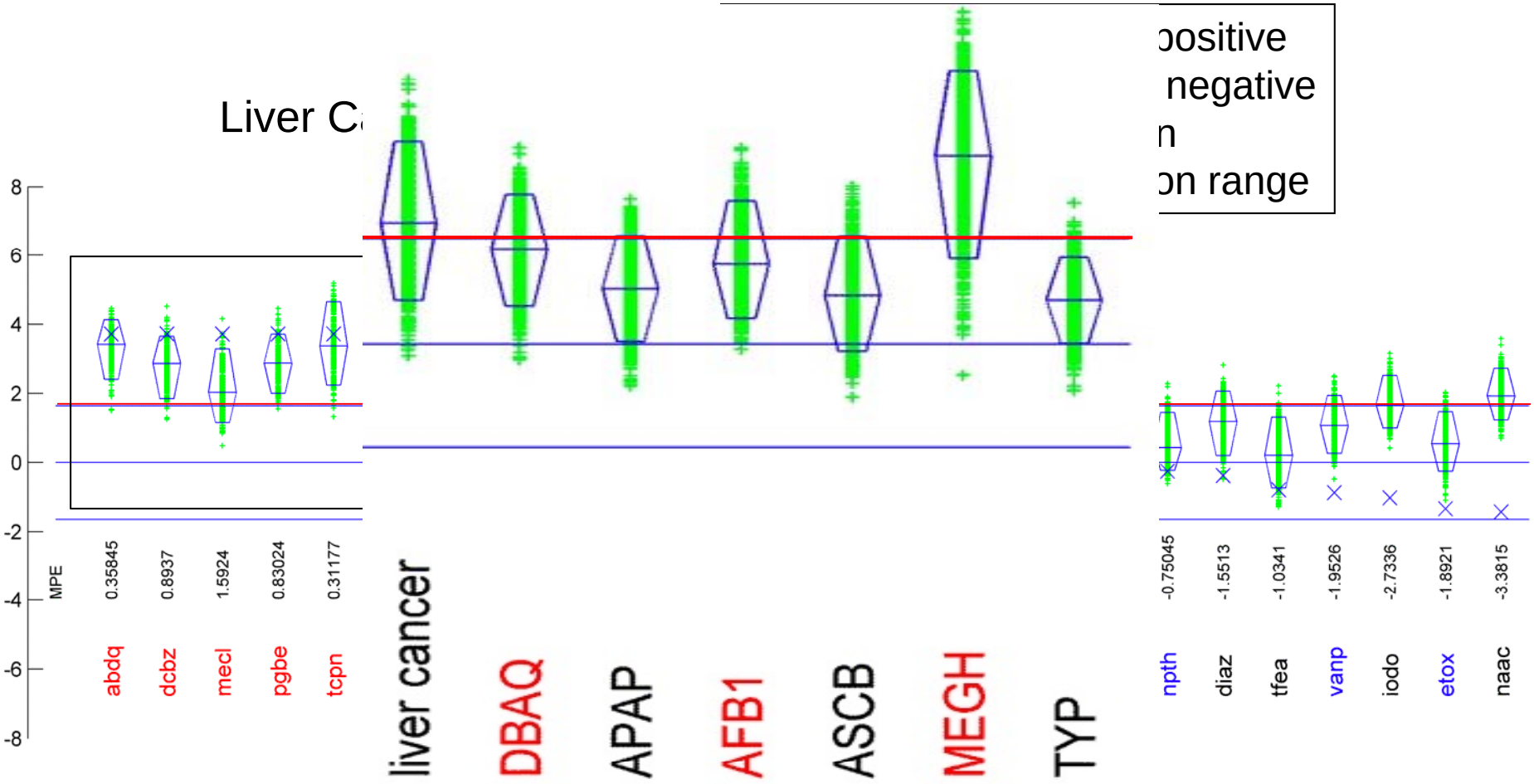
Predicted Liver Cancer from Microarray Data



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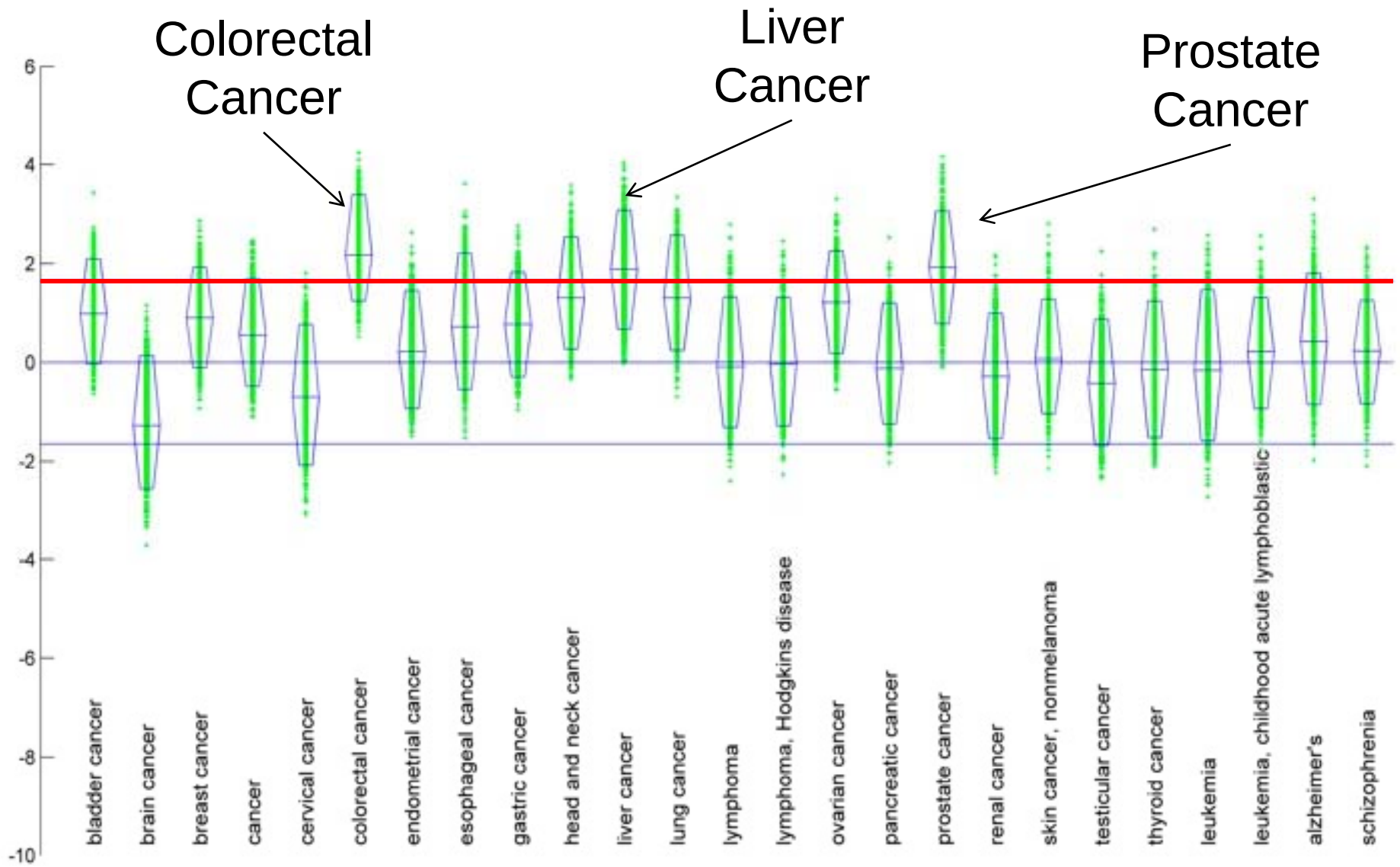

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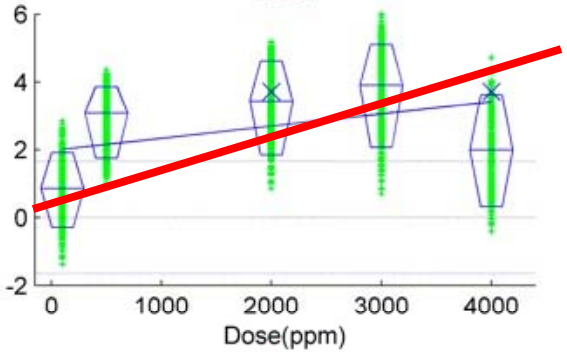
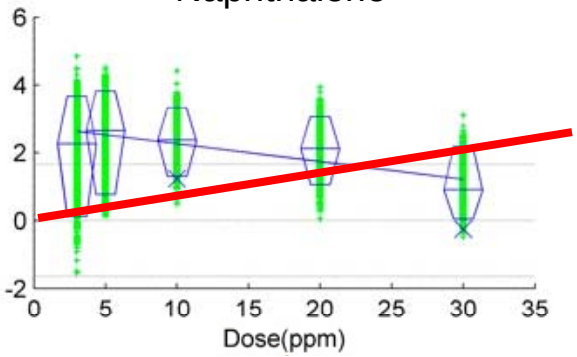
Evaluating Dose-Response Patterns

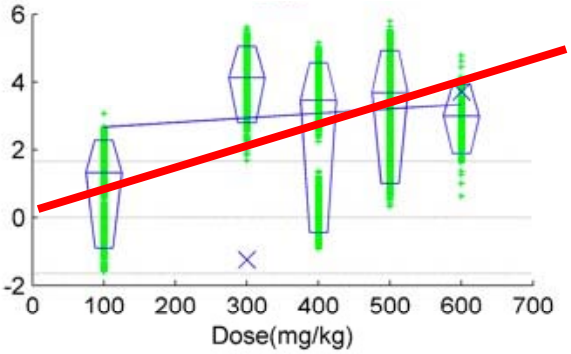
Methylene Chloride



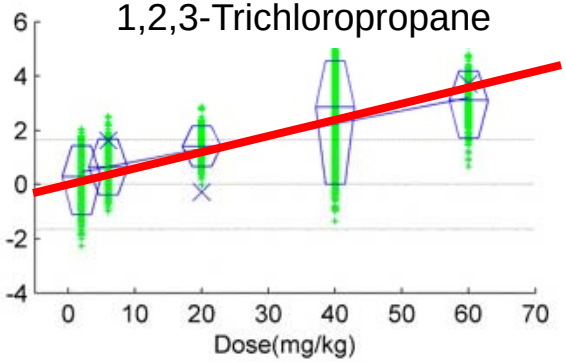
Naphthalene



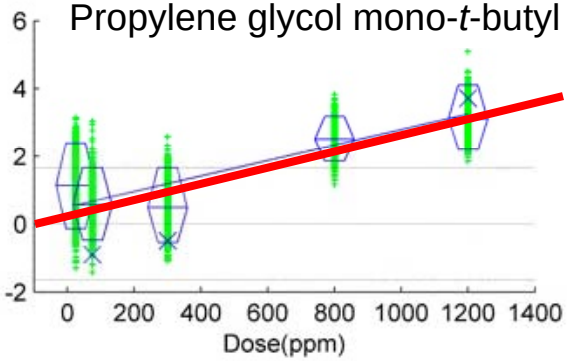
1,4-Dichlorobenzene



1,2,3-Trichloropropane



Propylene glycol mono-*t*-butyl ether



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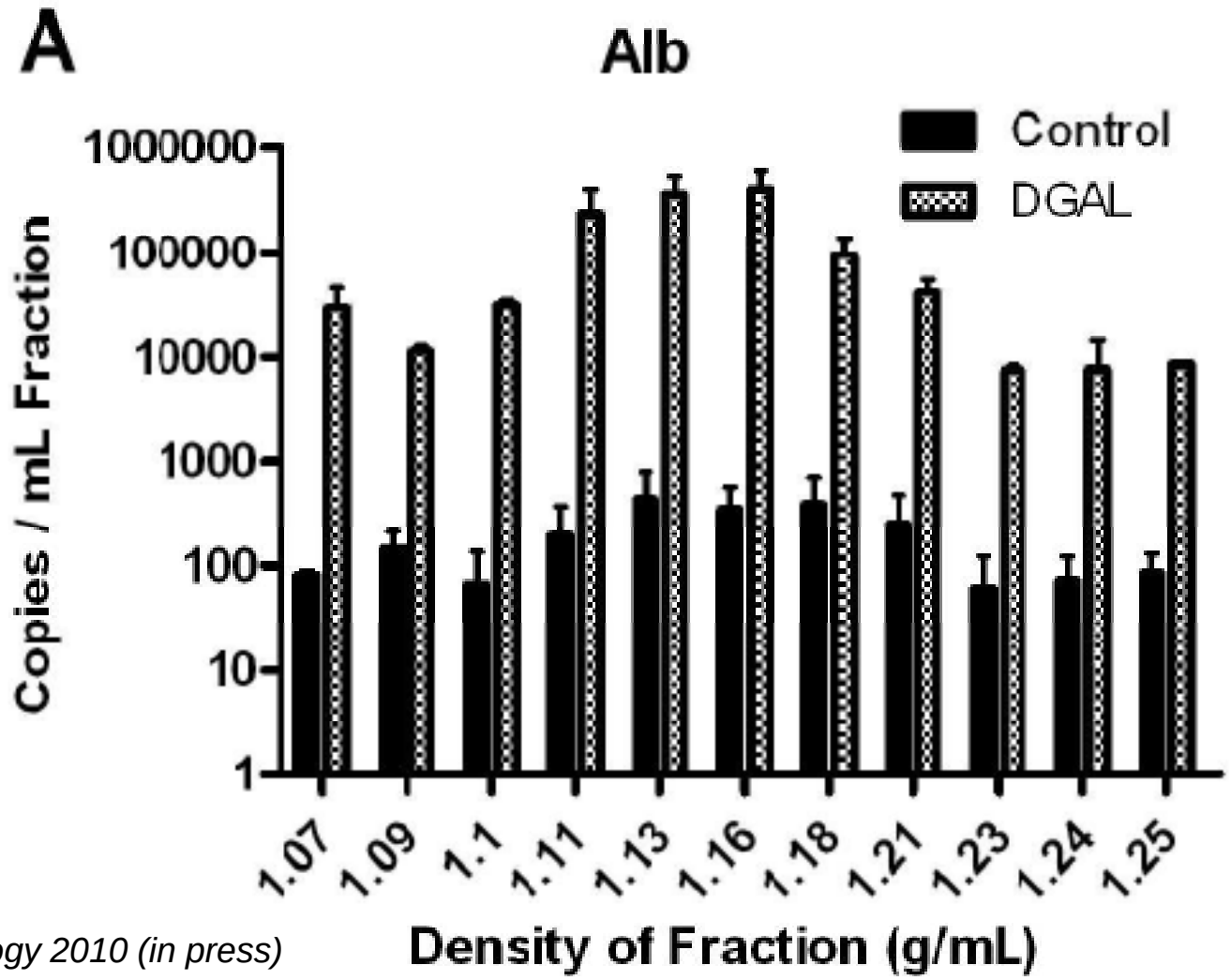
New types of biomarkers in blood: tissue-specific relase of mRNA into blood



Wetmore et al., Hepatology 2010 (in press)

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Issues to Consider

- Predictions are model dependent
  - Do we have a process for model review?
- Broaden the use of human data and human approaches in toxicology
  - Genetics
  - Epidemiology
  - Gene expression profiling
  - GWAS studies
- Validation?
  - Decisions are context dependent and data dependent
  - Is it a question of replacing an assay or not needing one because of other information?
- Scalable Approaches
  - Improved systems biology, improved human genetics, genomics, disease linkage, epidemiology data, etc.

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Advantages and Disadvantages to improving toxicity testing

- More compounds, more compounds, more compounds
- Focus intense testing on chemicals likely to be most important
- Screen new commercial formulations (e.g. green chemistry) before investing in a technology
- Dataset for risk assessment will be increasingly complex…
  - Need a complex measure for deciding if something is a hazard
  - NOEL's are unlikely to be useful in setting standards
  - Focus may finally come to complex exposures
  - Dose-response in itself will be complex
  - Societal decisions on whether a pattern of activity is adverse will be difficult
- Lots of public concern caused by misapplying models to product formulations