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Toxicogenomics: The Use of 'Omics' to Better Understand the Impact of Adverse Effects from Environmental Exposures on Human Health

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The mission of the National Center for Toxicogenomics of the National Institute of Environmental Health Sciences of the U.S. National Institutes of Health is to utilize genomic approaches to investigate environmental effects on the etiology and progression of injury and disease processes. Thus a key goal is to design and conduct seminal studies that provide definition to and stimulate development of the field of toxicogenomics, integrating global "omics" approaches into conventional studies of toxicity and disease processes. Toxicogenomics, as defined by the NCT, combines genetics, genome-wide mRNA expression analysis (transcriptomics), cell and tissue-wide protein expression analysis (proteomics), and metabolite profiling (metabolomics) with conventional biology, physiology, pathophysiology and toxicology in an effort to understand adverse affects of gene-environment interactions on human health. Core to the NCT research strategy is the concept of phenotypic anchoring in which studies are designed to relate specific alterations in gene expression to specific adverse effects of environmental stresses defined by conventional parameters of toxicity and pathology such as clinical chemistry, histopathology, etc. To accomplish this task, studies have been designed that utilize a variety of agents that elicit a similar adverse response at a variety of doses and a variety of times of treatment that would elicit the full range of biological responses to those agents. In addition, attempts have been made to incorporate exposures to related but non-adverse agents when possible and to analyze biological responses in additional tissues that do not seem to suffer the same adverse effect (non-target tissue). When analysis of the experimental results implicates a critical role of a particular biological process or a critical role of a particular gene in the response, additional experiments are designed to test those hypotheses concerning their roles. Studies therefore are designed both to gain insight into mechanisms of injury and disease initiation and progression, and to establish signatures of adverse effects, linking gene expression alterations to specific parameters of well-defined indices of injury and disease to develop putative biomarkers. One aspect of this is to develop biomarkers that are reflective of incipient injury or disease before the culmination of severe injury or disease in order to develop true "predictive toxicology" through the use of toxicogenomics. Our strategy to accomplish this is to build a compendium of signatures linked to environmentally important patho-biological endpoints. In order to learn more about processes involved in acute liver injury and to investigate the true power of genomics to provide insight into mechanisms of injury as well as to provide profiles of processes of injury, we selected a single agent to focus our research efforts initially. Acetaminophen (APAP) was selected as an appropriate model hepatotoxicant due to the well-defined adverse phenotypic endpoints in the liver following toxic exposures, the similarities in metabolism between rodent and human and the relevance of exposure to humans. Studies were designed to test several hypotheses including whether genomic analyses of the liver could reveal indicators of incipient injury before that injury was manifested in such a severe manner as to be detected by traditional indices of liver injury. We were able to demonstrate that gene expression analysis can yield signatures of incipient toxicity after exposure to sub-toxic doses of the toxicant of interest (Heinloth et al., Toxicol. Sci., 80:193ff, 2004). In this manner, the NCT program is striving to integrate conventional biology, genetics, pathology and toxicology with emerging "omics" technologies in order to develop useful insights and potential biomarkers to aid in improving human health.

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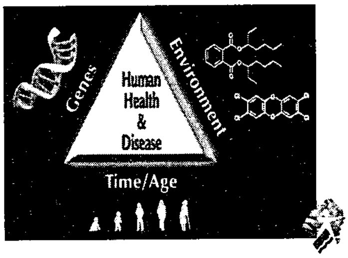


"Environment"

- Industrial chemicals
- Agricultural chemicals
- Physical agents (e.g., UV, IR)
- By-products of combustion and industrial processes (e.g., dioxin)
- Foods and nutrients
- Pharmaceuticals
- Lifestyle choices and substance abuse
- Social and economic factors
- Biological agents

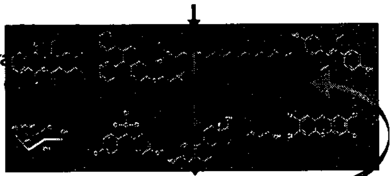
Problems in Human Environmental Health Risk Assessment

- Susceptibility (one-size-fits-all)
- Extrapolation from animal models to human
- Exposure is now measured using indirect surrogates
- Intrinsic toxicity is not known for most of the chemical agents in the environment
- Paucity of knowledge of mechanisms
- Use of default assumptions

Environment

Signals

Tr



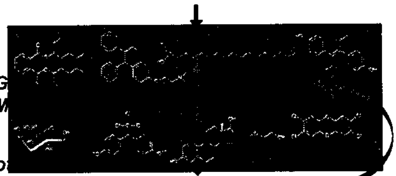
Protein Function
(Post-Translational Modifications)

Altered Phenotype

Environment

Signals

G
M



Pro
Metabonomics

Protein Function
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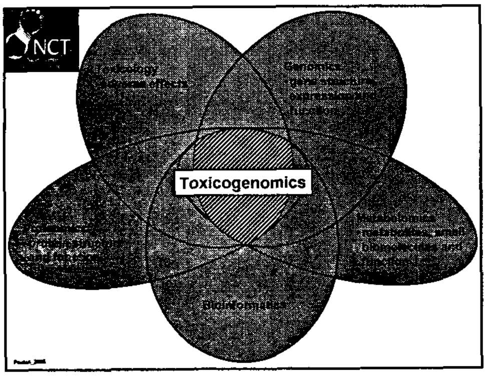
Altered Phenotype



September 2000
Ken Olden
Sam Wilson

Ray Tennant

NCT
National Center
for Toxicogenomics

Research Opportunities for Toxicogenomics

- Genomic technologies provide an unprecedented opportunity to address highly intractable problems of toxicology and environmental health
- Assess the objective value of surrogate models for prediction of human health risk
- Identify biomarkers of incipient adverse effects
- Harness the results of diverse research efforts for the collective benefit
- Provide a rational basis for risk assessment
- Facilitate the identification of specific susceptibility polymorphisms and relate them to environmental diseases

Research Challenges of Toxicogenomics

- Difficulty in analysis of high density data
- Difficulty in integration of data obtained by different technologies
- Difficulty in linking "omics" data to specific adverse effects (phenotypic anchoring)
- Difficulty in translating statistical assessments into biological understanding
- Limitations of incomplete functional annotation of genome data bases
- Incomplete knowledge of functional pathways and networks, particularly trans-genome relationships
- Inadequate accessible data sources for informatic analysis

Fundamental Hypotheses

- Analyzing global gene expression changes will provide new insight into the mechanisms underlying adverse effects.
- Profiling global gene expression changes will provide signatures that will be highly correlative with and predictive of incipient adverse health effects from environmental stresses.

Main Objectives for the Development of Toxicogenomics

- **Identify and understand mechanisms of toxicity - *Discovery Toxicology***
- **Identification of biomarkers of toxicity - *Predictive Toxicology***
- **Development of Knowledge-Base**

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Predictive Toxicogenomics Goals

- Develop Signature Patterns of Exposure from Toxicological Gene Expression Data Sets for use in Classifying Compounds
- Define Gene Expression Signatures Patterns of Toxicant-Induced Adverse Effects

Successful Application of Transcriptomics for Compound / Effect Classification

- Burczynski, ME, *et al.*, (2000) Toxicological Sciences. 58(2):399-415.
- Bartosiewicz, M, *et al.*, (2001) Environmental Health Perspectives. 109(1):71-4.
- Huang, Q, *et al.*, (2001) Toxicological Sciences. 63(2):196-207.
- Hughes, TR, *et al.*, (2000) Cell. 102(1):109-26.
- Hughes, TR, et al., (2001) Nature Biotechnology. 19(4):342-7.
- Hamadeh, HK, *et al.*, (2002) Toxicologic Pathology. 30(4):470-82.
- Hamadeh, HK, *et al.*, (2002) Toxicological Sciences. 67(2):232-40.
- Hamadeh, HK, *et al.*, (2002) Toxicological Sciences. 67(2):219-31.
- Steiner, G, *et al.*, (2004) Environmental Health Perspectives. 112(12):1236-48.
- Amin, RP, *et al.*, (2004) Environmental Health Perspectives. 112(4):465-79.
- Heinloth, AN, *et al.*, (2004) Toxicological Sciences. 80(1):193-202.
- Moggs, JG, *et al.*, (2004) Environmental Health Perspectives. 112(16):1589-606.
- Iconix (http://www.iconixpharm.com/)
- GeneLogic (http://www.genelogic.com/)

***Hamadeh, et al.*, Gene expression analysis reveals chemical-specific profiles. 2002. *Toxicological Sciences*, 67, 219-231.**

Peroxisome Proliferators
(Wyeth-14,643, Clofibrate, & Gemfibrozil)

Phenobarbital

d-Mannitol

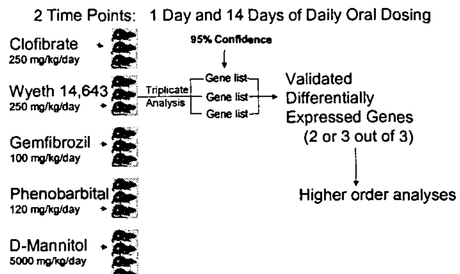
NIEHS / Boehringer-Ingelheim

***Hamadeh, et al.*, Prediction of compound signature using high density gene expression profiling. 2002. *Toxicological Sciences*, 67, 232-240.**

- RNA samples derived from individual livers of chemically exposed rats were sent to NIEHS with identity blinded
- Multiple bioinformatics tools were employed to classify compounds using previously derived data

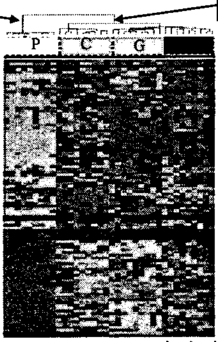
NIEHS / Boehringer-Ingelheim

Experimental Design



Hisham Hamadeh, NIEHS / Kerry Blanchard, BIPI

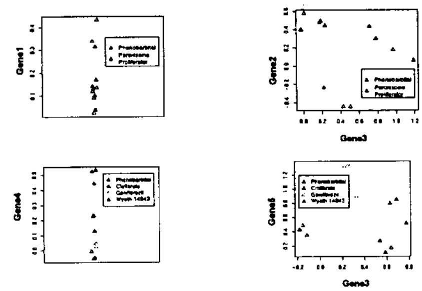
Genetic Algorithm coupled with K-Nearest Neighbor (GA-KNN) identifies top Discriminative or Informative genes (24 hr/ top 100 genes) and separates animal gene expression profiles by chemical class

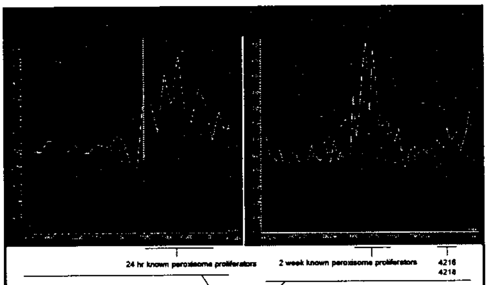


Node 1: P - Phenobarbital
Node 2: C - Clofibrate
G - Gemfibrozil
W - Wyeth 14,643

Leping Li, NIEHS

Single Gene ANOVA **Linear Discriminant Analysis**





None of the 24 hr blinded samples are Peroxisome Proliferators
4216 and 4218 are the only Peroxisome Proliferators (Wyeth-like) at 2 weeks
630, 632, 634, 690, 692, 694, 3462, 3463, 3466, 3468, 1838, 876 - Not Peroxisome Proliferators

Hisham Hamadeh, NIEHS

Blinded Samples: Predictions

Sample	Prediction	Time (days)
616	Phenobarbital-like	1
618	Phenobarbital-like	1
672	Phenobarbital-like	14
674	Phenobarbital-like	14
676	Phenobarbital-like	14
678	Phenobarbital-like, Weak	14
688	Phenobarbital-like, Weak	14
270	Clofibrate/Wyeth-like	3
272	Clofibrate/Wyeth-like	3
274	Clofibrate/Wyeth-like	3
276	Clofibrate/Wyeth-like	3
4216	Wyeth-like	14
4218	Wyeth-like	14
10 Others	Not Phenobarbital-like	1, 3, 14

Hisham Hamadeh, NIEHS

Blinded Samples: 22 / 23 Correctly Identified

Sample	Prediction	Actual		Time (days)
616	Phenobarbital-like	High Dose Phenytoin	✓	1
618	Phenobarbital-like	High Dose Phenytoin	✓	1
672	Phenobarbital-like	High Dose Phenytoin	✓	14
674	Phenobarbital-like	High Dose Phenytoin	✓	14
676	Phenobarbital-like	High Dose Phenytoin	✓	14
678	Phenobarbital-like, Weak	High Dose Phenytoin	✓	14
270	Clofibrate/Wyeth-like	High Dose DEHP	✓	3
272	Clofibrate/Wyeth-like	High Dose DEHP	✓	3
274	Clofibrate/Wyeth-like	High Dose DEHP	✓	3
276	Clofibrate/Wyeth-like	High Dose DEHP	✓	3
4216	Wyeth-like	High Dose DEHP	✓	14
4218	Wyeth-like	High Dose DEHP	✓	14
688	Phenobarbital-like, Weak	High Dose Hexobarbital		14
10 Others	Not Phenobarbital-like	Hexobarbital, Low DEHP, Controls	✓	1, 3, 14

Hisham Hamadeh, NIEHS

Limitations of Compound Classification

- Are all members of a "class" equivalent hazards?
- What to do with compounds that belong to more than one "class"?
- What to do with novel compounds?
- What to do with mixtures of compounds?

Predictive Toxicogenomics Goals

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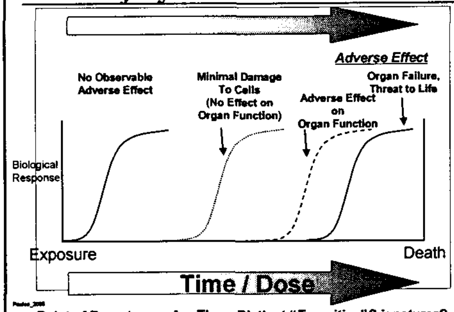
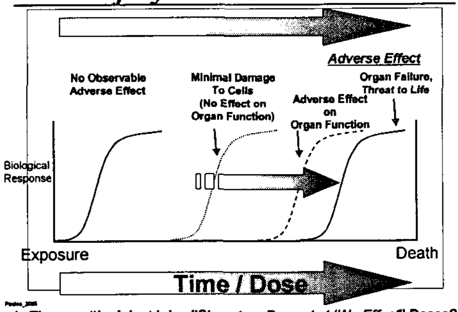
Injury / Disease Process



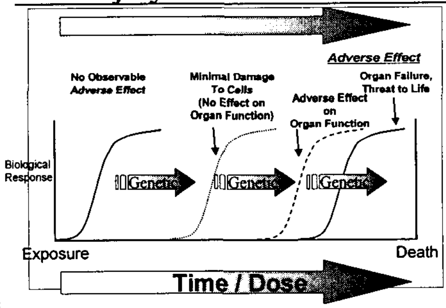
Point of Departure - Are There Distinct *"Transition"* Signatures?

Injury / Disease Process

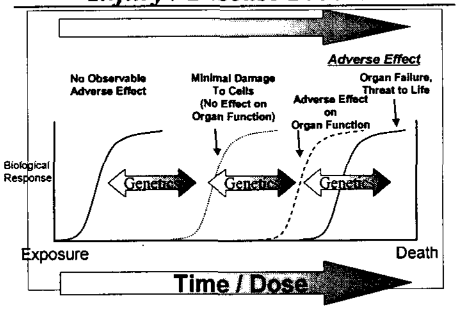


Is There an *"Incipient Injury"* Signature Present at *"No Effect"* Doses?

Injury / Disease Process



Injury / Disease Process



Phenotypic Anchoring of Expression Profiles

Hamadeh, et al., Methapyrilene toxicity: anchorage of pathologic observations to gene expression alterations. 2002. *Toxicologic Pathology* **30**, 470-482.

NIEHS / Boehringer-Ingelheim

Heinloth, et al., Gene expression profiling of rat livers reveals indicators of potential adverse effects. 2004. *Toxicological Sciences*, **80**, 193-202.

NIEHS ToxPath Team

Prototype: ACETAMINOPHEN

- Human and rodent hepatotoxicant well-studied mechanism
- Significant human exposure
- Human dose range from pharmacological to lethal dose
- 56,000 Emergency room admissions (FDA; 2002)
- > 500 life-threatening exposures with 100 fatalities per year
- Clinical chemistry poor prognosticator of survival
- Rodents and humans metabolize acetaminophen similarly

Prototype: ACETAMINOPHEN
Hypotheses

1. A gene expression signature can be identified that will allow for discrimination between **mild** and **severe** liver injury from APAP exposure in rats.

   1.1. A gene expression signature can be identified that reveals **incipient liver injury** that manifests itself only at higher doses and/or at later times.

2. A gene expression signature can be identified in **rat blood** (similar or dissimilar to that in liver) that will allow for discrimination between mild and severe liver injury from APAP exposure.

3. A gene expression signature can be identified in **human blood** that will be similar to that identified in rat blood that will allow discrimination between mild injury, severe injury and irreversible liver failure following APAP intoxication.

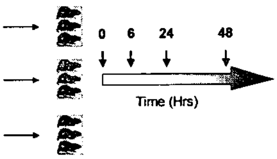
Experimental Design

3 Time Points: 6, 24, 48 Hrs following Single Oral Dosing

Low
50 mg/kg/day

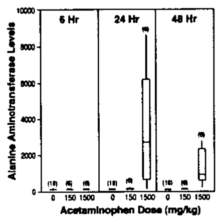
PreToxic
150 mg/kg/day

Toxic, Recoverable
1500 mg/kg/day

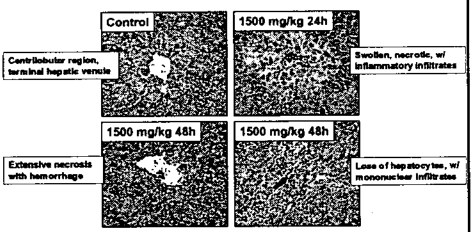


NIEHS ToxPath Team

Alanine Aminotransferase

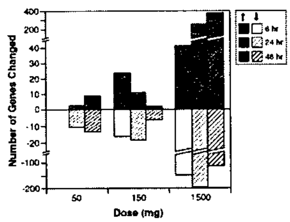


Histopathological Lesions



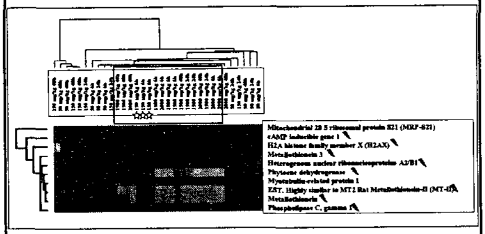
Differentially Expressed Genes

Genes and EST's Differentially Expressed by Dose and Time



Early Indicators of Adverse Effects

Implicated in Oxidative Stress or DNA Damage Responses:

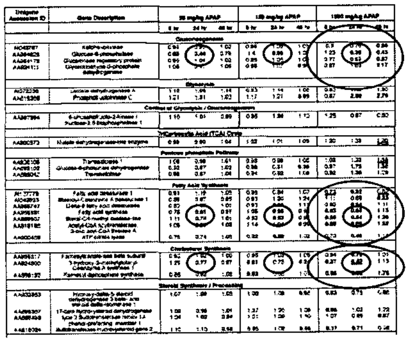


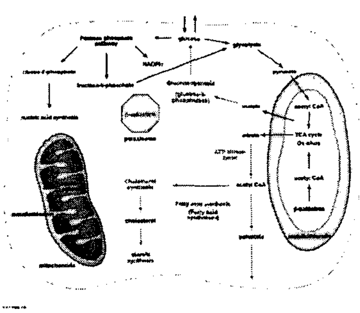
Cholesterol Synthesis

Acetyl-CoA
+
Aceto-Acetyl-CoA
↓ **3-Hydroxymethyl-Glutaryl-CoA-Synthase**
S-3-Hydroxy-3-Methyl-Glutaryl-CoA
↓
Geranylpyrophosphate
↓ **Farnesyl-diphosphate synthase**
Farnesyl Diphosphate
↓ **Farnesyl-diphosphate Farnesyl-transferase**
Squalene

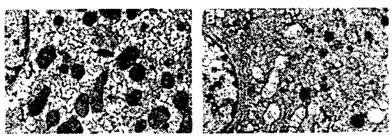
Glycolysis

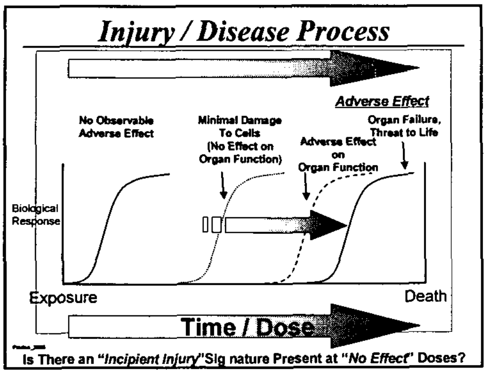
Glucose
↓
Glucose-6-P
↓
Fructose-6-P ⟷ Fructose-2,6-P_2 (**6-PhosphoFructo-2-Kinase / Fructose-2,6 Bisphosphatase**)
↓ **Phospho-FructoKinase C**
Fructose-1,6-P
↓
Pyruvate
↓ **Lactate Dehydrogenase**
Lactate

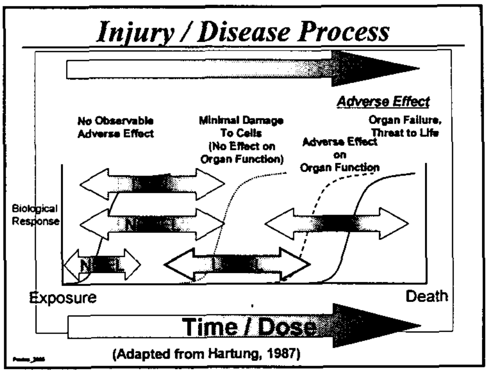


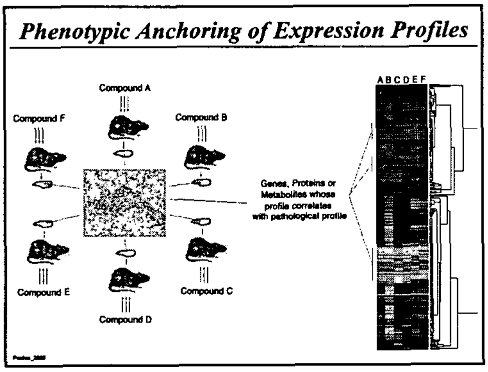


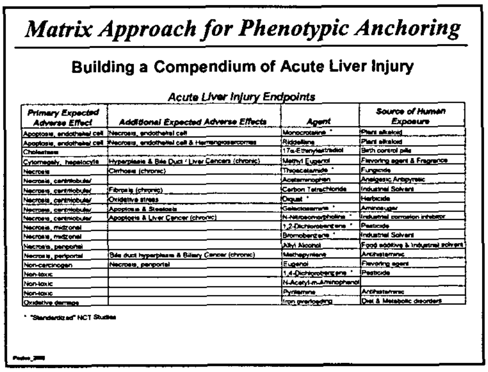
Mitochondrial Damage after Exposure to 150 mg/kg APAP











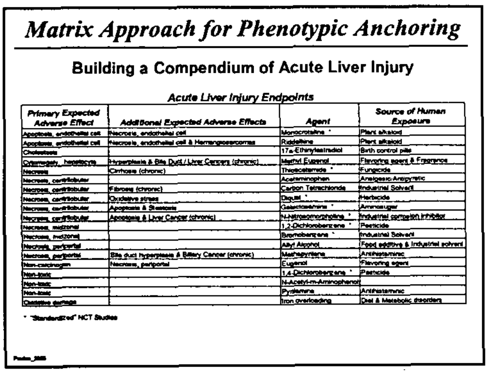
Matrix Approach for Phenotypic Anchoring

Building a Compendium of Acute Liver Injury

Acute Liver Injury Endpoints

Primary Expected Adverse Effect	Additional Expected Adverse Effects	Agent	Source of Human Exposure
Apoptosis, endothelial cell	Necrosis, endothelial cell	Monocrotaline *	Plant alkaloid
Apoptosis, endothelial cell	Necrosis, endothelial cell & Hemangiosarcomas	Riddelliine	Plant alkaloid
Cholestasis		17α-Ethinylestradiol	Birth control pills
Cytomegaly, hepatocyte	Hyperplasia & Bile Duct / Liver Cancers (chronic)	Methyl Eugenol	Flavoring agent & Fragrance
Necrosis	Cirrhosis (chronic)	Thioacetamide *	Fungicide
Necrosis, centrilobular		Acetaminophen	Analgesic Antipyretic
Necrosis, centrilobular	Fibrosis (chronic)	Carbon Tetrachloride	Industrial Solvent
Necrosis, centrilobular	Oxidative stress	Diquat *	Herbicide
Necrosis, centrilobular	Apoptosis & Steatosis	Galactosamine *	Aminosugar
Necrosis, centrilobular	Apoptosis & Liver Cancer (chronic)	N-Nitrosomorpholine *	Industrial corrosion inhibitor
Necrosis, midzonal		1,2-Dichlorobenzene *	Pesticide
Necrosis, midzonal		Bromobenzene *	Industrial Solvent
Necrosis, periportal		Allyl Alcohol	Food additive & industrial solvent
Necrosis, periportal	Bile duct hyperplasia & Biliary Cancer (chronic)	Methapyrilene	Antihistaminic
Non-carcinogen	Necrosis, periportal	Eugenol	Flavoring agent
Non-toxic		1,4-Dichlorobenzene *	Pesticide
Non-toxic		N-Acetyl-m-Aminophenol	
Non-toxic		Pyrilamine	Antihistaminic
Oxidative damage		Iron overloading	Diet & Metabolic disorders

* "Standardized" NCT Studies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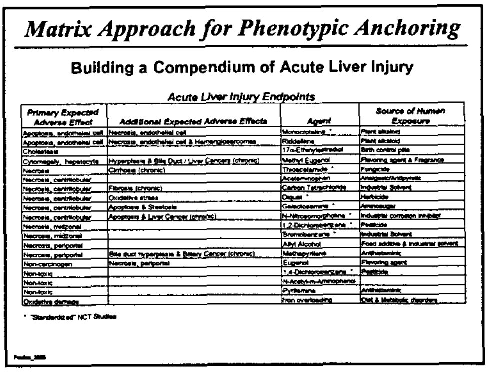
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Necrosis, midzonal		Bromobenzene *	Industrial Solvent
Necrosis, periportal		Allyl Alcohol	Food additive & industrial solvent
Necrosis, periportal	Bile duct hyperplasia & Biliary Cancer (chronic)	Methapyrilene	Antihistaminic
Non-carcinogen	Necrosis, periportal	Eugenol	Flavoring agent
Non-toxic		1,4-Dichlorobenzene *	Pesticide
Non-toxic		N-Acetyl-m-Aminophenol	
Non-toxic		Pyrilamine	Antihistaminic
Oxidative damage		Iron overloading	Diet & Metabolic disorders

* "Standardized" NCT Studies

Matrix Approach for Phenotypic Anchoring

Building a Compendium of Acute Liver Injury

Acute Liver Injury Endpoints

Primary Expected Adverse Effect	*Additional Expected Adverse Effects*	*Agent*	*Source of Human Exposure*
Apoptosis, endothelial cell	Necrosis, endothelial cell	Monocrotaline *	Plant alkaloid
Apoptosis, endothelial cell	Necrosis, endothelial cell & Hemangiosarcomas	Riddelliine	Plant alkaloid
Cholestasis		17α-Ethinylestradiol	Birth control pills
Cytomegaly, hepatocyte	Hyperplasia & Bile Duct / Liver Cancers (chronic)	Methyl Eugenol	Flavoring agent & Fragrance
Necrosis	Cirrhosis (chronic)	Thioacetamide *	Fungicide
Necrosis, centrilobular		Acetaminophen	Analgesic/Antipyretic
Necrosis, centrilobular	Fibrosis (chronic)	Carbon Tetrachloride	Industrial Solvent
Necrosis, centrilobular	Oxidative stress	Diquat *	Herbicide
Necrosis, centrilobular	Apoptosis & Steatosis	Galactosamine *	Aminosugar
Necrosis, centrilobular	Apoptosis & Liver Cancer (chronic)	N-Nitrosomorpholine *	Industrial corrosion inhibitor
Necrosis, midzonal		1,2-Dichlorobenzene *	Pesticide
Necrosis, midzonal		Bromobenzene *	Industrial Solvent
Necrosis, periportal		Allyl Alcohol	Food additive & Industrial solvent
Necrosis, periportal	Bile duct hyperplasia & Biliary Cancer (chronic)	Methapyrilene	Antihistaminic
Non-carcinogen	Necrosis, periportal	Eugenol	Flavoring agent
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Non-toxic		N-Acetyl-m-Aminophenol	
Non-toxic		Pyrilamine	Antihistaminic
Oxidative damage		Iron overloading	Diet & Metabolic disorders

* "Standardized" NCT Studies

Collecting Liver, Kidney, Blood/Serum for Transcriptomics, Proteomics and Metabolomics
In-life data; Clin. Chem., Hematology, Pathology

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Matrix Approach for Phenotypic Anchoring

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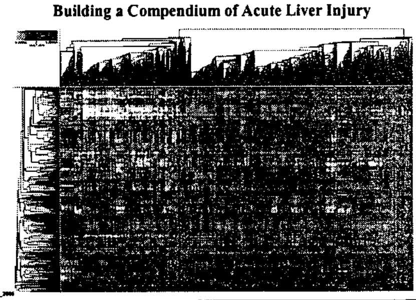
Matrix Approach for Phenotypic Anchoring

Building a Compendium of Acute Liver Injury



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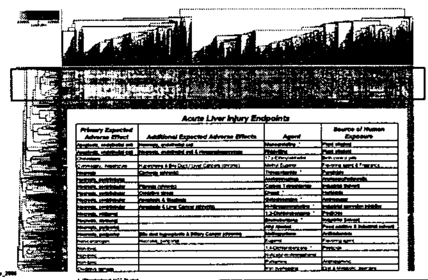
Matrix Approach for Phenotypic Anchoring

Building a Compendium of Acute Liver Injury



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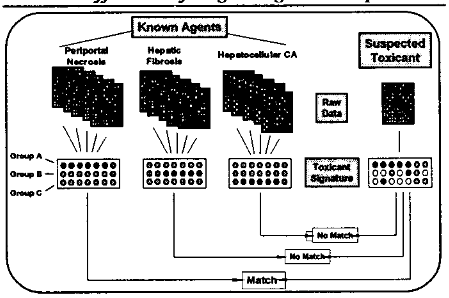
Adverse Effects Profiling Using Gene Expression



NCT CEBS Knowledge Base

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Chemical Effects in Biological Systems

The Concept

Combine genomic annotation, molecular expression analysis and literature mining, together with toxicological information, to enable global query, using gene and protein sequence, for the purpose of building a knowledge base.

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Conclusions

- Genome-based technologies provide an unprecedented opportunity to explore the biological complexity underlying adverse effects.
- While the opportunities are great, so are the challenges of being able to explore high density data.
- Progress will occur slowly and incrementally; a solid foundation for high quality data assimilation and analysis must be established.
- The opportunity now exists for creating an information base which compiles the results of diverse individual studies into a compendium of chemical effects in biological systems that can be a resource for the scientific community.

NCT

- Ray Tennant, NCT Director
- Mike Waters, Assist. Dir. CEBS

NMG

Current

- **Jeff Tucker**
- Jennifer Collins
- Danica Ducharme
- Rick Fannin
- Sherry Grissom
- Stella Sieber
- Todd Auman
- Kevin Gerrish
- Alexandra Heinloth
- **Pierre Bushel**
- Jeff Chou
- Jianying Li
- Jonathan Miller

Past

- **Cindy Afshari**
- Hisham Hamadeh
- Emile Nuwaysir
- Rupesh Amin
- Lee Bennett
- J. Carl Barrett

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- Ray Stoll
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- Russ Wolfinger

Molecular Mining

- Steve Misener