New Technologies To Solve Old Problems and Address Issues In Risk Assessment

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Genomics

The study of an organism's entire genome which includes:

The sequencing of the genome

The patterns of gene expression

The patterns of protein expression

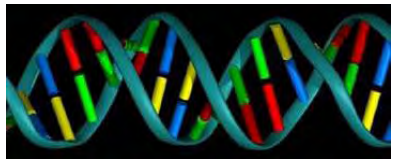
The functional cellular changes and activities

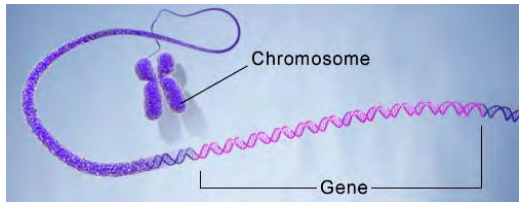
The patterns of metabolic products.

GENOMICS

DNA

Gene

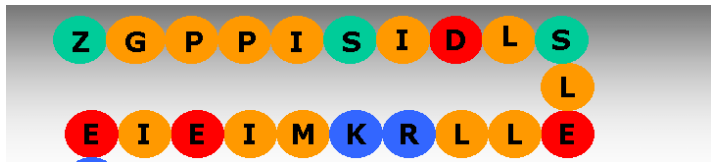




mRNA

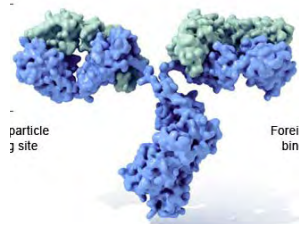
Transcriptomics

Amino Acid Chain



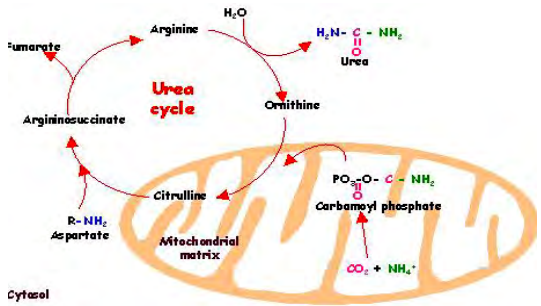
Protein structure

Post-translational modification



Proteomics

Protein function



Metabolites

Metabolomics

Toxicogenomics

Examines how the entire genome is involved in biological responses to environmental toxicants and stressors

Combines information from

- mRNA profiling
- cell or tissue protein profiling
- genetic susceptibility
- computational models

Goal: Understand how environmental stressors induce disease

Transcriptomics

Frequently called genomics (little g).

Transcriptome

- Total mRNA produced from the DNA.
- Varies with conditions.
- Represents the genes actively expressed.

Expression of mRNAs in a given cell population using high-density data techniques.

Proteomics

The large-scale study of proteins.

Includes both structure and function.

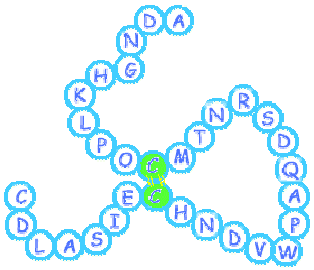
More complicated than genomics.

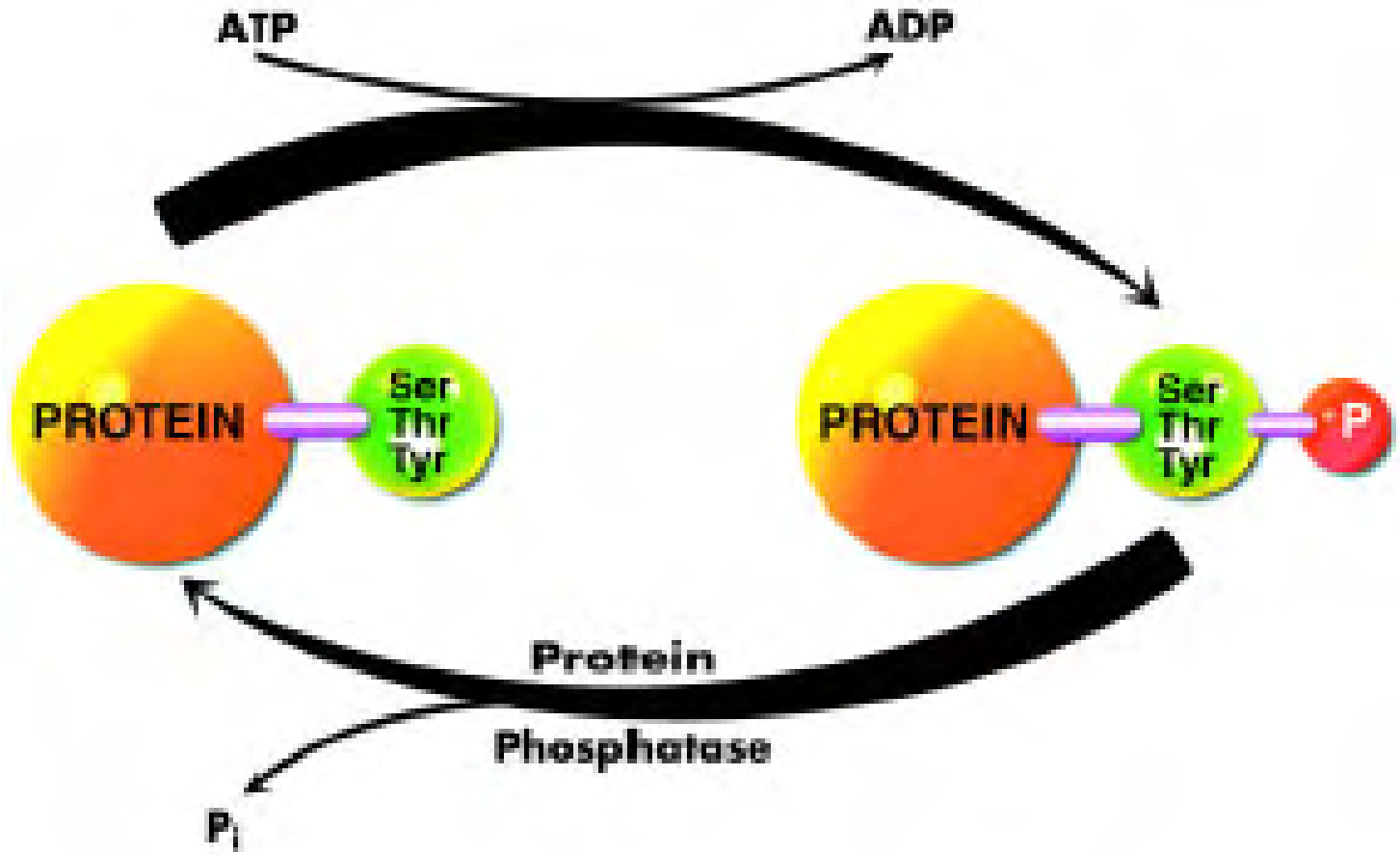
The genome is considered constant while the proteome is constantly changing.

Proteome is different from cell to cell and in response to the cellular environment.

Protein expression is dependent on organ, developmental state, and micro- or macro-environment.

Levels Of Information Provided By The Protein Analytic Methods





Addition of Functional Groups

Metabolomics

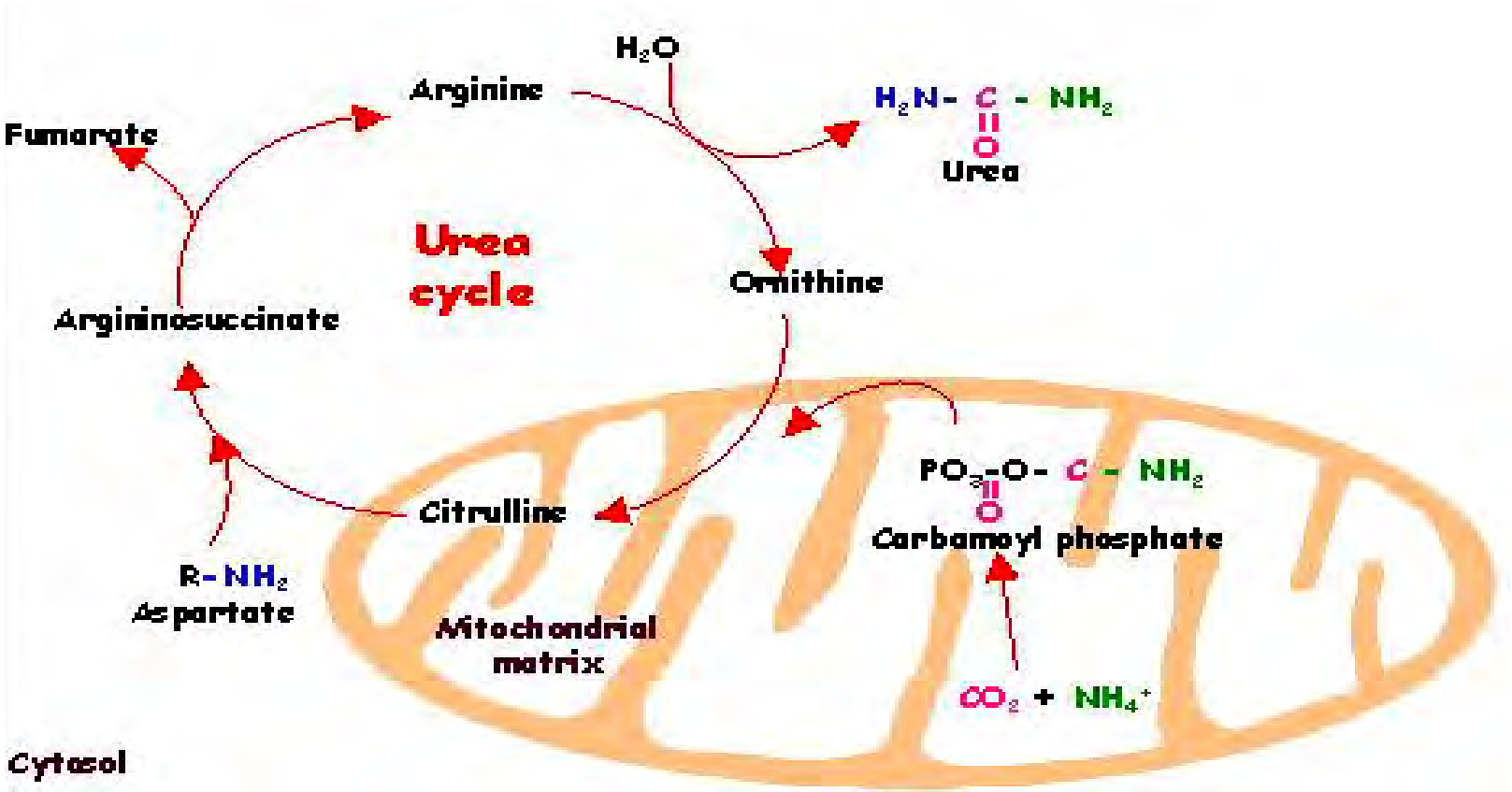
The study of small-molecule metabolite profiles.

Metabolome

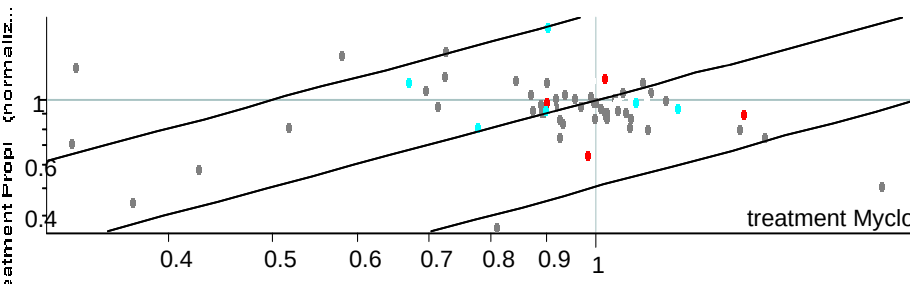
- All metabolites in a biological organism.
- End products of gene expression.
- Result of specific cellular processes.

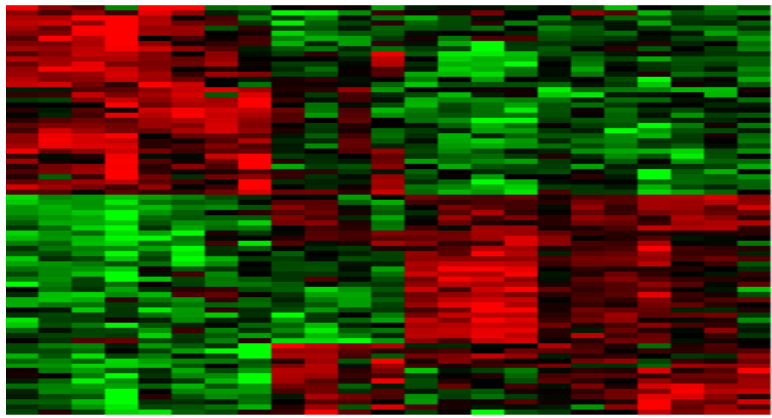
Metabolic profiling provides an instantaneous snapshot of the physiology of the cell, organ, or organism.

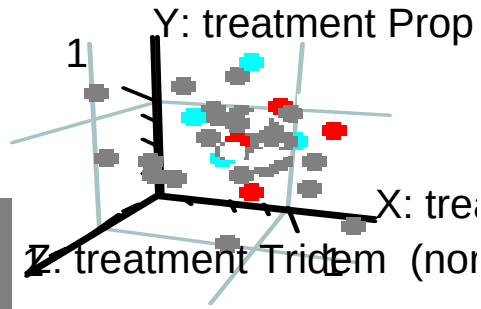
From Protein Structure to Function to Metabolism

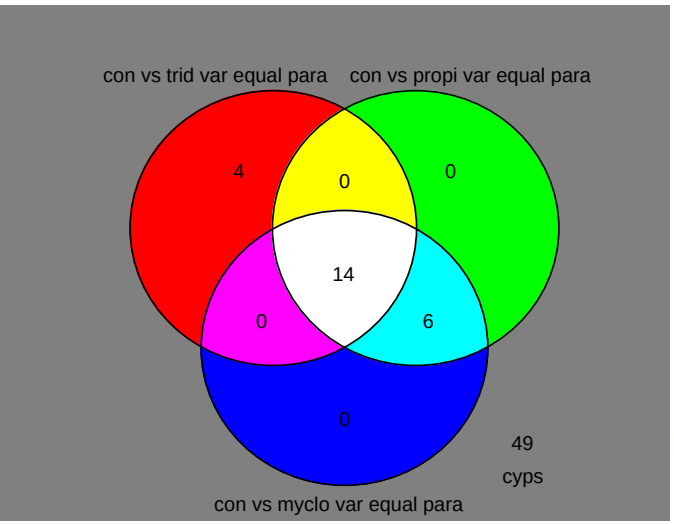


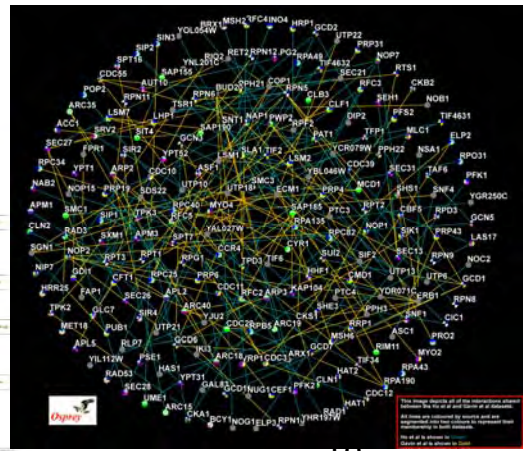
Data Analysis: Patterns, Numbers, and Pathways











Risk Assessment Applications

Exposure assessment

Hazard identification

Prioritization and Screening

Mode of action

Dose-response assessment

Extrapolation - dose, species

Susceptible population

Mixtures assessments

Exposure Assessment

Exposure Assessment: Microbial Source Tracking

- Microbial host-specific gene sequence databases
- Microbial host-specific markers
- Novel approach - total DNA from entire fecal community to find microbial host-specific markers
- Water Quality:
  - Bioremediation
  - Biotechnology
  - Ecosystem effects
  - Virulence factors

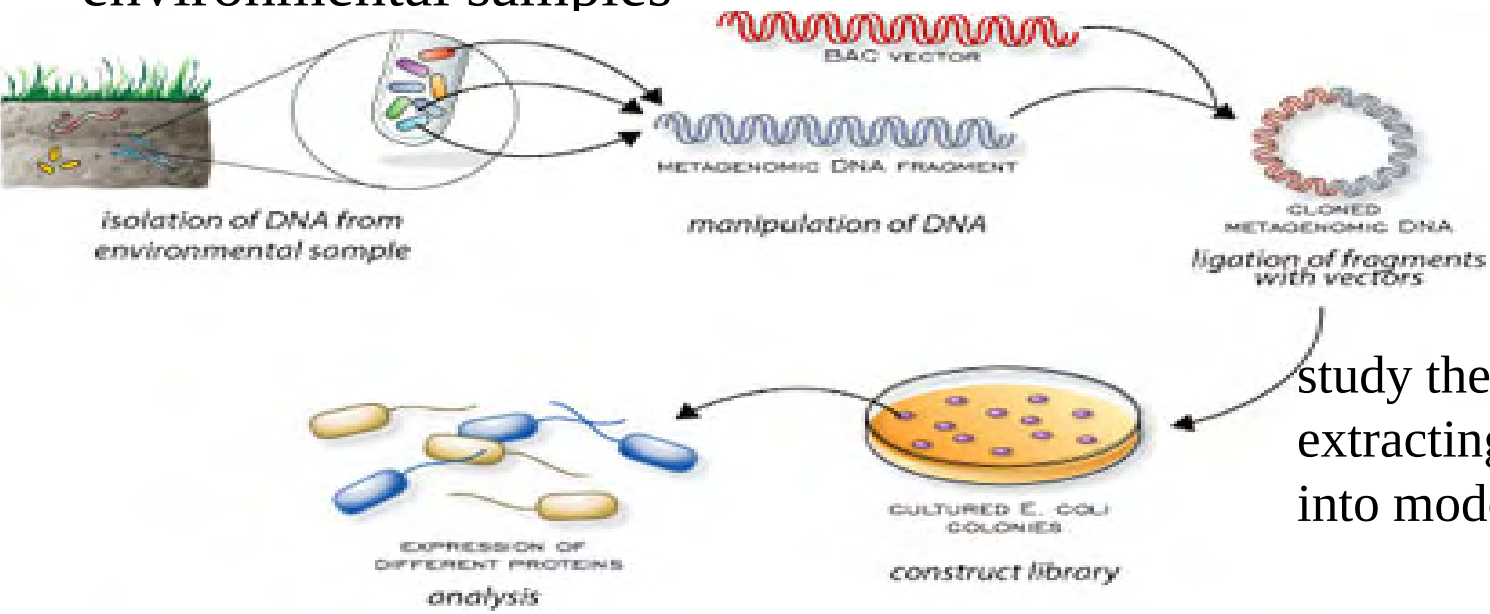
Exposure Assessment

Metagenomics

Genomics on a huge scale

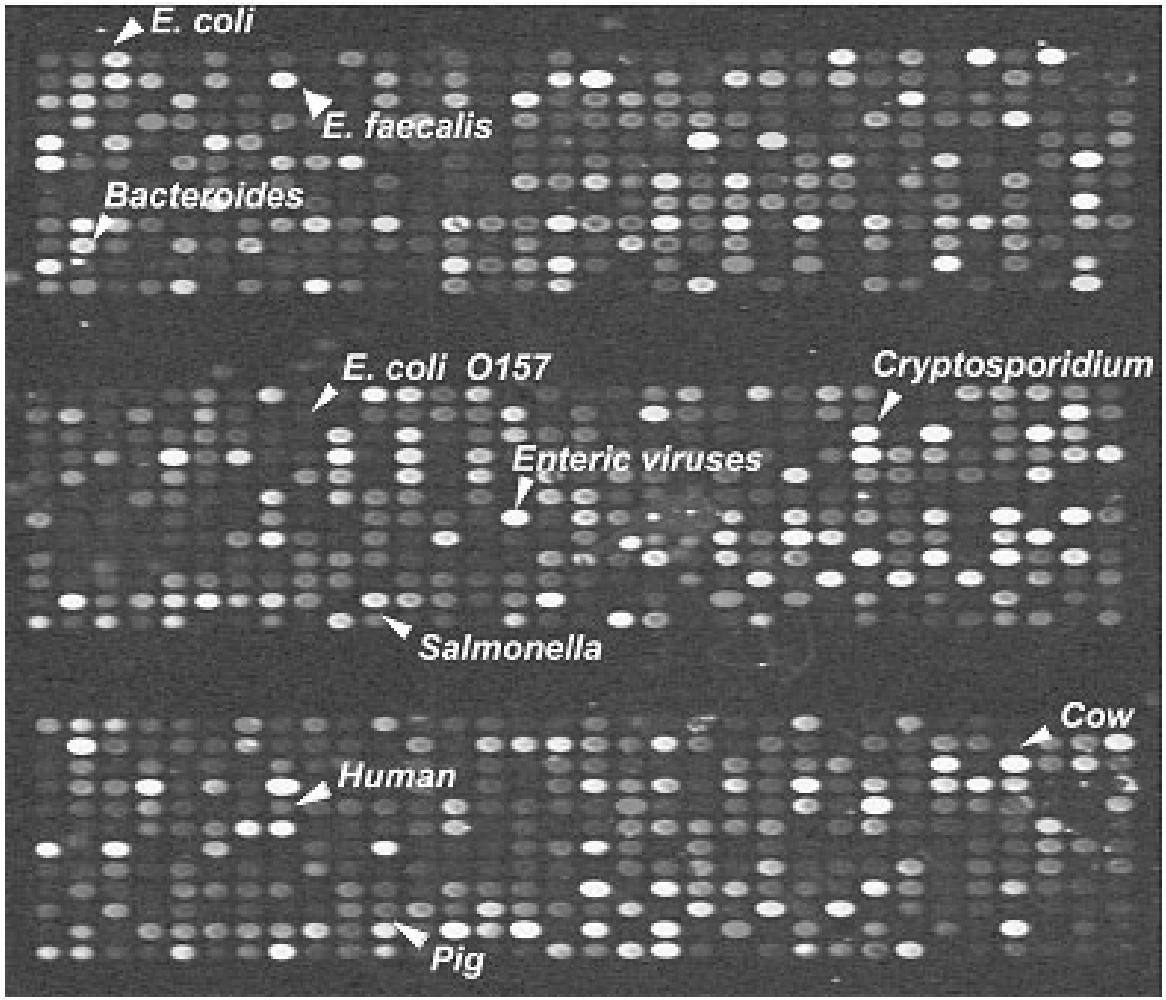
Comprehensive study of nucleotide sequence, structure, regulation, and function

Genomic analysis of microbial DNA extracted directly from environmental samples



study the components by extracting DNA and insert into model organism.

Future Exposure Assessment : Microbial Water Quality Biochip



Indicators

Pathogens

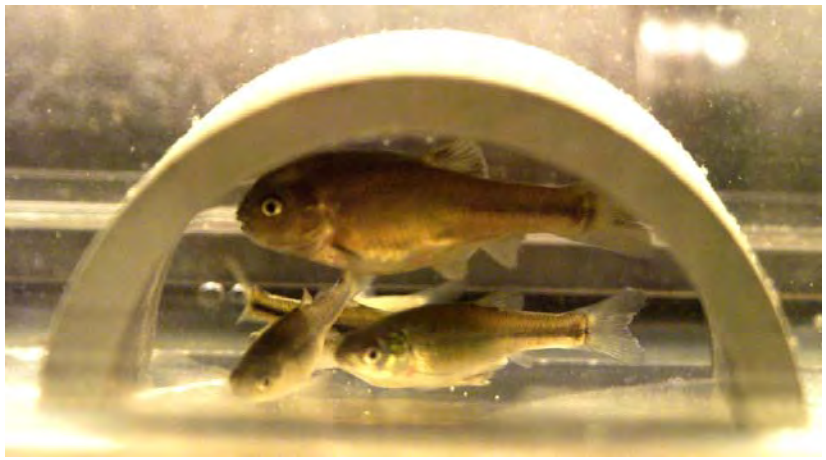
MST

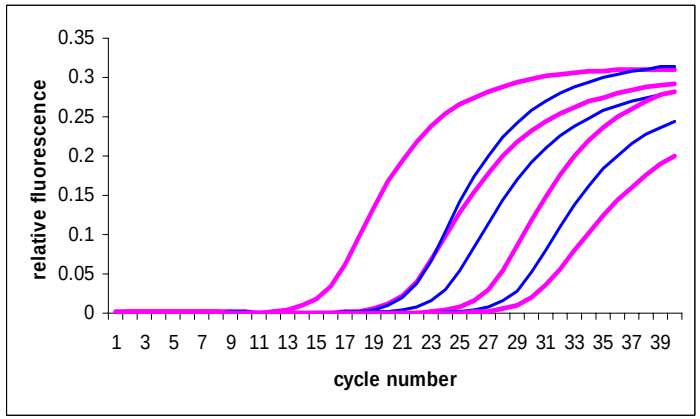
Stewart, Santo Domingo, and Wade (2007)
In Microbial Source Tracking, ASM Press

Screening for Potential Hazard

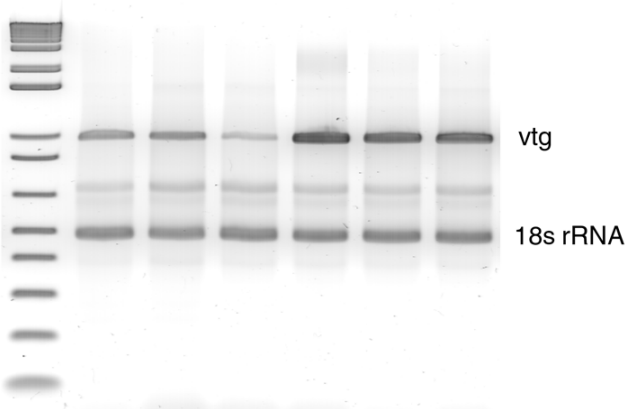
Vitellogenin RT-PCR Fathead Minnow Bioindicator Assay

Male Fathead Minnows are exposed to contaminated water for 7 days



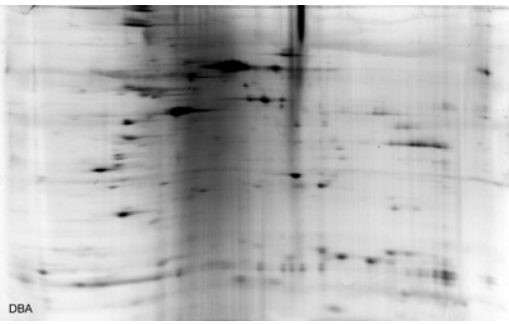


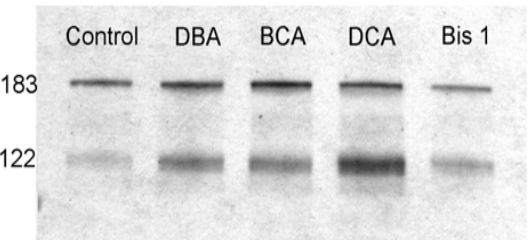
Expression of egg yolk protein Vitellogenin gene mRNA in livers of males exposed to estrogenic chemicals



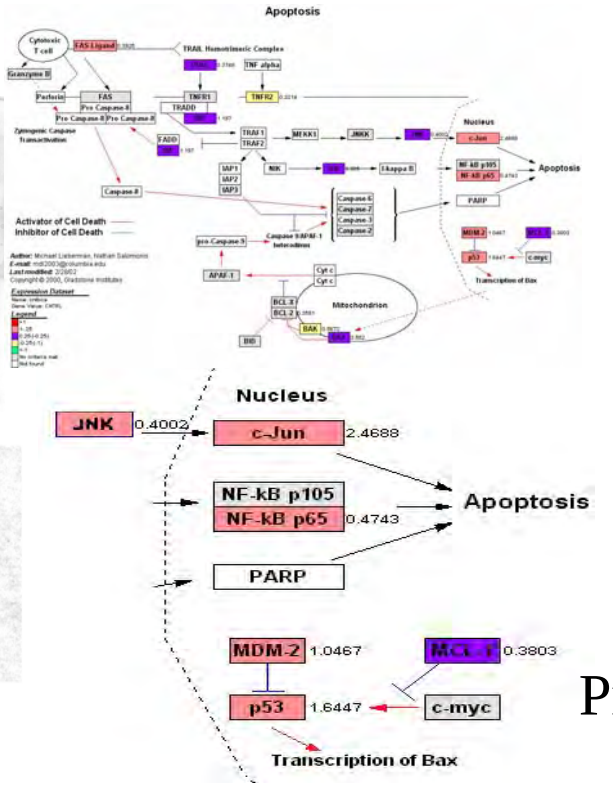
Semi-quantitative estimates of Vitellogenin gene mRNA used to demonstrate estrogenic effects and feminization of male fish

Molecule to Pathway to Function to Phenotype





Haloacetic Acid induces changes in Protein Phosphorylation



Result in changes in transcription factor activation and gene expression

Produce alterations in cellular differentiation and induces cell death

Leads to Birth Defects

Hunter et al., Reprod Toxicol. 2006 21:260-6

Prioritization

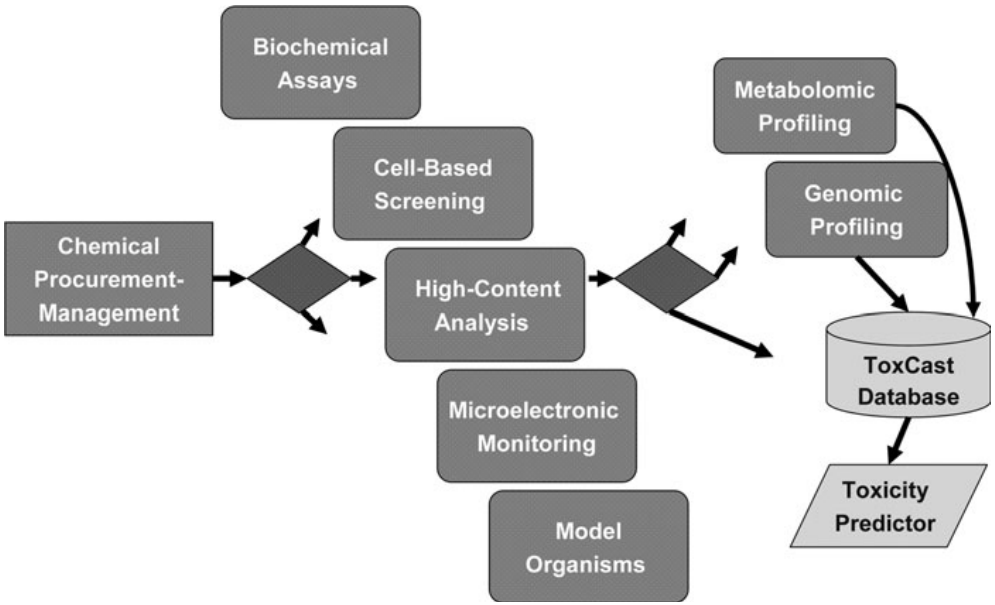
Screening for testing and decision-making

(*e.g.*, voluntary HPV Challenge, EDC, CCL, PMN, REACH).

Approaches for prioritizing chemicals for subsequent screening and testing

Prioritization

ToxCast™ Program

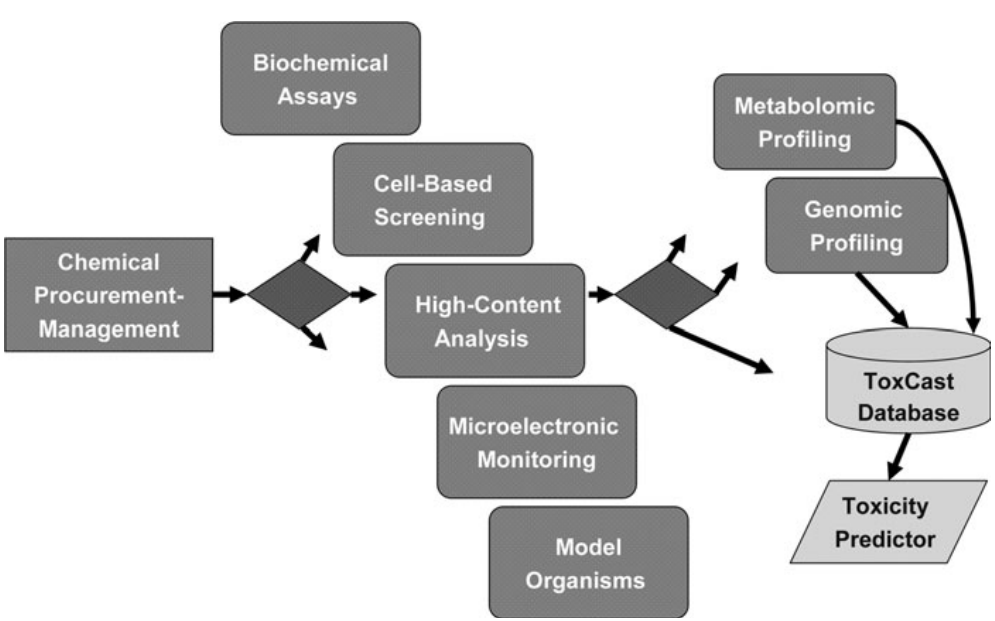


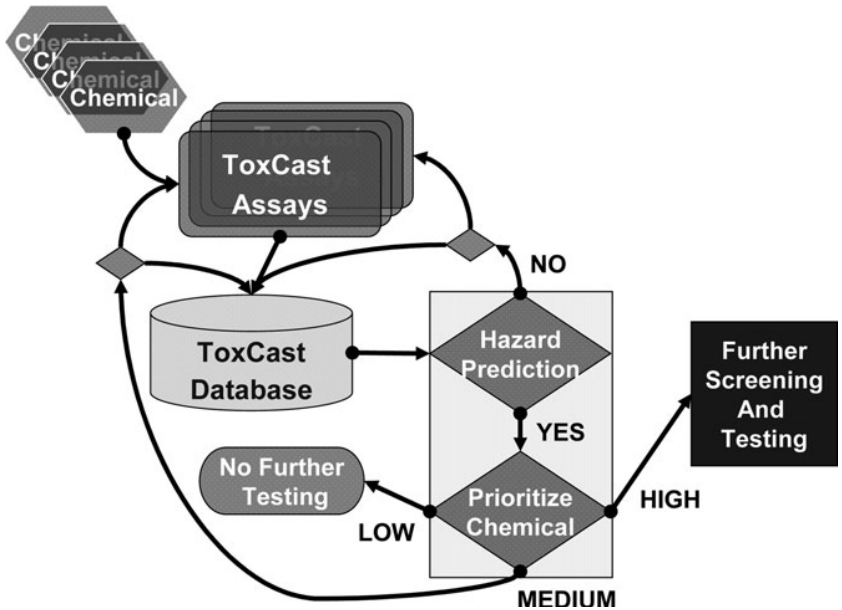
Managed chemical library and integrated analyses interpretively linked within the ToxCast ™ database to predict toxicity.

Dix et al., Toxicol Sci 95:5-12, 2007

Prioritization

ToxCast™ Program





Managed chemical library and integrated analyses interpretively linked within the ToxCast ™ database to predict toxicity.

Prioritizing environmental chemicals based on hazard prediction.

Dix et al., Toxicol Sci 95:5-12, 2007

Inform Mode of Action Analysis

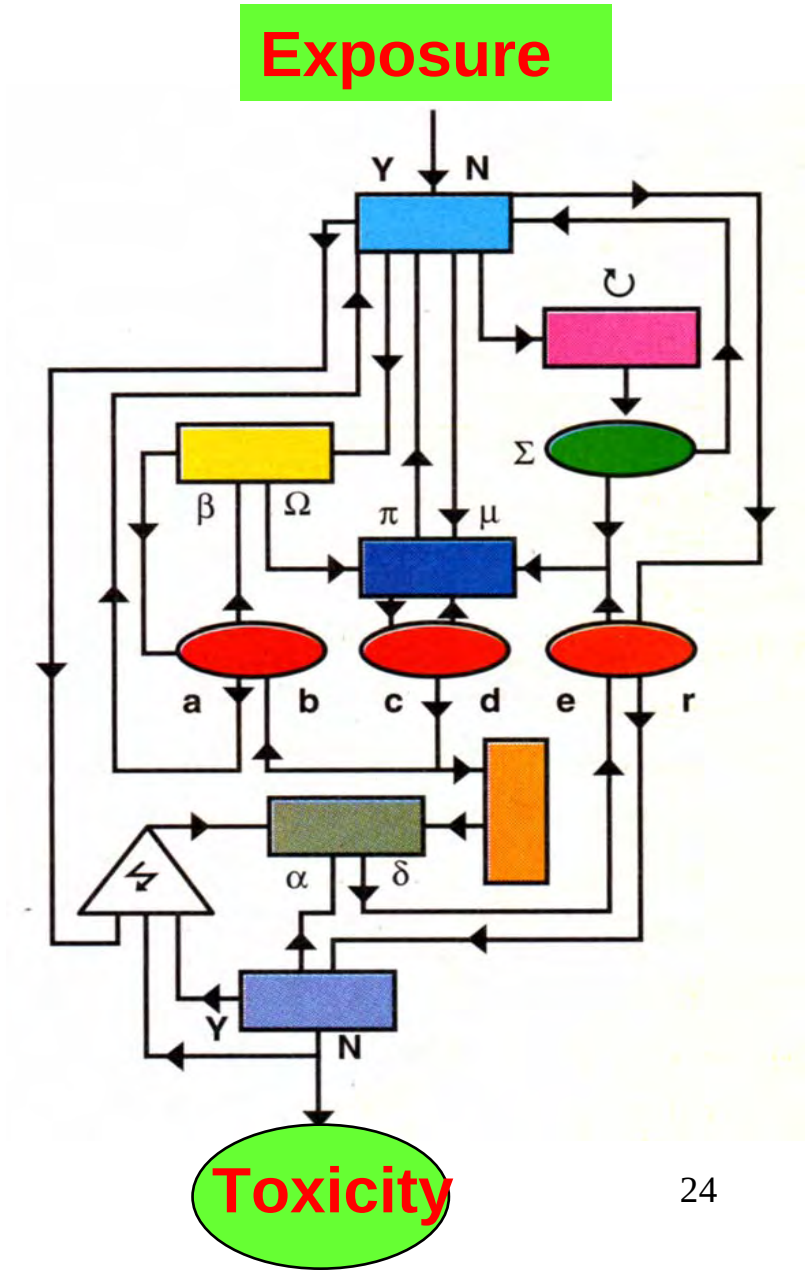
Important Definitions

Mode of Action: Key events and processes, starting with the interaction of an agent with the target cell through functional and anatomical changes resulting in adverse health effect

Key Event: Empirically observable precursor step that is a necessary element of the mode of action or is a biological marker for such an element

mechanism of action

detailed understanding at biochemical & molecular level

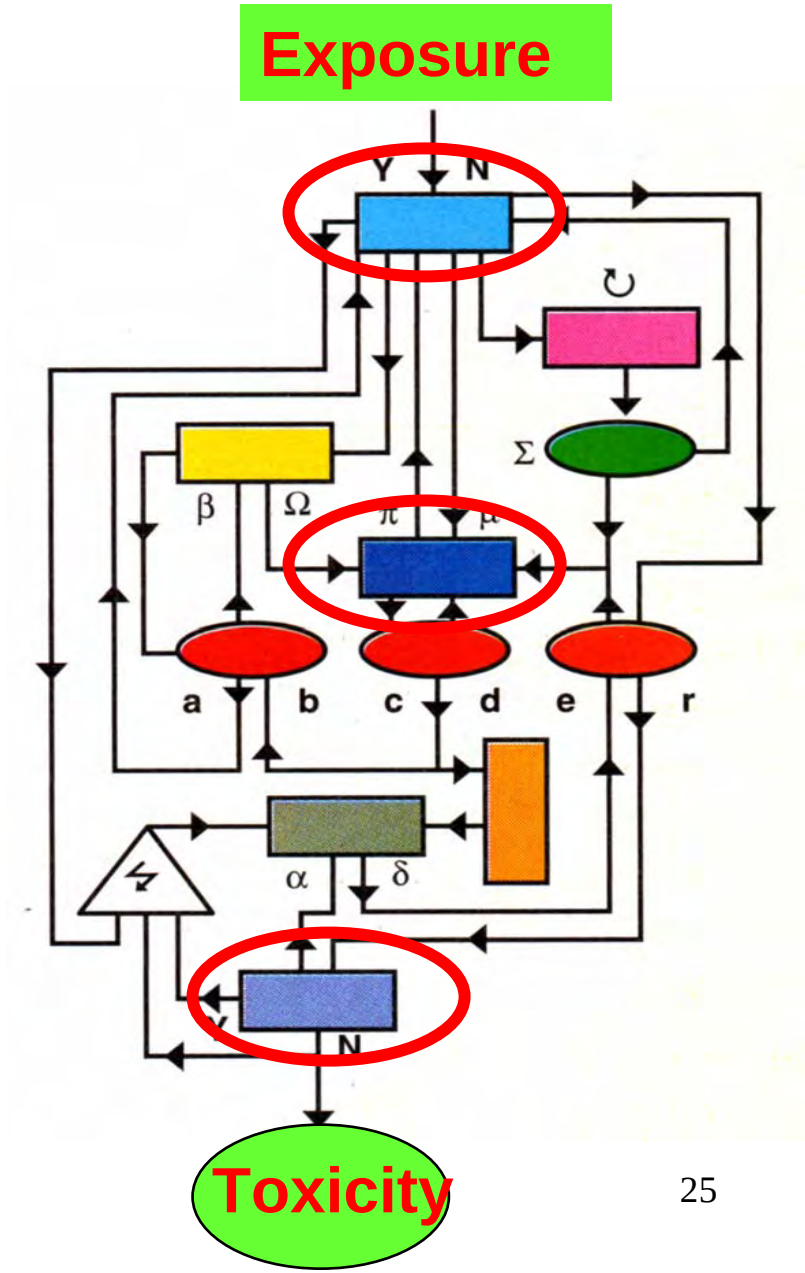


mechanism of action

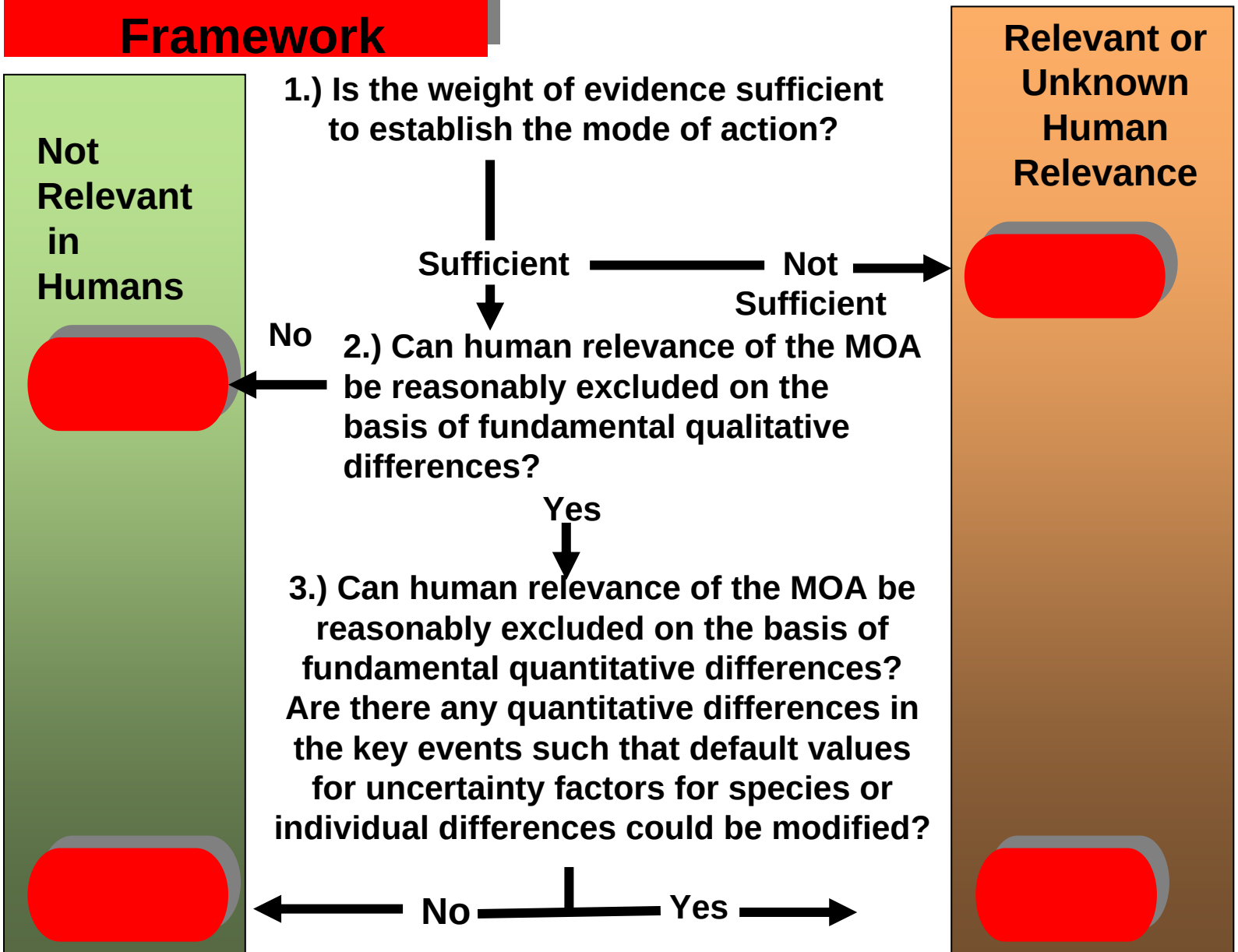
detailed understanding at biochemical and molecular level

mode of action

identification of key events, quantifiable obligatory steps



Human Relevance Framework



CONAZOLE FUNGICIDE EXAMPLE

- Conazoles are azole-containing compounds with anti-fungal activity that are used in agriculture and medicine.

- Conazoles can also induce follicular cell adenomas of the thyroid in rats and hepatocellular tumors in mice.

Wolf et al., Toxicol Pathol 34:895-902, 2006
Hester et al., Toxicol Pathol 34:879-894, 2006
Allen et al., Toxicol Pathol 34:853-862, 2006
Ward et al., Toxicol Pathol 34:863-878, 2006

Propiconazole-Induced Hepatocellular Tumors in Male CD Mice after Dietary Exposure

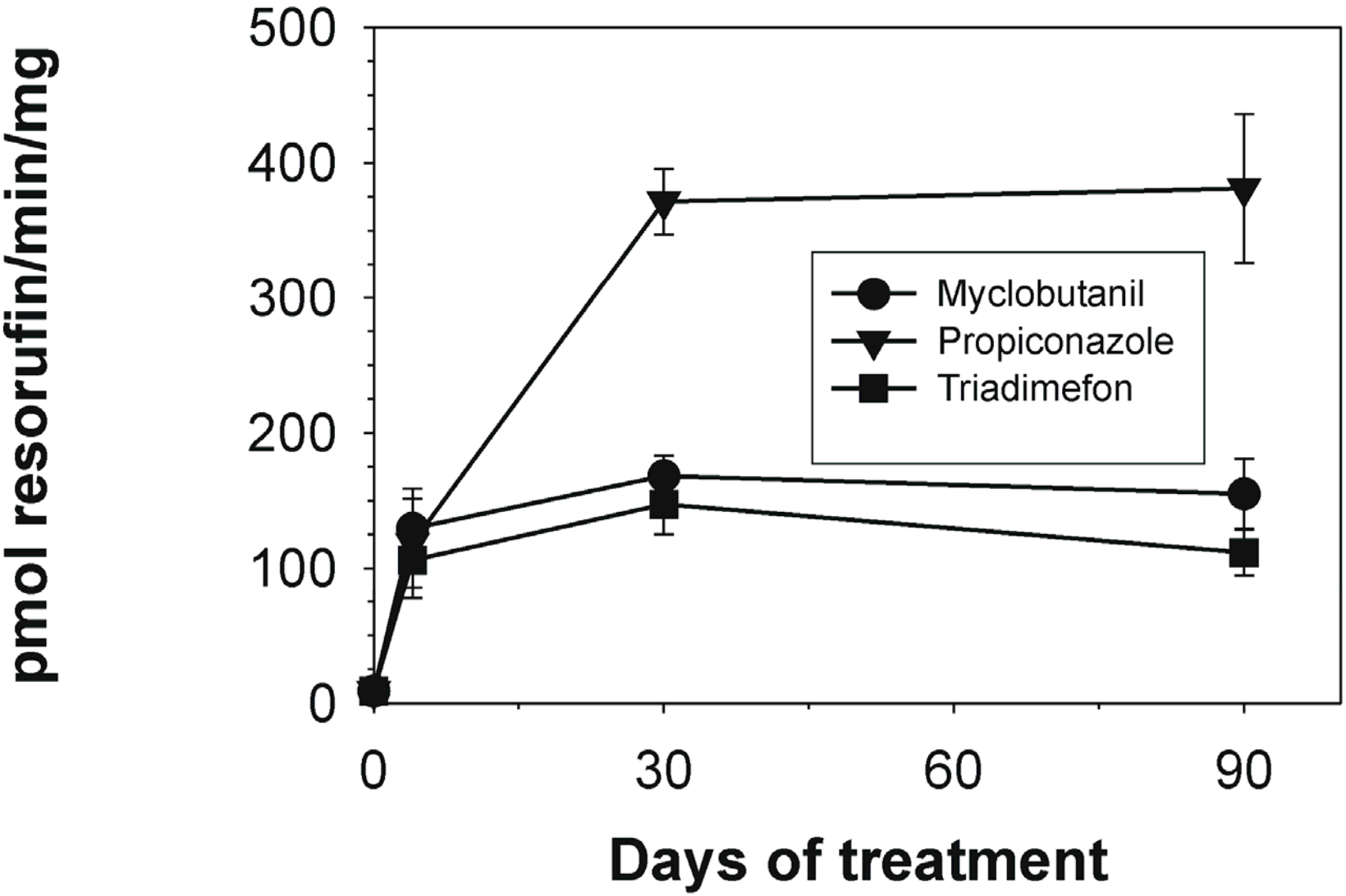
	Control	100 ppm	500 ppm	2500 ppm
Foci of cellular atypia	16/51 31%	11/51 22%	12/50 24%	**31/52** **60%**
adenomas	12/51 24%	7/51 14%	8/50 16%	**21/52** **40%**
adenomas and carcinomas	15/51 29%	7/51 14%	14/50 28%	**23/52** **44%**

INCHEM (IPCS) Monograph 768, 1987

Mouse Liver Hepatocyte Hypertrophy And Vacuolation Summary Data

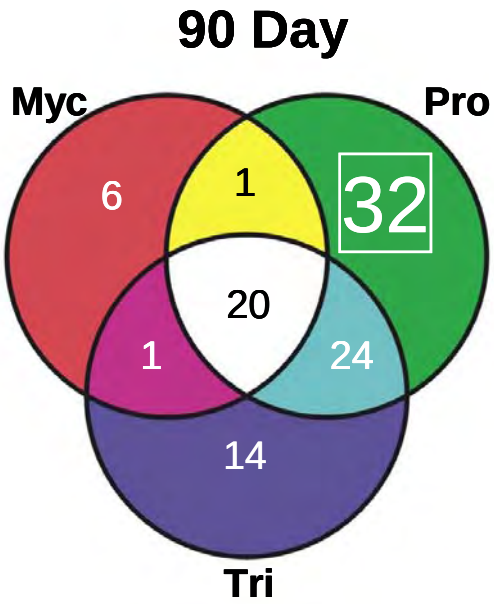
	4 Days of Treatment	30 Days of Treatment	90 Days of Treatment
Control	0/5 0	1/5 0.2 ± 0.5 (1.0)	1/5 0.2 ± 0.5 (1.0)
Myclobutanil			
100 ppm	3/5 2.0 ± 1.0^{TD} (1.7 ± 1.2)	3/5 0.8 ± 0.8 (1.3 ± 0.6)	3/5 0.8 ± 0.8 (1.3 ± 0.6)
500 ppm	1/5 0.2 ± 0.5 (1.0)	3/5 1.0 ± 1.0 (1.7 ± 0.6)	4/5 1.4 ± 1.1^{T} (1.8 ± 1.0)
2000 ppm	5/5 1.4 ± 0.6^{T}	5/5 1.8 ± 0.5^{TD}	5/5 2.4 ± 0.6^{TD}
Propiconazole			
100 ppm	4/5 1.2 ± 0.8^{T} (1.5 ± 0.6)	3/5 1.0 ± 1.0 (1.7 ± 0.6)	3/5 1.0 ± 1.0 (1.7 ± 0.6)
500 ppm	3/5 1.0 ± 0.5 (1.7 ± 0.6)	4/5 1.4 ± 0.9T (1.0 ± 0.5)	**5/5 1.4 ± 0.6^{TD}**
2500 ppm	**5/5 2.0 ± 0^{TD}**	**5/5 3.2 ± 0.8^{TD}**	**5/5 3.0 ± 0.7^{TD}**
Triadimefon			
100 ppm	3/5 1.4 ± 1.3T (2.3 ± 0.6)	1/5 0.2 ± 0.5 (1.0)	3/5 0.6 ± 0.6 (1.0)
500 ppm	5/5 1.6 ± 0.6^{TD}	5/5 1.6 ± 0.5^{TD}	4/4 1.5 ± 1.0^{TD}
1800 ppm	5/5 2.4 ± 1.1^{TD}	5/5 2.2 ± 0.5^{TD}	5/5 2.8 ± 0.5^{TD}

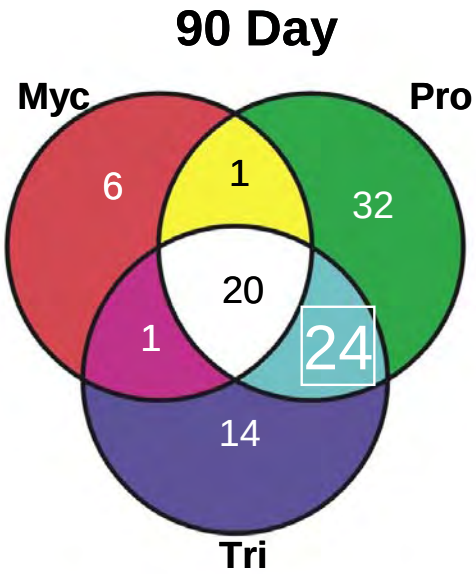
Alkoxyresorufin O-dealkylation (AROD) to measure P450 activities.



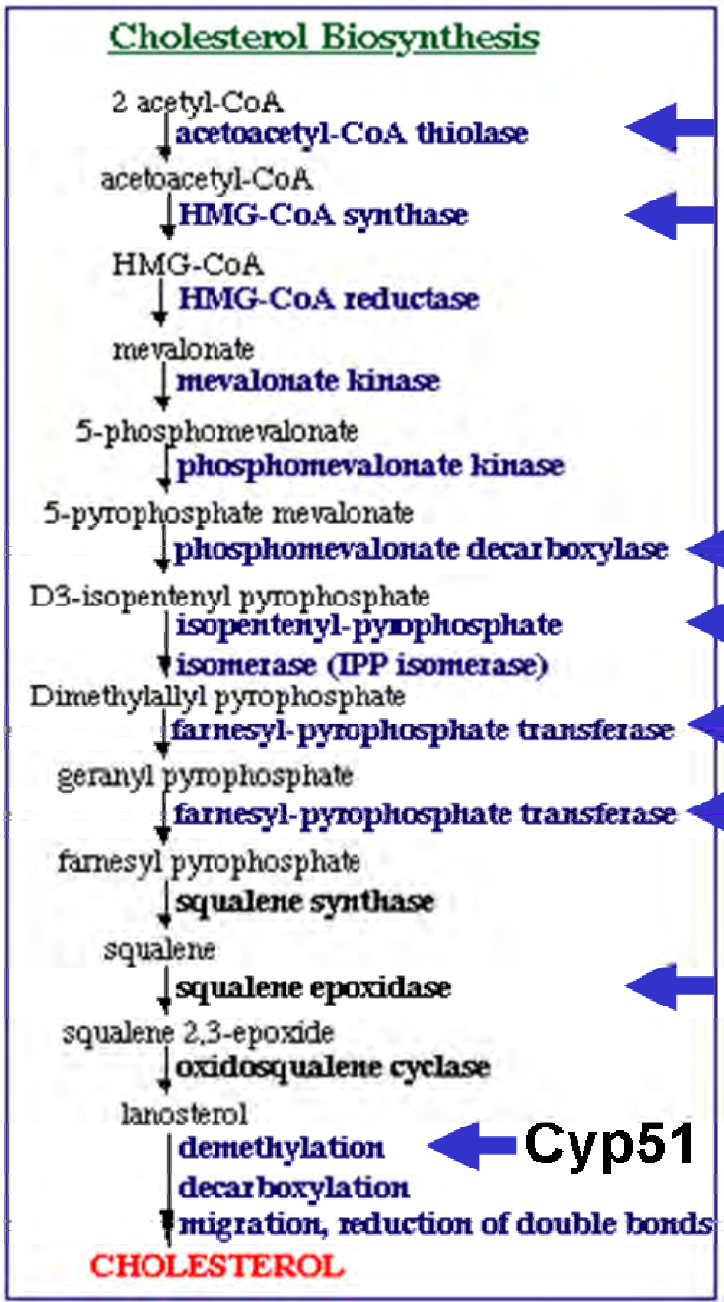
Cell Proliferation In Mouse Liver

	4 Days of Treatment	30 Days of Treatment	90 Days of Treatment
Control	0.490 ± 0.39	0.076 ± 0.12	0.210 ± 0.27
Myclobutanil			
Low-dose	1.410± 1.30	0.287 ±0.34	0.218 ± 0.16
Mid-dose	0.154 ± 0.11	0.279 ± 0.22	0.019 ± 0.04
High-dose	2.460 ± 0.81[T]	0.603 ± 0.59	0.358 ± 0.25
Propiconazole			
Low-dose	0.155 ± 0.30	0.230 ± 0.30	0.094 ± 0.11
Mid-dose	2.720 ± 3.60	0.272 ± 0.34	0.167 ± 0.10
High-dose	**4.380 ± 4.42 [TD]**	0.458 ± 0.26	0.381 ± 0.22
Triadimefon			
Low-dose	0.266 ± 0.32	0.575 ± 0.41	0.193 ± 0.22
Mid-dose	0.233 ± 0.24	0.628 ± 0.75 [T]	[a] 0.173± 0.28
High-dose	1.210 ± 0.82	0.769 ± 0.54 [T]	0.247 ± 0.39





BAD phosphorylation
PTEN pathway
AKT signaling
Insulin receptor signaling
BRCA1 as transcription regulator
Estrogen receptor signaling
PDGF signaling via STATs and NF-kB
Regulation of lipid metabolism via LXR
Role of CD28 in cytoskeleton reorganization
TGF-b Signaling
WNT Signaling
Apoptosis
Cell Cycle: G2/M Checkpoint
EGFR signaling
Regulation of Lipid Metabolism via PPAR,RXR and VDR
Urea Cycle



Specific Pathway Identified from Genomic Profile

Serum Cholesterol and Triglyceride

	4 days	30 days	90 days
Cholesterol (mg/dl)			
Control	**143.0 ± 28.3 (1)**	**134.6 ± 12.7 (1)**	**138.4 ± 24.4 (1)**
Myclobutanil High-dose	123.4 ± 2.8	77.2 ± 14.3 (0.57) *†	87.6 ± 34.4 (0.63) *†
Propiconazole High-dose	**114.2 ± 39.0**	**75.6 ± 20.7 (0.56)***	**76.6 ± 20.7 (0.55)***
Triadimefon High-dose	101.8 ± 15.0 (0.71)*	102.2 ± 12.7 (0.76)*	108.4 ± 31.7
Triglycerides (mg/dl)			
Control	183.4 ± 26.0	184.4 ± 53.8 (1)	233.8 ± 53.3
Myclobutanil High-dose	267.4 ± 136.2	329.6 ± 44.4 (1.79)*	264.4 ± 61.9
Propiconazole High-dose	295.6 ± 114.7	274.0 ± 61.0 (1.49)*	374.2 ± 193.2
Triadimefon High-dose	299.4 ± 103.4	335.4 ± 74.1 (1.82)*	250.4 ± 63.6

A proposed Set of Key Events Based on the Data

Not DNA reactive (standard *in vitro* and *in vivo* screening assays)

Nuclear receptor activation (transcriptional profile)

Induction of P450 enzymes (transcriptional profile confirmed by biochemistry)

Inhibition of Cyp 51 (site of action of fungicide)

Decreased cholesterol synthesis (transcriptional profile confirmed by clinical chemistry)

Mitogenesis (histology)

Altered mitosis (suggested by inhibition of cholesterol synthesis)

Oxidative stress (transcriptional profile)

Hypothesis Generation

Triadimefon-induced Thyroid Tumors

Altered metabolism in the liver is associated with the development of thyroid hormone disruption.

Circulating T4 is decreased at 4 and 30 days of treatment, T3 at 30 days, back to control levels at 90 days and no change in TSH at any time point.

These data suggest that an alternative toxicity pathway (not TSH dependent) may contribute to thyroid tumor development in rats treated with triadimefon.

Data Analysis

Rat 230_2 genechips

↓ gc-RMA

GSEA

TRG genesets ↓ **NRG genesets**

core genes

FDR 0.25 ↙ ↘ FDR 0.06

GeneGO® ↓

functional pathways

Ingenuity® ↓

gene networks

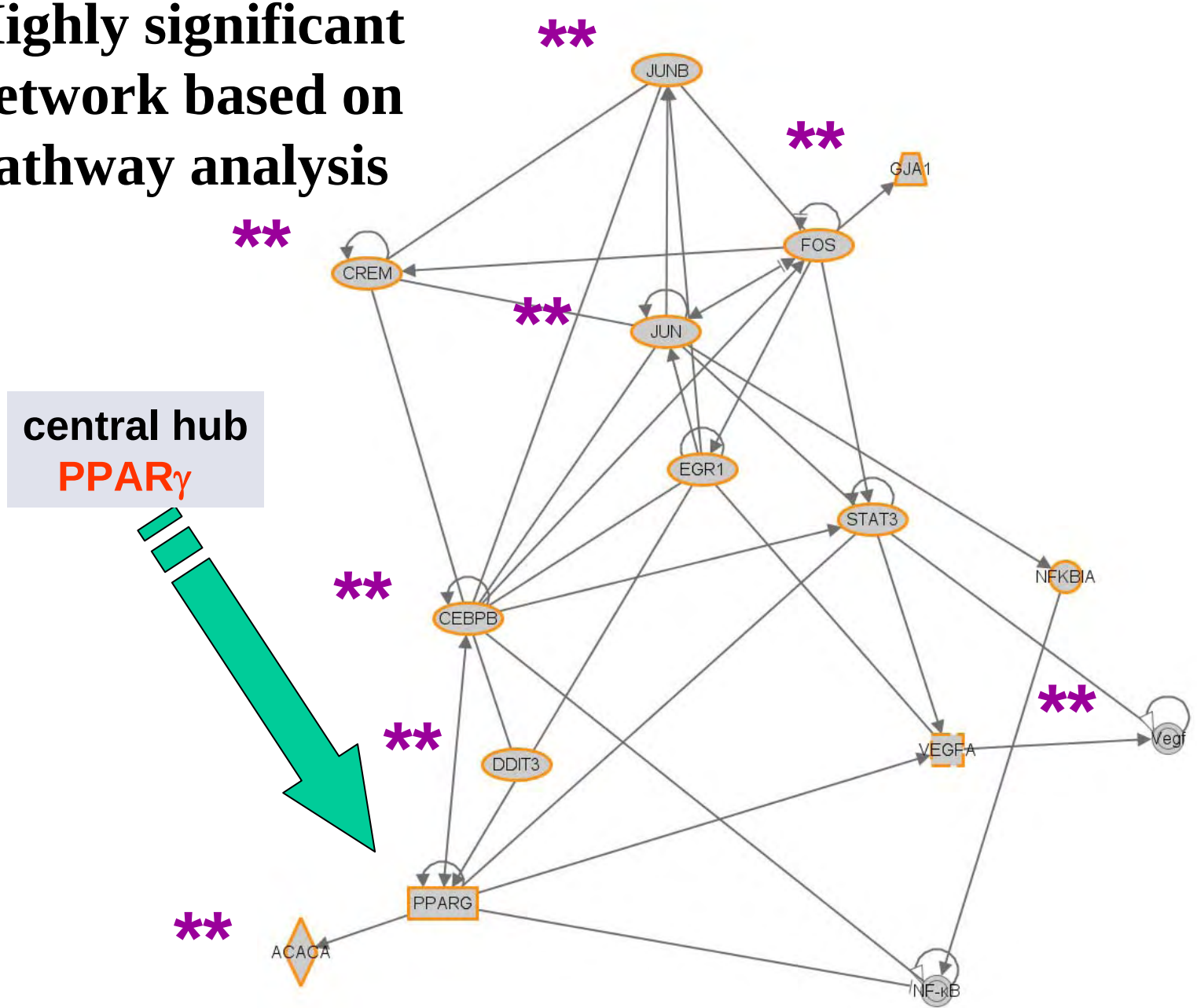
query literature ↓

Thyroid Human gene cancer database ↓

genes identified ↓

compare to rat genes

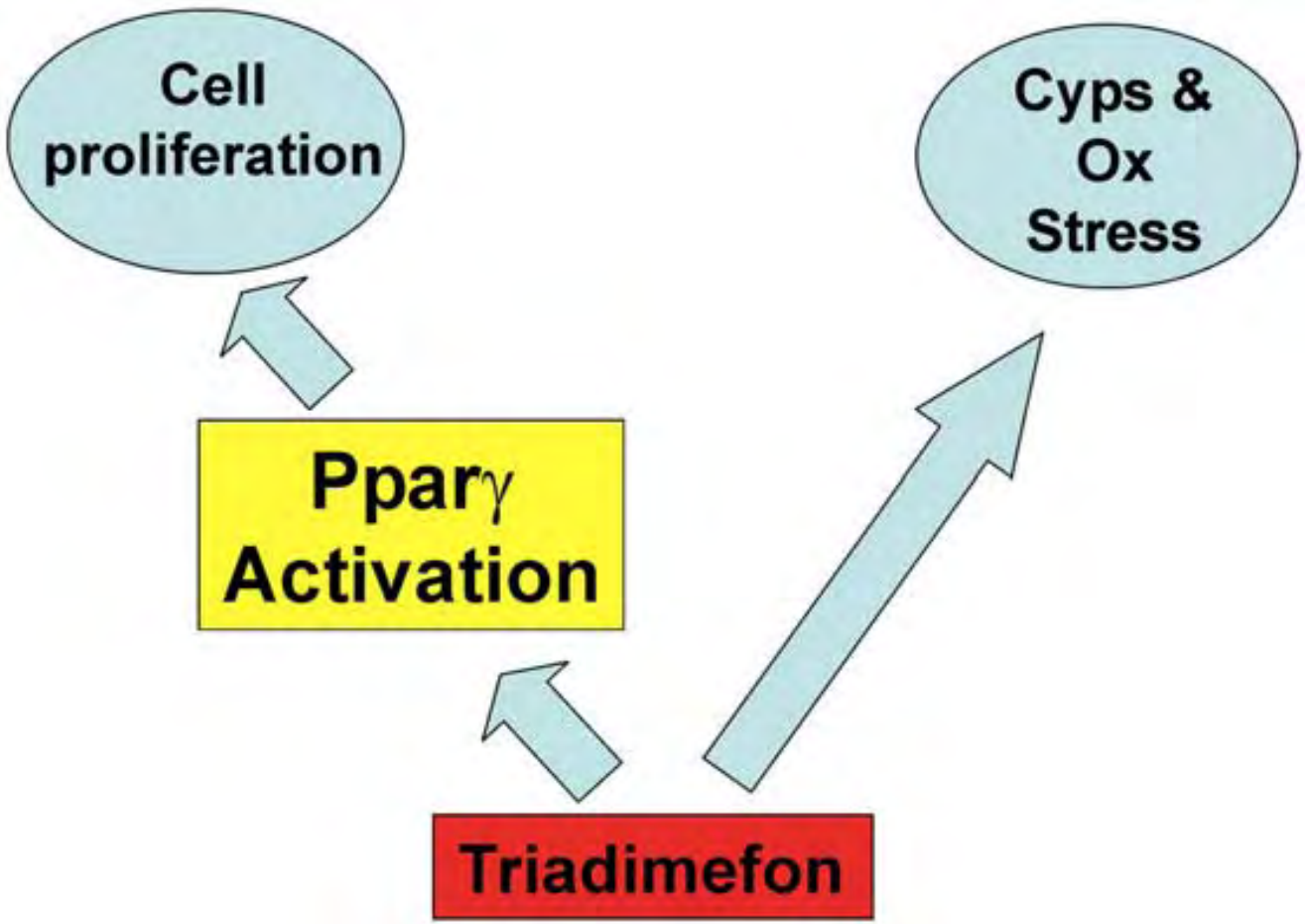
Highly significant network based on pathway analysis



central hub
PPARγ

**genes in both rat & human datasets

Hypothesized key events for triadimefon-induced thyroid tumorigenesis



Hester, SD and Nesnow, S. TAAP, 2007, Vol 227/3 pp 357-369.

Cumulative Risk Assessment

Genomics: Establishing Modes-Of-Action and Common Toxicity Pathways

1996 US Food Quality Protection Act

Consider cumulative effects of pesticide residues that have a common mechanism of toxicity

Cumulative risk

Based on a common toxic effect to exposure from a group of chemicals that share a common mechanism of toxicity by the same, or essentially the same, sequence of major biochemical events.

Conazole-Induced Hepatocellular Tumors in Male CD Mice after Dietary Exposure

Triadimefon

mouse: hepatocellular adenoma

Propiconazole

mouse: hepatocellular adenoma/carcinoma

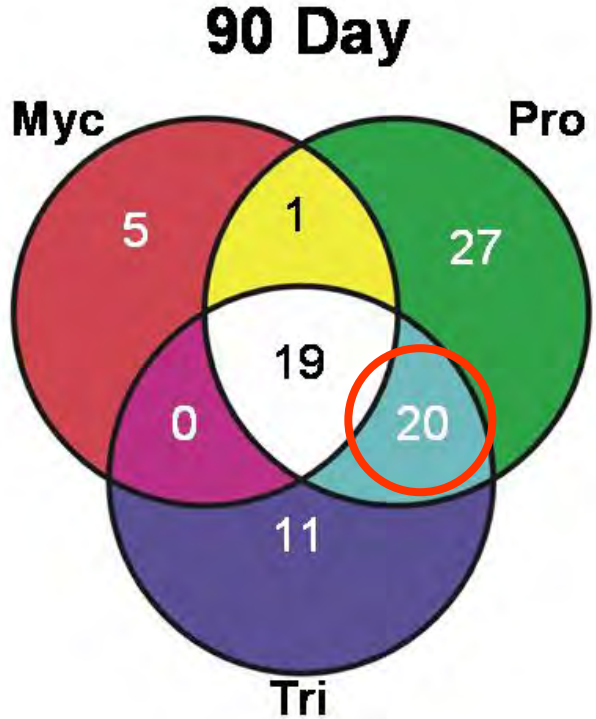
Myclobutanil

mouse: no liver tumors

Conazole	Mouse liver tumors
Triadimefon	+
Propiconazole	+
Myclobutanil	-

Dose	Tumor formation
High	+
Mid	-
Low	-

Triadimefon and Propiconazole Common Pathways



Apoptosis

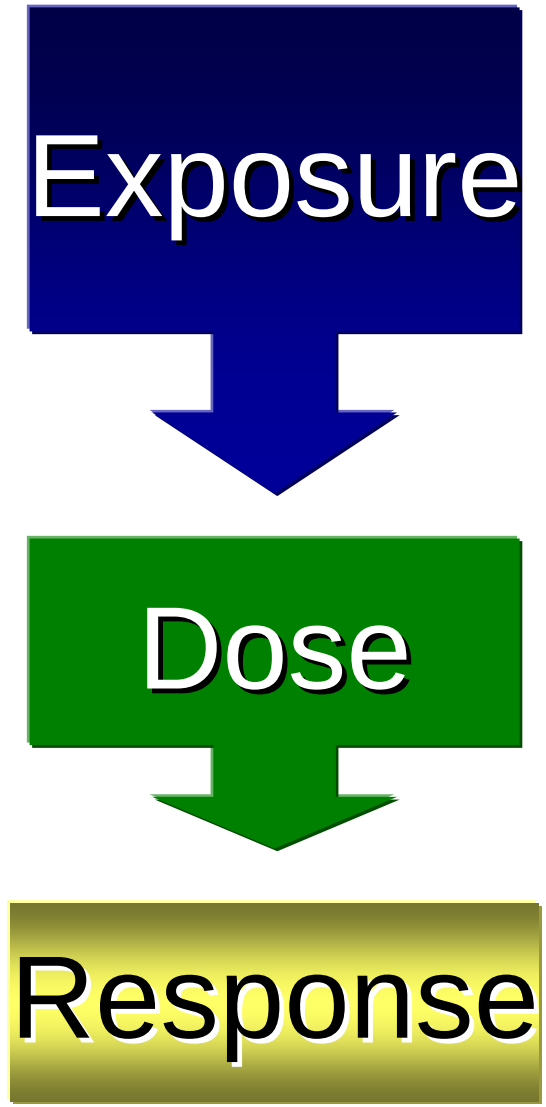
Cell Cycle: G2/M Checkpoint

EGFR signaling

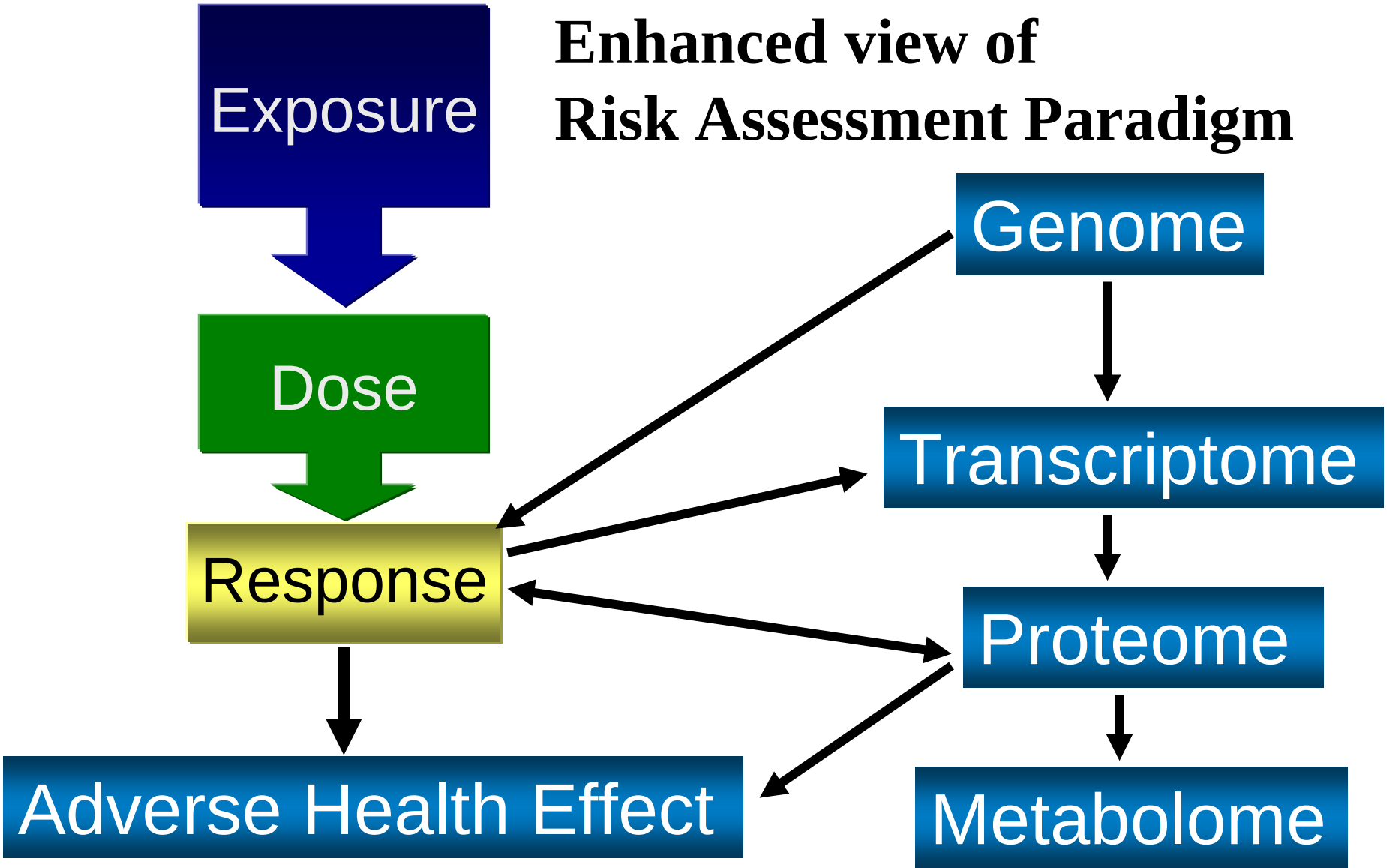
Regulation of Lipid Metabolism via PPAR,RXR and VDR

Urea Cycle

Traditional view of Risk Assessment Paradigm



Enhanced view of Risk Assessment Paradigm



Summary

Genomics is not a tool of the future – it is available now

Current applications include

- Informing mechanistic research
- Improving approaches to cumulative risk assessment
- Determining source water contamination through microbial source tracking
- Informing mode of action analysis in risk assessment
- Screening and predicting adverse effects
- Testing prioritization