The Use of Genomics in Cancer Risk Characterization and Safety Assessment.

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Guidelines for Carcinogen Risk Assessment

Risk Assessment Forum
U.S. Environmental Protection Agency
Washington, DC

http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=116283

Features of Cancer Guidelines

- Hazard Identification
- Framework for mode of action
- Weight-of-evidence narrative
- Two-step dose-response process
  - model the observed data
  - extrapolation to lower doses
- Linear and nonlinear extrapolations
- Differential risks to susceptible populations and lifestages

Important Definitions

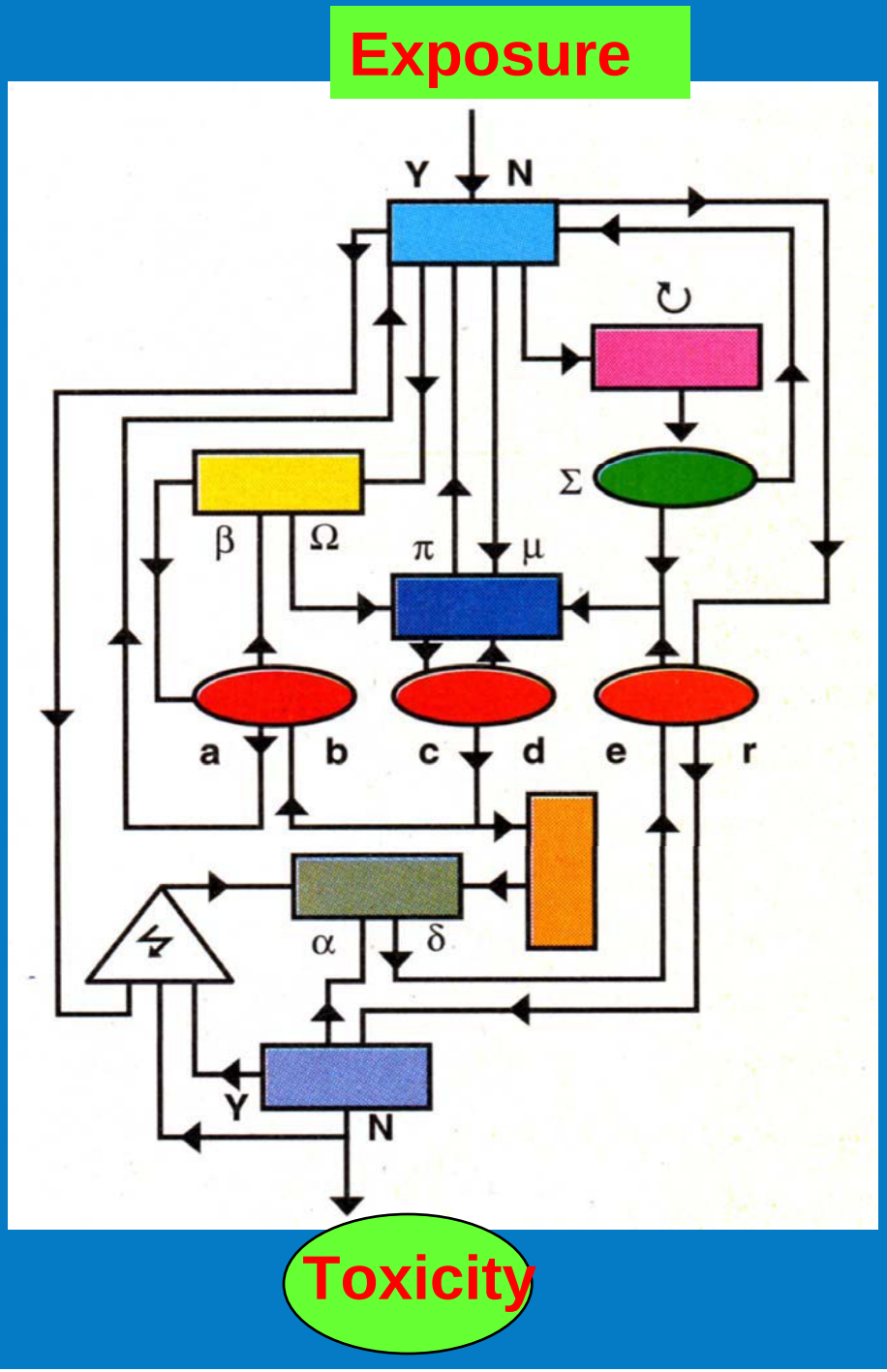
Mode of Action: Key events and processes, starting with the interaction of an agent with the target cell, through functional and anatomical changes, resulting in cancer or other adverse health effects

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

mode of action

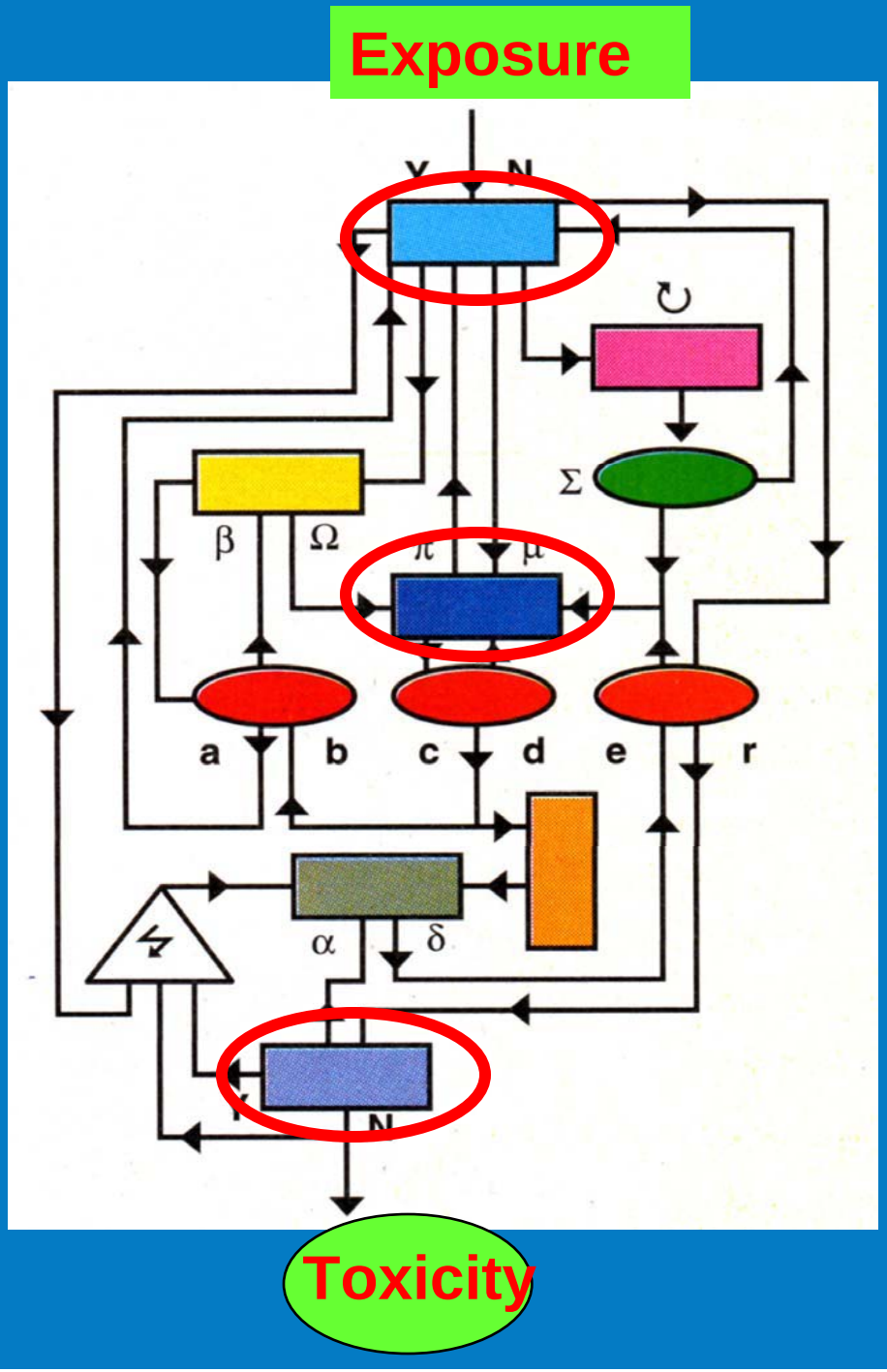


mechanism of action

detailed understanding at biochemical & molecular level

mode of action

identification of key & obligatory steps



Use of Mode of Action Information

Relevance of laboratory animal results to human environmental exposures

Shape of dose-response curve

Low-dose extrapolation

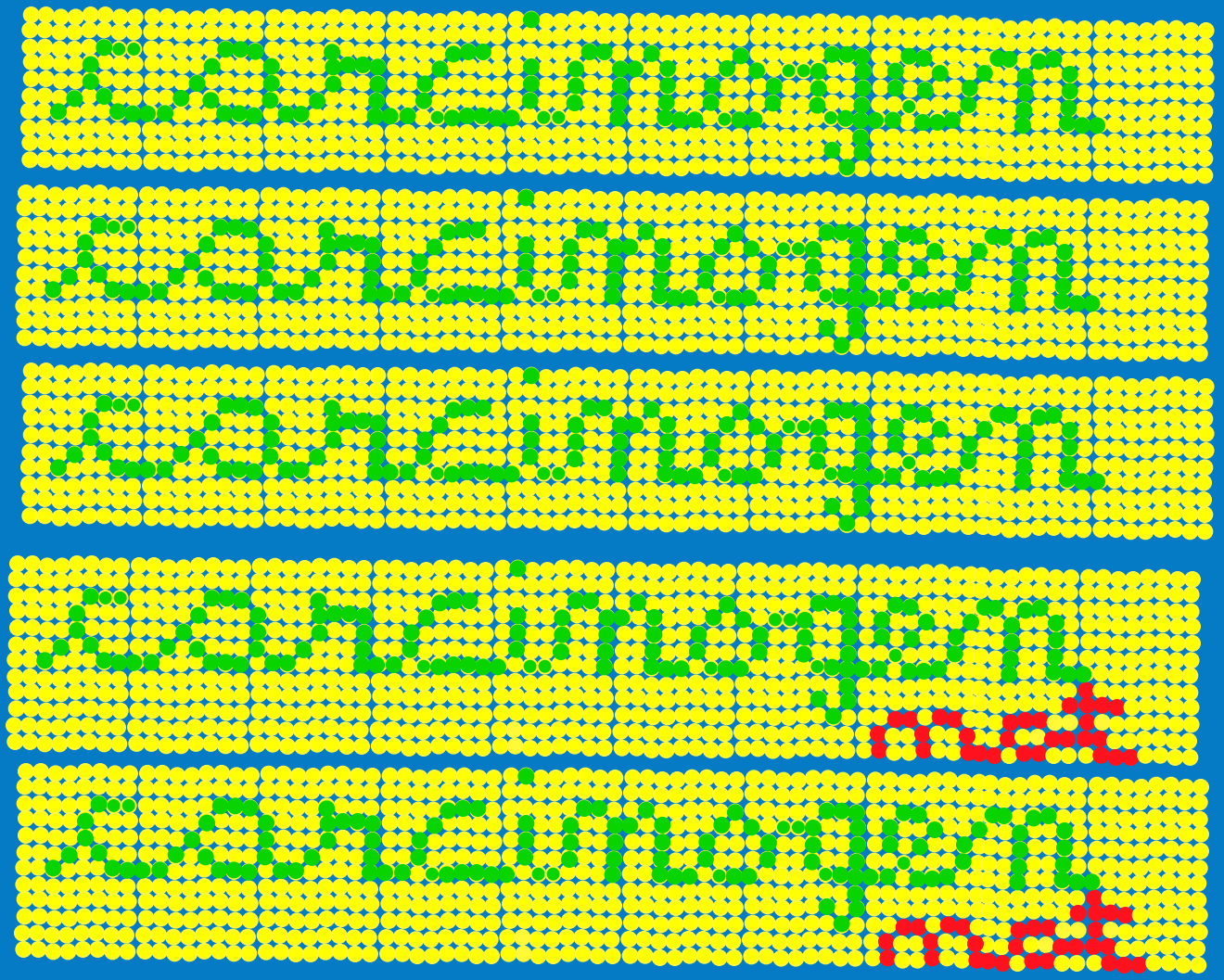
Identify susceptible populations and lifestages

Species Extrapolation

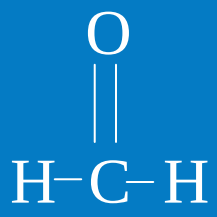
Differentiating a Carcinogenic from a Non-carcinogenic Aldehyde

Work Conducted by Susan Hester, Ph.D.

All purpose carcinogen screen

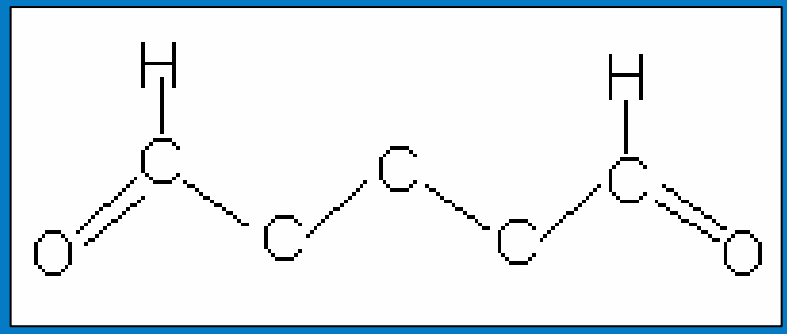


Formaldehyde



one aldehyde group

Glutaraldehyde



two aldehyde groups (bi-functional)

Aldehydes

Aldehydes are very reactive compounds, participating in oxidation, reduction, addition, and polymerization reactions

Reacts with proteins and DNA

Induces DNA-protein and protein-protein cross-links

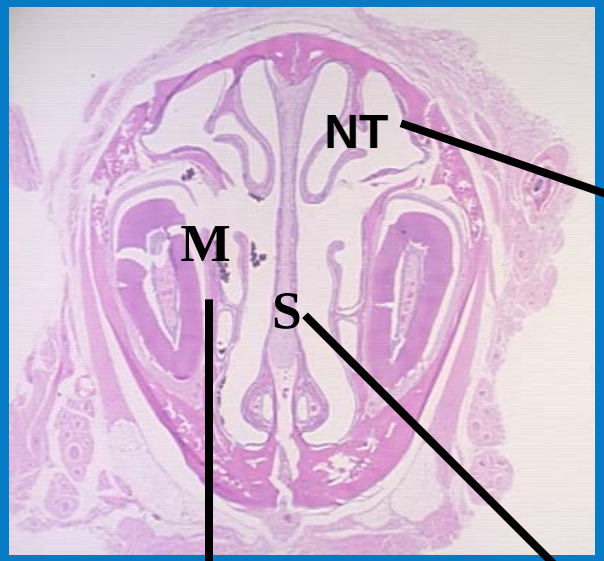
Induces mutations

Induces cytotoxicity with subsequent increased cell proliferation

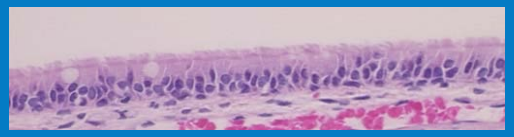
Formaldehyde induces squamous cell carcinoma in the rat nose and human respiratory tract cancer

Glutarlaldehyde long term exposure in rodents results in no cancer

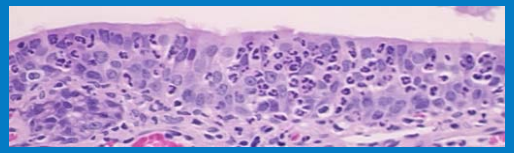
Formaldehyde and Glutaraldehyde Nasal Lesions



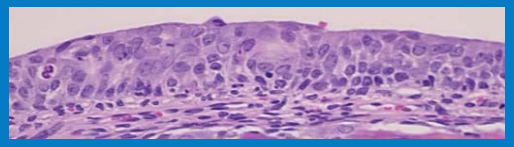
Nasooturbinate



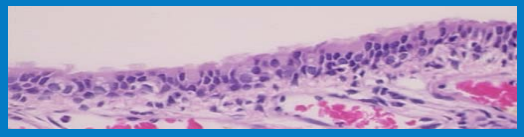
Control

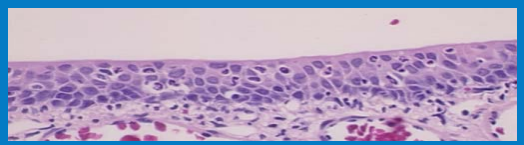


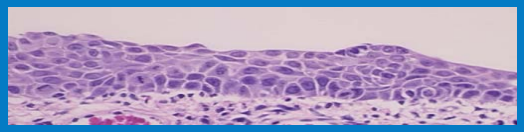
Formaldehyde



Glutaraldehyde





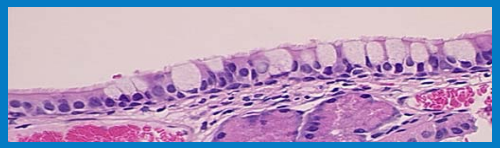


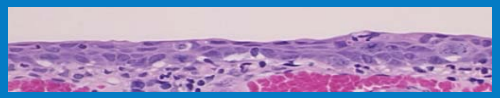
Maxilloturbinate

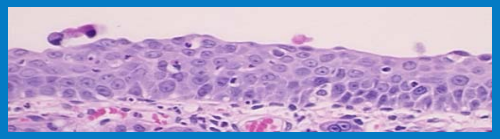
Control

Formaldehyde

Glutaraldehyde







Nasal Septum

Experimental Design

- 3 time points: 3 treatment groups (4 animals each)

for histologic examination: (only 1 time point done as acute exposures previously reported)

28 d of FA (400 mM); GA (20 mM); distilled water

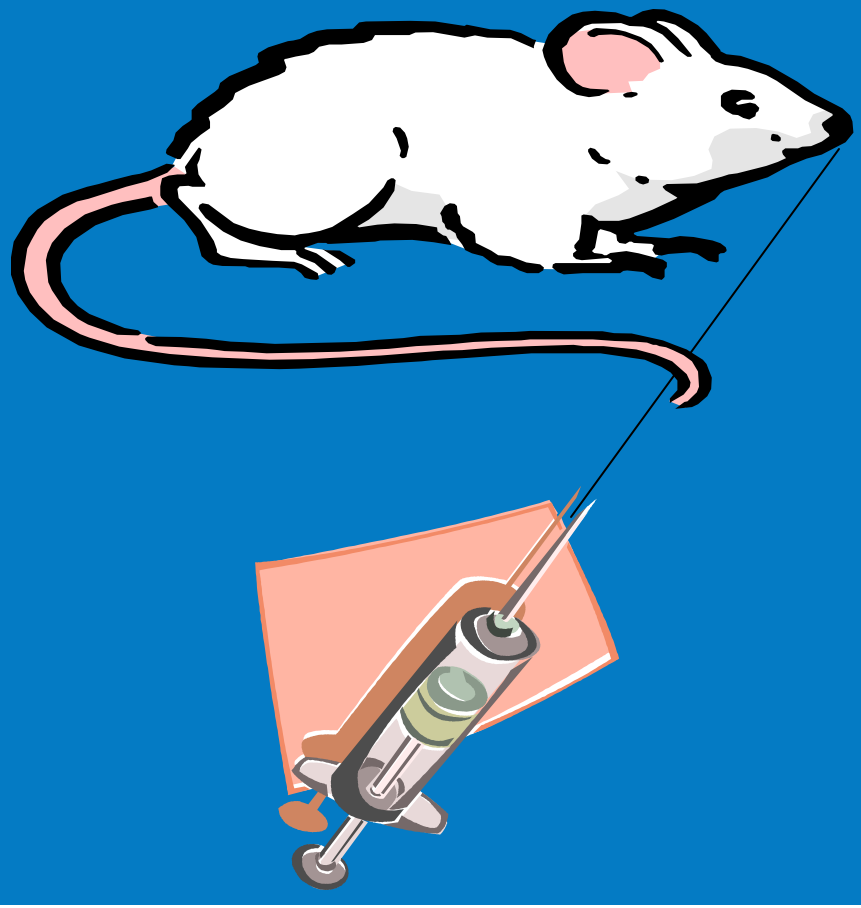
for gene expression studies:

1, 5, and 28 d of nasal instillation of FA (400 mM); GA (20 mM); distilled water

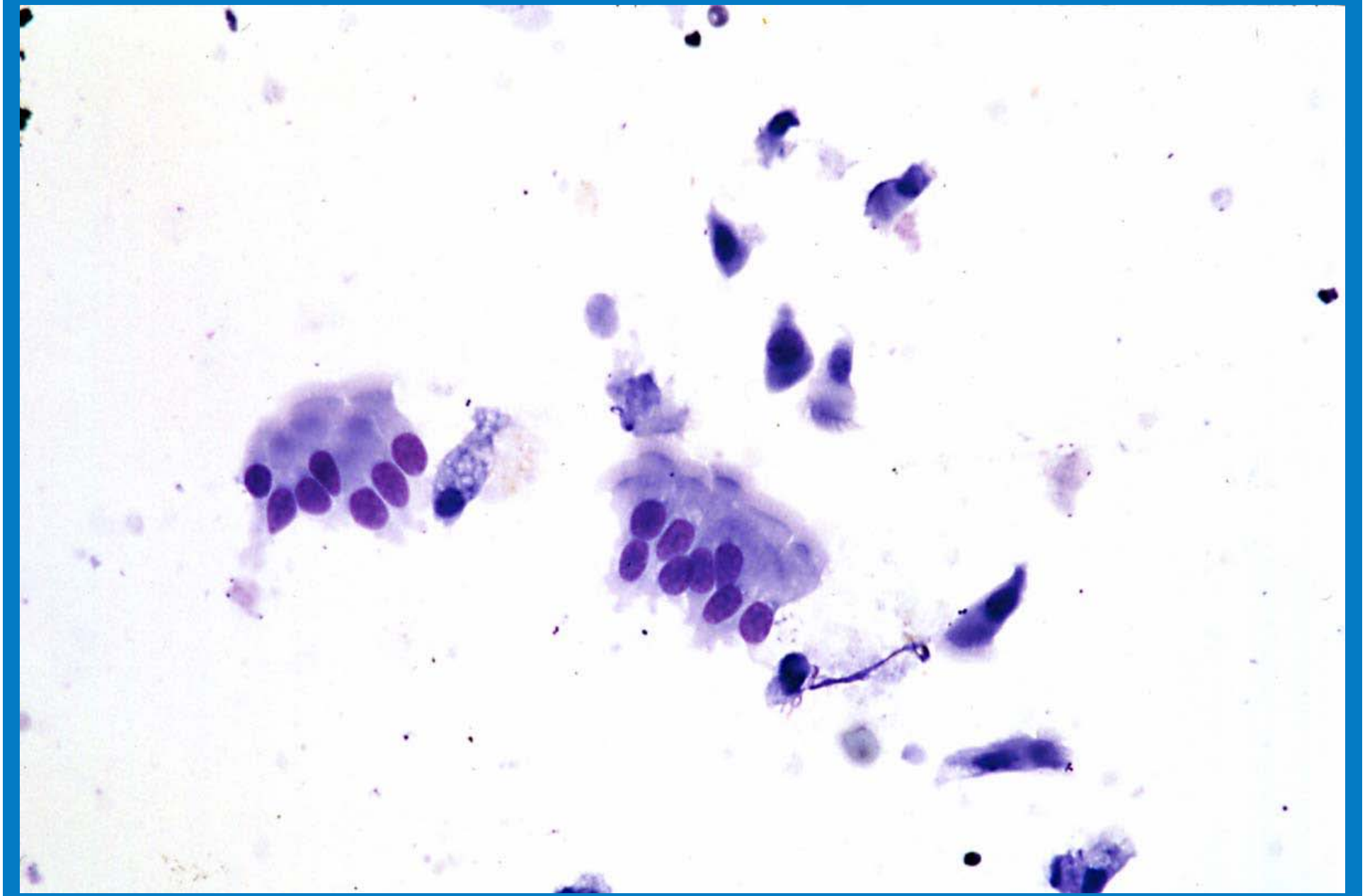
- Perform *in vivo* removal of nasal epithelium (RNA reagent-Trizol)
- Isolate total RNA for probe generation

Method for extraction of Nasal lining cells

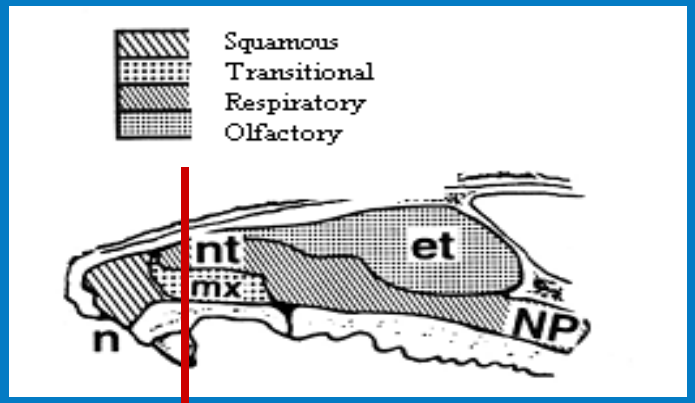
A Method to recover respiratory and transitional epithelial cells from the rostral nose up to the level of the septal window and isolation of total RNA for molecular analysis was developed.



Cytospin prep of nasal epithelium recovered

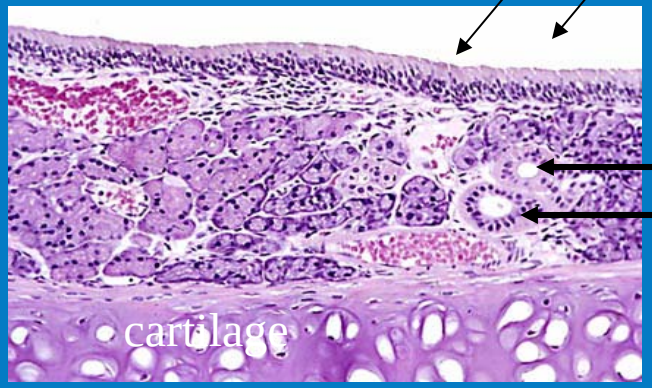


Recovered nasal respiratory and transitional cells from where formaldehyde tumors arise.



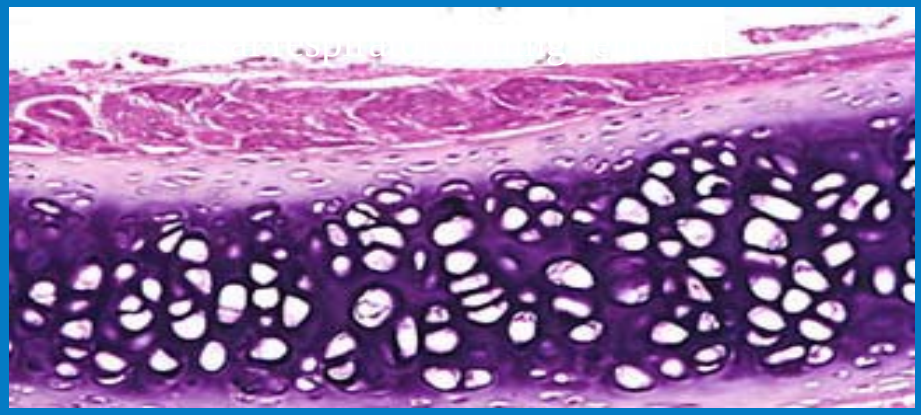
area for cell recovery

nasal respiratory lining

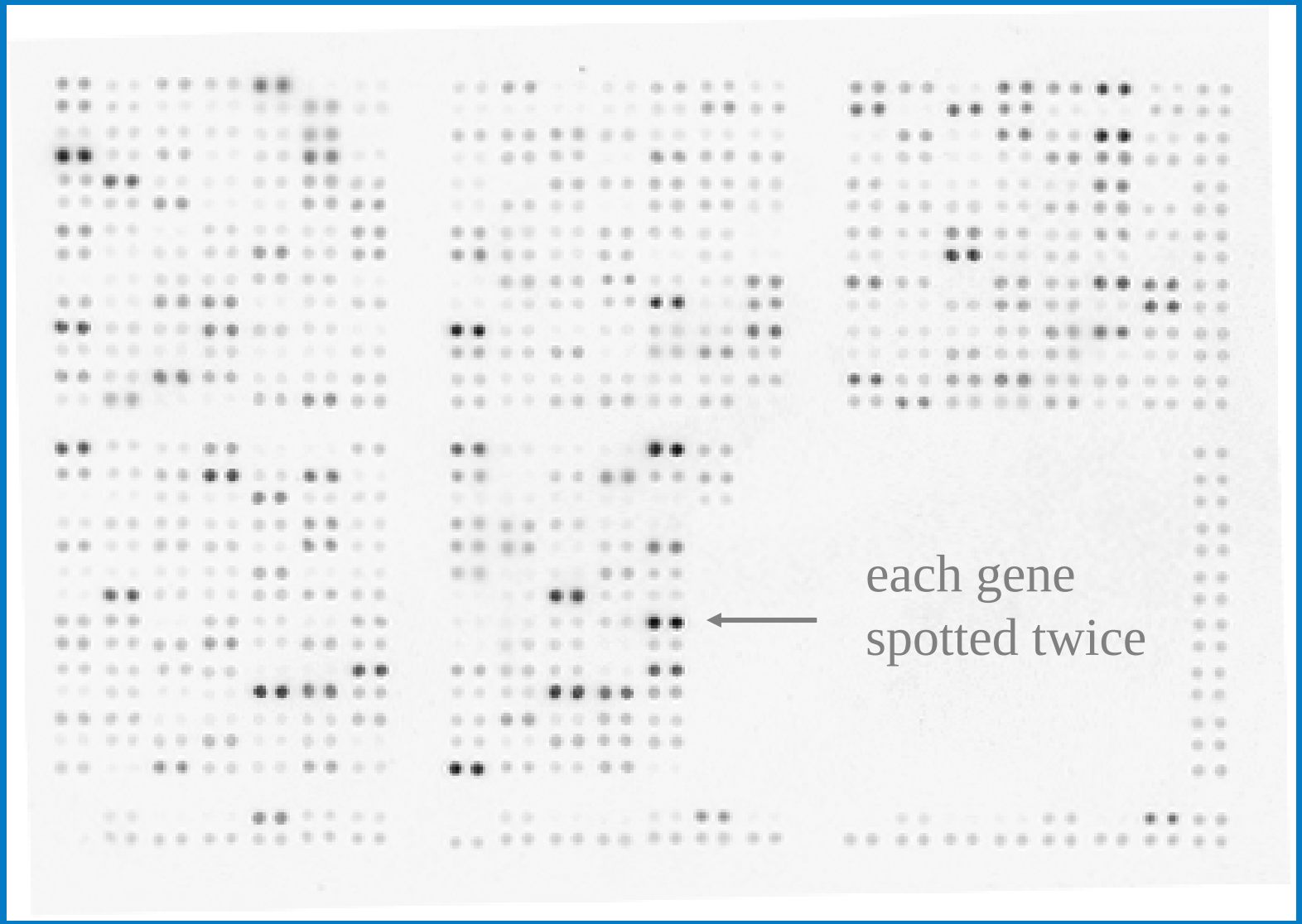


septal sero-
mucous gland

Trizol

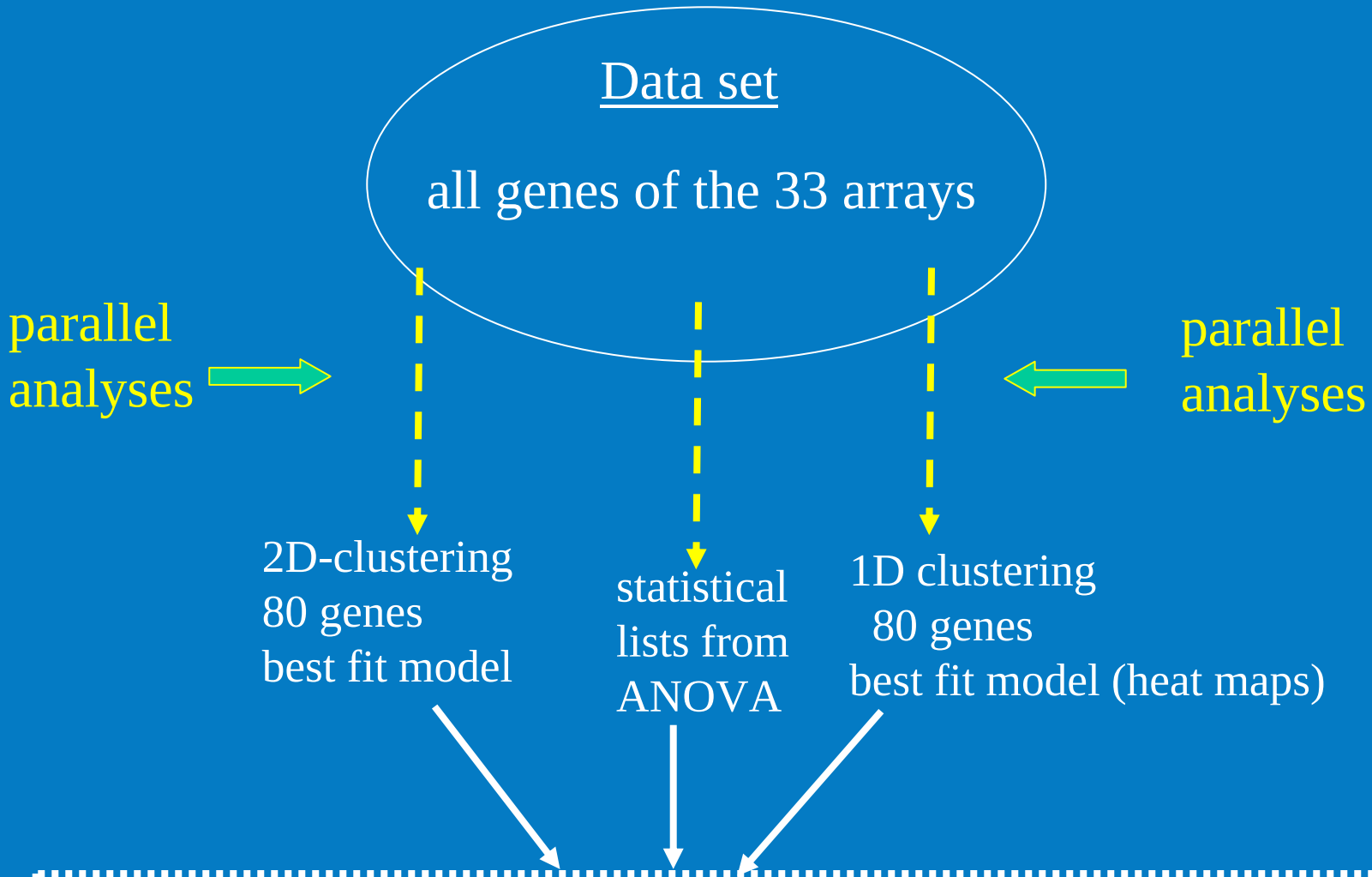


Gene Expression



Rat Toxicology II Array plus ^{33}P-cDNA Formaldehyde Rat Nose

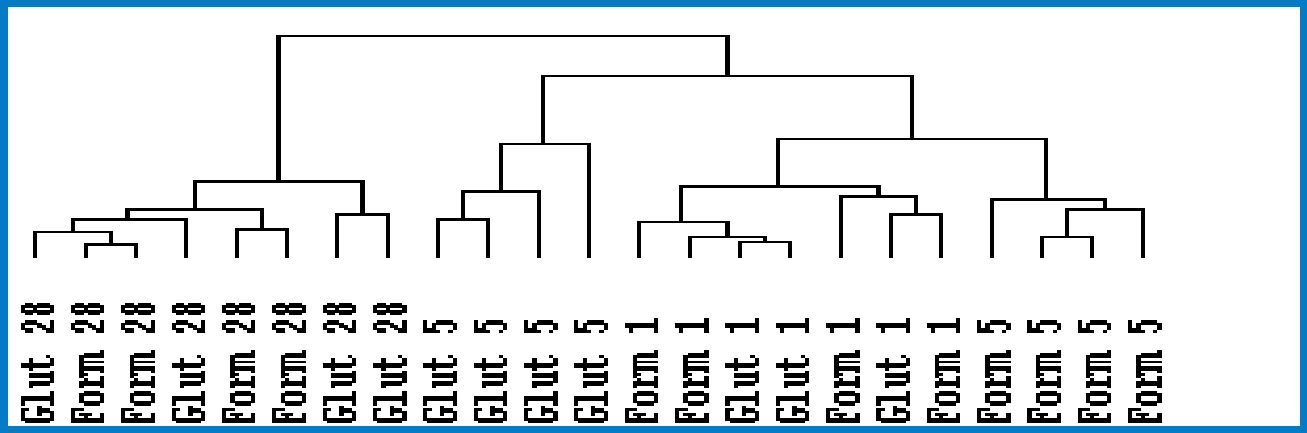
Data Filtering Strategy Used to Perform the Analysis



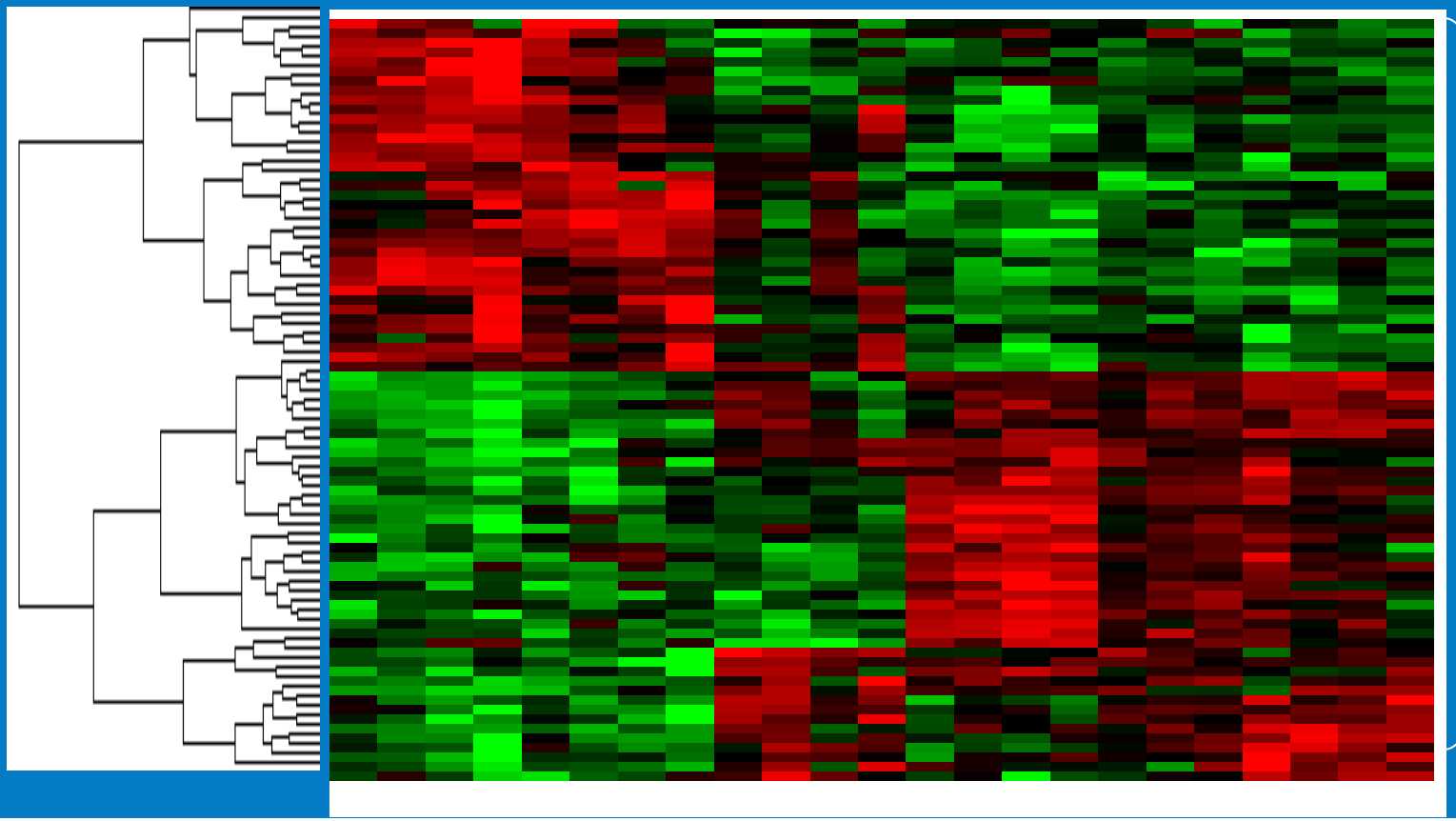
ALL agree that 3 categories of genes are altered by exposures:
DNA repair, metabolism, and apoptosis

80 Genes best fit the ANOVA model: search for patterns

arrays →

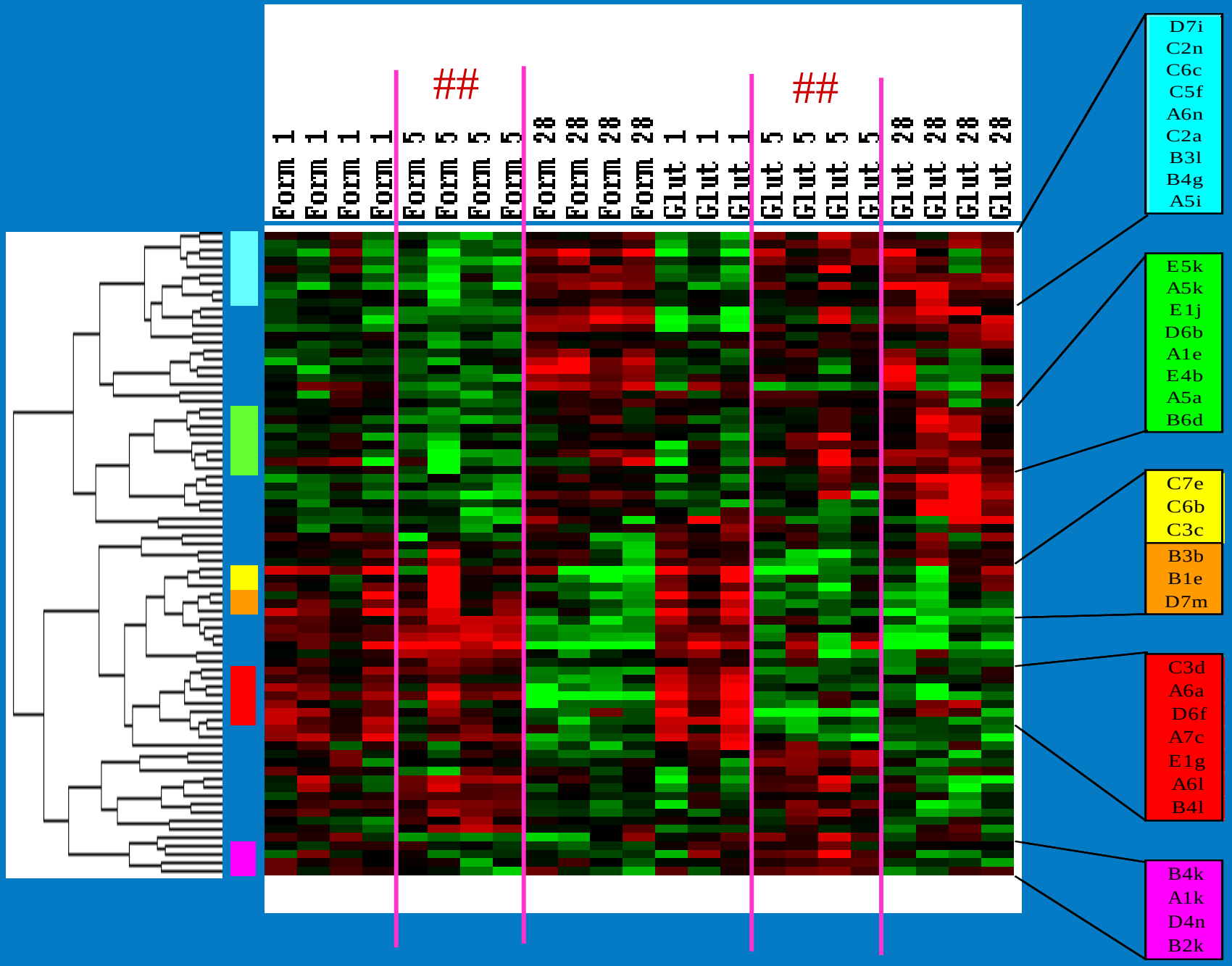


each row is a gene

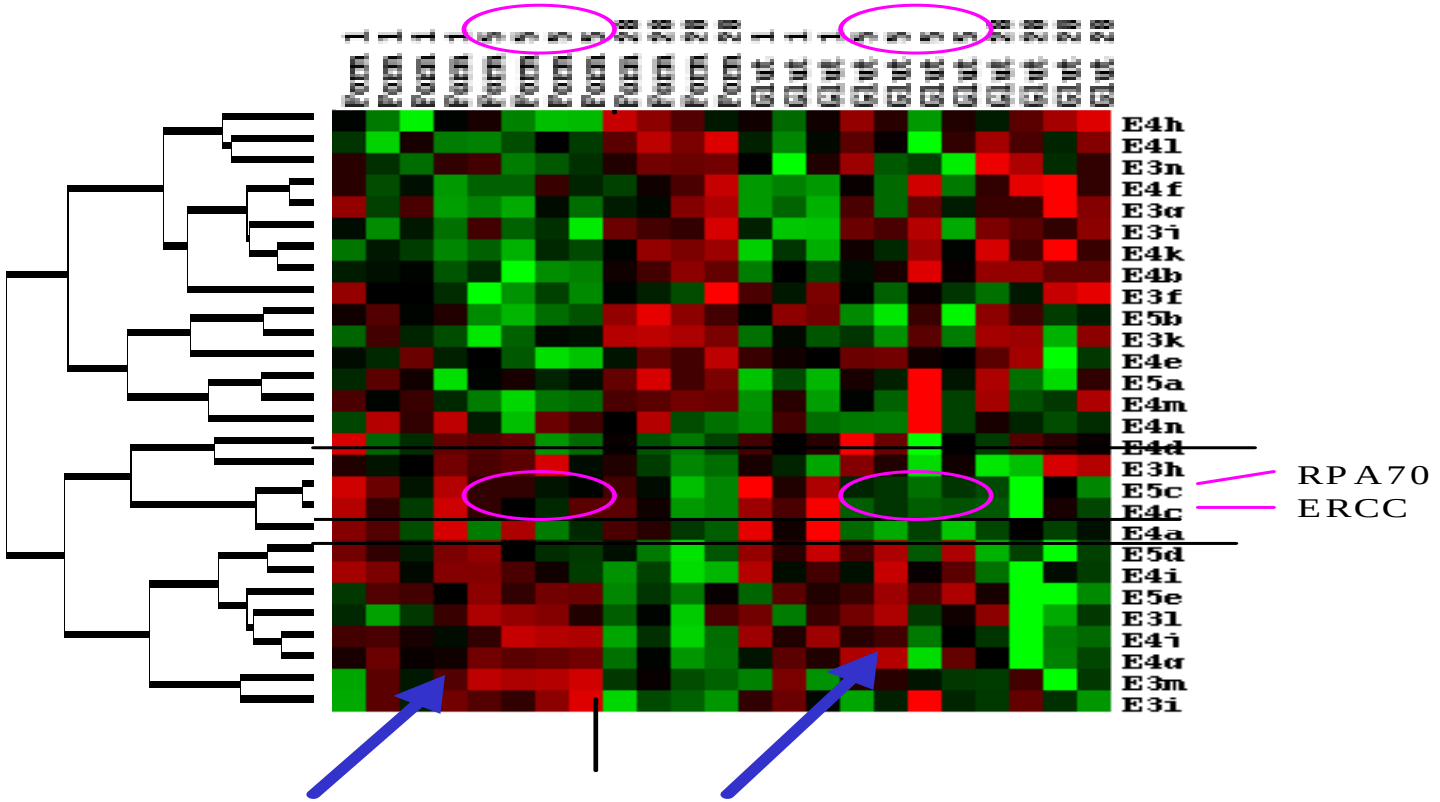


color shows response

Differential Gene Expression of Formaldehyde and Glutaraldehyde



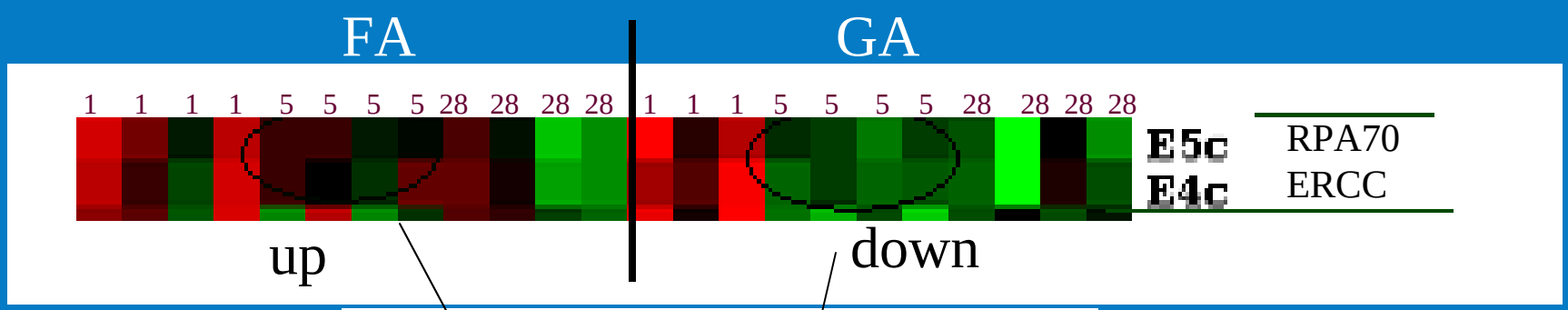
Heat Maps for Repair Genes: Loss of Induction at 5d



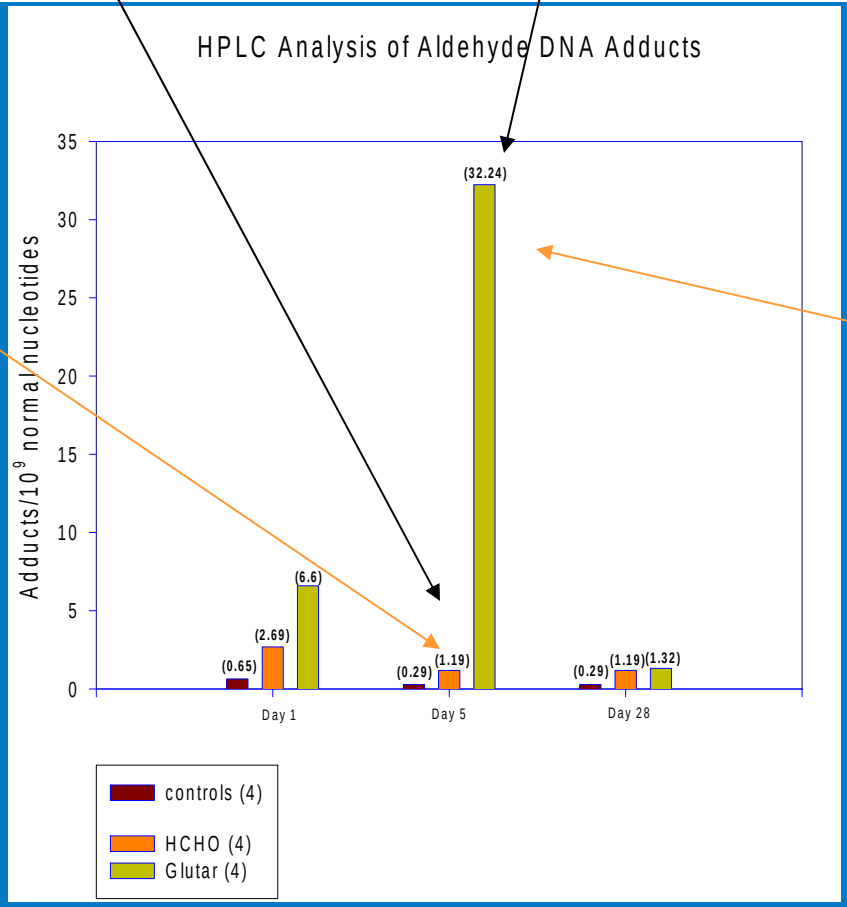
DNA repair genes: increased expression at 1&5 days for FA&GA

DNA-BP
RAD-52
3MA-glycosylase
Pol-delta1
VDJ-recom
O-6 methyltransferase
Top2a
PCNA

Heat Map for 2 nucleotide excision repair genes



FA adducts



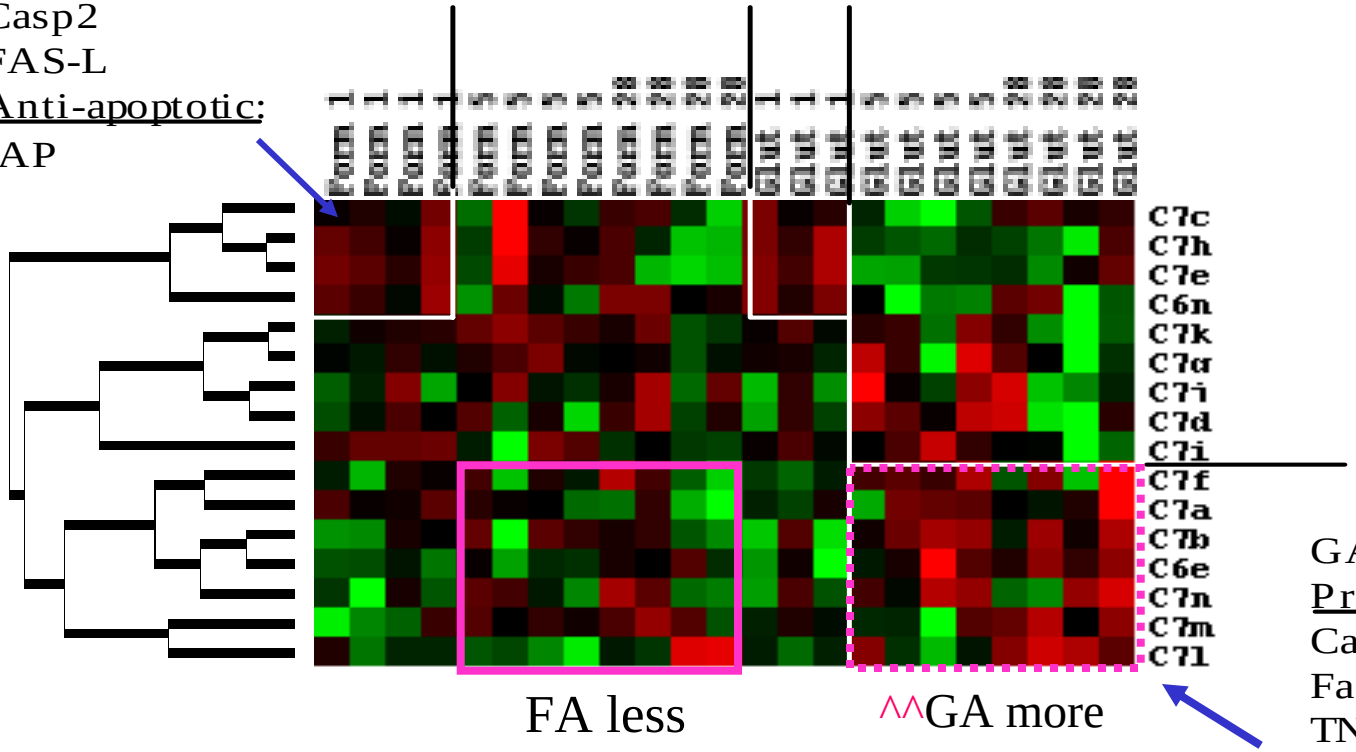
GA adducts

GA induces proapoptotic genes

1d- FA&GA

Proapoptotic:
Casp7
Casp2
FAS-L

Anti-apoptotic:
IAP



FA less

^^GA more

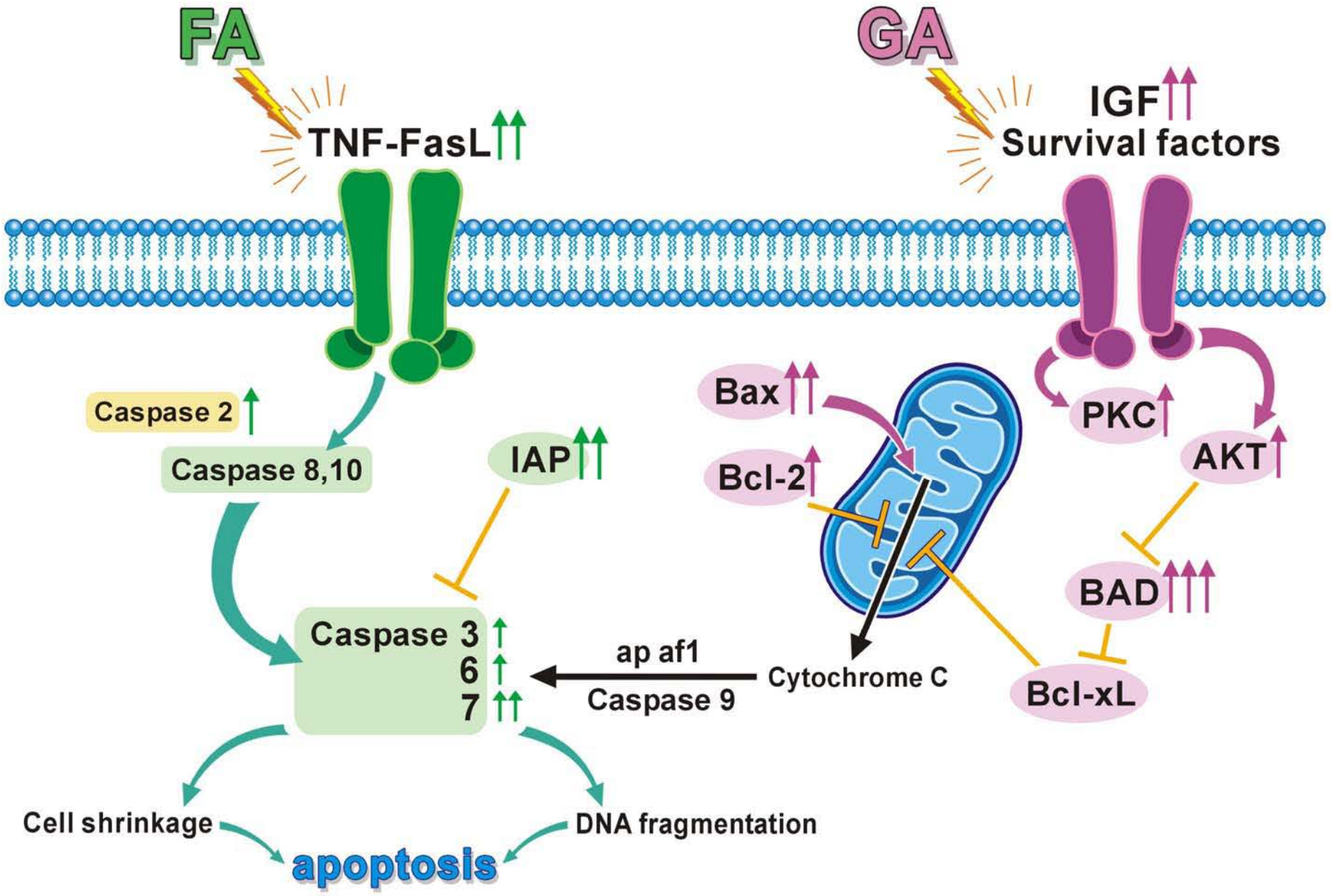
GA>FA 5&28d

Proapoptotic:
Casp1
Fas antigen
TNFR1
BAD
BOK

Anti-apoptotic:
Bcl-2
DAD

^^GA induces proapoptotic genes at 5 & 28 d to a greater extent than FA

Overview of Apoptosis Regulation



Conclusions

Histology:

Both compounds induced similar lesions characterized by hyperplasia, squamous metaplasia, inflammation, and scattered apoptotic bodies at 28 days.

Gene expression:

Formaldehyde induced less expression of genes controlling apoptosis compared to glutaraldehyde.

Formaldehyde induced a different apoptotic pathway than glutaraldehyde.

Formaldehyde induced greater expression of DNA repair.

Overall Biologic Conclusion

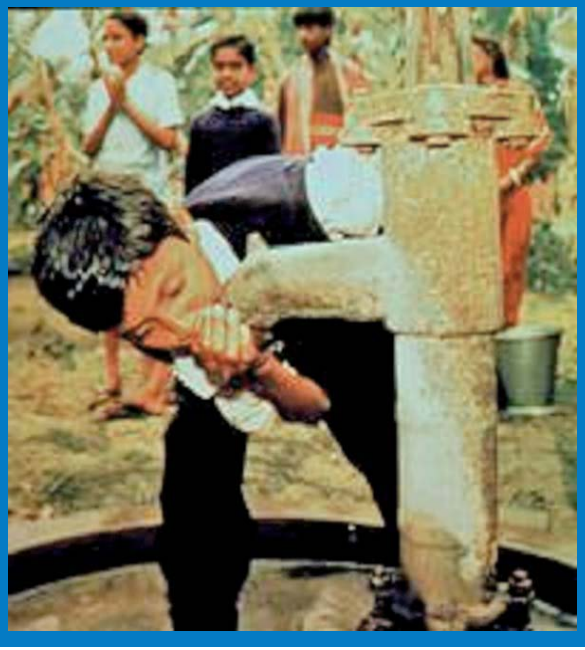
Glutaraldehyde has markedly greater cytoxicity than formaldehyde thereby inhibiting its ability induce tumors.

Gene Expression To Identify Interspecies Concordance Of The Mechanisms Of Arsenic Induced Bladder Cancer

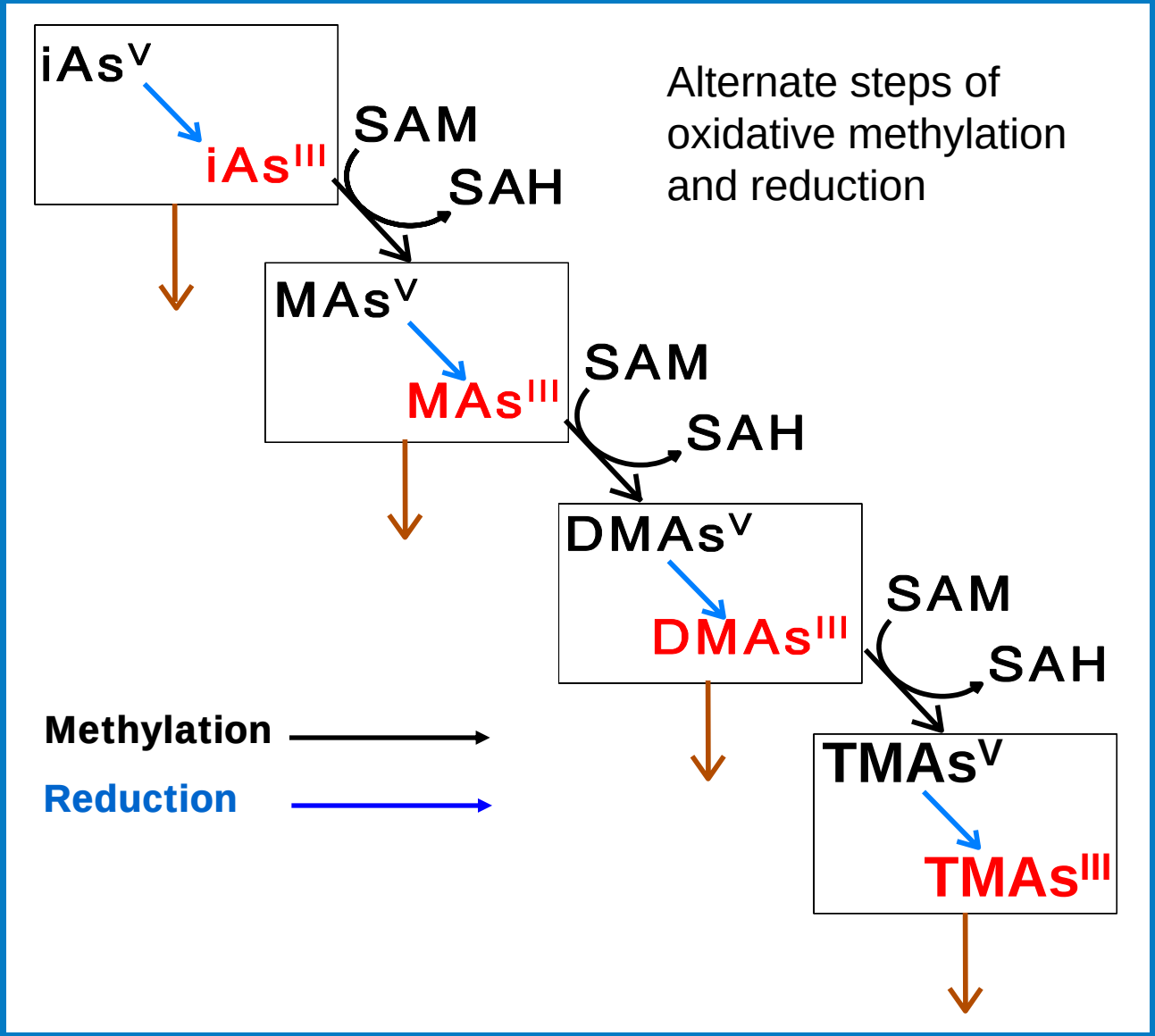
Work Conducted by Banalata Sen, Ph.D.

Arsenic (iAs)
a significant environmental concern worldwide
millions of people are at risk from drinking arsenic contaminated water.
increased incidence of skin, lung and urinary bladder cancer.

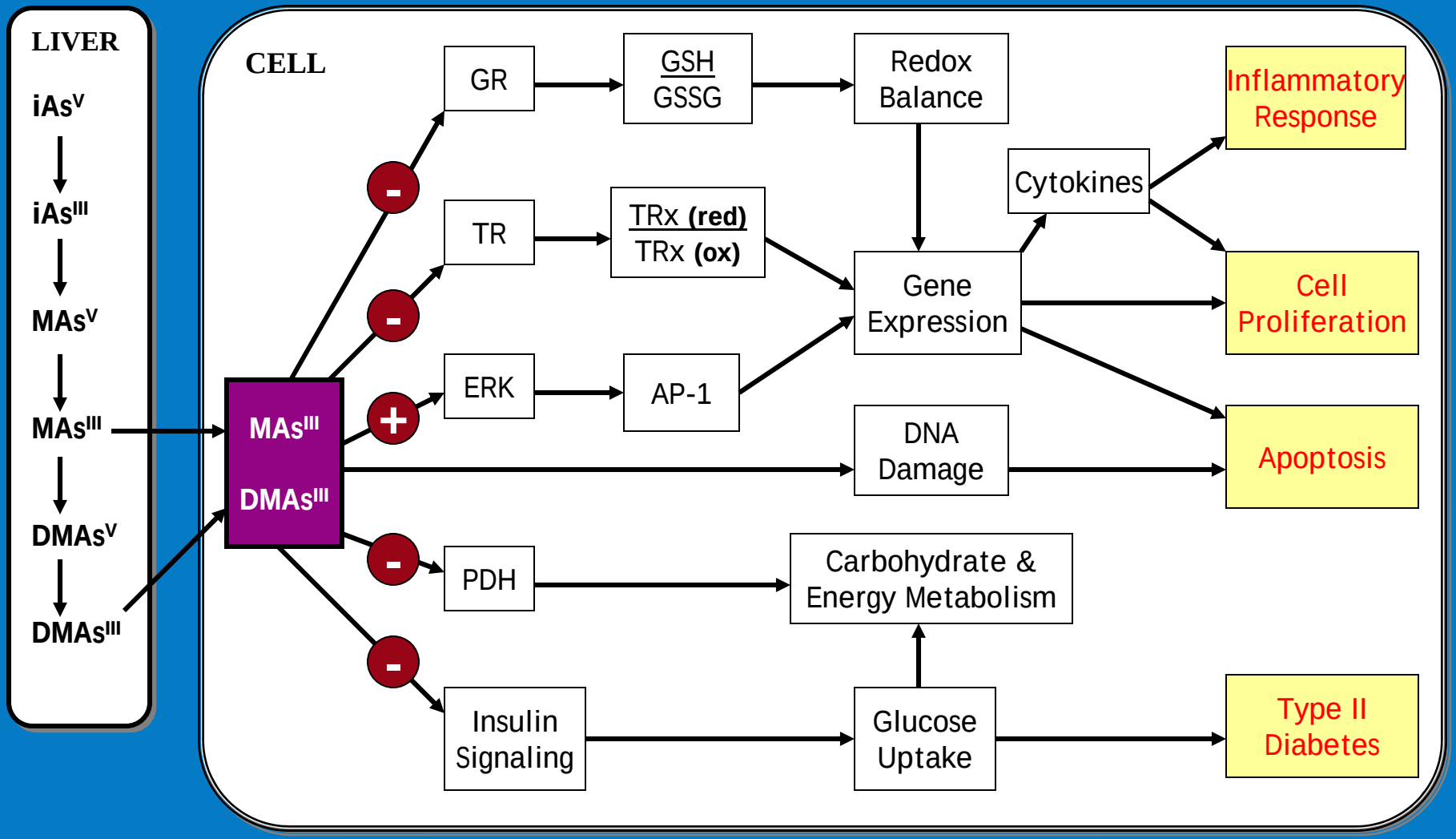
Dimethylarsinic acid (cacodylic acid, DMA^{V})
major metabolite of iAs
Herbicide
increase in transitional cell tumors of the urinary bladder in rats.



Challenger's Scheme for the Methylation of As



Toxic (and Carcinogenic) Actions of Methylated Trivalent Arsenicals

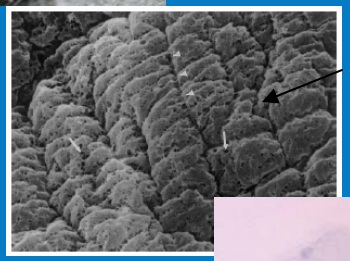


From Miroslav Styblo, UNC

Cacodylic Acid (DMA^V) Key Events in Mode of Action

DMA^V → DMA^{III}

(sustained) **Cytotoxicity**

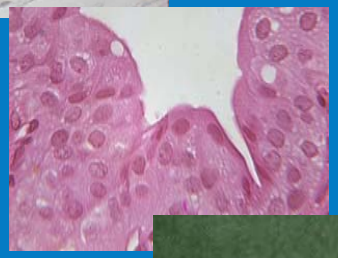


Urinary bladder from a female F344 treated with 100 ppm DMAV

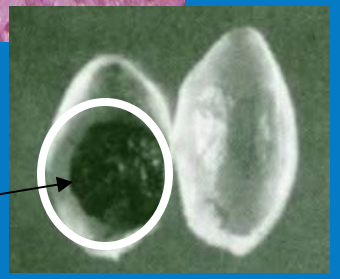
(sustained) **Enhanced Cell Proliferation**

BrdU Labeling

Hyperplasia



Tumors



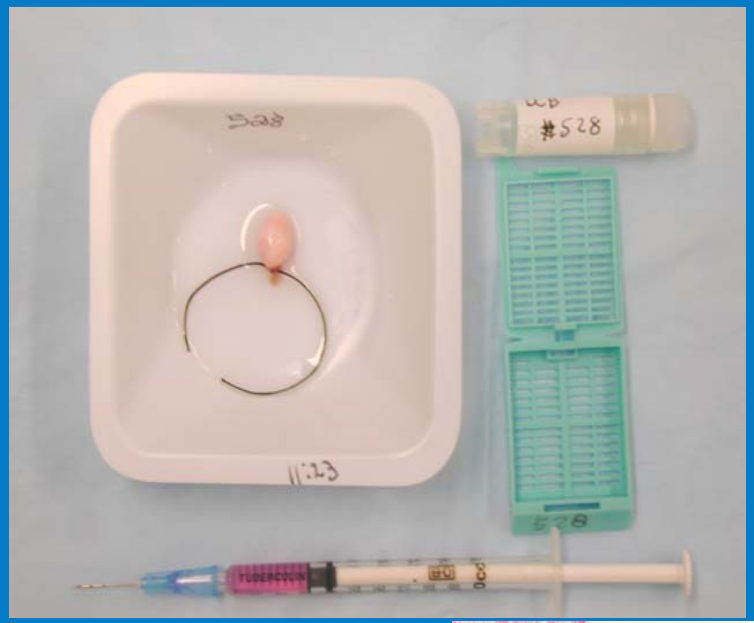
Urinary bladder tumors

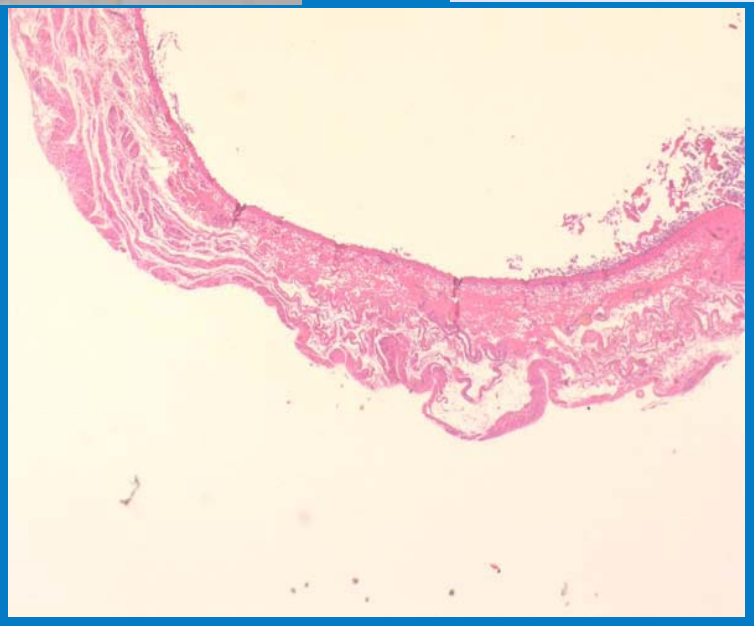
Methods

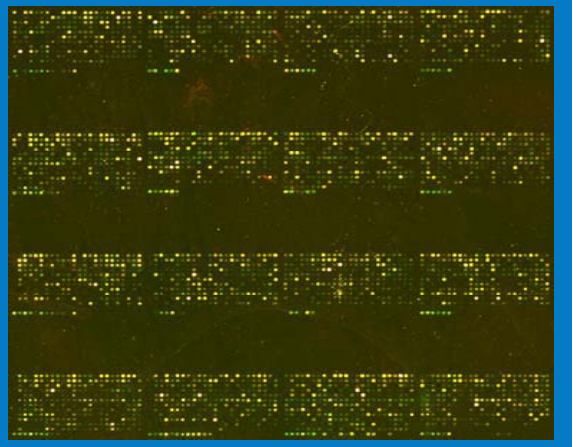
Exposure of F344 rats to DMA(V) at 100, 40, 4 and 1 ppm for 4 weeks through the drinking water

Immortalized human urothelial (UROtsa) and rat urothelial (MYP3) cells were treated for 18h with 8000, 200, 20 or 2 ppb DMA in serum free media. The selected doses were equivalent to the DMA present in the urine of rats exposed to 40, 1, 0.1 and 0.01 ppm DMA in drinking water for 1week. These doses were selected to mimic cytotoxic (40ppm) and non-cytotoxic (1ppm) doses to the rat bladder, as well as environmentally relevant (100 and 10 ppb) doses of DMA. Each exposure was done in triplicate.

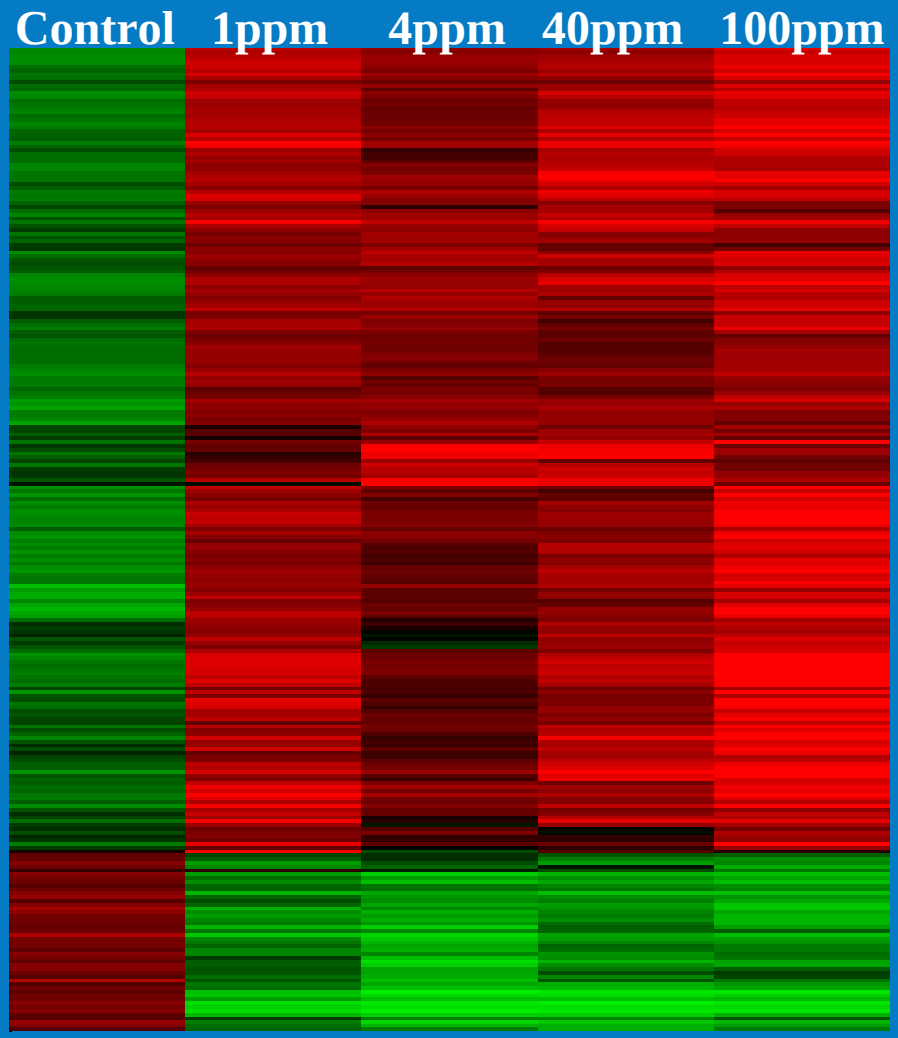
Technique to Collect Transitional Epithelium for RNA Isolation and Expression Profiling





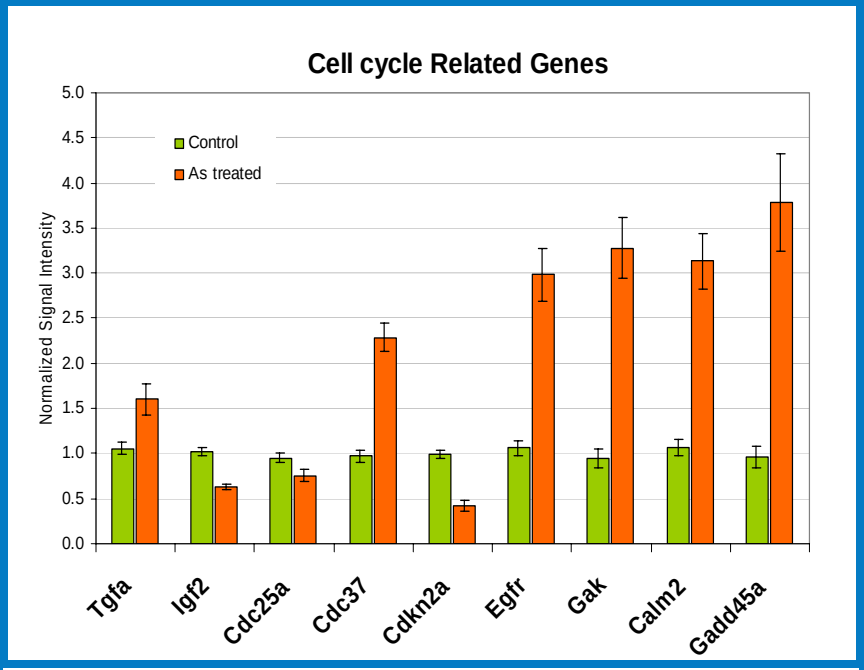


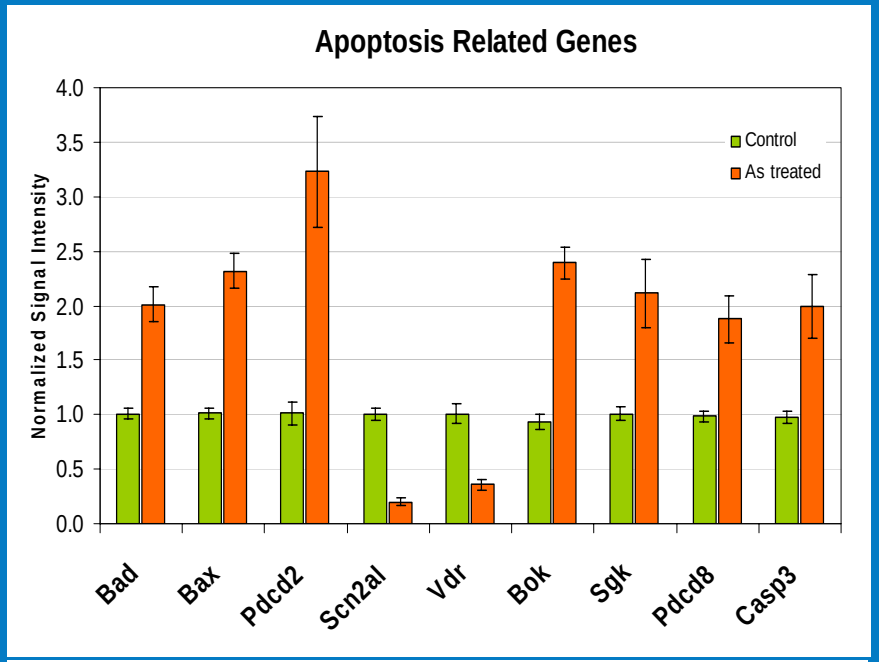
In Vivo Results

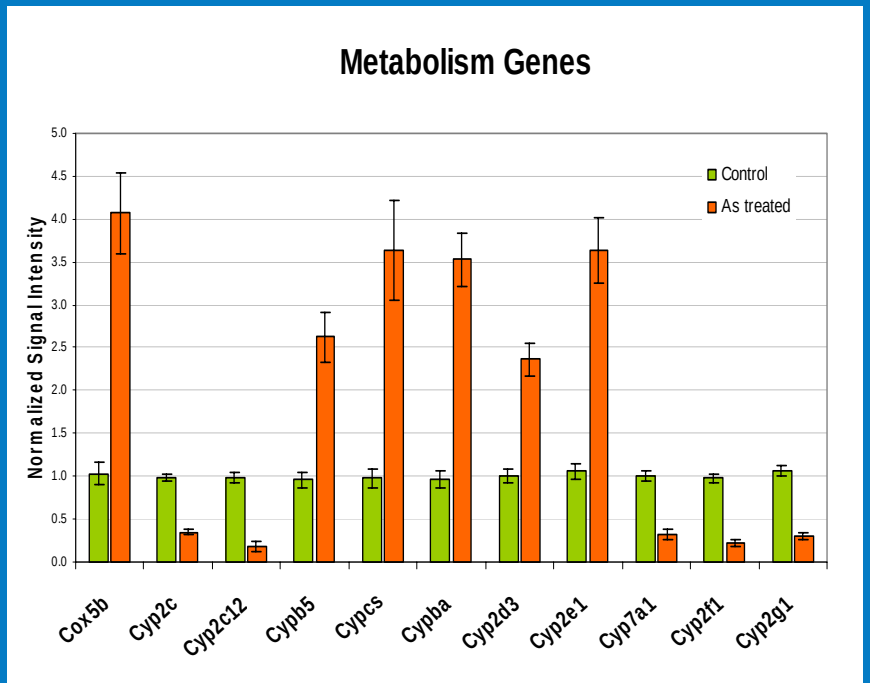


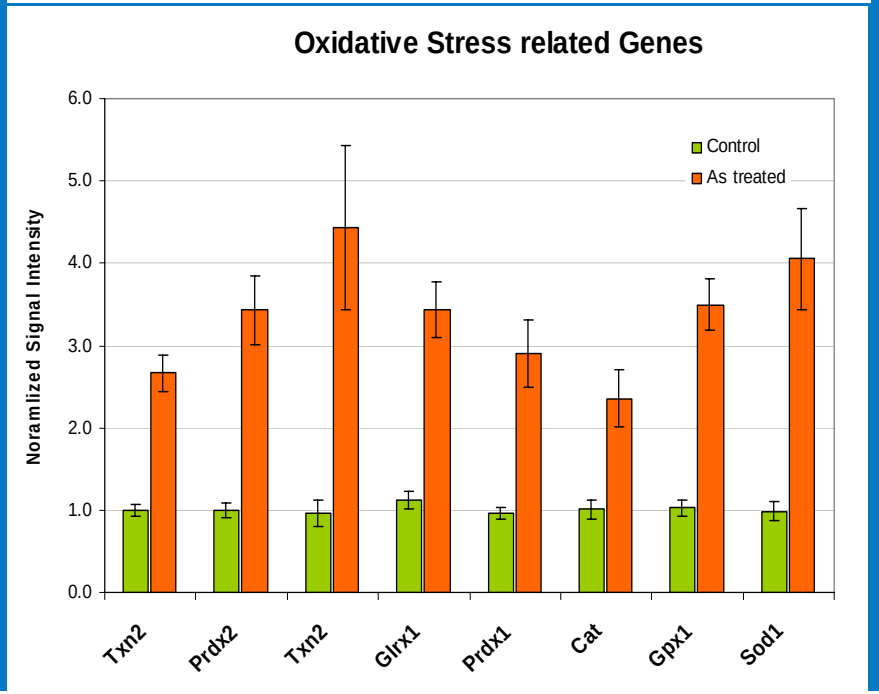
Global analysis of ~4,300 genes by microarray showed that 46% were expressed. Of the 510 genes that were significantly altered, 48% were induced (196 transcripts) or suppressed (47 transcripts) by at least 3 fold upon treatment with DMA(V).

In Vivo Results



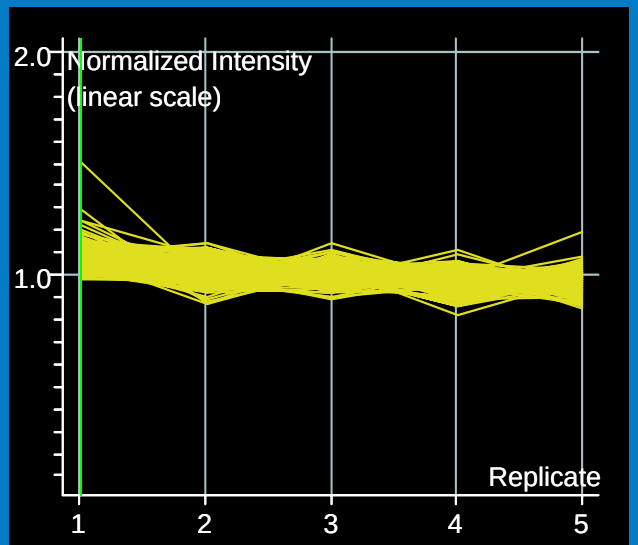




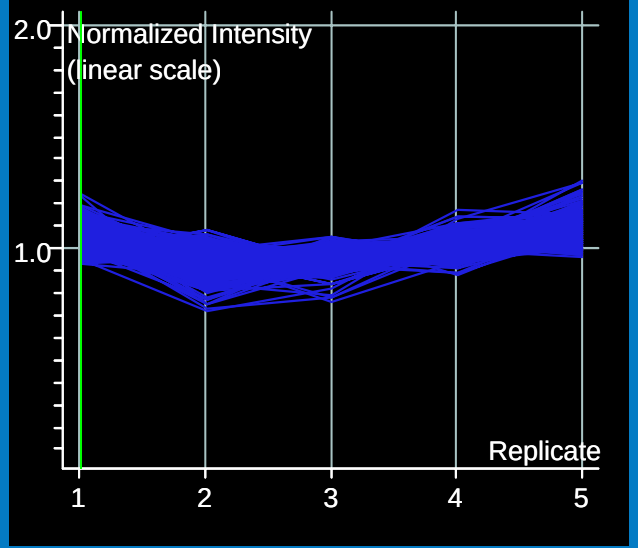


In Vitro Results

HUMAN (UROtsa)

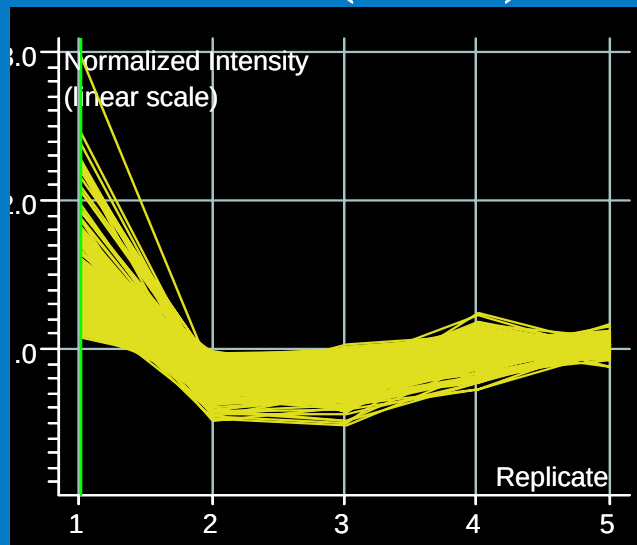


Set 2: 230 genes, 230 in list

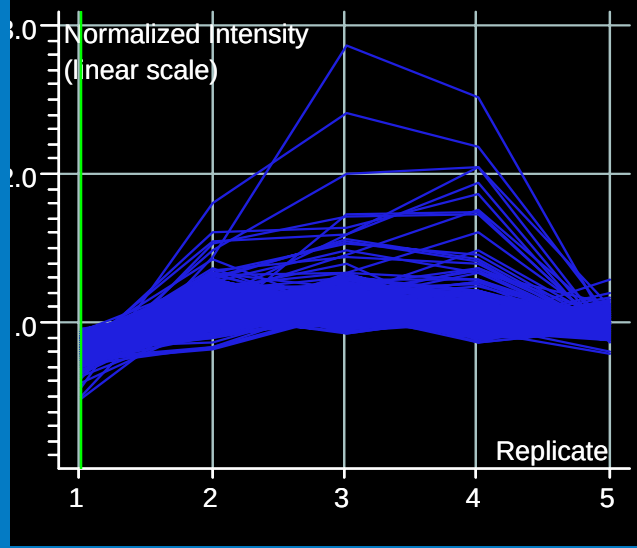


Cell Growth
Proliferation
Apoptosis
Stress

RAT (MYP3)

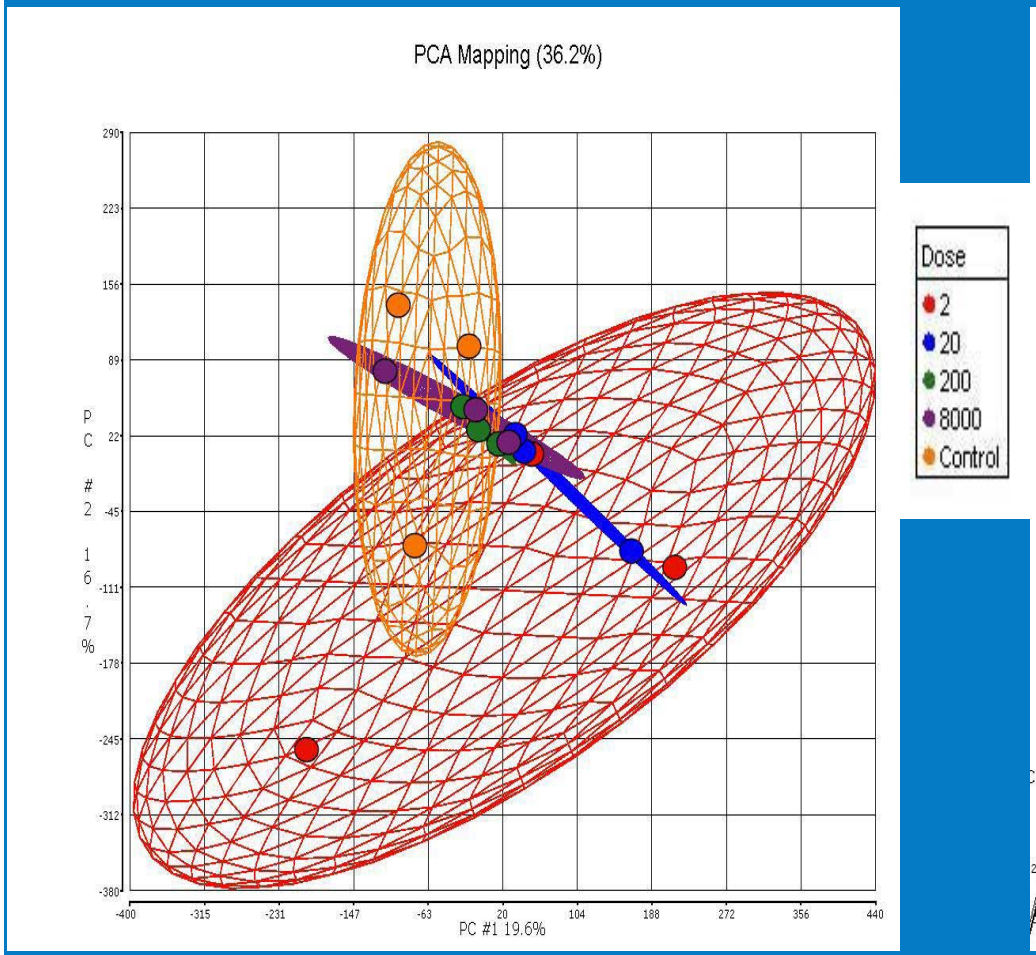


Set 2: 287 genes, 287 in list

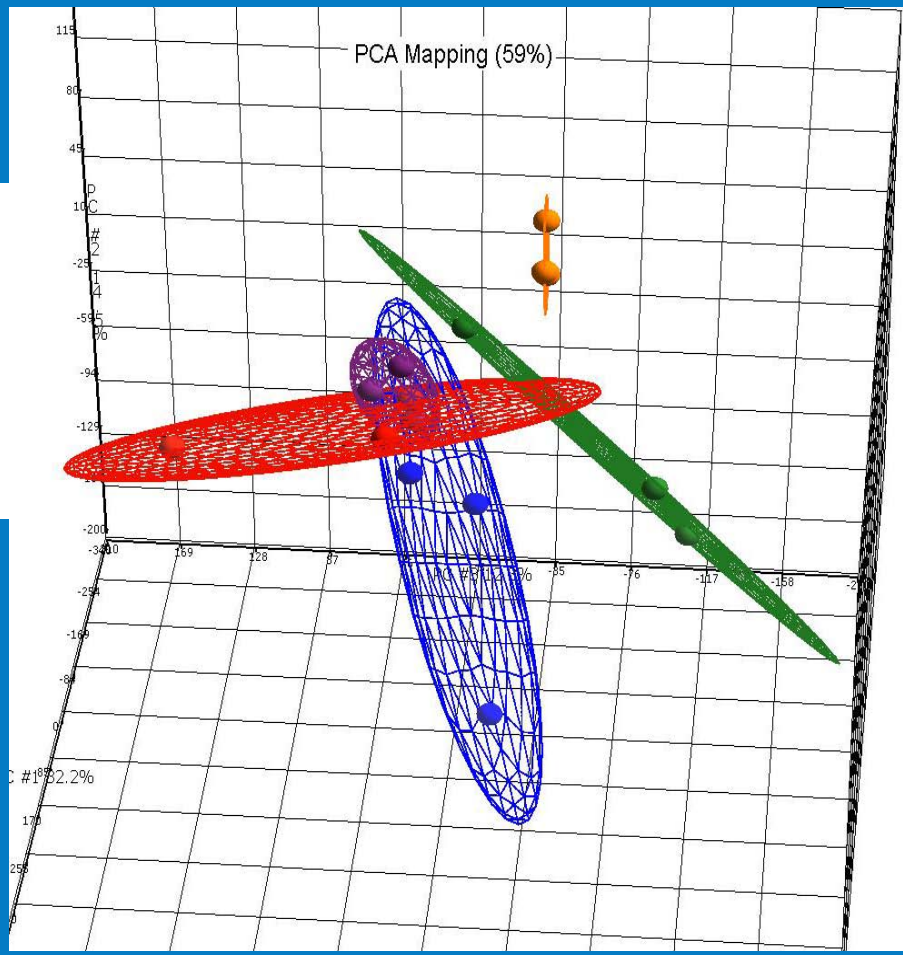


In Vitro Results

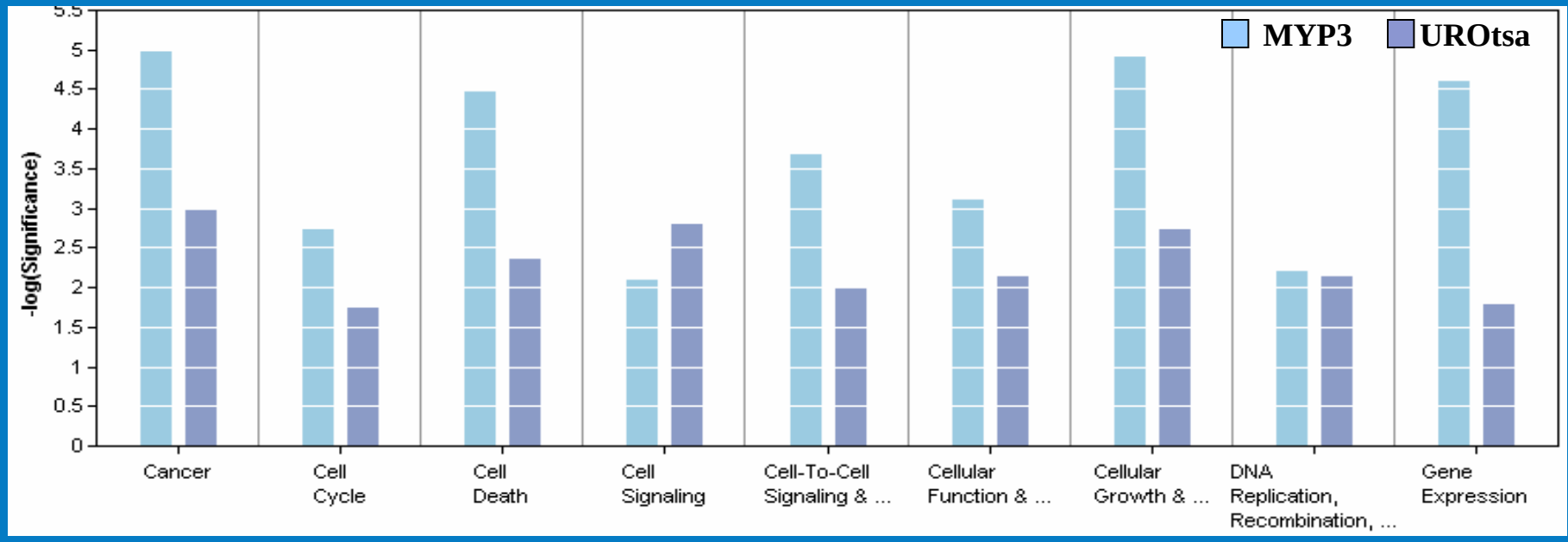
HUMAN (UROtsa)

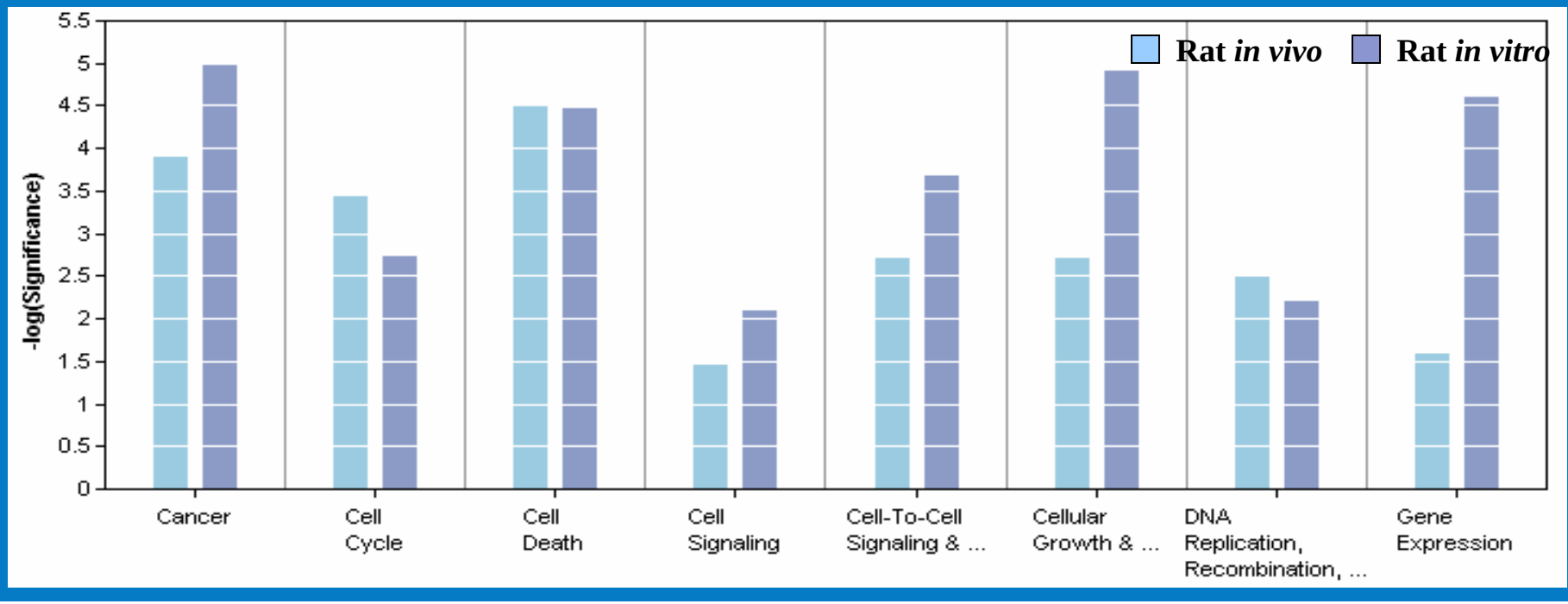


RAT (MYP3)

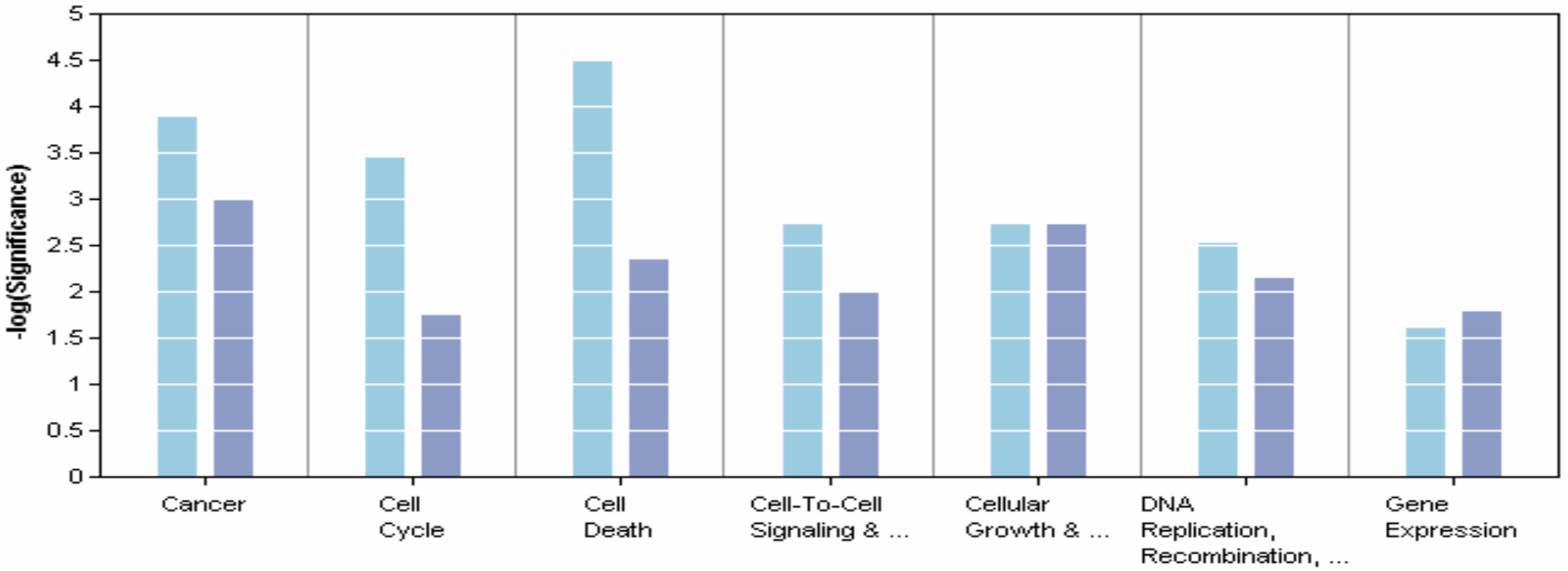


Comparisons





Comparisons



Rat *in vivo*

Human *in vitro*

Dimethylarsenic (V) Acid in Drinking Water

Morphological changes observed at carcinogenic doses of DMA(V) exposure are indicative of cellular toxicity.

Functional categories of genes altered after DMA(V) exposure were consistent with reported mechanisms associated with arsenic-induced carcinogenicity.

A distinct treatment response as well as a dose response is evident from the expression profile of the rat bladder cells (MYP3) following exposure to DMA.

The dose and treatment response to DMA exposure are not as prominent in human bladder cells (UROtsa). This difference could be due to differences in uptake and/or lower susceptibility.

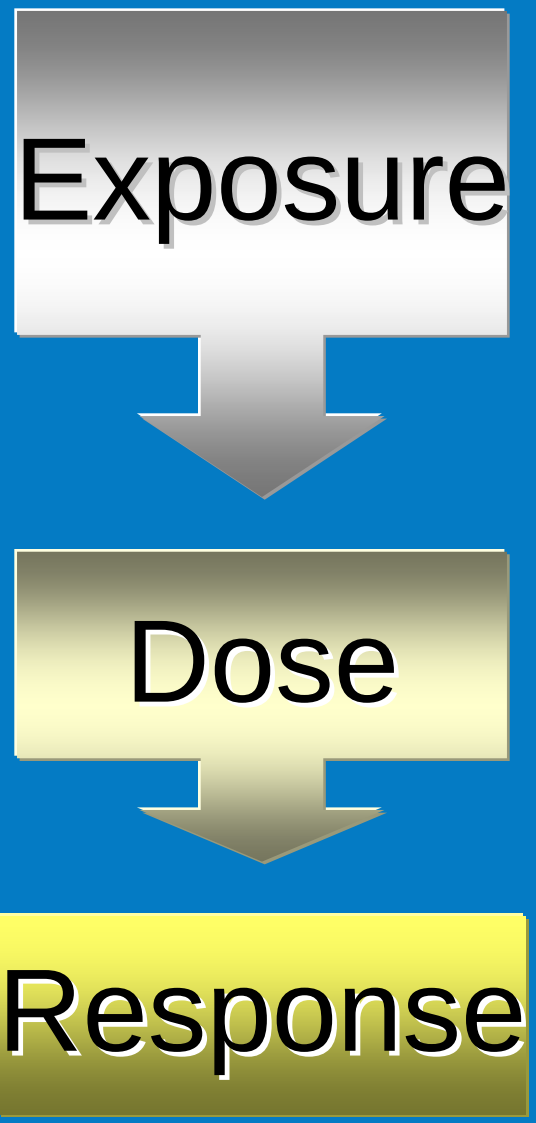
Overall Biologic Conclusion

Changes in gene expression of cancer control pathways alone is not sufficient to drive the cancer process, cellular toxicity present at higher doses is also necessary.

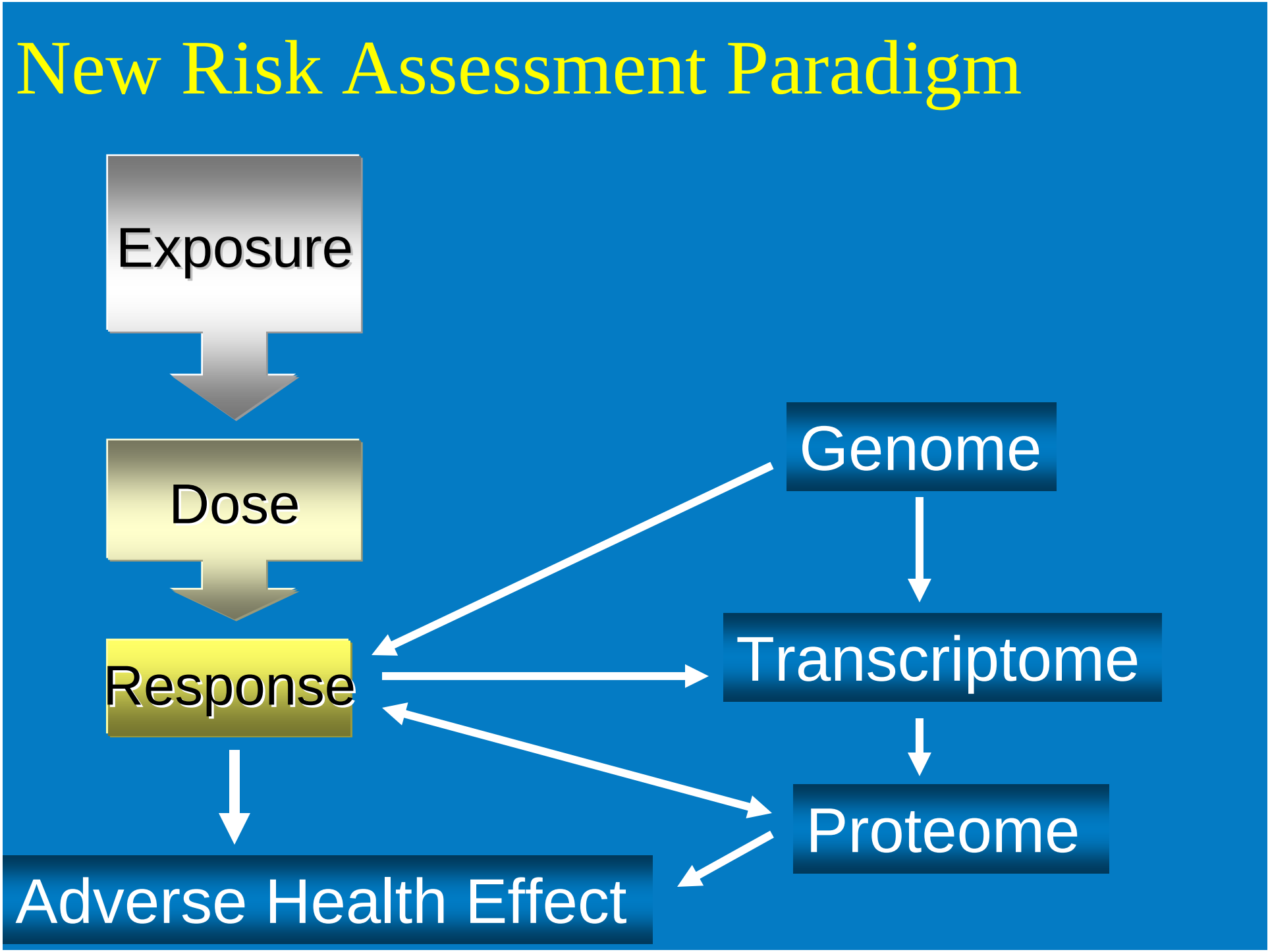
Gene expression profiles can predict interspecies concordance of mechanism as well as *in vitro* to *in vivo* response.

Integration of Genomics and Proteomics into Risk Assessment

Risk Assessment Paradigm



New Risk Assessment Paradigm



Integration of Biology with Chemistry

"…to integrate modern computing and information technology with molecular biology to improve Agency prioritization of data requirements and risk assessment of chemicals"

Bioinformatics….. meets Chemoinformatics

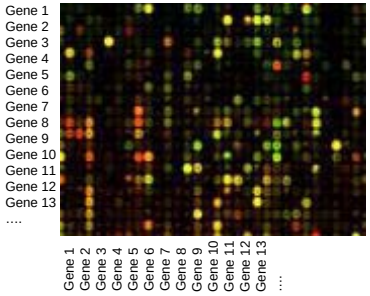
Single Chemical Exposure

Test animal

Target tissue

RNA

Gene Expression Pattern



Chemical Toxicity Databases:

chemical 1	Toxicity bioassay result 1	
chemical 2	result 2	
chemical 3	result 3	
chemical 4	result 4	
…chemical n	… result n	

Carcinogenicity
Mutagenicity
Develop. Tox.
Acute Toxicity
Ecotoxicity
…

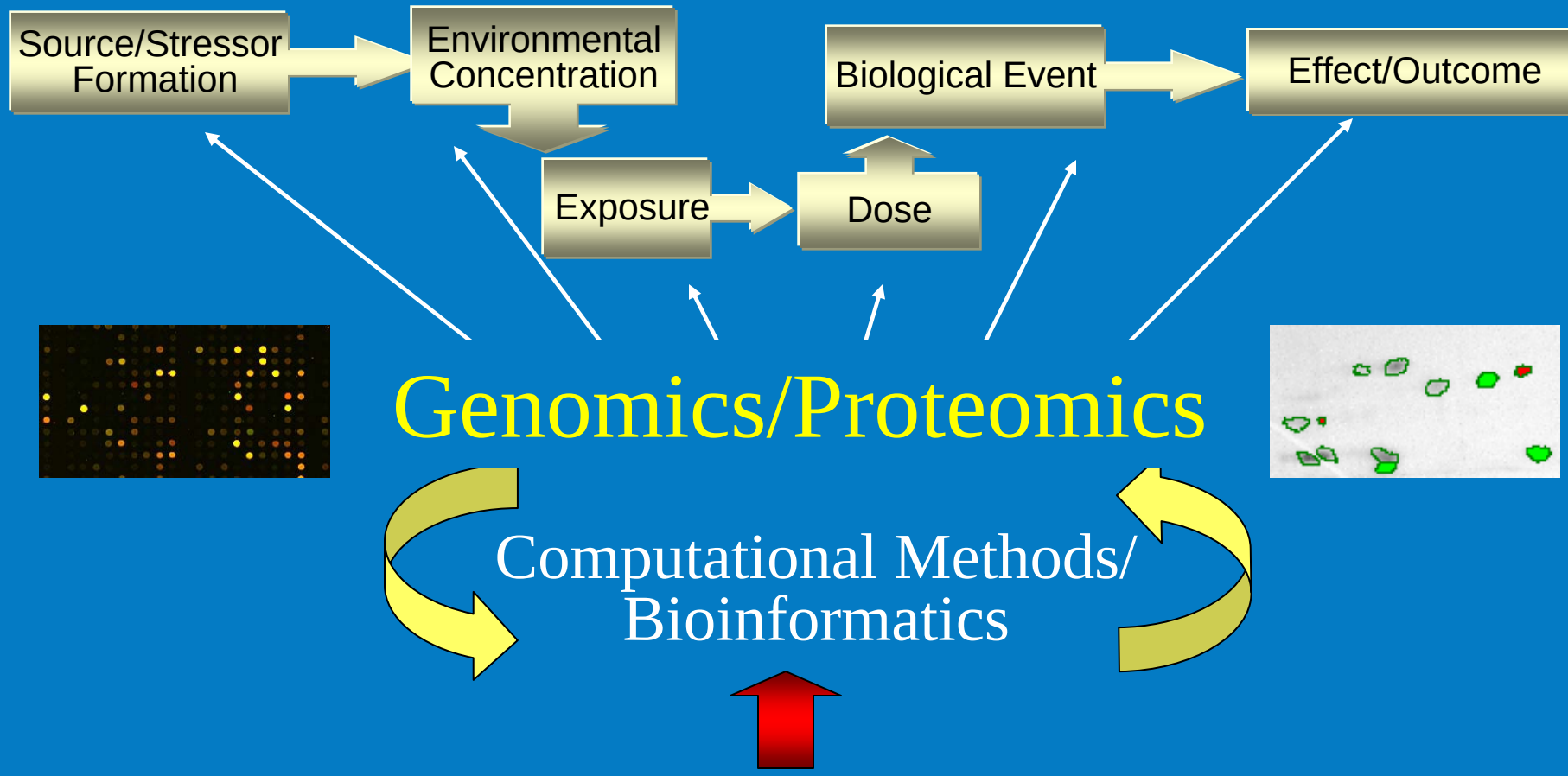
Chemical structures, physico-chemical properties →

Structure-Activity Relationships
Data-mining
Predictive Toxicology

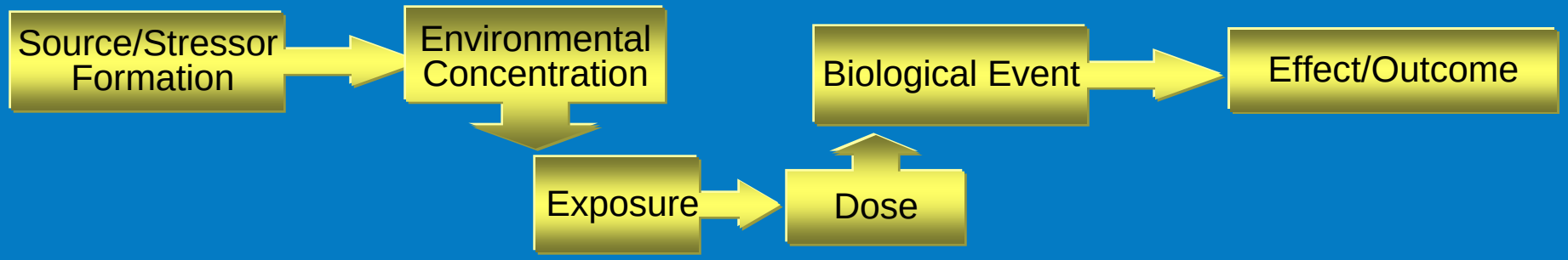
Informatics Exploration Axes:

- *Chemical Structure*
- *Gene*
- *Bioassay*

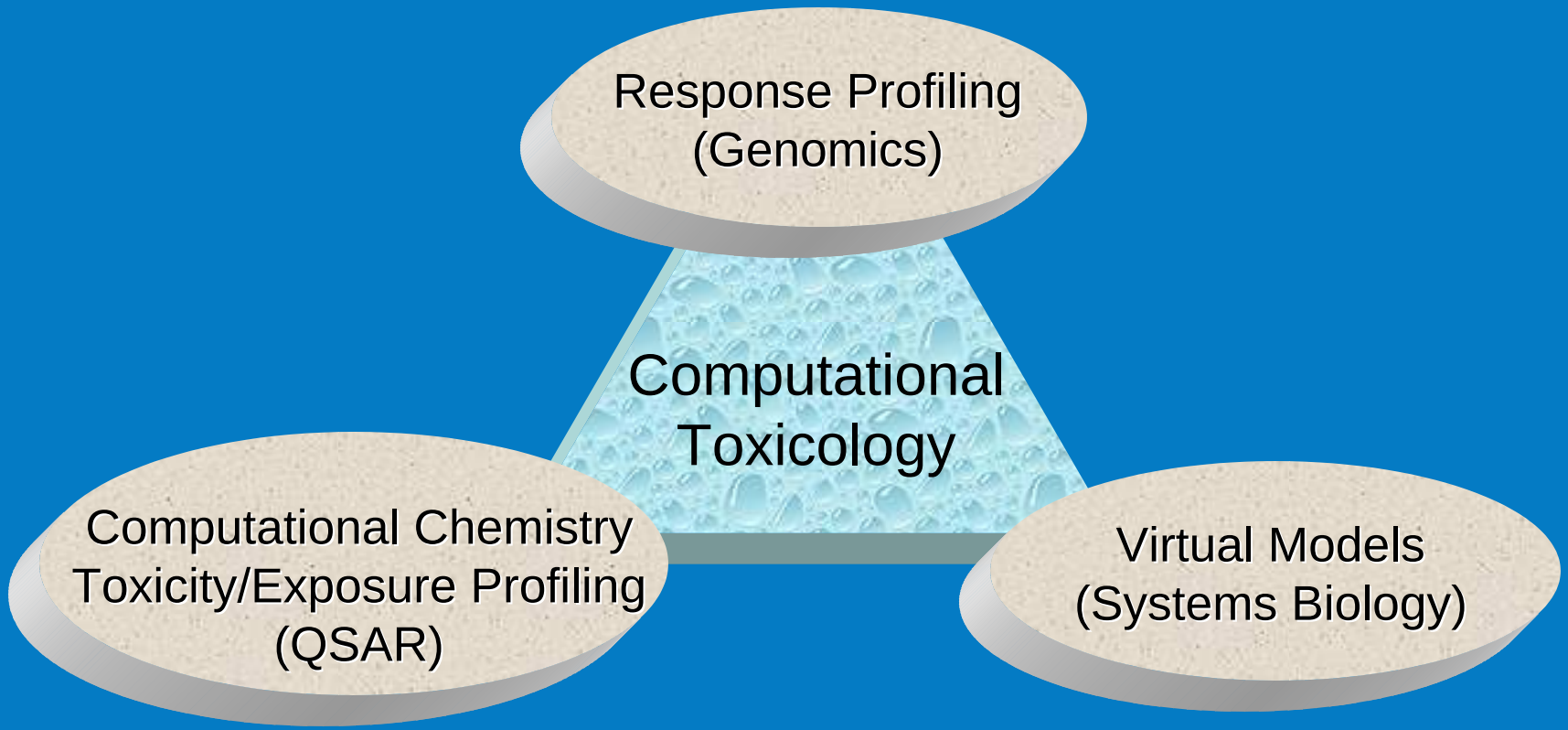
Quantitative Risk Assessments



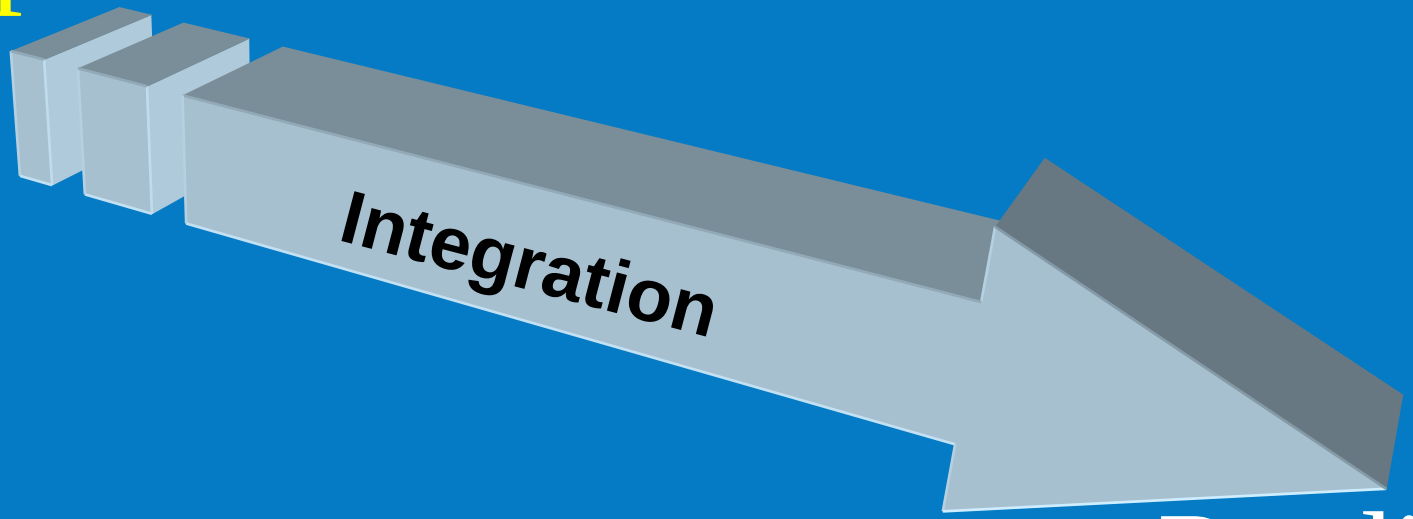
Integrate molecular biology and chemistry to prioritize data requirements and improve risk assessment



Coordinated High-throughput screening



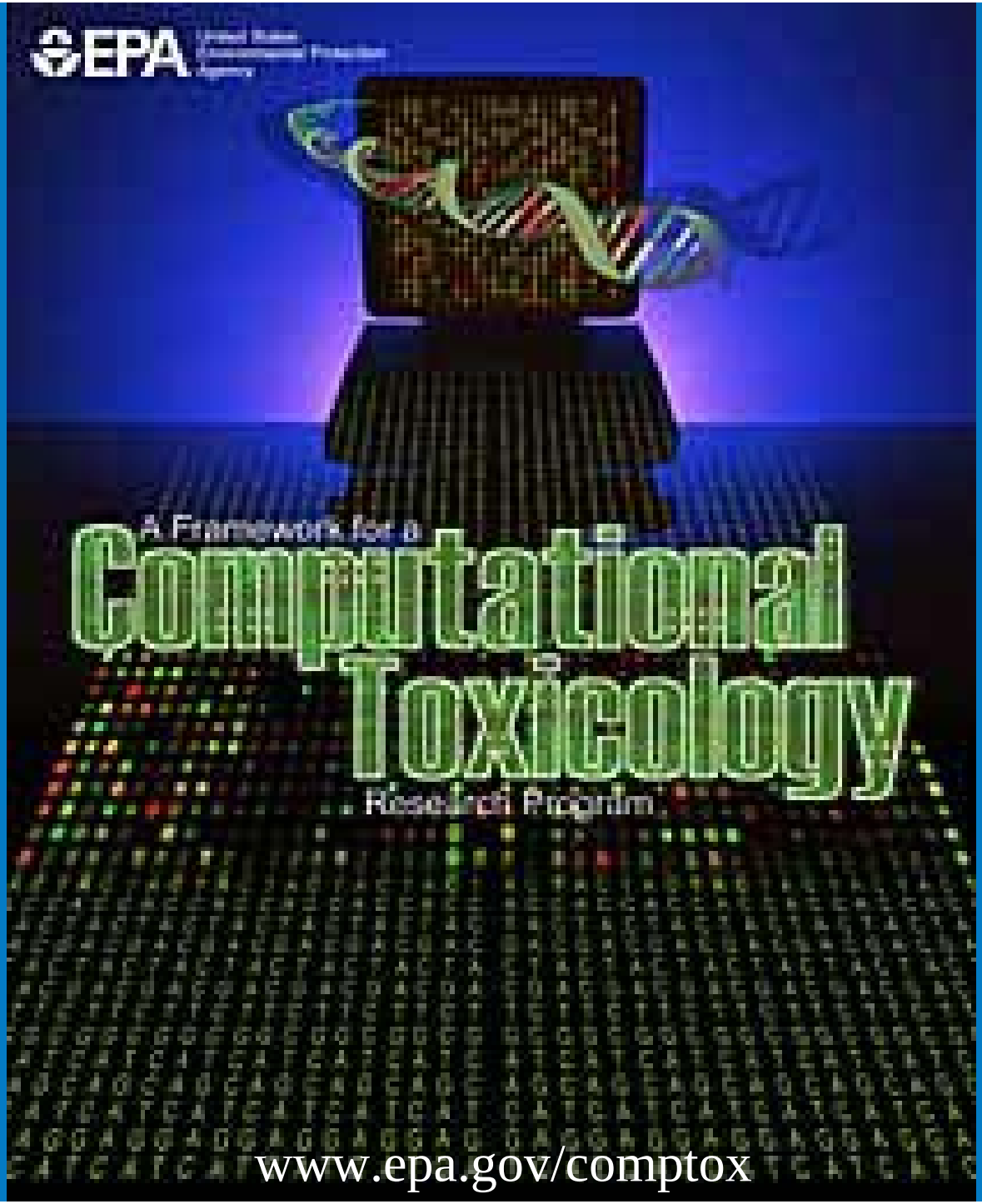
Computational Toxicologic Approach



Prediction

Prioritization

Quantitative Risk Assessment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Toxicology
Research Program
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