Toxicological Considerations in Interpreting Epigenetic Data

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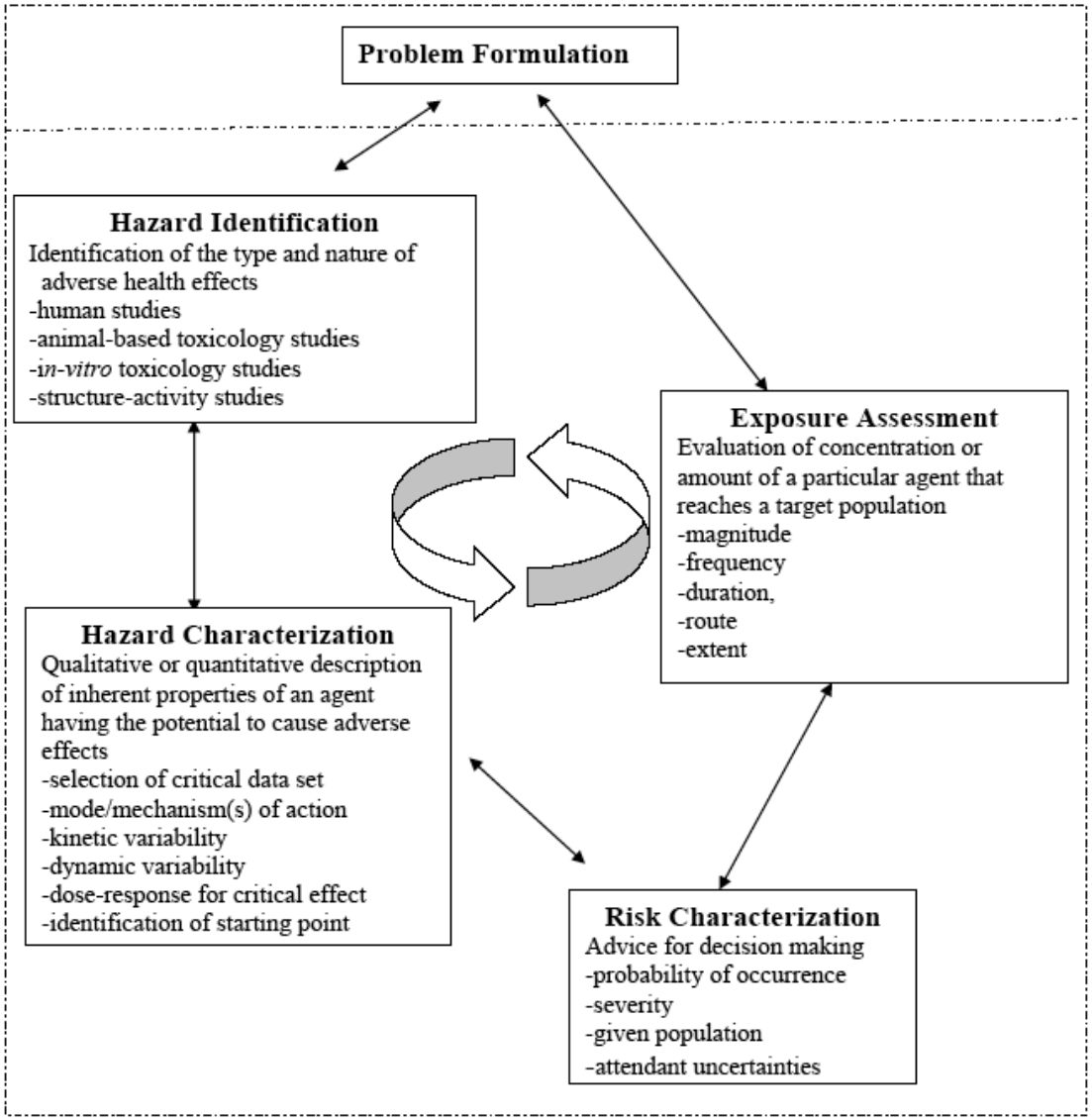
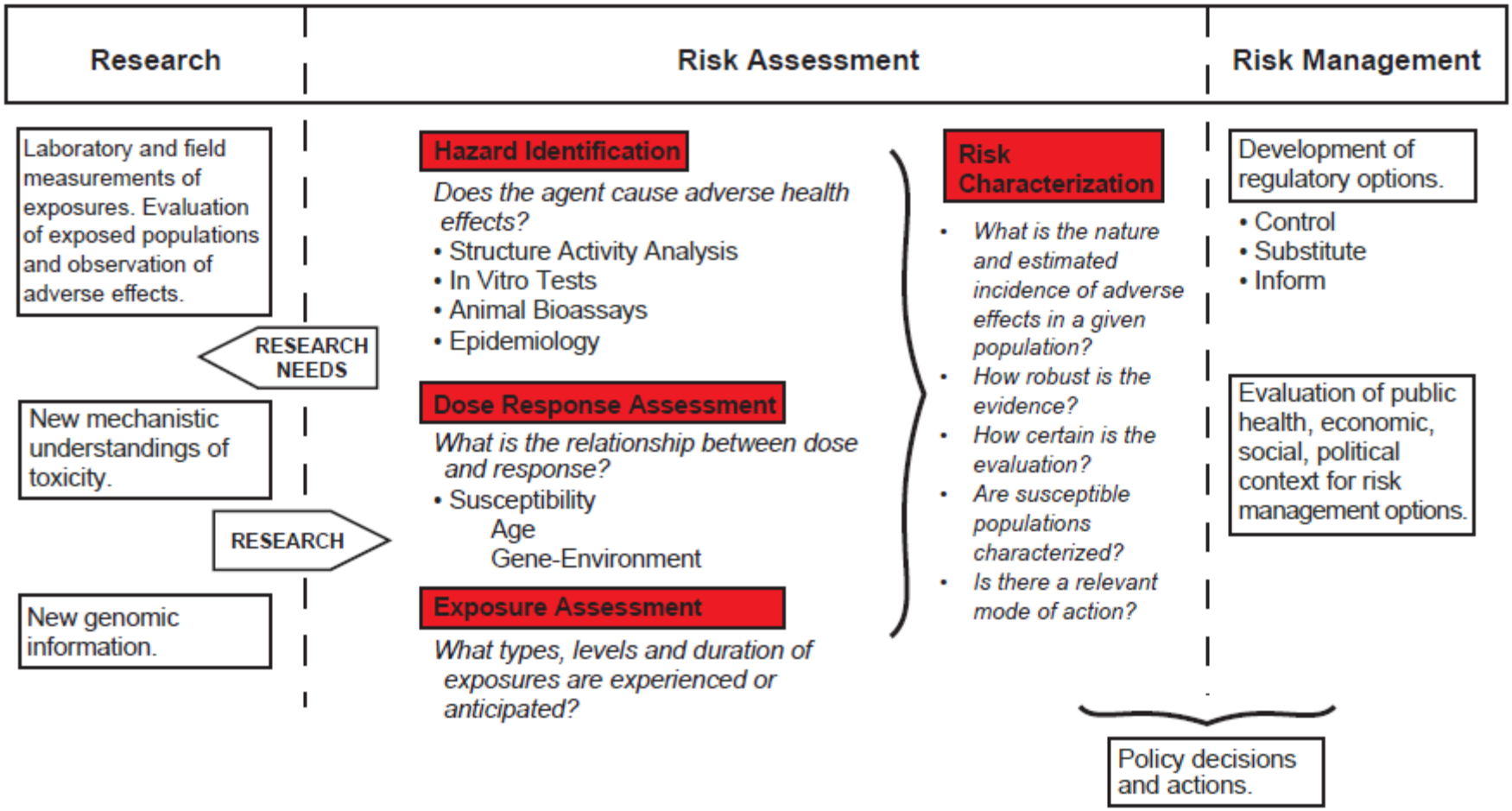
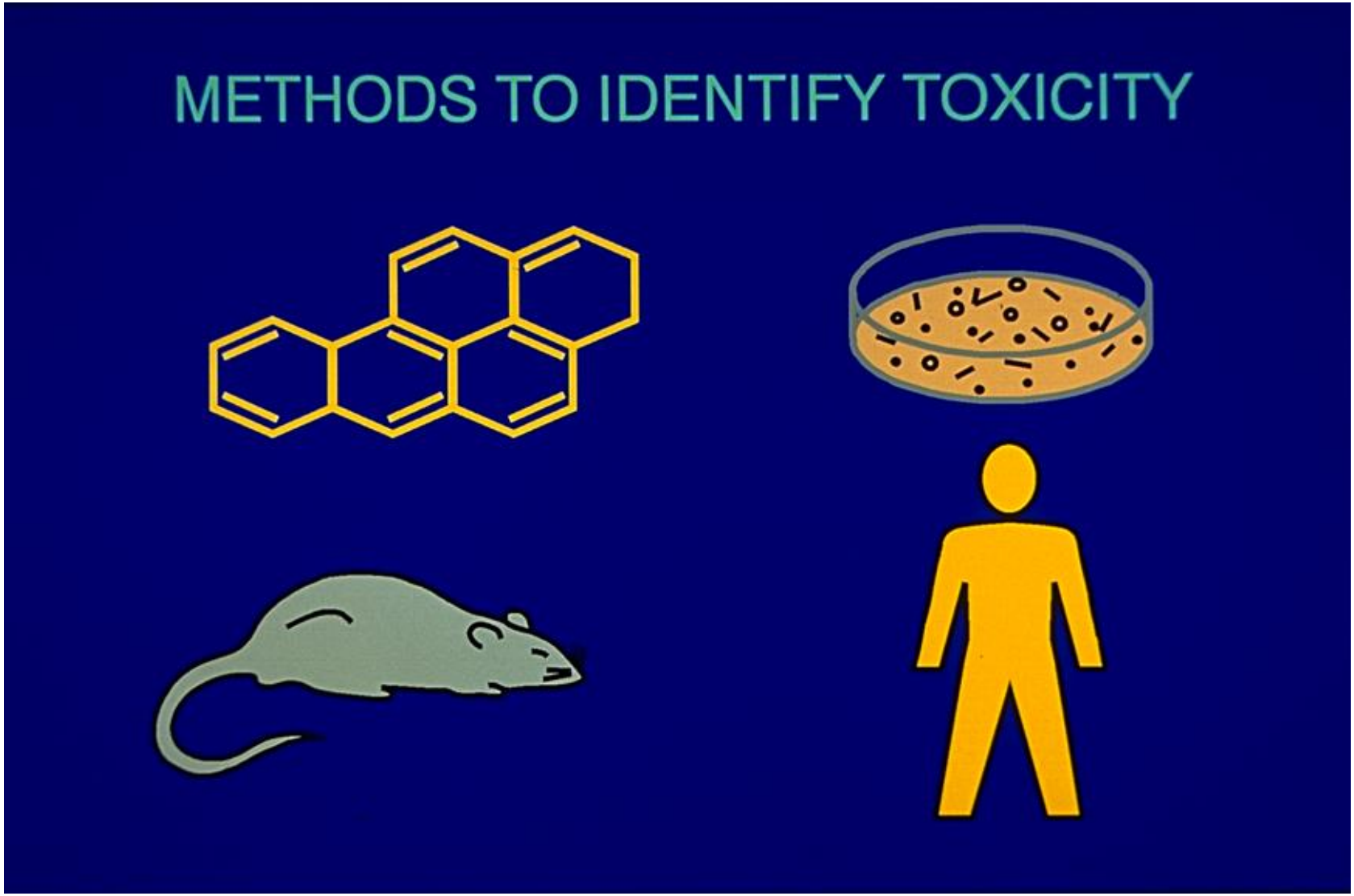
Risk Assessment



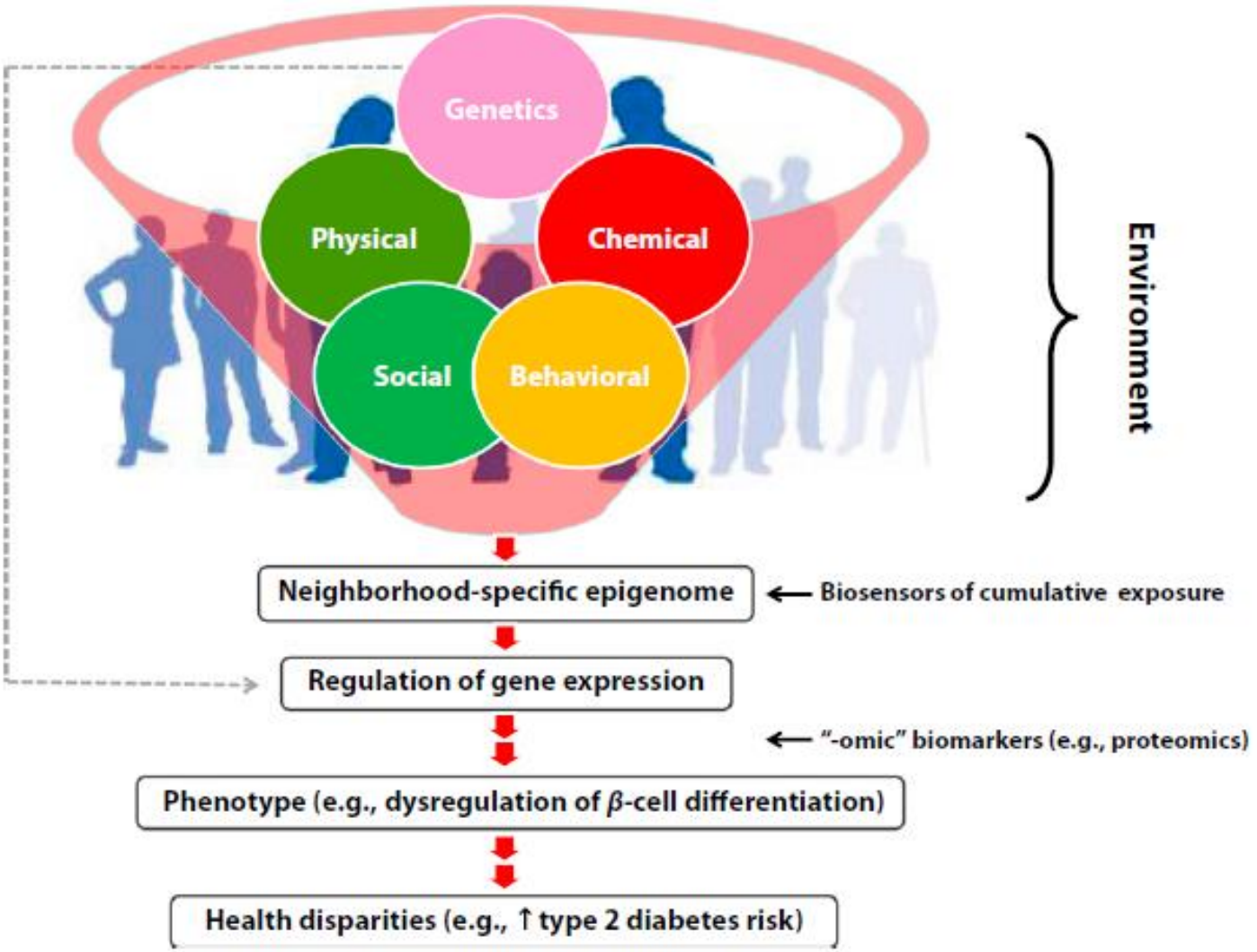
WHO, 2006


Research
Risk Assessment
Risk Management
Laboratory and field measurements of exposures. Evaluation of exposed populations and observation of adverse effects.
RESEARCH NEEDS
New mechanistic understandings of toxicity.
RESEARCH
New genomic information.
Hazard Identification
Does the agent cause adverse health effects?
• Structure Activity Analysis
• In Vitro Tests
• Animal Bioassays
• Epidemiology
Dose Response Assessment
What is the relationship between dose and response?
• Susceptibility
Age
Gene-Environment
Exposure Assessment
What types, levels and duration of exposures are experienced or anticipated?
Risk Characterization
• What is the nature and estimated incidence of adverse effects in a given population?
• How robust is the evidence?
• How certain is the evaluation?
• Are susceptible populations characterized?
• Is there a relevant mode of action?
Development of regulatory options.
• Control
• Substitute
• Inform
Evaluation of public health, economic, social, political context for risk management options.
Policy decisions and actions.



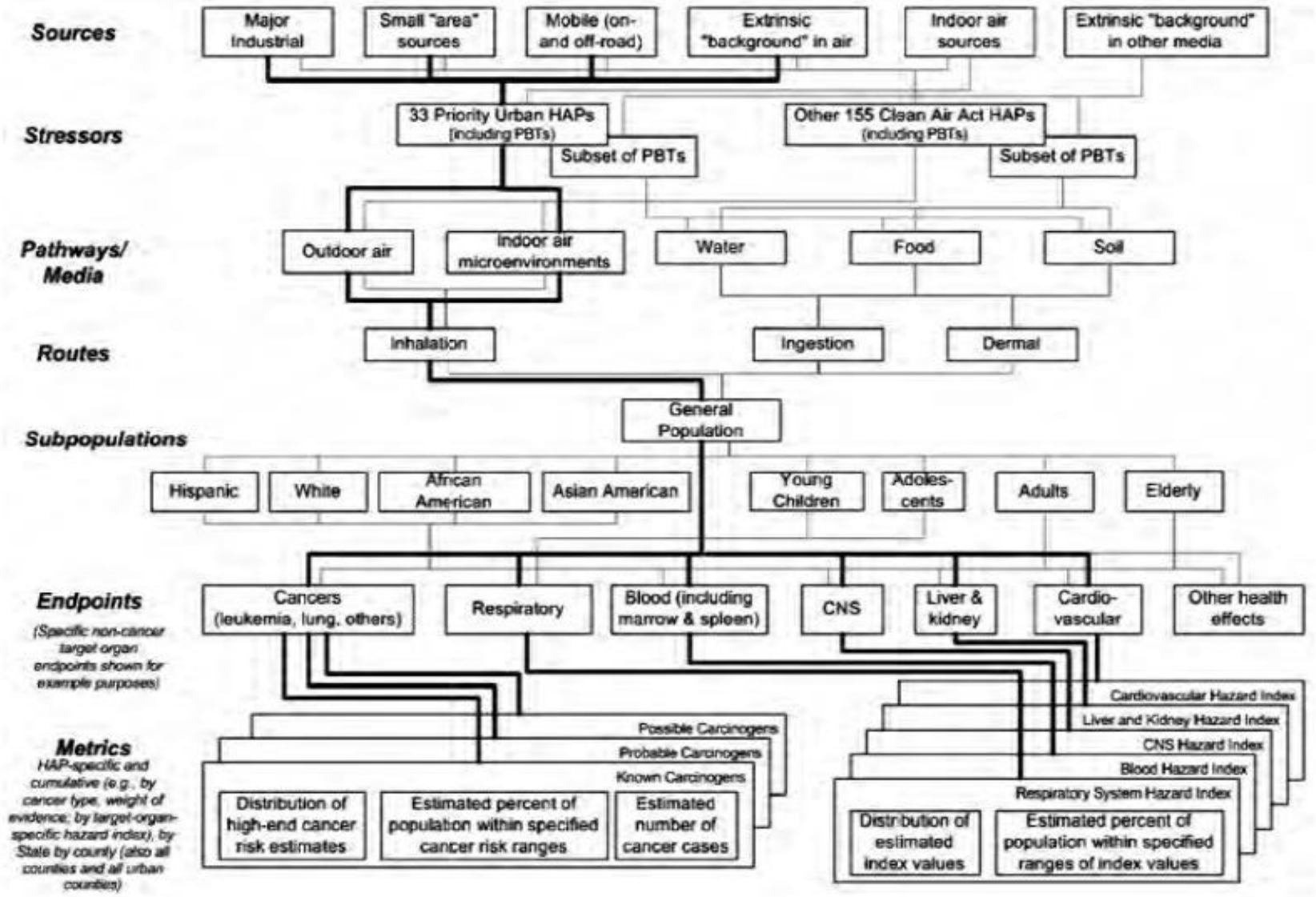
Faustman et al, 2010

Epigenome: Biosensor of Cumulative Exposure to Chemical and Nonchemical Stressors Related to Environmental Justice



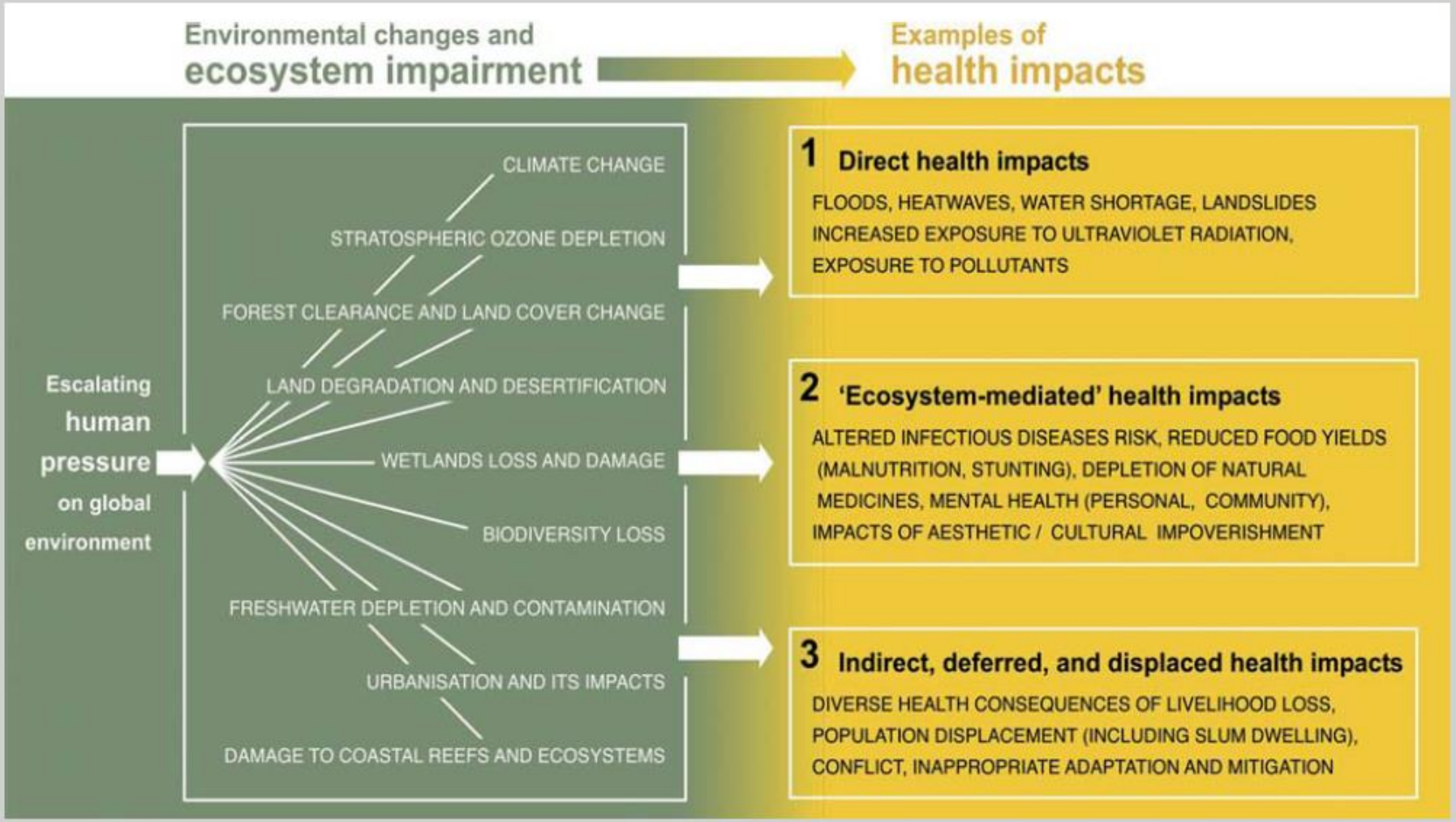
K. Olden et al, American Journal of Public Health | October 2014, Vol 104, No. 10

Broadening the scope of a risk assessment



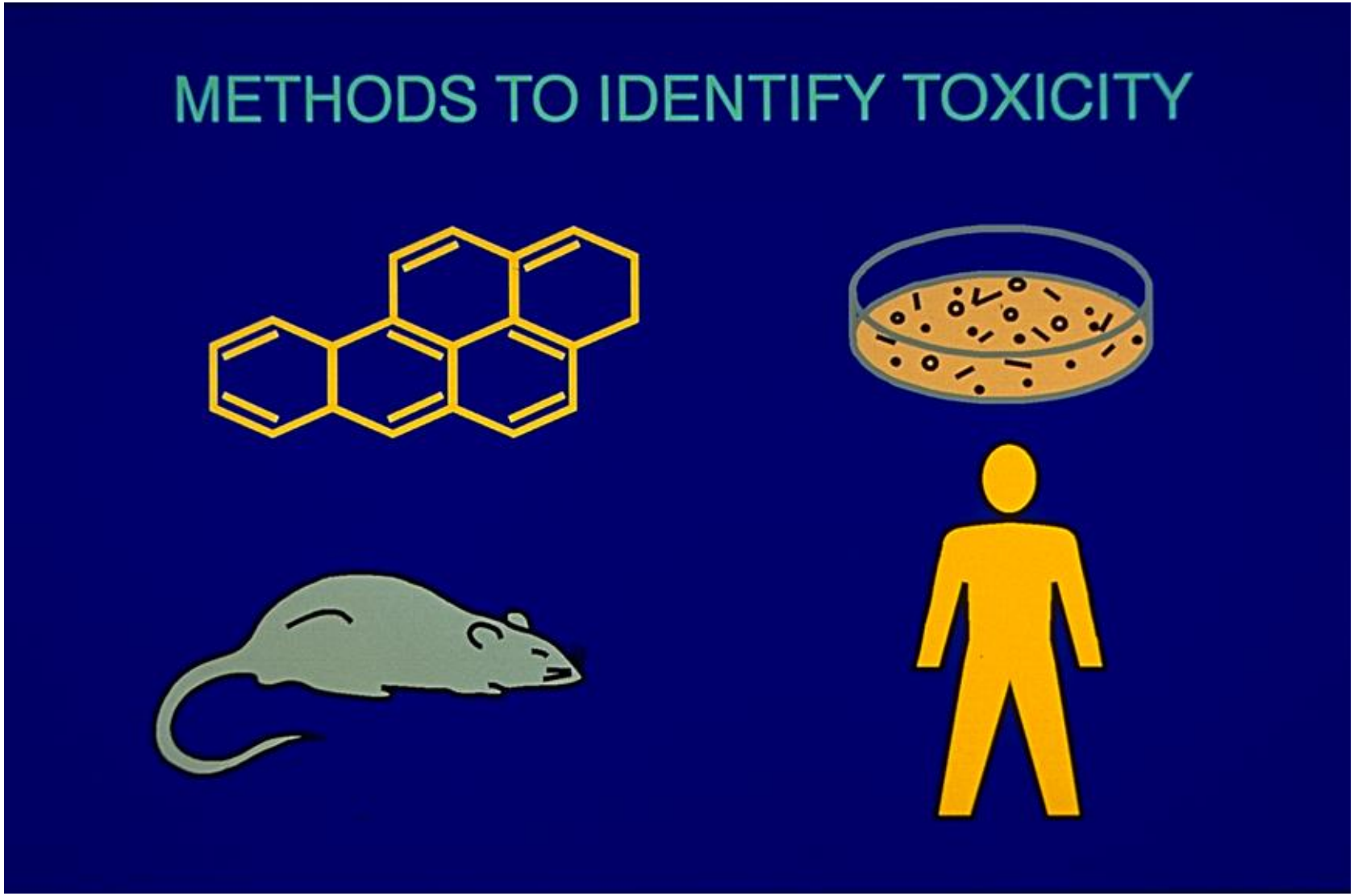
Science and Decisions: Advancing Risk Assessment; 2009

Figure SDM1. HARMFUL EFFECTS OF ECOSYSTEM CHANGE ON HUMAN HEALTH



This figure describes the causal pathway from escalating human pressures on the environment through to ecosystem changes resulting in diverse health consequences. Not all ecosystem changes are included. Some changes can have positive effects (e.g. food production).

Source: World Health Organization. (2005). Ecosystems and Human Well-Being: Health Synthesis.



Faustman et al, 2010

Challenges and Opportunities

for using epigenetic information and approaches for understanding mechanisms of toxicity and dose-response

- Intra- and cross-species extrapolation
- Facilitate use of model systems
- Characterize low dose and early temporal response
- Facilitate in vitro to in vivo extrapolation
- Extrapolation across levels of biological complexity
- Identify actual pathways of disease

Example Environmental Factors Known to Impact Epigenomic Profiles; Includes Chemical and Non-Chemical Stressors

- Tobacco Smoke
- Infectious pathogens (H. pylori)
- Particulate matter
- Diesel exhaust particles
- Air pollutants
- Dust mites
- Fungi/mold

- Polycyclic aromatic hydrocarbons (PAH)
- Heavy metals (As, Cd, Pb, Ni, Mg)
- Pesticides (OPs, vinclozolin)
- Endocrine disruptors
- Hormones (DES)
- Plasticizers (BPA, phthalates)
- Phytoestrogens

- Changes in stress
- Changes in cortisol
- Changes in neighborhood

- Famine
- Racial disparities
- Glucocorticoid homeostasis

Sources: Sweatt et al (2001), Hu et al (2014), Osborne-Maynard et al (2013), Rivera and Bennett (2010)

Factors Affecting Differences in Susceptibility

- Age
- Sex
- Strain
- Enzyme Induction
- Genetic
- Predisposition

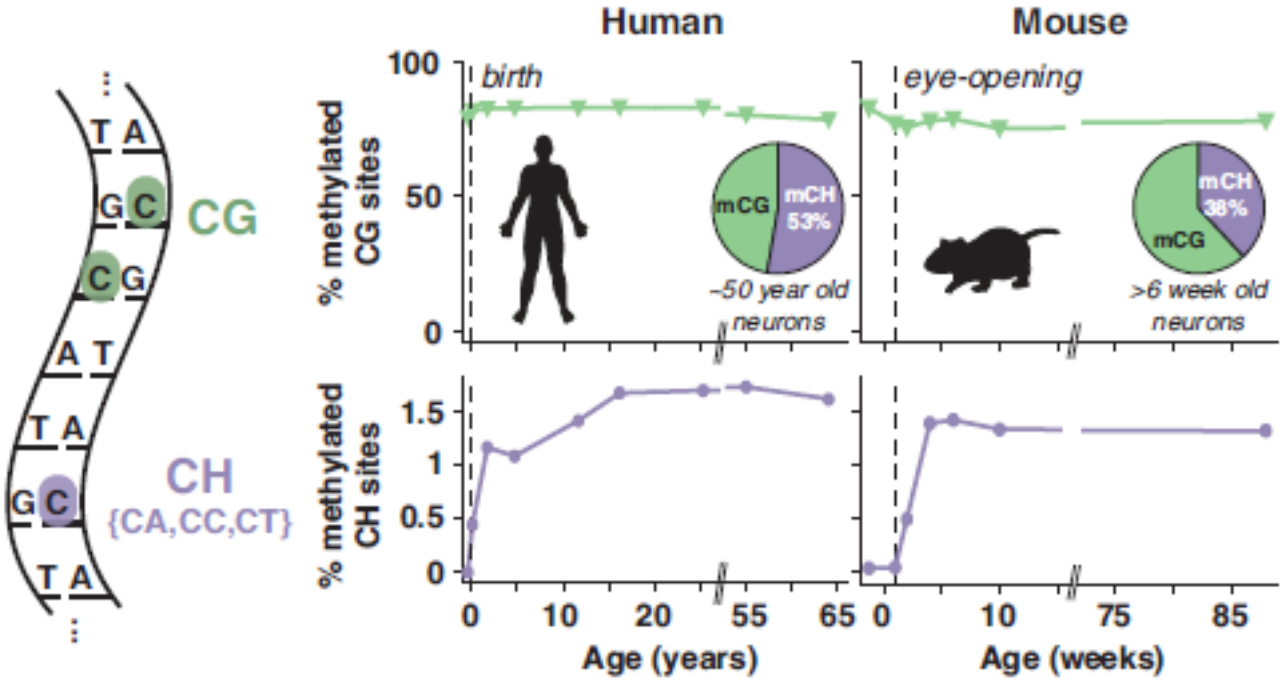
- Hormonal Status
- Nutritional Status
- Disease
- Circadian Variation
- Stress

Global Epigenomic Reconfiguration During Mammalian Brain Development

Ryan Lister,* Eran A. Mukamel, Joseph R. Nery, Mark Urich, Clare A. Puddifoot, Nicholas D. Johnson, Jacinta Lucero, Yun Huang, Andrew J. Dwork, Matthew D. Schultz, Miao Yu, Julian Tonti-Filippini, Holger Heyn, Shijun Hu, Joseph C. Wu, Anjana Rao, Manel Esteller, Chuan He, Fatemeh G. Haghighi, Terrence J. Sejnowski, M. Margarita Behrens,* Joseph R. Ecker*

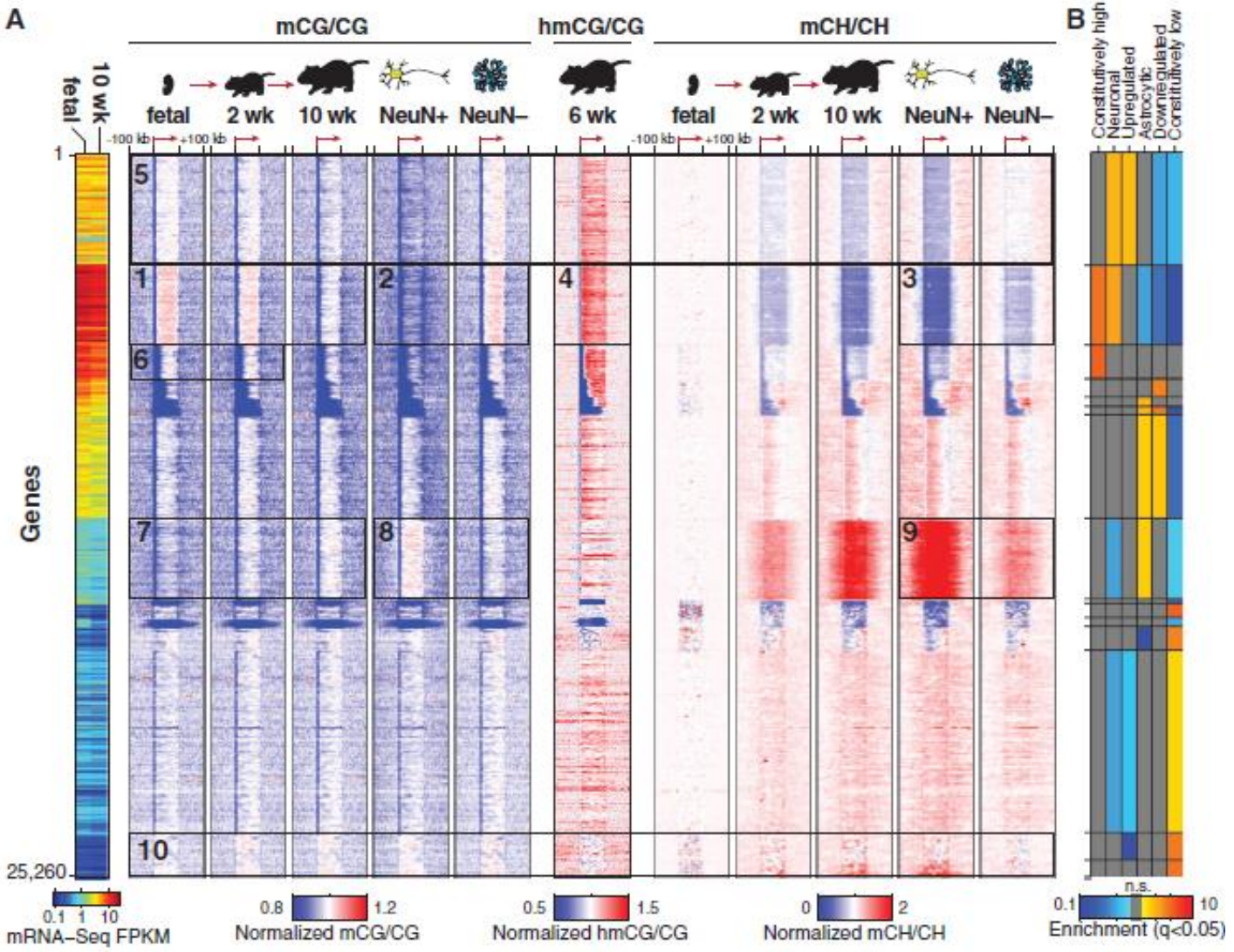
Lister R et al, 2013. Science. 341(64146): 1237905.

The DNA methylation landscape of human and mouse neurons is dynamically reconfigured through development



Lister R et al, 2013. Science. 341(64146): 1237905.

Cell type–specific and developmental differences in mC between mouse neurons and glia



Lister R et al, 2013. Science. 341(64146): 1237905.

Ontology application and use at the ENCODE DCC

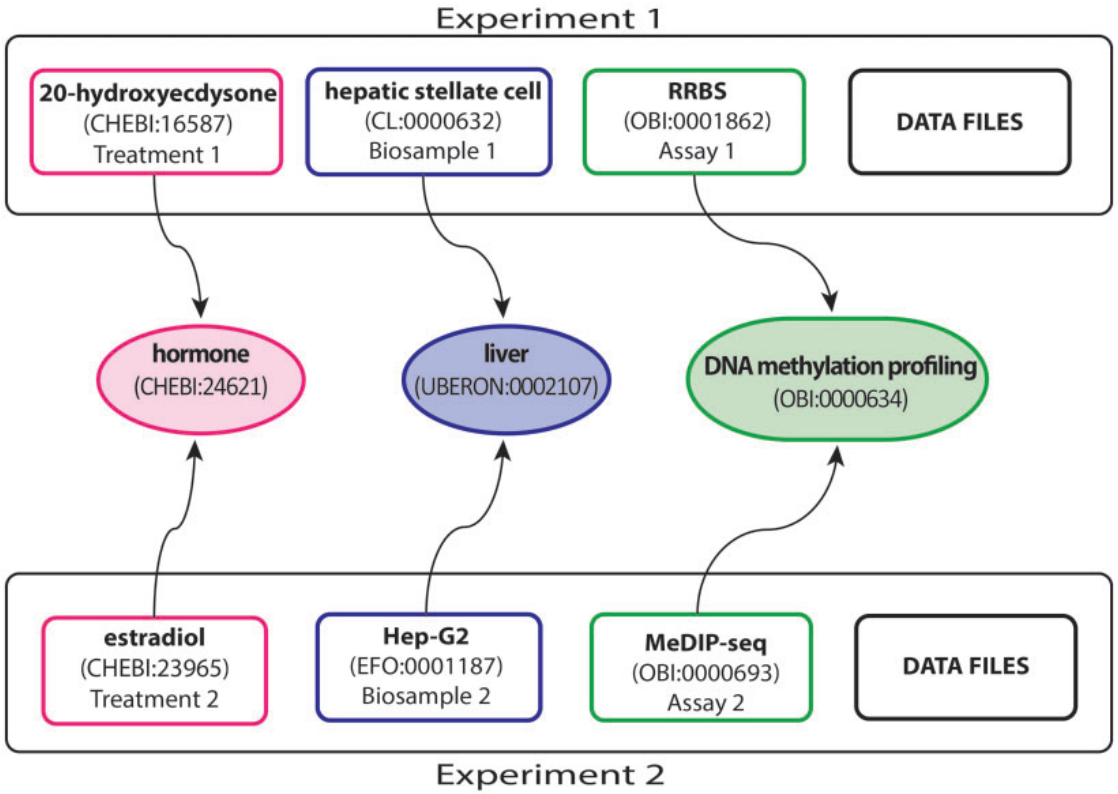
Venkat S. Malladi[1], Drew T. Erickson[1], Nikhil R. Podduturi[1], Laurence D. Rowe[1], Esther T. Chan[1], Jean M. Davidson[1], Benjamin C. Hitz[1], Marcus Ho[1], Brian T. Lee[2], Stuart Miyasato[1], Gregory R. Roe[1], Matt Simison[1], Cricket A. Sloan[1], J. Seth Strattan[1], Forrest Tanaka[1], W. James Kent[2], J. Michael Cherry[1] and Eurie L. Hong[1,*]

[1]Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305, USA and [2]Center for Biomolecular Science and Engineering, School of Engineering, University of California Santa Cruz, Santa Cruz, CA 95064, USA

Citation details: Malladi,V.S., Erickson,D.T., Podduturi,N.R., *et al.* Ontology application and use at the ENCODE DCC. *Database* (2015) Vol. 2015: article ID bav010; doi:10.1093/database/bav010

Received 16 November 2014; Revised 13 January 2015; Accepted 19 January 2015

Experimental metadata annotated with appropriate ontology terms



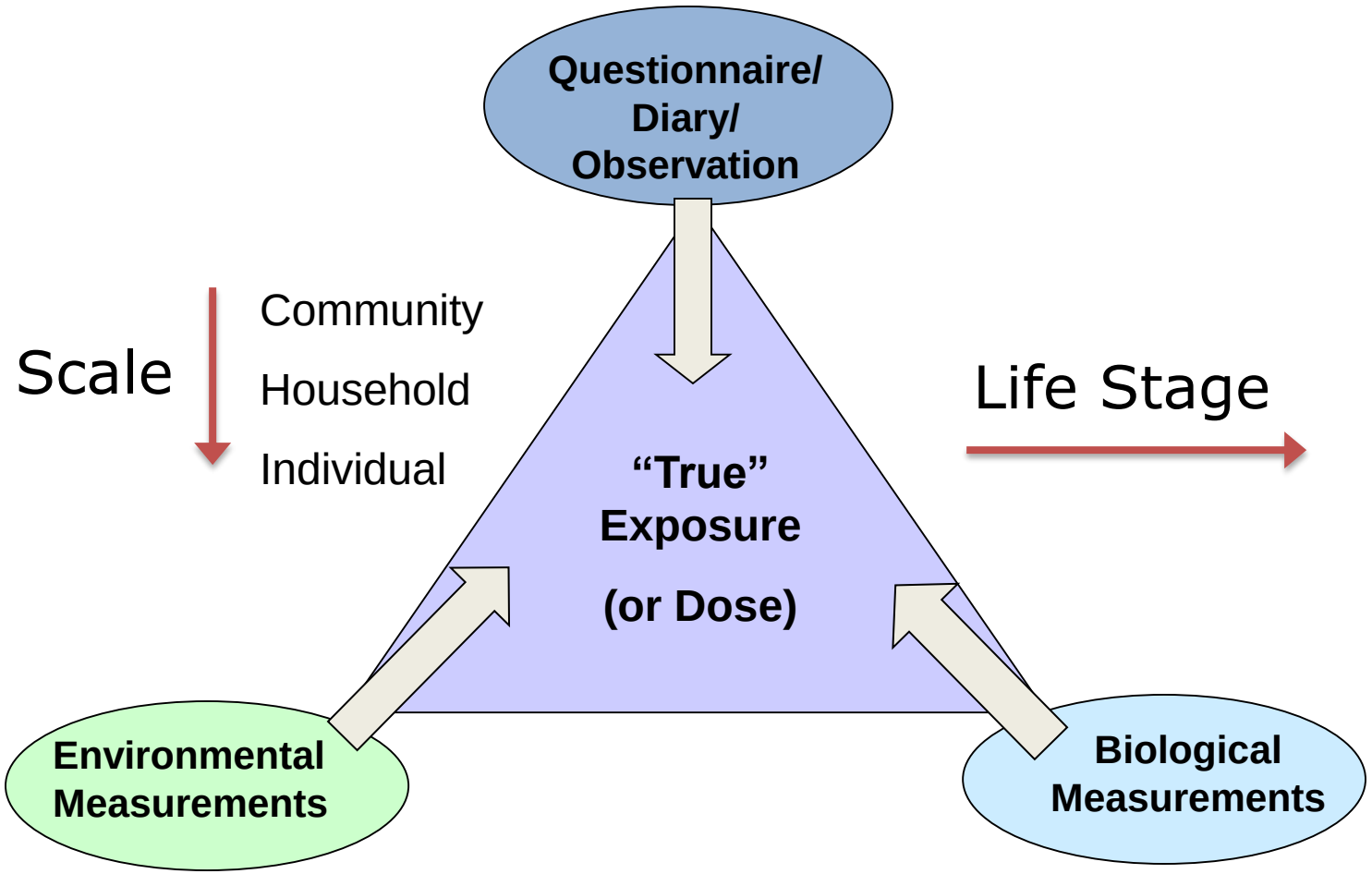
Malladi VS et al. 2015

Can we make a difference?

"Environment" Broadly Defined in Longitudinal Studies

- **Physical** environment:
  - housing, neighborhoods and communities, climate, radiation…
- **Chemical** exposures:
  - air, water, soil, food, dust, industrial products, pharmaceuticals…
- **Biological** environment:
  - womb, infection, nutrition; inflammatory and metabolic response…
- **Genetics**:
  - influence of genetics on disease; relationships between genes and the environment
- **Psychosocial**:
  - influence of family, socio-economics, community, culture, stress…

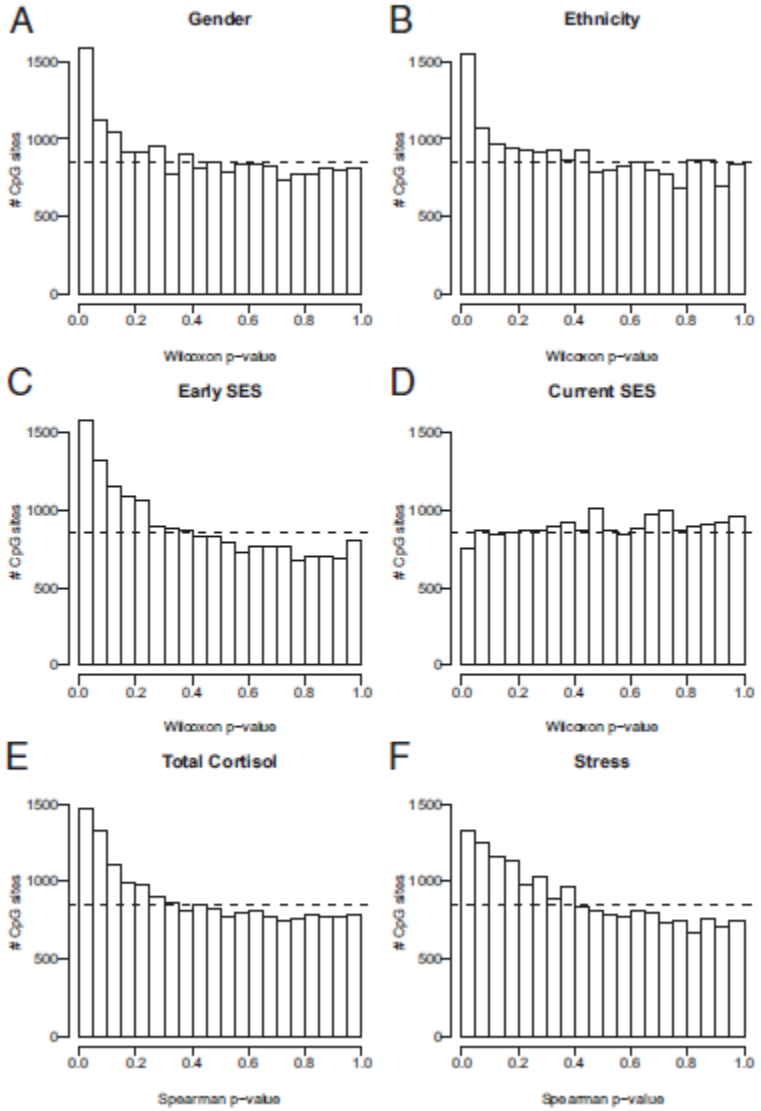
Combined Approaches Developed for Children's Exposure Assessment—Resource s from NCS EHM workgroups



NCS 2010

Factors underlying variable DNA methylation in a human community cohort

Lucia L. Lam[a], Eldon Emberly[b], Hunter B. Fraser[c], Sarah M. Neumann[a], Edith Chen[d], Gregory E. Miller[d,1],



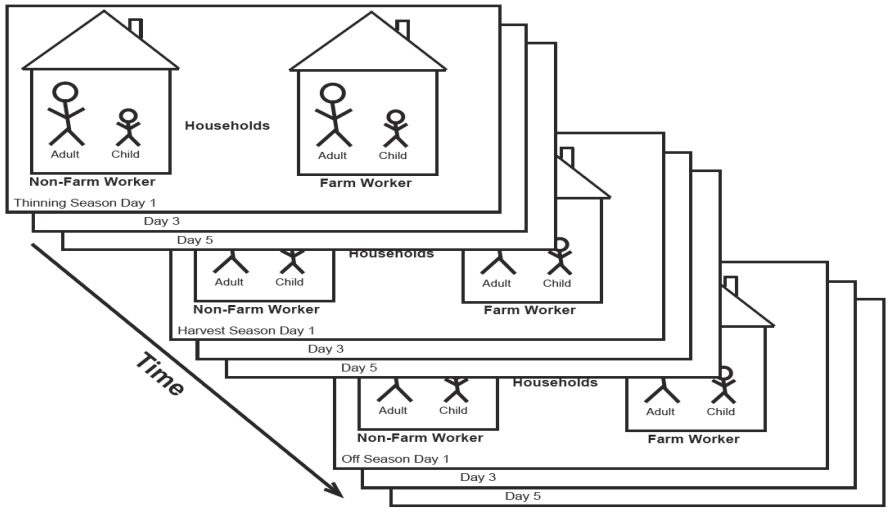
PNAS | October 16, 2012 | vol. 109 | suppl. 2 | 17253–17260

Demographic and psychosocial factors were associated with DNA methylation. (Graphical presentations of P-value distributions)

Study Population

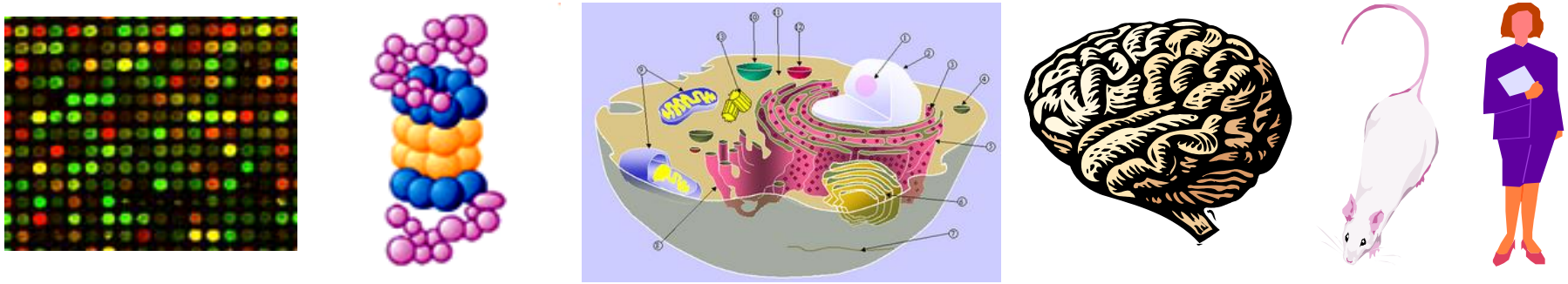
Children's Health Center Cohort:
Yakima Valley, Washington

Farmworker and Non-Farmworker households
Adults and Children in each household
Sampled during spray seasons and non-spray season



Cross-Platform Evaluation Needed for Risk Assessment

Molecular* → Organelle → Cellular* → Organ* → Organism*



Child Health Research Center (CHC) has been investigating epigenomic changes at multiple biological levels of assessment. * indicates where measurements are made

Integrating Genetic and Toxicogenomic Information for Determining Underlying Susceptibility to Developmental Disorders

Joshua F. Robinson,[1,3] Jesse A. Port,[1,3] Xiaozhong Yu,[1,3] and Elaine M. Faustman[1,2,3,4,5]*

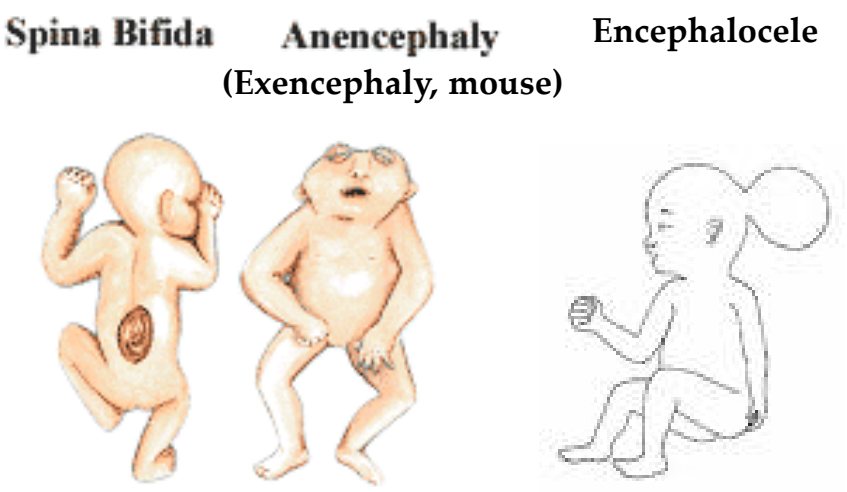
[1]Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, Washington
[2]Center for Ecogenetics and Environmental Health, Seattle, Washington
[3]Institute for Risk Analysis and Risk Communication, Seattle, Washington
[4]Center on Human Development and Disability, Seattle, Washington
[5]Center for Child Environmental Health Risks Research, Seattle, Washington

Birth Defects Research (Part A) 88:920–930 (2010)

Neural Tube Defects (NTDs)

Definition

NTDs represent a group of defects where the neural tube fails to develop properly



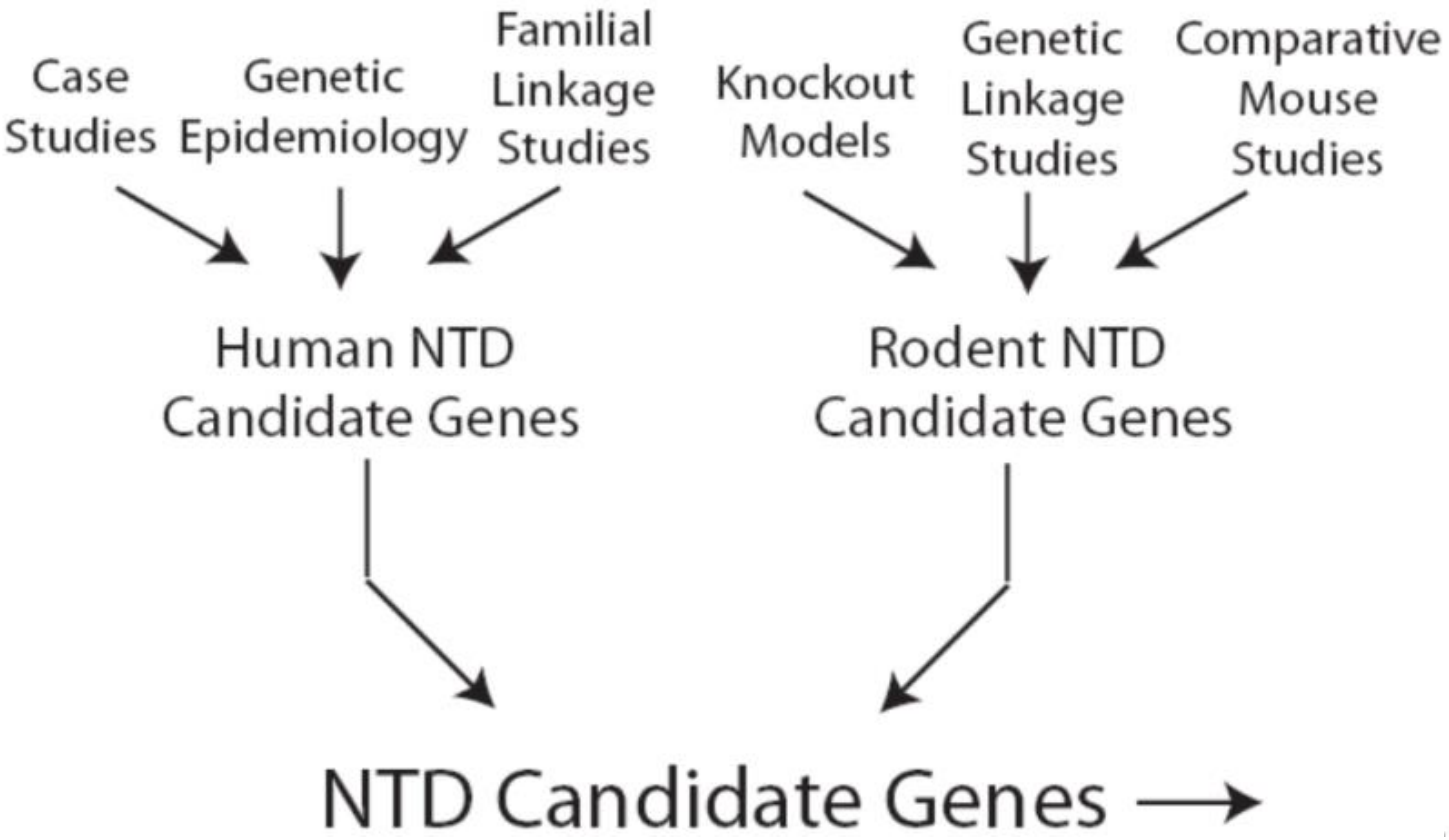
The second most common human birth defect

Consequences range in severity
- developmental delays
- physical limitations
- behavioral problems
- facial abnormalities
- early death

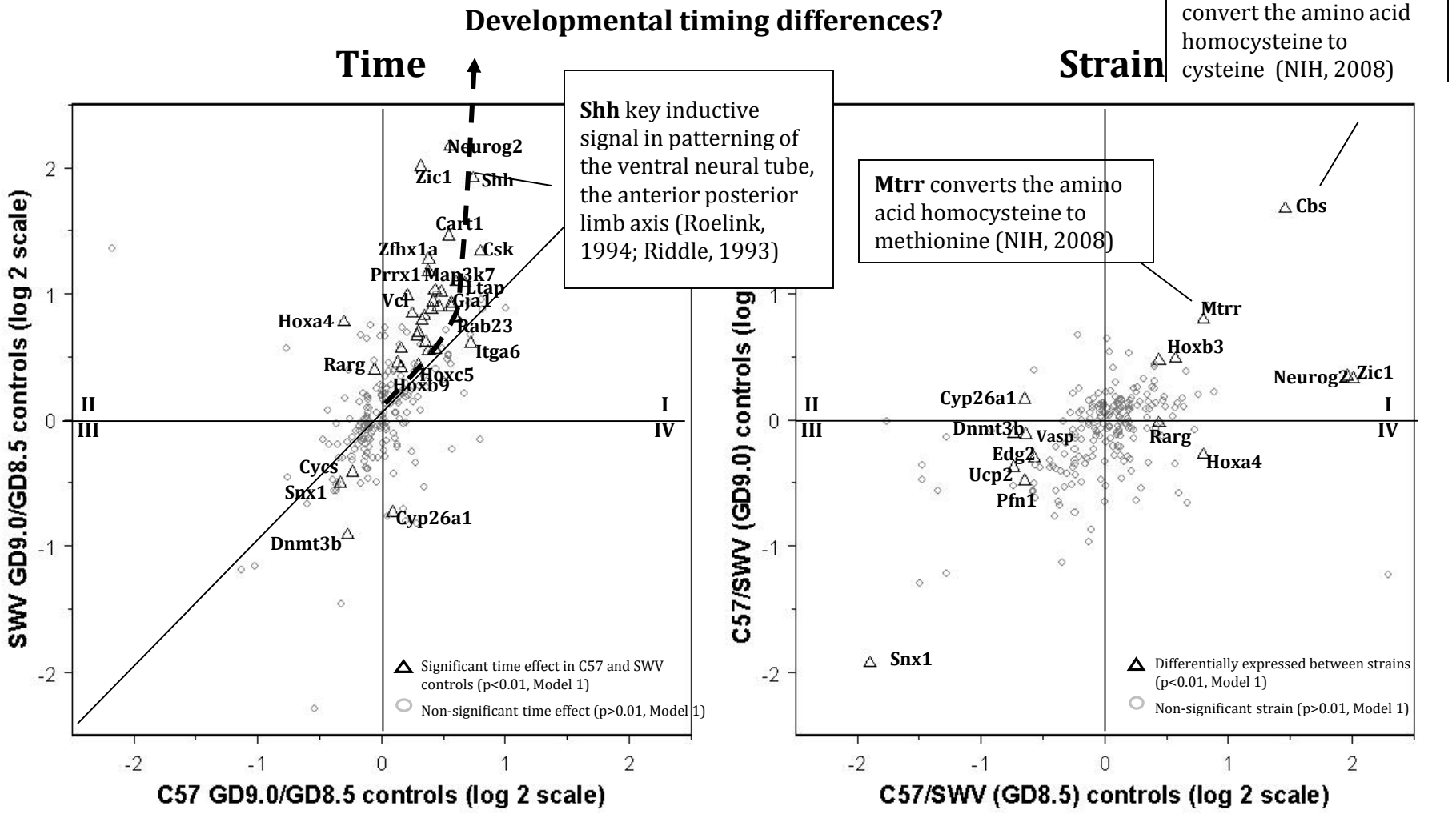
Health care cost: ~$1.4 million per case of spina bifida (Detrait *et al.,* 2005)

60-70% of birth defects have unknown origin (March of Dimes, 2006)

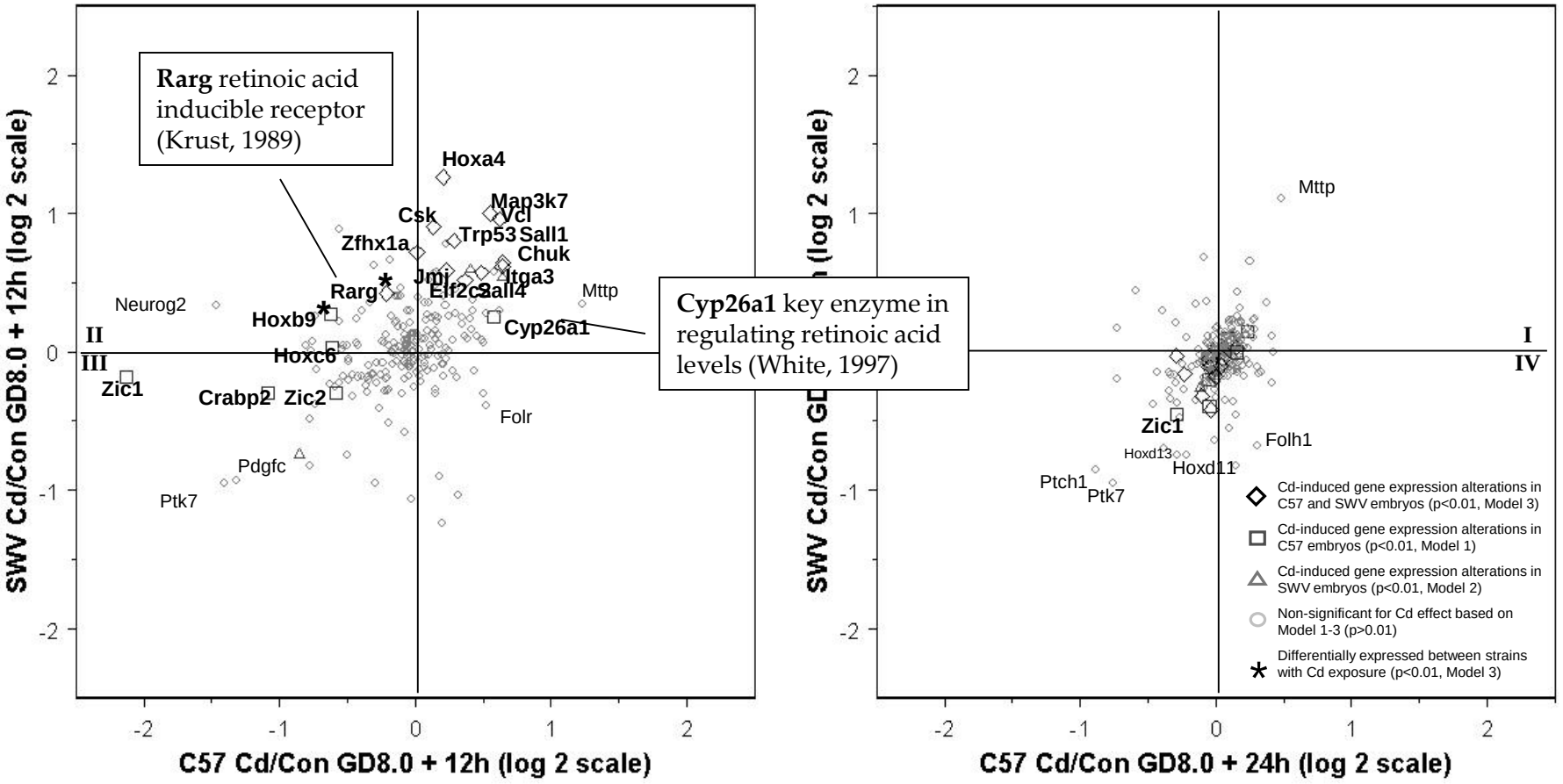
Genetics


Case Studies
Genetic Epidemiology
Familial Linkage Studies
Knockout Models
Genetic Linkage Studies
Comparative Mouse Studies
Human NTD Candidate Genes
Rodent NTD Candidate Genes
NTD Candidate Genes

NTD candidate genes display strain and time dependent differences in expression in C57 and SWV embryos



Cd-induced gene expression alterations in NTD candidate genes in C57 and SWV embryos



Why is social stress relevant to children's environmental health?

- Both physical toxicants and social stress exposure during development can have life-long impacts
- Social stress can be an effect modifier of physical toxicant exposure and disease pathway
- Social stress and environmental toxicants often have overlapping exposure profiles, potentially impacting low income and minority populations most heavily

From Wright, *Curr Opin Pediatr*. 2009 April ; 21(2): 222–229

Integrating Measures of Nonchemical Stress Exposure in the UW's Children's Health Center and the National Children's Study

Marissa Smith

William Griffith, Melinda Vredevoogd, Eric Vigoren, Shirley Beresford, Carly Strecker, Beti Thompson and Elaine Faustman

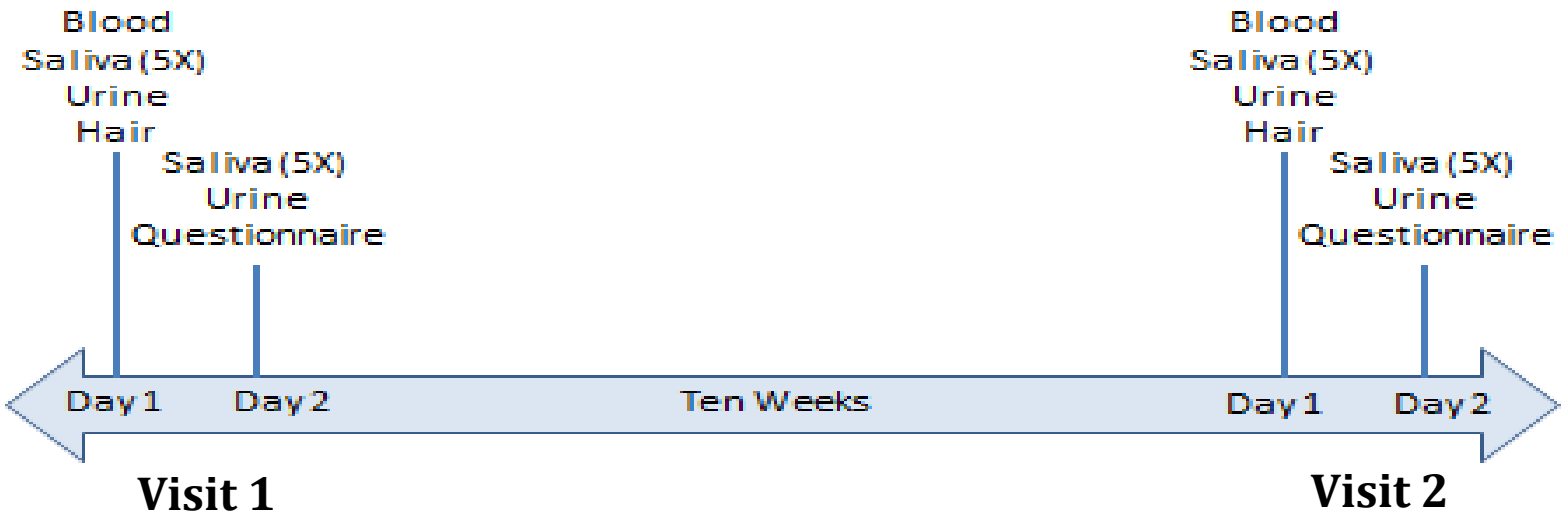
Institute for Risk Analysis and Risk Communication

University of Washington

Stress Study Sampling Framework

For CHC Mothers we have:

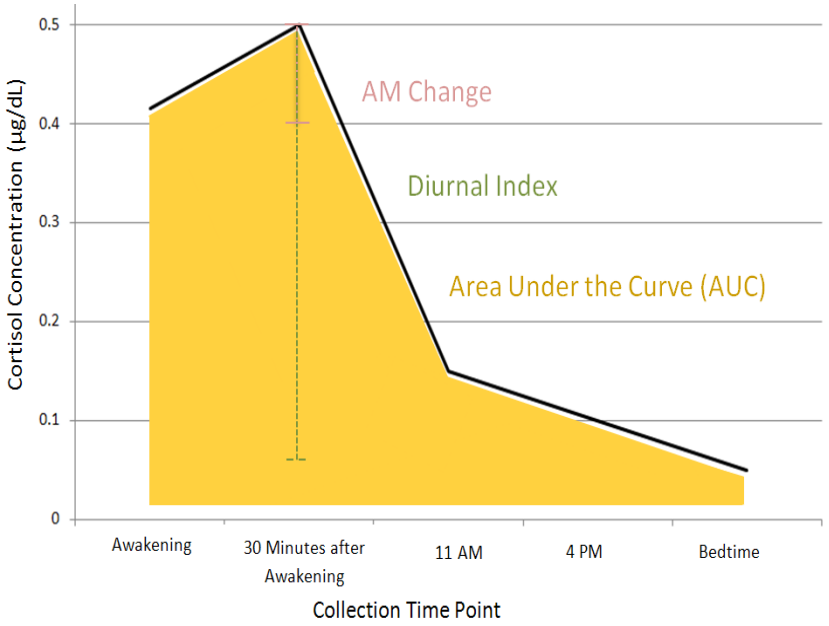
- Two blood samples
- Twenty saliva samples
- Two stress questionnaires
- Four urine samples
- Two hair samples representing at least three months growth



Stress Questionnaires

- Neighborhood Satisfaction (11 questions)
- Social Ladder
- Health Status
- Cohen's 10-item Perceived Stress Scale (PSS-10) (Cohen S, 1983)
- Culturally-appropriate stress scale that was developed for assessing stress among Mexican immigrant farmworkers (Snipes 2007)

Saliva Cortisol Metrics



Term	Definition
Area Under the Curve (AUC)	Measure of total daily response calculated as the area under the daily curve normalized for hours awake
AM Change	Difference between the first (wake) and second (30 minutes post wake) time points of the day
Diurnal Index	Difference between highest morning time point (time 1 or 2) and bedtime level

Relating Biomarkers and Questionnaires from the CHC

AM Change Increased With:

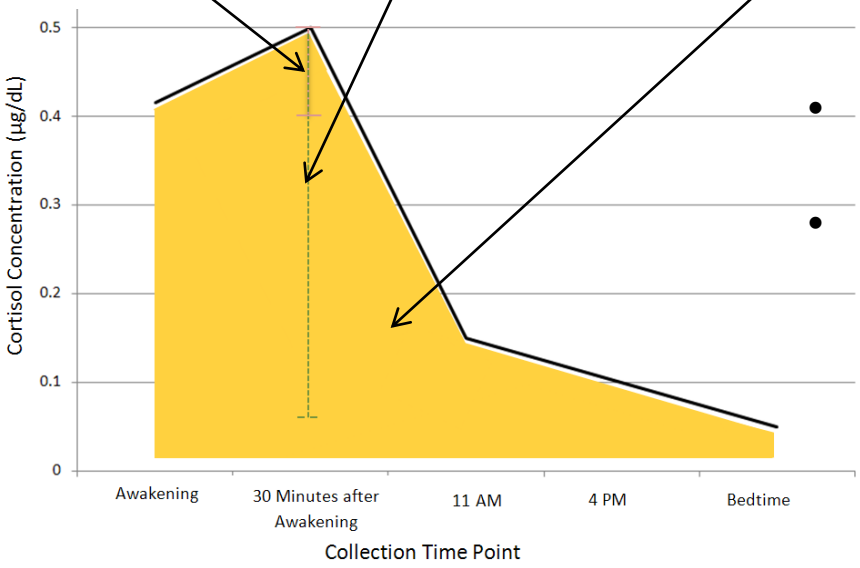
- Increased neighborhood satisfaction
- Increased stress from lack of work

Diurnal Index Increased With

- Decreased neighborhood services
- Increased stress about lack of work
- Increased stress about medical bills

AUC Increased With

- Increased perceived stress
- Increased social ladder status
- Increased neighborhood satisfaction



Urinary microRNA Profiles as Potential Biomarkers of Pesticide Exposure

Brittany A. Weldon[1,2], Sara E. Pacheco[1,2], Kirk Van Ness[1,2], Tomomi Workman[1,2], Beti Thompson[3], and Elaine M. Faustman[1,2]

[1]Institute for Risk Analysis and Risk Communication, University of Washington, Seattle, WA [2]Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, [3]Fred Hutchinson Cancer Research Center, Seattle, WA

Optimization Methods

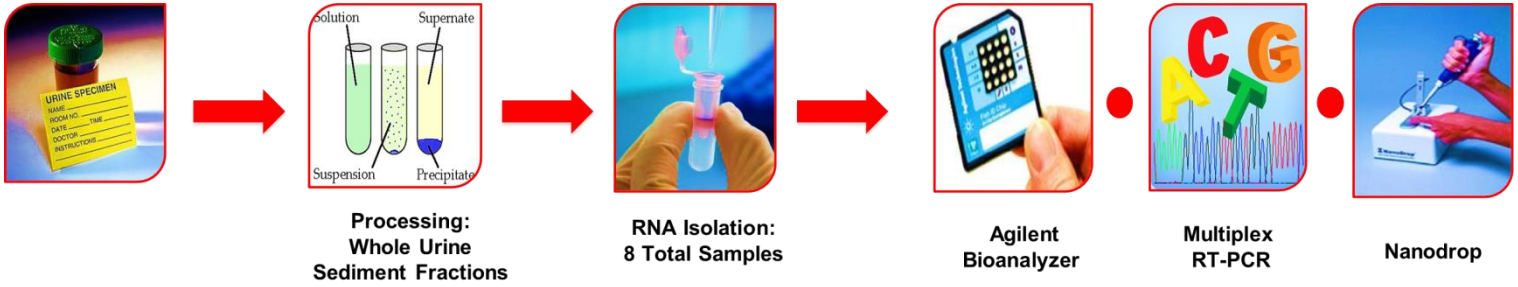


Figure 2. Experimental Workflow. Urine was thawed and processed prior to RNA extraction. The RNA was used for Nanodrop Spectrophotometer and Agilent Bioanalyzer analyses and multiplex RT-PCR reactions using the TaqMan MicroRNA Cards.

Optimization Results

Table 1: Optimization Nanodrop Sample Characteristics

Sample ID	Group	Fraction	Concentration (ng/ul)	260/280, 260/230
1W	Fresh	Whole Urine	97.79	1.92, 1.14
1S	Fresh	Sediment	163.90	1.94, 1.60
2W	Field	Whole Urine	45.53	1.82, 1.17
2S	Field	Sediment	101.49	1.77, 1.06
3W	Field	Whole Urine	12.36	1.64, 1.09
3S	Field	Sediment	22.62	1.65, 0.67
4W	Field	Whole Urine	7.93	1.37, 0.22
4S	Field	Sediment	8.64	1.08, 0.27

Note: A 260/280 and 260/230 of 2 is considered optimal.

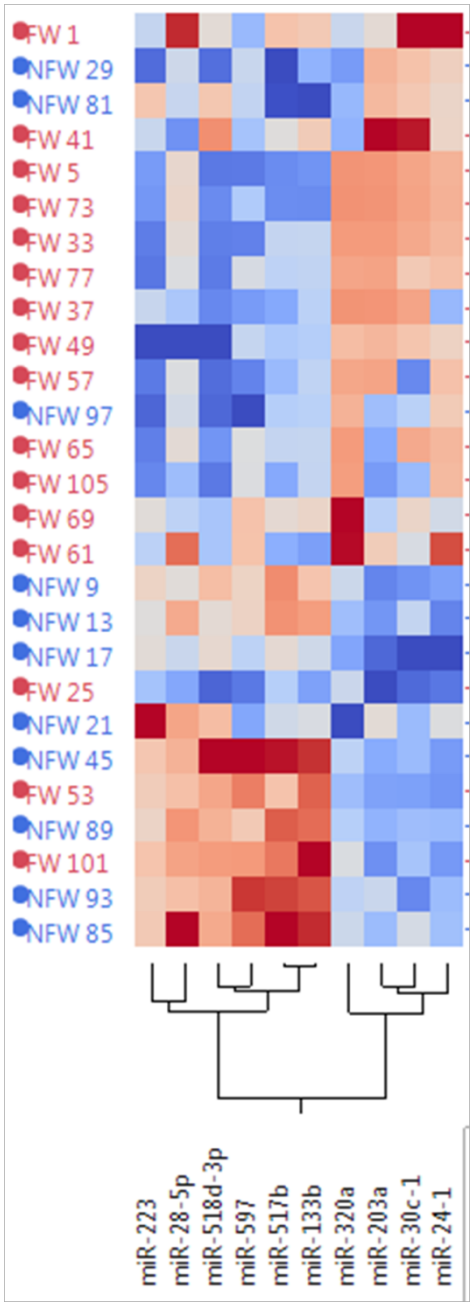
Multiplex RT-PCR Results:

Table 2: The Number of miRNAs shared among Urine Samples.

Samples	Whole Urine	Sediment
All 4 Samples	32 miRNAs	39 miRNAs
Top 3 Samples (1-3)	116 miRNAs	47 miRNAs
Fresh Samples Only	12 miRNAs	23 miRNAs
None	145 miRNAs	141 miRNAs

Table 3: Top 3 miRNAs in Both Whole Urine and Sediment Samples

miR-223
miR-203
miR-222



Hierarchical clustering analysis of top 10 urinary microRNAs shows clustering of four miRNAs (miR320, miR203, miR24, &miR30c) in adult farmworkers during spray season.

Results

Optimized methods applied to 108 urine samples from the CHC study cohort

MicroRNA Observations

- MicroRNAs can be successfully extracted from archived CHC urine samples from adult and child farmworkers and non-farmworkers.
- Of 380 microRNAs investigated, 297 (78%) were detectable in at least one sample in urine.
- 7 miRNAs were found present in at least 50% of the samples, and 1 miRNA (miR-223) was present in 97% of the samples.
- MicroRNAs observed match commonly observed mircoRNAs in urine.
- Households (parent and child combined) expressed fewer miRNAs in their urine during thinning than during non-spray (mean, 31 [range, 3-169] vs. 57 [6-197]). This difference was more drastic in adults (mean, 25 [range, 3-130] vs. 74 [6-169]) than children (mean, 38 [6-169] vs. 40 [9-173]) when analyzed separately.

Conclusions

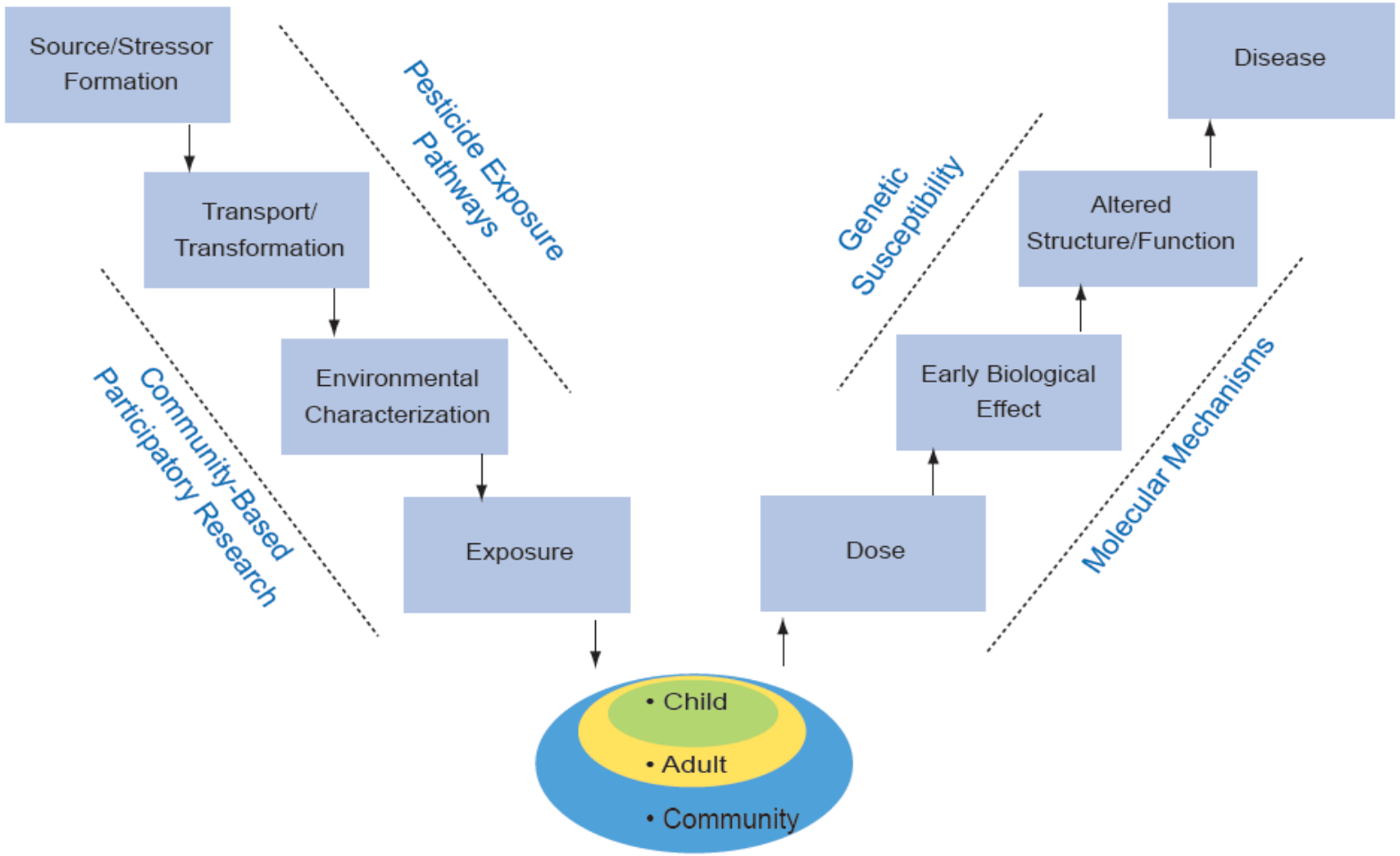
- Principal Components and hierarchical clustering analyses indicate significant differences in microRNA profiles between farmworker and non farmworker groups.

- Further investigation of microRNA profiles and associated post transcriptional regulatory targets will inform potential endpoints of OP exposure.

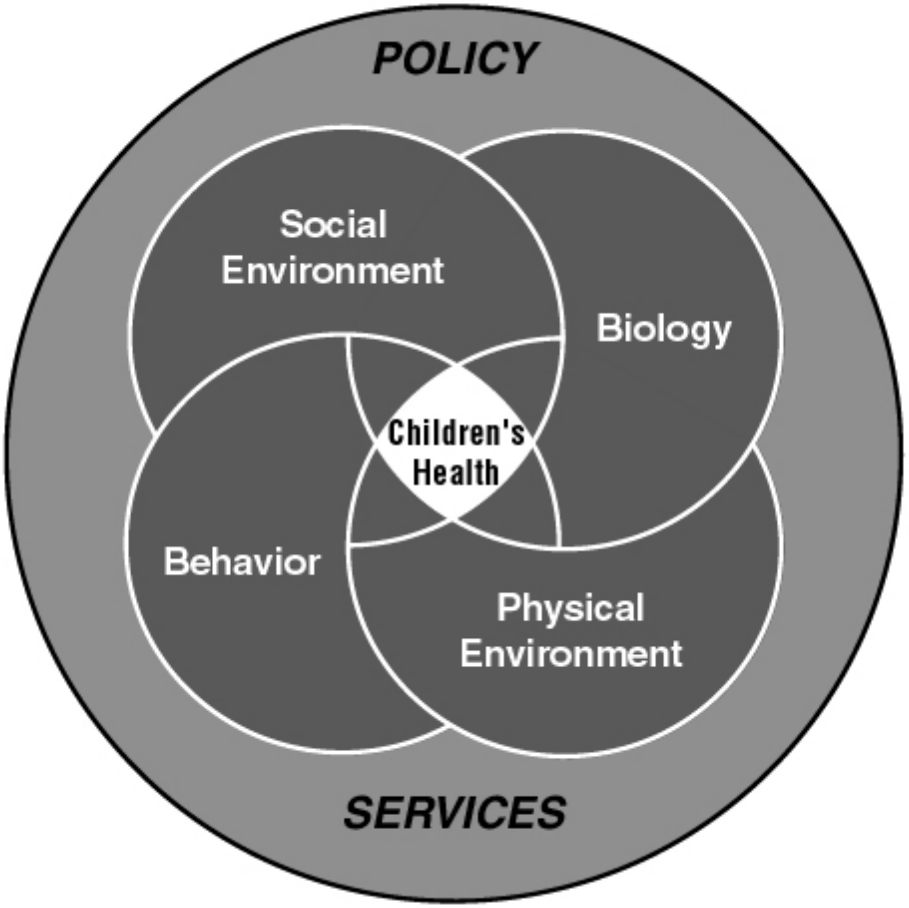
- These results provide valuable insight on the utility of archived field samples for the future development of urinary biomarkers.

This work made possible by HHSN2672007023C and HHSN2752008015C (NICHD), P01-ES009601 and P30-ES007033 (NIEHS) and RD-834514 (EPA)

Environmental Public Health Continuum

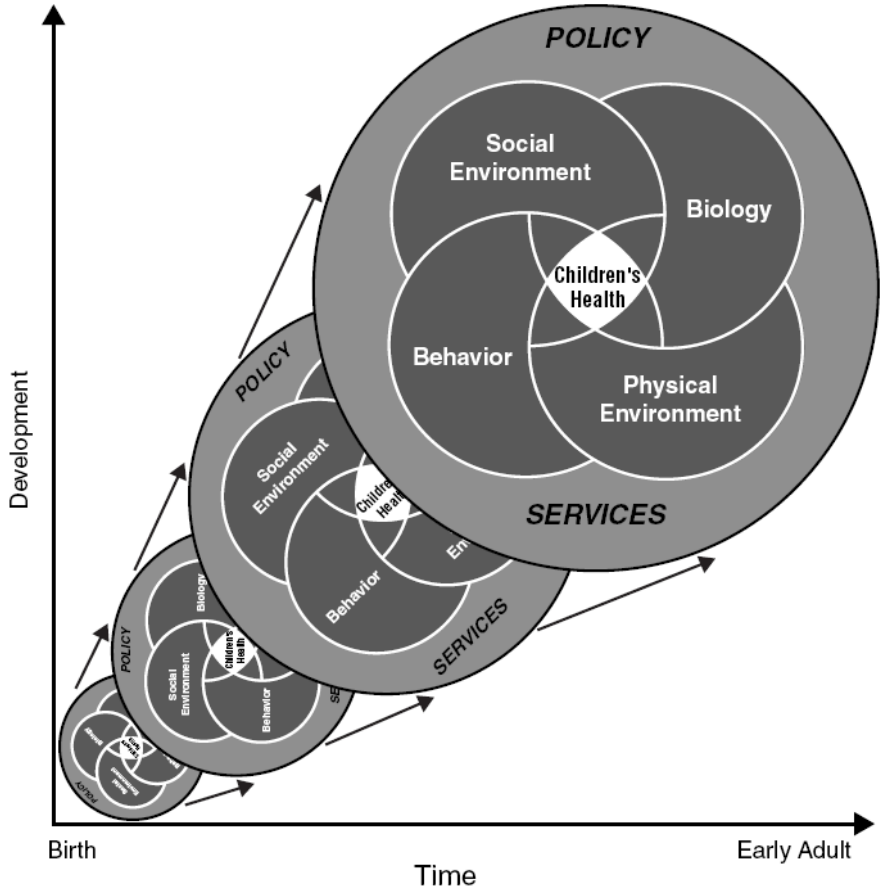


Multiple, Interacting Influences Affect Children's health including Chemical and Non Chemical Stressors



IOM, 2004

Life Course Based Model of Children's Health and its Influences:



Source: Children's Health, Nation's Health, IOM report, 2004

Epigenomic Factors that Effect our Risk Assessment Approaches

- Epigenomic changes are known to be affected by both chemical and non-chemical stressors
- Epigenomic changes occur after exposure to many chemicals, not related by structure
- Epigenomic or stress changes can be inherited and affect multiple generations.
- Epigenomic changes after chemical and non-chemical stressors can affect the same pathways.
- There are many different types of epigenomic pathways that can be changed by stressor exposure and these occur differentially across time (lifestage) in various biological tissues and can be species, organic and organism specific.
- There are known genetic polymorphisms that affect epigenomic responses

Information on Epigenomics Informs Multiple Aspects of Risk Assessment

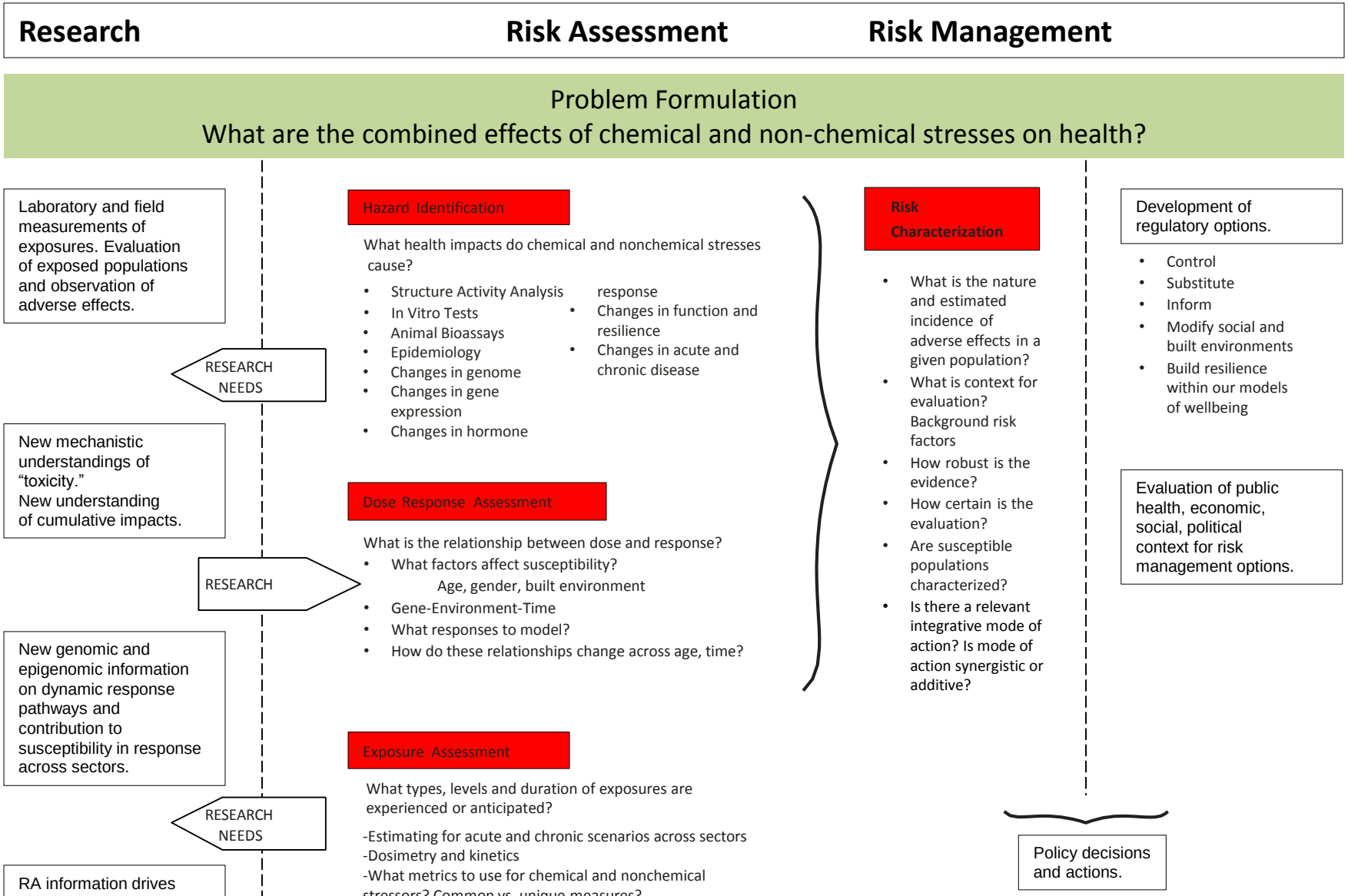
Problem formulation
- Determination of risk assessment context and scope
- Definition of scope provides context for risk assessment and leads to the identification of relevant life stages, systems, or processes of interest for the risk assessment
- Determination of relevant exposure pathways/scenarios will provide context for identifying relevant developmental life stages
- Determination of chemical-specific factors will also provide context for the identification of potential life stages for evaluation, as it will identify potential toxicological processes of interest and hence identify developmental systems for potential evaluation
- Identification of cross-species relevancy of potential responses

Analysis
- Identification of uniquely susceptible dynamic processes
- Identification of developmental milestones and/or end points for testing/assessment
- Identification of functional consequences of processes if altered
- Illustrate the interrelatedness of dynamic developmental processes and thus identify impacts that could occur at later life stages and within other organ systems
- Identification of immediate or delayed responses

Risk characterization
- Define dose–response relationships, especially dose, time, and response relationships
- Characterize potential magnitude of effect, reversibility, repair, functional reserve, etc., of dynamic developmental processes

al stressors

Daston et al (2004) A Framework for Assessing Risks to Children from Exposure to Environmental Agents. Environ Health Perspect 112:238-256


Research
Risk Assessment
Risk Management
Problem Formulation
What are the combined effects of chemical and non-chemical stresses on health?
Laboratory and field measurements of exposures. Evaluation of exposed populations and observation of adverse effects.
RESEARCH NEEDS
New mechanistic understandings of "toxicity."
New understanding of cumulative impacts.
RESEARCH
New genomic and epigenomic information on dynamic response pathways and contribution to susceptibility in response across sectors.
RESEARCH NEEDS
RA information drives design of new sustainable communities.
Hazard Identification
What health impacts do chemical and nonchemical stresses cause?
Structure Activity Analysis
In Vitro Tests
Animal Bioassays
Epidemiology
Changes in genome
Changes in gene expression
Changes in hormone response
Changes in function and resilience
Changes in acute and chronic disease
Dose Response Assessment
What is the relationship between dose and response?
What factors affect susceptibility?
Age, gender, built environment
Gene-Environment-Time
What responses to model?
How do these relationships change across age, time?
Exposure Assessment
What types, levels and duration of exposures are experienced or anticipated?
-Estimating for acute and chronic scenarios across sectors
-Dosimetry and kinetics
-What metrics to use for chemical and nonchemical stressors? Common vs. unique measures?
Risk Characterization
What is the nature and estimated incidence of adverse effects in a given population?
What is context for evaluation? Background risk factors
How robust is the evidence?
How certain is the evaluation?
Are susceptible populations characterized?
Is there a relevant integrative mode of action? Is mode of action synergistic or additive?
Development of regulatory options.
Control
Substitute
Inform
Modify social and built environments
Build resilience within our models of wellbeing
Evaluation of public health, economic, social, political context for risk management options.
Policy decisions and actions.

Challenges and Opportunities

for using epigenetic information and approaches for understanding mechanisms of toxicity and dose-response

- Intra- and cross-species extrapolation
- Facilitate use of model systems
- Characterize low dose and early temporal response
- Facilitate in vitro to in vivo extrapolation
- Extrapolation across levels of biological complexity
- Identify actual pathways of disease

Risk Management and Policy Considerations

Genomics and the EPA: Interim Policy

- Encourages and supports continued genomic research.
- Limited use of genomics while the Agency gains experience in assessing quality, accuracy and reproducibility and relevance of the data.
- Genomics data alone are currently insufficient as a basis for risk assessment and management decisions.
- May be used in a “weight-of-evidence” approach.
- Policy outlined at: ww.epa.gov/osp/spc/genomics.htm

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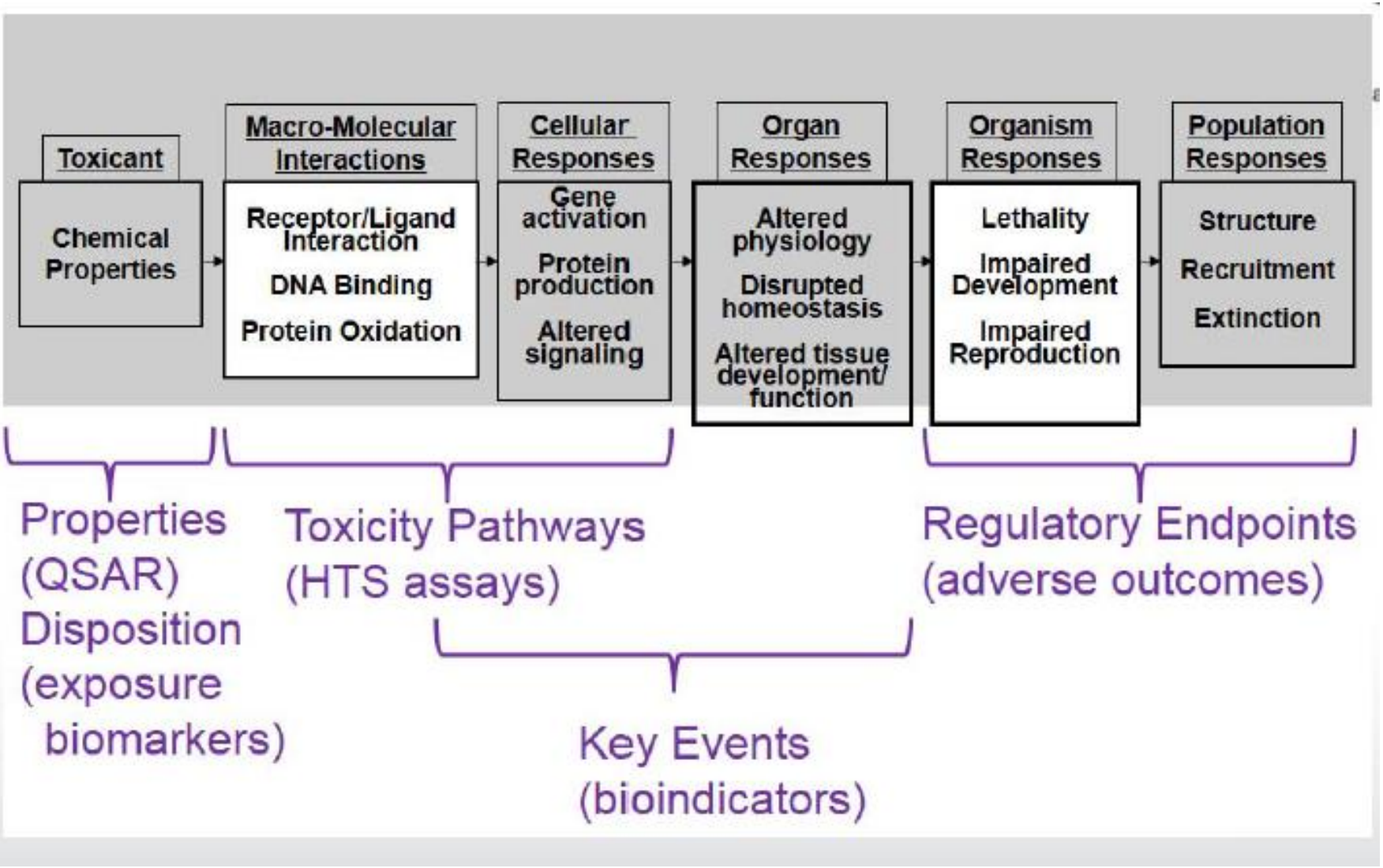
- Microarray data has already been received by an EPA Office of Pesticide Programs
- A pesticide registrant cited a published genomics article (Genter, Burman et al, 2002) as part of a mode-of-action data package submission for product registration

Dix et al, 2006

AOP and biomarkers serve to link elements and describe disease pathogenesis



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Mary Cassatt

Acknowledgements

Funding: Thank you to the FDA (1U01FD004242-01), CHC Center NIEHS (5 P01 ES009601) and EPA (RD-83170901) and a new EPA Predictive Toxicology Center Grant
NIEHS Environmental Pathology/Toxicology Training Grant (ES07032)

Challenges and Opportunities

for using systems biology information and approaches for understanding mechanisms of toxicity and dose-response

- Intra- and cross-species extrapolation
- Facilitate use of model systems
- Characterize low dose and early temporal response
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Gene Ontology: Tool for the unification of biology. The Gene Ontology Consortium

Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G.

Nat Genet. 2000 May;25(1):25-9.

Gene Ontology Hierarchy: Based on the AmiGO, the GO Consortium's annotation and ontology toolkit

Carbon S, Ireland A, Mungall CJ, Shu S, Marshall B, Lewis S, AmiGO Hub, Web Presence Working Group. AmiGO: online access to ontology and annotation data.

Bioinformatics. Jan 2009;25(2):288-9

http://www.geneontology.org/

Ontology application and use at the ENCODE DCC

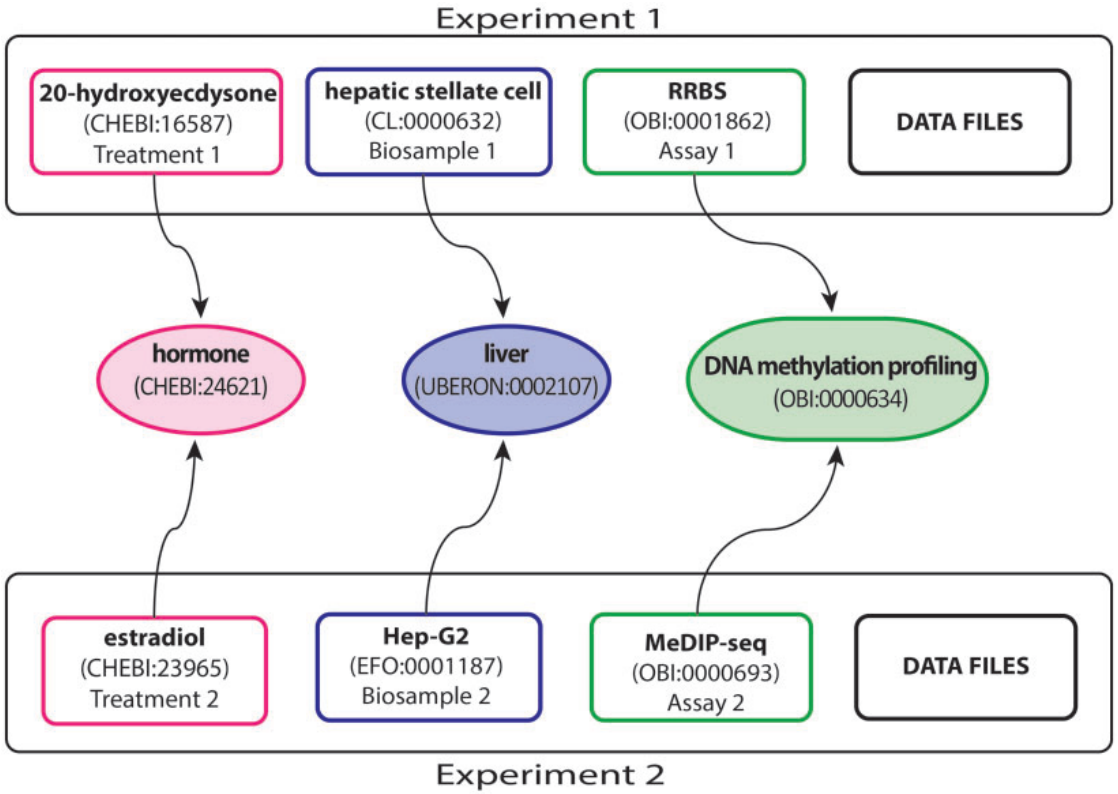
Venkat S. Malladi[1], Drew T. Erickson[1], Nikhil R. Podduturi[1], Laurence D. Rowe[1], Esther T. Chan[1], Jean M. Davidson[1], Benjamin C. Hitz[1], Marcus Ho[1], Brian T. Lee[2], Stuart Miyasato[1], Gregory R. Roe[1], Matt Simison[1], Cricket A. Sloan[1], J. Seth Strattan[1], Forrest Tanaka[1], W. James Kent[2], J. Michael Cherry[1] and Eurie L. Hong[1,*]

[1]Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305, USA and [2]Center for Biomolecular Science and Engineering, School of Engineering, University of California Santa Cruz, Santa Cruz, CA 95064, USA

Citation details: Malladi,V.S., Erickson,D.T., Podduturi,N.R., *et al.* Ontology application and use at the ENCODE DCC. *Database* (2015) Vol. 2015: article ID bav010; doi:10.1093/database/bav010

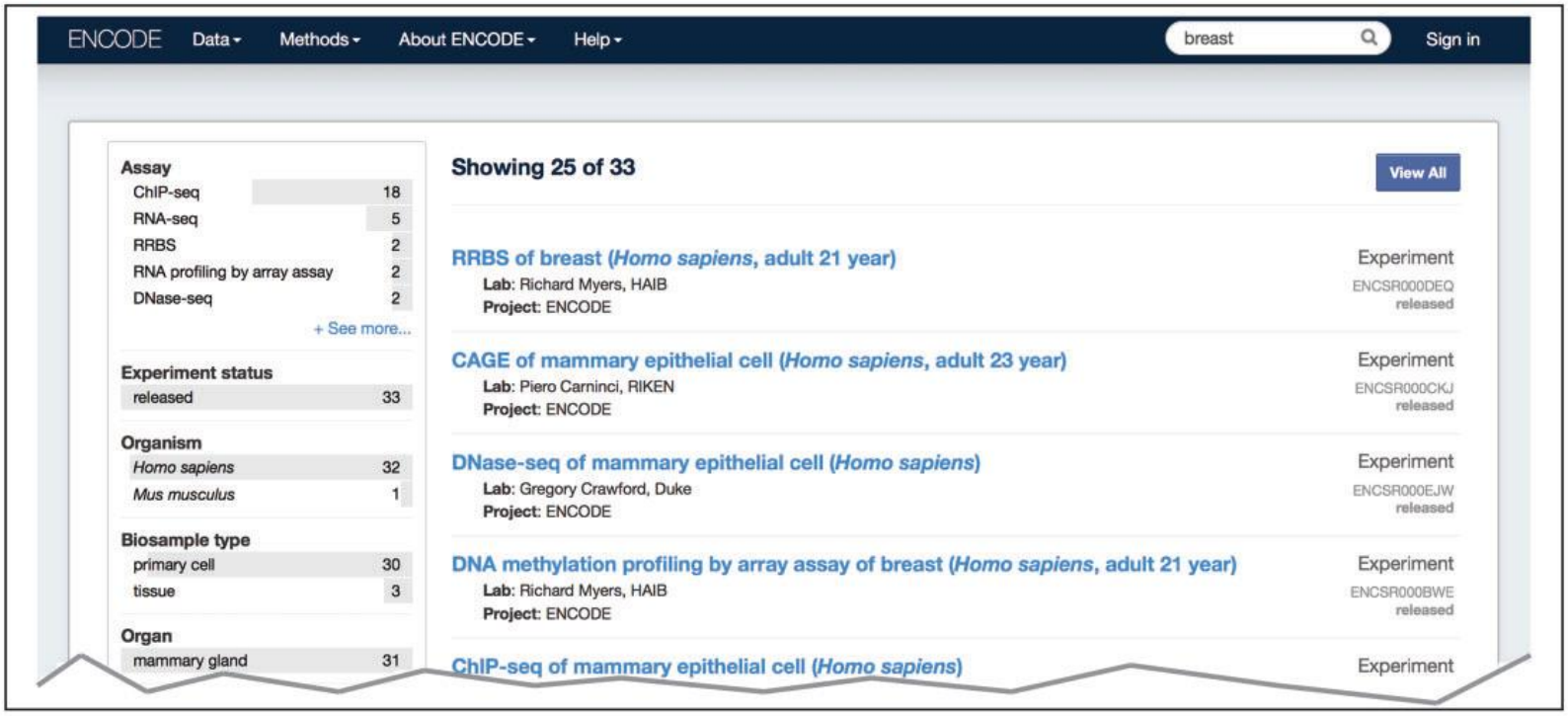
Received 16 November 2014; Revised 13 January 2015; Accepted 19 January 2015

Experimental metadata annotated with appropriate ontology terms



Malladi VS et al. 2015

Search at the ENCODE portal (https://www.encodeproject.org/). In this example, a free text search is done for ‘breast’



Malladi VS et al. 2015

Research | **Risk Assessment** | **Risk Management**

Problem Formulation
What are the combined effects of chemical and non-chemical stresses on health?

Laboratory and field measurements of exposures. Evaluation of exposed populations and observation of adverse effects.

RESEARCH NEEDS

New mechanistic understandings of "toxicity."
New understanding of cumulative impacts.

RESEARCH

New genomic and epigenomic information on dynamic response pathways and contribution to susceptibility in response across sectors.

RESEARCH NEEDS

RA information drives design of new sustainable communities.

Hazard Identification

What health impacts do chemical and nonchemical stresses cause?

- Structure Activity Analysis
- In Vitro Tests
- Animal Bioassays
- Epidemiology
- Changes in genome
- Changes in gene expression
- Changes in hormone response
- Changes in function and resilience
- Changes in acute and chronic disease

Dose Response Assessment

What is the relationship between dose and response?

- What factors affect susceptibility?
  Age, gender, built environment
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Development of regulatory options.

- Control
- Substitute
- Inform
- Modify social and built environments
- Build resilience within our models of wellbeing

Evaluation of public health, economic, social, political context for risk management options.

Policy decisions and actions.

Factors to consider for Hazard Identification

1. For Epigenetic changes how strong is the database supporting QSAR like approaches for both chemical and non-chemical stressors?
2. For Epigenetic changes how strong is the database that our assessment of impacts will be the same across assessment tools– for example across in vitro systems (primary and established cell lines), organ specific cell lines, life stage of cell lines?
3. For in vivo assessment using animal models, how much do we know about species variability?
4. For epidemiological studies do we know the consistency of epigenetic responses? Especially for biomarkers we would use to assess prior to impacts versus organ specific info that might be obtained from cancer specimens? What would be the best epigenetic endpoints for RA versus in the clinic or for mechanistic studies?

Integrating Genetic and Toxicogenomic Information for Determining Underlying Susceptibility to Developmental Disorders

Joshua F. Robinson,[1,3] Jesse A. Port,[1,3] Xiaozhong Yu,[1,3] and Elaine M. Faustman[1,2,3,4,5*]

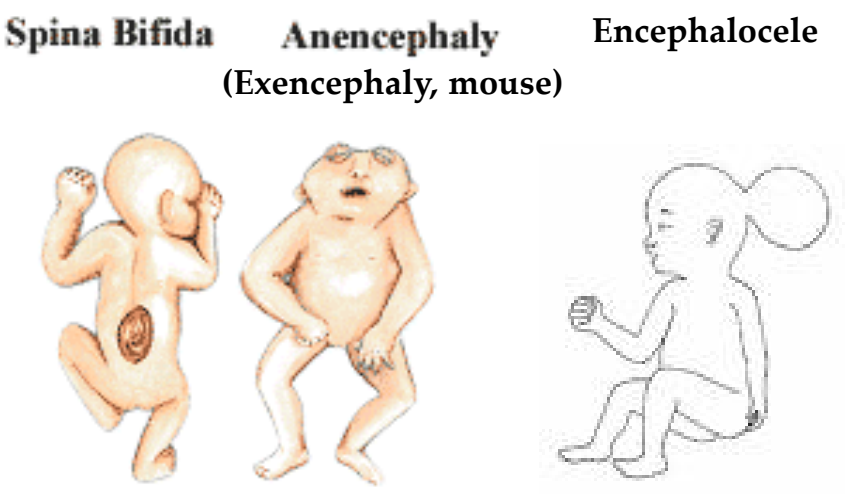
[1]Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, Washington
[2]Center for Ecogenetics and Environmental Health, Seattle, Washington
[3]Institute for Risk Analysis and Risk Communication, Seattle, Washington
[4]Center on Human Development and Disability, Seattle, Washington
[5]Center for Child Environmental Health Risks Research, Seattle, Washington

Birth Defects Research (Part A) 88:920–930 (2010)

Neural Tube Defects (NTDs)

Definition

NTDs represent a group of defects where the neural tube fails to develop properly



The second most common human birth defect

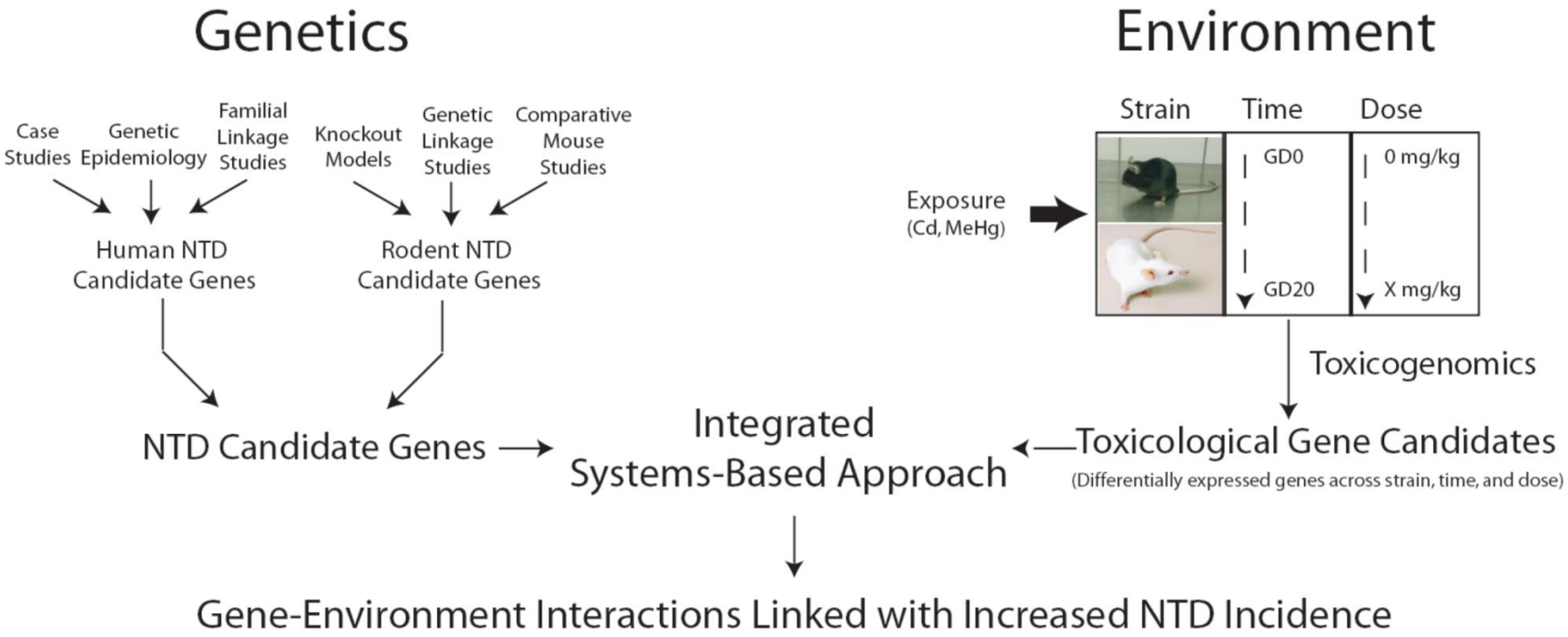
Consequences range in severity
- developmental delays
- physical limitations
- behavioral problems
- facial abnormalities
- early death

Health care cost: ~$1.4 million per case of spina bifida (Detrait *et al.,* 2005)

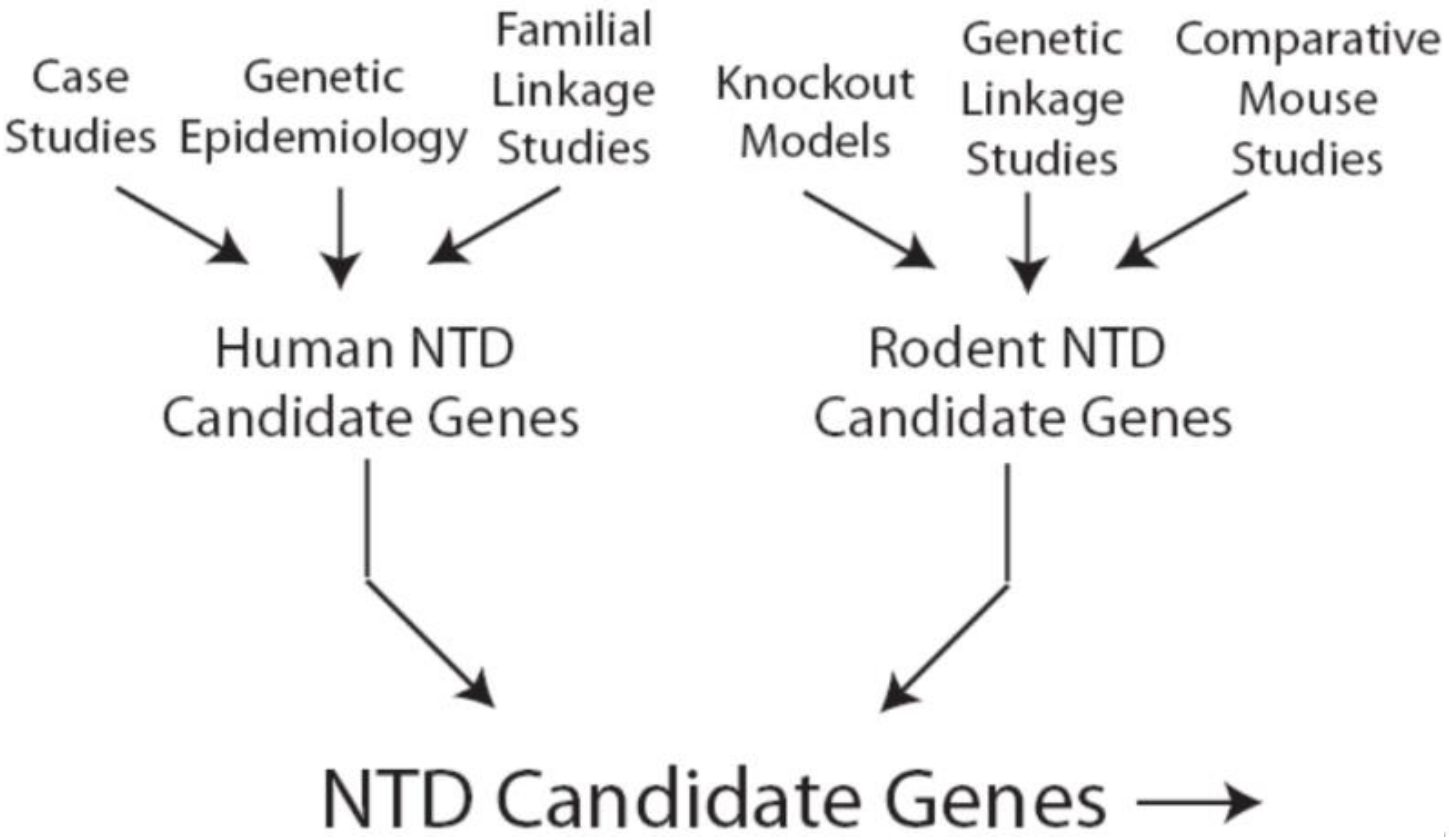
60-70% of birth defects have unknown origin (March of Dimes, 2006)

Integrating Genetic and Toxicogenomic Information for Determining Underlying Susceptibility to Developmental Disorders

The Integration of Gene-Disease Databases and Environmental Toxicogenomic Studies



Genetics


Case Studies
Genetic Epidemiology
Familial Linkage Studies
Knockout Models
Genetic Linkage Studies
Comparative Mouse Studies
Human NTD Candidate Genes
Rodent NTD Candidate Genes
NTD Candidate Genes

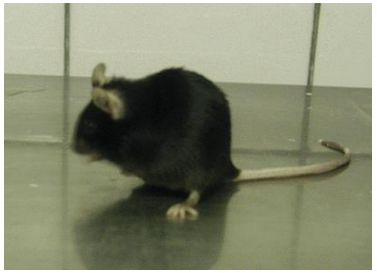
Differential Sensitivity to Environmental Teratogens between the C57 and SWV during neurulation

	SWV	C57		
Hyperthermia	++	-	Exencephaly	Finnell *et al*. 1986
Phenobarbital	+/-	-	Malformations (including NTDs)	Finnell *et al*. 1986
Valproic Acid	++++	-	Malformations (including NTDs)	Naurse *et al*. 1988
Arsenite	-	+	Exencephaly	Machado *et al*. 1999
Cadmium	+	++++	Exencephaly (GD 7-9) Limb Malformations (>GD9)	Hovland *et al*. 1999

+ = 20% difference in sensitivity based on study results at specific timepoints (GD7-10)

SWV

C57BL/6



Human and mouse NTD genetic studies

American Journal of Medical Genetics Part C (Semin. Med. Genet.) 135C:9–23 (2005)

ARTICLE

Candidate Gene Analysis in Human Neural Tube Defects

ABEE L. BOYLES, PRESTON HAMMOCK, AND MARCY C. SPEER*

Biochemical and developmental pathways, mouse models, and positional evidence have provided numerous candidate genes for the study of human neural tube defects. In a survey of 80 studies on 38 candidate genes, few found significant results in human populations through case-control or family-based association studies. While the folate pathway has been explored extensively, only the *MTHFR* 677C > T polymorphism was significant, and only in an Irish population. Developmental pathways such as the Wnt signaling pathway and *Hox* genes have also been explored without positive results. More than 90 mouse candidates have been identified through spontaneous and knockout mutations, but only the T locus (mouse *Brachyury* gene) showed association in an initial study that was not confirmed on follow-up. Positional candidates have been derived from cytogenetic evidence, but preliminary genomic screens have limited power due to small sample sizes. Future studies would

Extensive literature review of over 80 human NTD studies

Few associations identified between gene candidates and NTD risk

REVIEW ARTICLE

Mouse Mutants With Neural Tube Closure Defects and Their Role in Understanding Human Neural Tube Defects

Muriel J. Harris* and Diana M. Juriloff
Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada

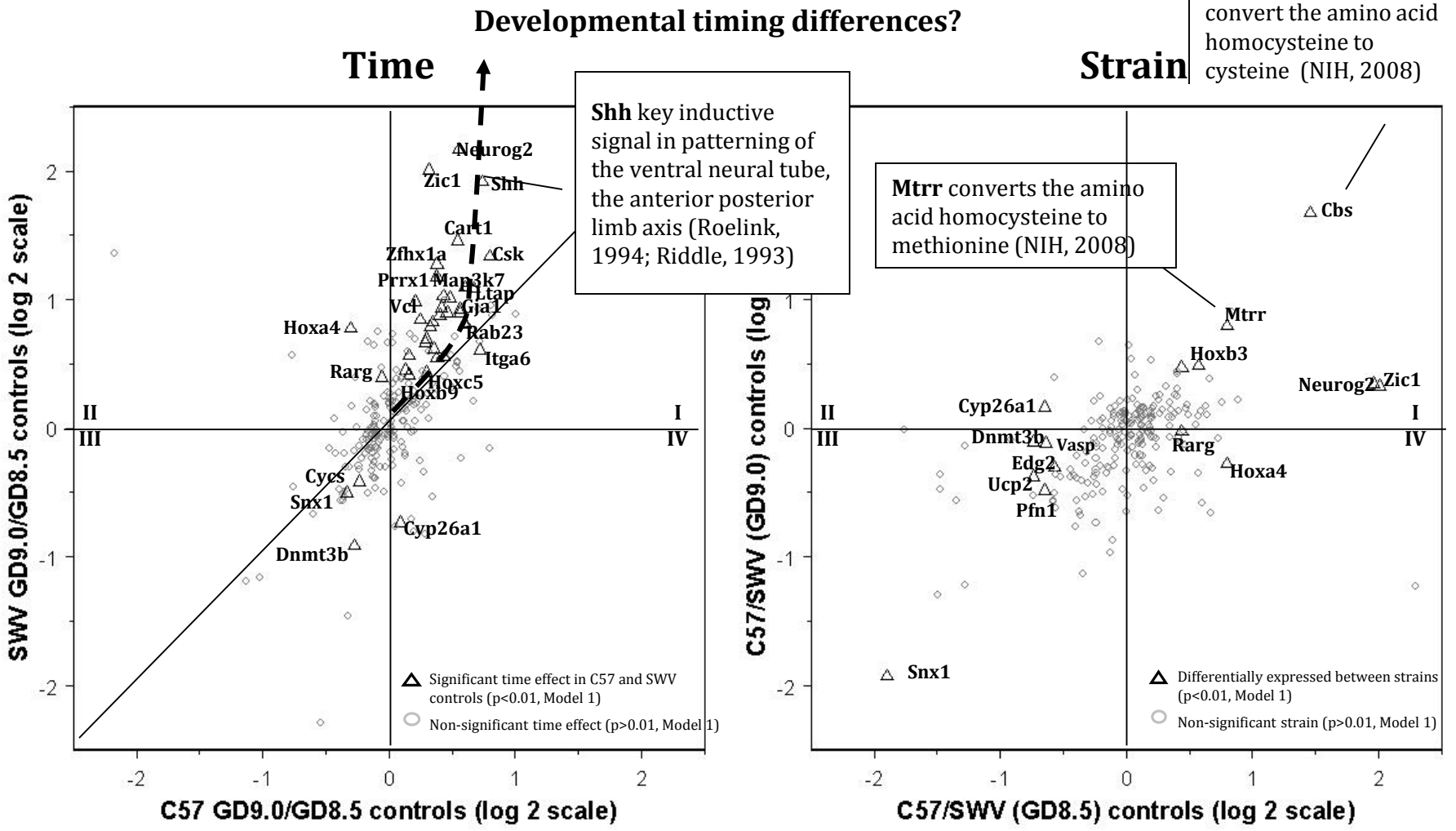
Received 15 September 2006; Revised 20 October 2006; Accepted 20 October 2006

BACKGROUND: The number of mouse mutants and strains with neural tube closure defects (NTDs) now exceeds 190, including 155 involving known genes, 33 with unidentified genes, and eight "multifactorial" strains. **METHODS:** The emerging patterns of mouse NTDs are considered in relation to the unknown genet-

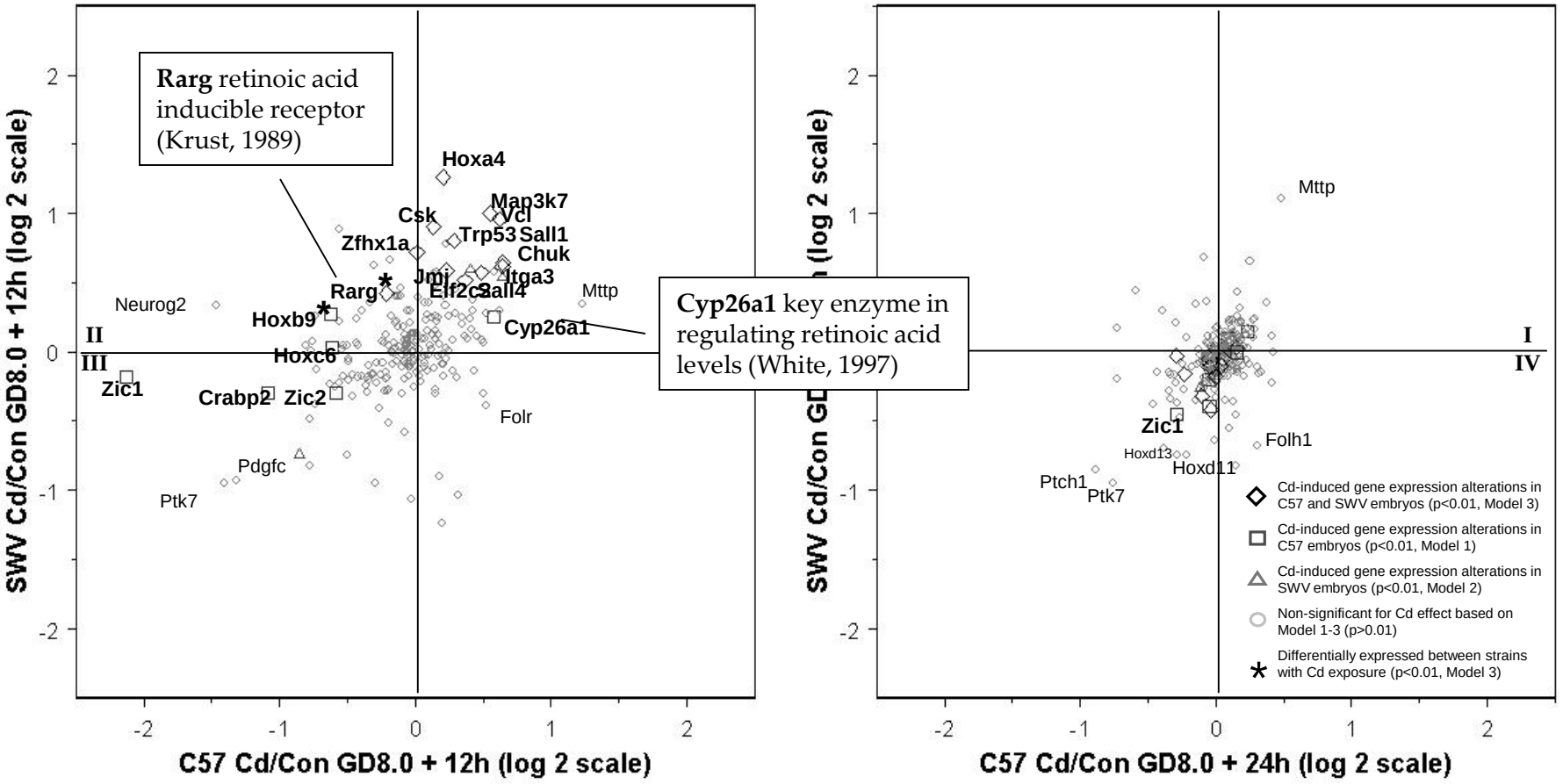
Extensive literature review

Over 200+ mouse mutants identified to be linked with NTD incidence

NTD candidate genes display strain and time dependent differences in expression in C57 and SWV embryos



Cd-induced gene expression alterations in NTD candidate genes in C57 and SWV embryos



Overall Conclusions

- Differential gene expression response within CNS development and environmental stress pathways correlates with increased sensitivity to metal-induced NTDs
- Increased Cd accumulation contributes to observed differing sensitivities between resistant (C57) and sensitive (SWV) strains
- Metals may commonly (cell cycle, development, transcription) and uniquely (methylation, one-carbon metabolism) disrupt processes associated with NTD development
- Using a toxicogenomic approach and available independent genetic mouse model data, we have identified a potential mechanism to screen for potential gene-environmental interactions that may identify susceptible populations

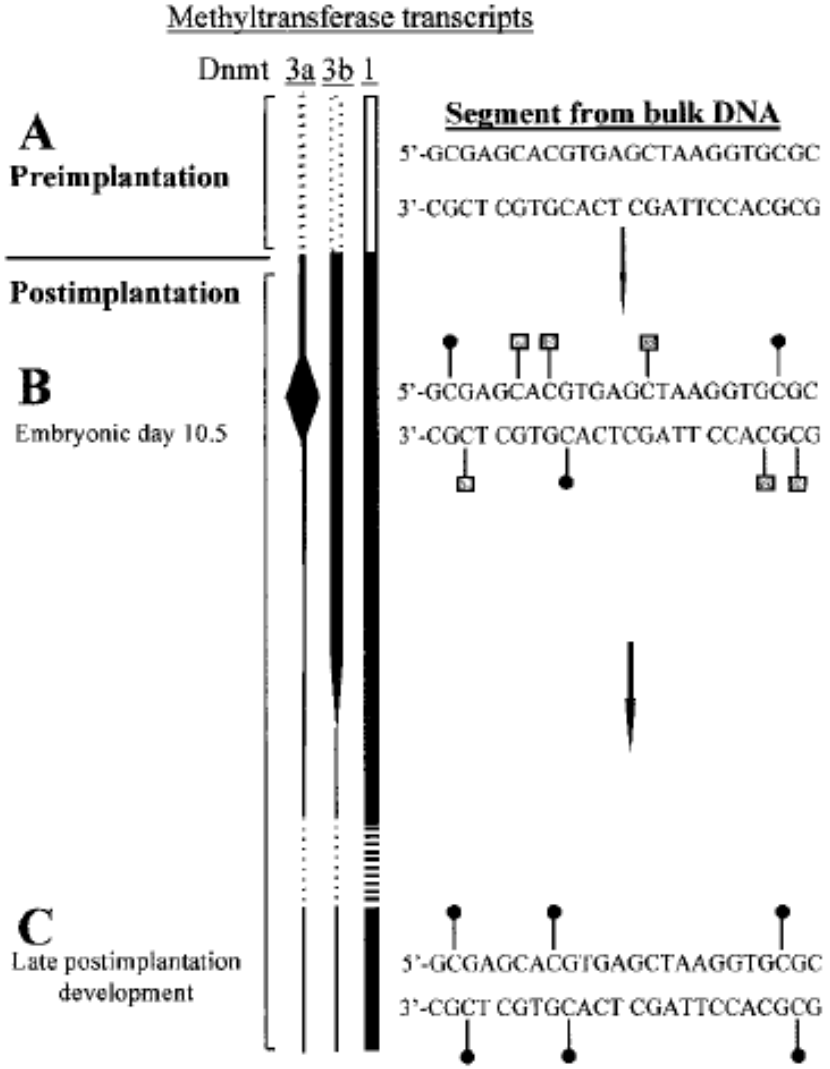
Non-CpG methylation is prevalent in embryonic stem cells and may be mediated by DNA methyltransferase 3a

Bernard H. Ramsahoye*†, Detlev Biniszkiewicz‡, Frank Lyko‡, Victoria Clark§, Adrian P. Bird§, and Rudolf Jaenisch‡

*Department of Hematology, Western General Hospital, EH4 2XU Edinburgh, United Kingdom; ‡Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142; and §University of Edinburgh, Institute of Cell and Molecular Biology, Darwin Building, Kings Buildings, Mayfield Road, EH9 3JR Edinburgh, United Kingdom

Ramsahoye BH et al, 2000. PNAS. 97(10): 5237-5242.

Model for the reestablishment of DNA methylation after implantation



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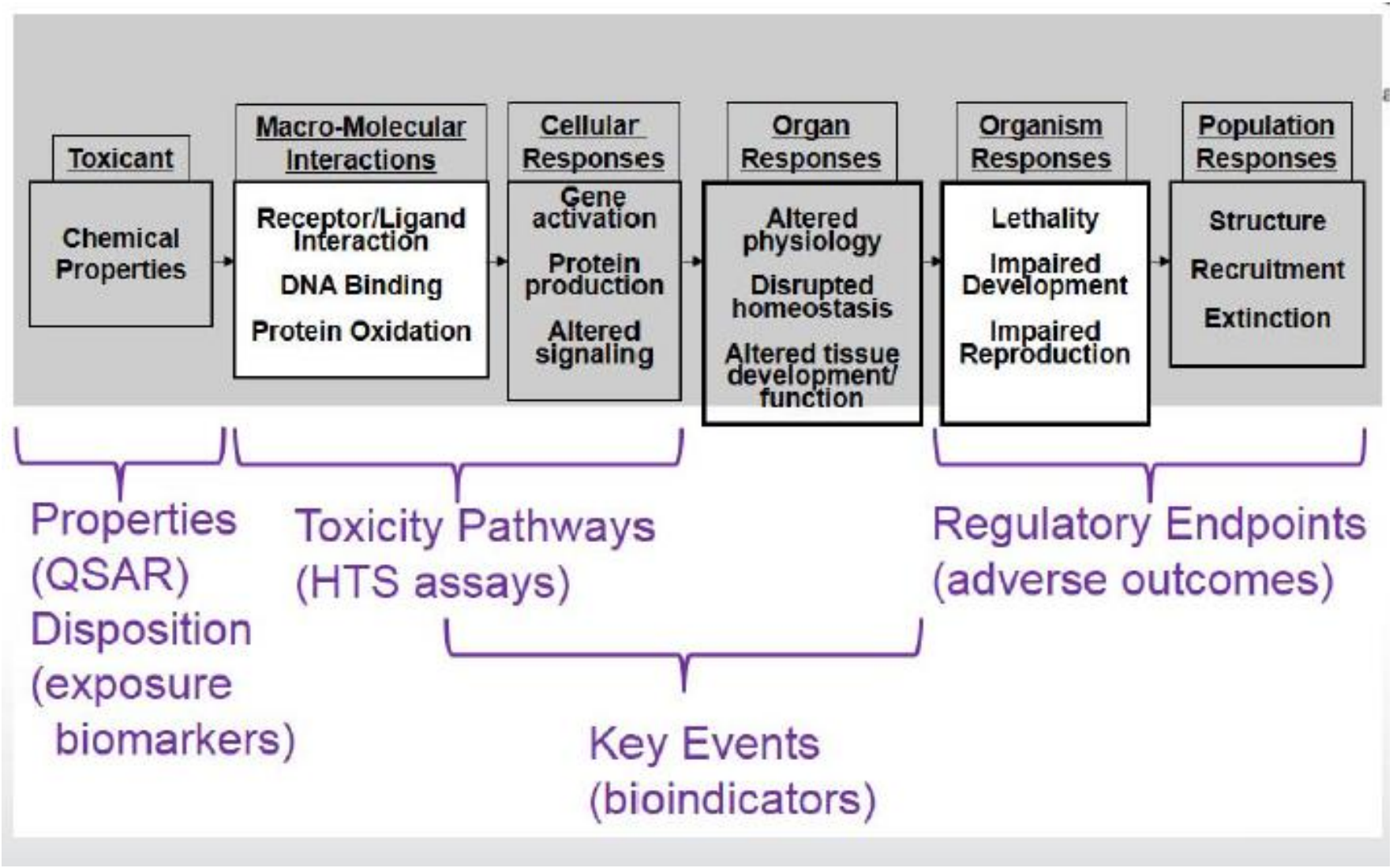
AOP and biomarkers serve to link elements and describe disease pathogenesis



John Vandenberg, Annie M. Jarabek 2014

Urinary microRNA Profiles as Potential Biomarkers of Pesticide Exposure

Brittany A. Weldon[1,2], Sara E. Pacheco[1,2], Kirk Van Ness[1,2], Tomomi Workman[1,2], Beti Thompson[3], and Elaine M. Faustman[1,2]

[1]Institute for Risk Analysis and Risk Communication, University of Washington, Seattle, WA [2]Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, [3]Fred Hutchinson Cancer Research Center, Seattle, WA

Objectives

Optimization Objectives:

- Isolate miRNA from both whole urine and urine sediments
- Determine the quantity and quality of urinary RNA using Nanodrop spectrophotometer
- Analyze RNA profiles from whole urine and urine sediments using Agilent Bioanalyzer
- Identify specific miRNAs in the samples using TaqMan® microRNA RT-PCR analysis (384 miRNAs probed)

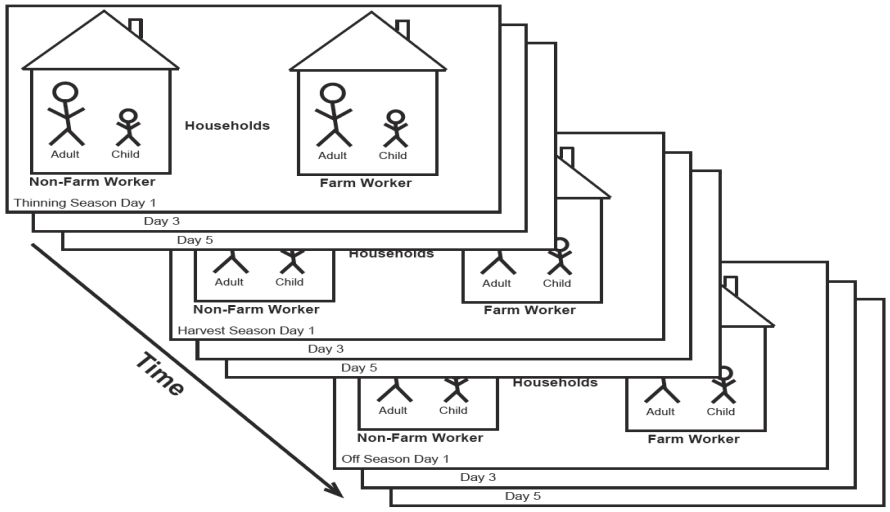
Experimental Objectives:

- Apply optimized methods to Children's Health Center (CHC) sub cohort (n=108)
- Investigate differential miRNA expression levels between:
  - Farmworker/Non Farmworker
  - Adult/Child
  - Spray season/ Non spray season
- Determine whether urinary miRNA expression will reflect anticipated exposure status of farmworker and non-farmworker families and could thus serve as a biomarker of exposure.

Study Population

Children's Health Center Cohort:
Yakima Valley, Washington

Farmworker and Non-Farmworker households
Adults and Children in each household
Sampled during spray seasons and non-spray season



Optimization Methods

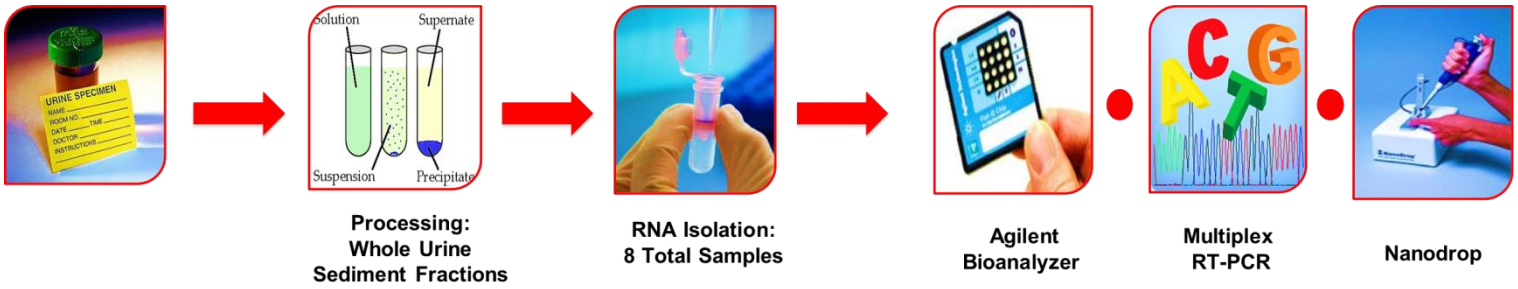


Figure 2. Experimental Workflow. Urine was thawed and processed prior to RNA extraction. The RNA was used for Nanodrop Spectrophotometer and Agilent Bioanalyzer analyses and multiplex RT-PCR reactions using the TaqMan MicroRNA Cards.

Optimization Results

Table 1: Optimization Nanodrop Sample Characteristics

Sample ID	Group	Fraction	Concentration (ng/ul)	260/280, 260/230
1W	Fresh	Whole Urine	97.79	1.92, 1.14
1S	Fresh	Sediment	163.90	1.94, 1.60
2W	Field	Whole Urine	45.53	1.82, 1.17
2S	Field	Sediment	101.49	1.77, 1.06
3W	Field	Whole Urine	12.36	1.64, 1.09
3S	Field	Sediment	22.62	1.65, 0.67
4W	Field	Whole Urine	7.93	1.37, 0.22
4S	Field	Sediment	8.64	1.08, 0.27

Note: A 260/280 and 260/230 of 2 is considered optimal.

Multiplex RT-PCR Results:

Table 2: The Number of miRNAs shared among Urine Samples.

Samples	Whole Urine	Sediment
All 4 Samples	32 miRNAs	39 miRNAs
Top 3 Samples (1-3)	116 miRNAs	47 miRNAs
Fresh Samples Only	12 miRNAs	23 miRNAs
None	145 miRNAs	141 miRNAs

Table 3: Top 3 miRNAs in Both Whole Urine and Sediment Samples

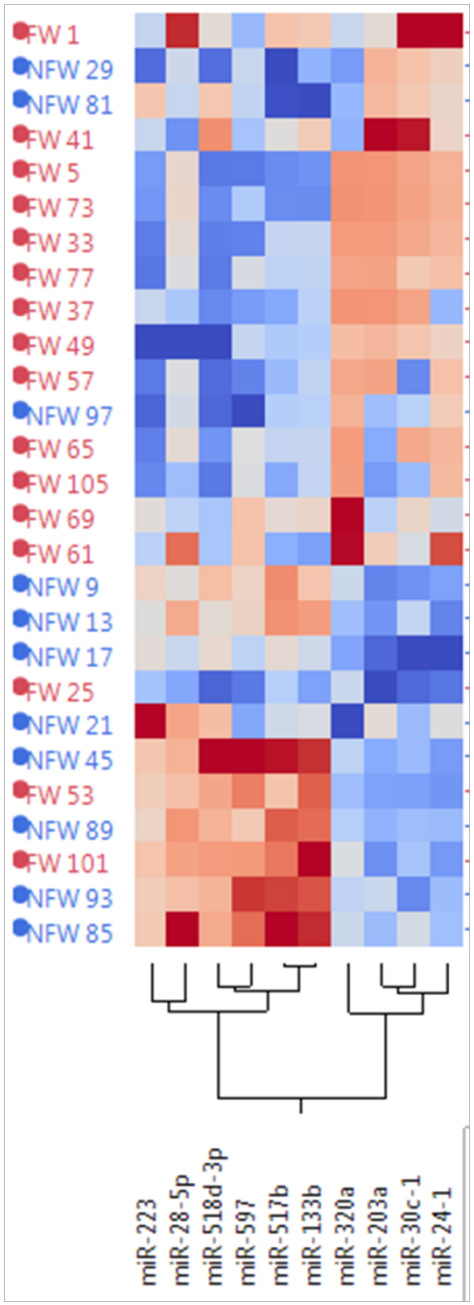
miR-223
miR-203
miR-222

Results

Optimized methods applied to 108 urine samples from the CHC study cohort

MicroRNA Observations

- MicroRNAs can be successfully extracted from archived CHC urine samples from adult and child farmworkers and non-farmworkers.
- Of 380 microRNAs investigated, 297 (78%) were detectable in at least one sample in urine.
- 7 miRNAs were found present in at least 50% of the samples, and 1 miRNA (miR-223) was present in 97% of the samples.
- MicroRNAs observed match commonly observed mircoRNAs in urine.
- Households (parent and child combined) expressed fewer miRNAs in their urine during thinning than during non-spray (mean, 31 [range, 3-169] vs. 57 [6-197]). This difference was more drastic in adults (mean, 25 [range, 3-130] vs. 74 [6-169]) than children (mean, 38 [6-169] vs. 40 [9-173]) when analyzed separately.



Hierarchical clustering analysis of top 10 urinary microRNAs shows clustering of four miRNAs (miR320, miR203, miR24, &miR30c) in adult farmworkers during spray season.

miRNA	Selected cellular processes and tissue sources
miR-24-1	Cell proliferation, DNA repair and apoptosis
miR-30c-1	Tumor suppression, innate immunity, highly expressed in heart cells
miR-203a	Specifically expressed in keratinocytes and promotes epidermal differentiation
miR-320a	Regulates PTEN-controlled tumor-suppressive axis

Selected cellular processes associated with farmworker associated microRNAs.

Conclusions

- Principal Components and hierarchical clustering analyses indicate significant differences in microRNA profiles between farmworker and non farmworker groups.

- Further investigation of microRNA profiles and associated post transcriptional regulatory targets will inform potential endpoints of OP exposure.

- These results provide valuable insight on the utility of archived field samples for the future development of urinary biomarkers.

This work made possible by HHSN2672007023C and HHSN2752008015C (NICHD), P01-ES009601 and P30-ES007033 (NIEHS) and RD-834514 (EPA)

Risk Management and Policy Considerations

Genomics and the EPA: Interim Policy

- Encourages and supports continued genomic research.
- Limited use of genomics while the Agency gains experience in assessing quality, accuracy and reproducibility and relevance of the data.
- Genomics data alone are currently insufficient as a basis for risk assessment and management decisions.
- May be used in a “weight-of-evidence” approach.
- Policy outlined at: ww.epa.gov/osp/spc/genomics.htm

Environmental Protection Agency

Potential Implications of Genomics for Regulatory and Risk Assessment Applications at EPA (2004)

- "Genomics data alone are currently insufficient as a basis for risk assessment and management decisions."
- "Genomics data may be useful in a weight of evidence approach for human and ecological health risk assessments and can be used in concert with all the other information the EPA considers for a particular assessment or decision."

Dix *et al.*, 2006

Table 1 US EPA development of science policy for the use of genomics data in regulatory and risk assessment applications

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Dix et al, 2006

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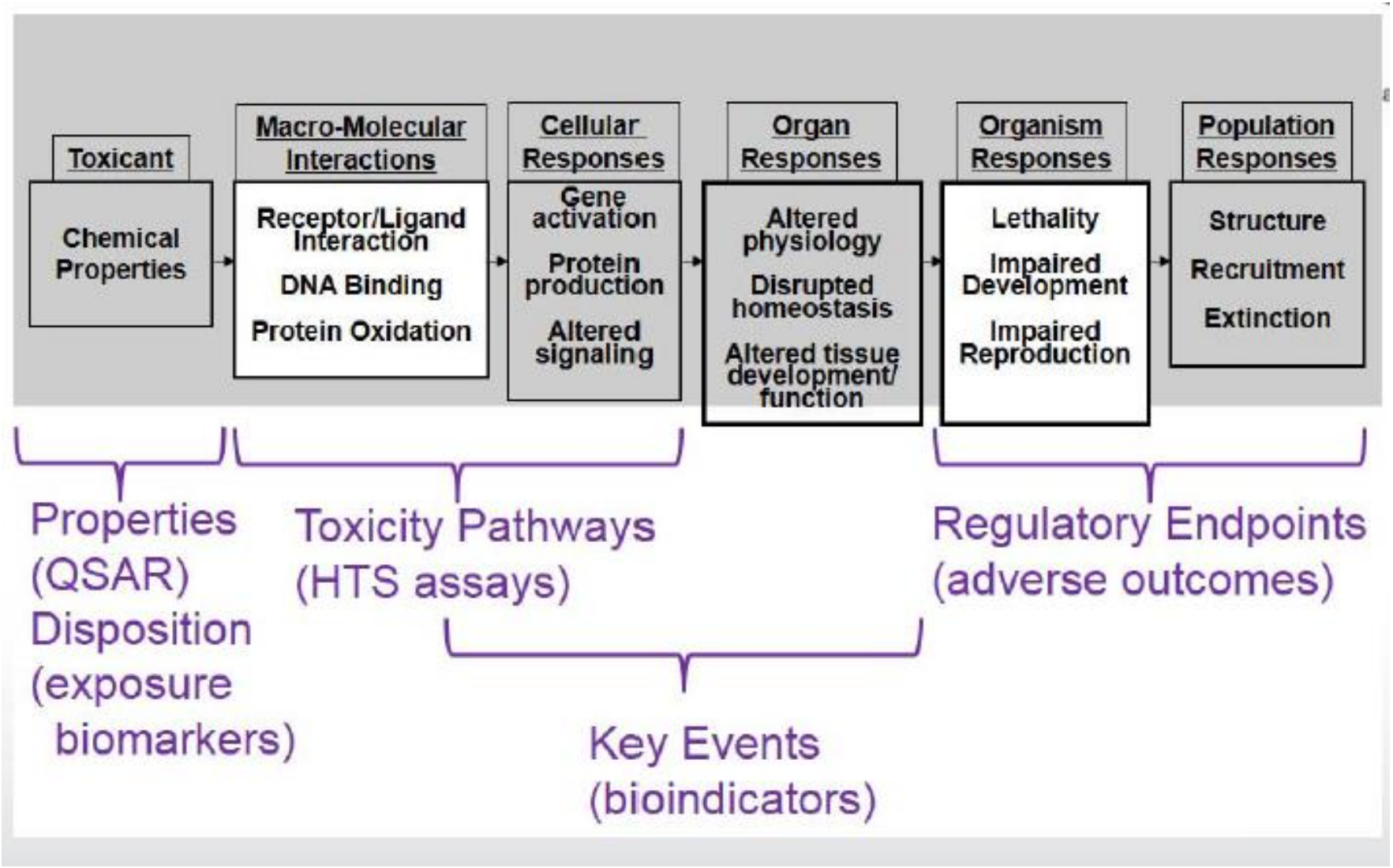

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Potential Implications of Genomics for Regulatory and Risk Assessment Applications at EPA (2004)

Identified four areas likely to be influenced by genomics:

1. Prioritization of contaminants and contaminated sites
   - Testing to more fully identify hazard and predictions based on testing
   - HPV program –can help group chemicals and ID hazardous ones
2. Environmental monitoring
   - Chemical and physical analyses of air, water, soil and sediment
   - Toxicity testing
   - Analysis of tissue residues
   - Ecological community structure analysis
   - Microbial and pathogenic analysis

*Potential Implications of Genomics for Regulatory and Risk Assessment Applications at EP*A (2004) – CONT.

3. Reporting provisions
   - To have an effect on reporting provisions, linkage of genomic changes to adverse effects or response pathways must be established and addressed
4. Risk assessment
   - Establishing mode-of-action
   - Comparative genomics might aid in interpreting human relevance of animal toxicity

Paper: Interim Guidance for Microarray-Based Assays: Regulatory and Risk Assessment Applications at EPA

- Currently in development
- In context of current possible applications by EPA and the academic and industrial community, guidance will address:
  - Genomics data submission
  - Quality assurance
  - Analysis
  - Management
  - Future actions needed to incorporate genomics more fully into EPA's risk assessments and regulatory decision making

Dix et al, 2006

Paper: Interim Guidance for Microarray-Based Assays: Regulatory and Risk Assessment Applications at EPA

- Purpose is to provide information regarding submission of microarray data to EPA and provide guidance for reviewers
- Will not prescribe specific methods to be used in microarray experiments beyond compliance with MIAME
- Will propose a "data evaluation template" as a tool for extraction and organization of data from genomic studies
- Emphasis on importance of data management

Dix et al., 2006

EPA's recommendations for followup activities

- Further development of genomic training materials and modules
- Continued collaboration of EPA with other federal agencies and stakeholders in tool development
- Application of this guidance to a series of case studies
- Updating of guidance as needed

Dix et al, 2006

Gene Expression and Mode of Action

- Toxicants/drugs can impact expression of genes
  - Alterations to normal function of cellular pathways leads to toxicity
- "Fingerprints" of expression reflect commonalities within mode of action:
  - e.g. classification through clustering (NIEHS 'toxchip')
- Gene expression changes are a sensitive way to evaluate response
  - Potential biomarkers of exposure/disease
  - Drug discovery
- Classification of chemicals with common MOA

Challenges and Limitations

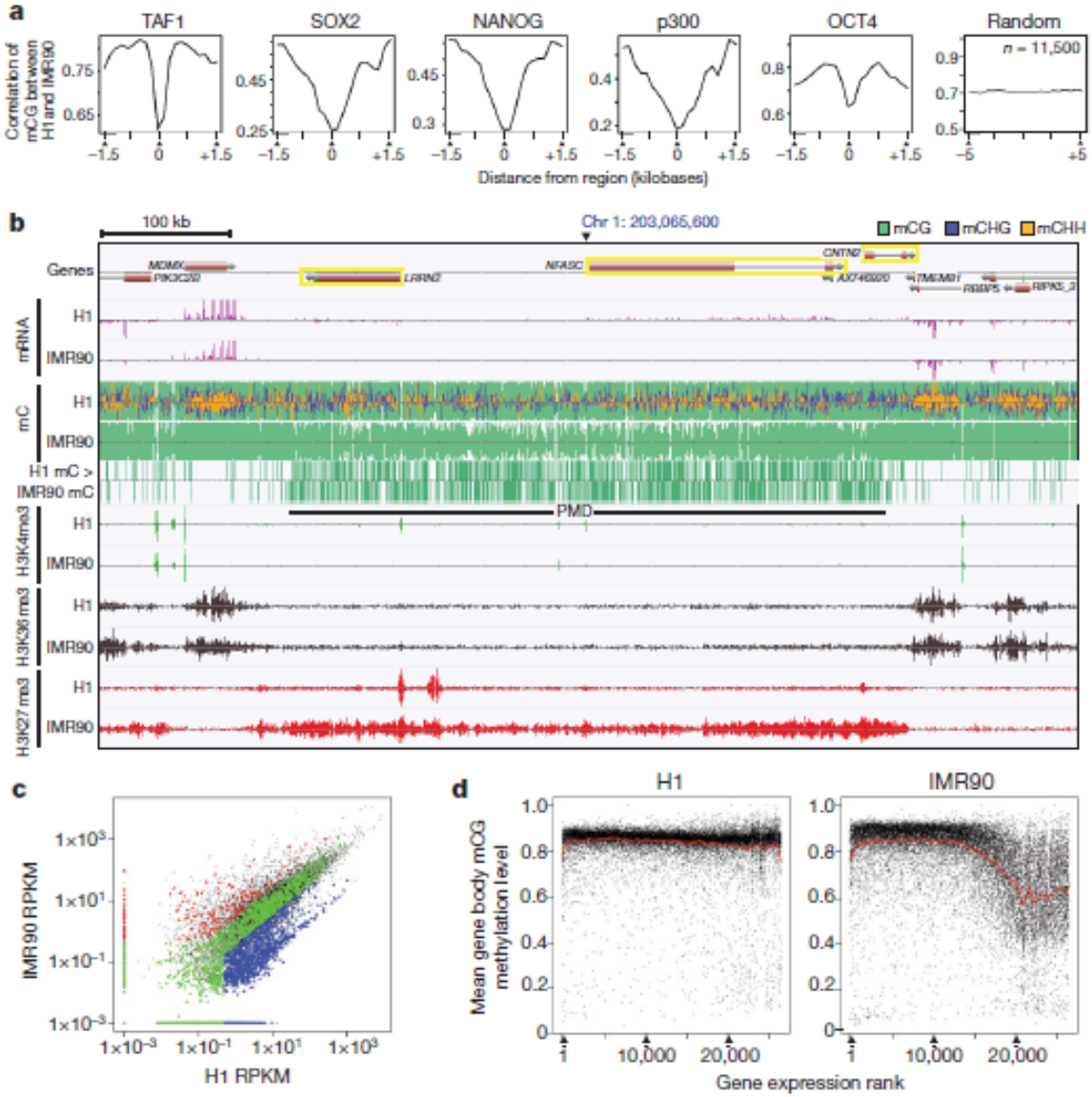
- Assays must be reliable, rapid, accurate.
- Need to recognize that gene expression is not equal to functional or protein changes.
- Need to distinguish between normal gene response and toxic response….are these methods predictive of toxicity?
- Standardization needed for genotype information and database construction; sharing across agencies and research groups.
- Privacy, discrimination, stigma and psychological stress issues.
- Ethical, social and legal issues require active involvement of stakeholders.
- “omics” is a powerful tool but should be considered on conjunction with traditional risk assessment practices.

Human DNA methylomes at base resolution show widespread epigenomic differences

Ryan Lister[1]*, Mattia Pelizzola[1]*, Robert H. Dowen[1], R. David Hawkins[2], Gary Hon[2], Julian Tonti-Filippini[4], Joseph R. Nery[1], Leonard Lee[2], Zhen Ye[2], Que-Minh Ngo[2], Lee Edsall[2], Jessica Antosiewicz-Bourget[5,6], Ron Stewart[5,6], Victor Ruotti[5,6], A. Harvey Millar[4], James A. Thomson[5,6,7,8], Bing Ren[2,3] & Joseph R. Ecker[1]

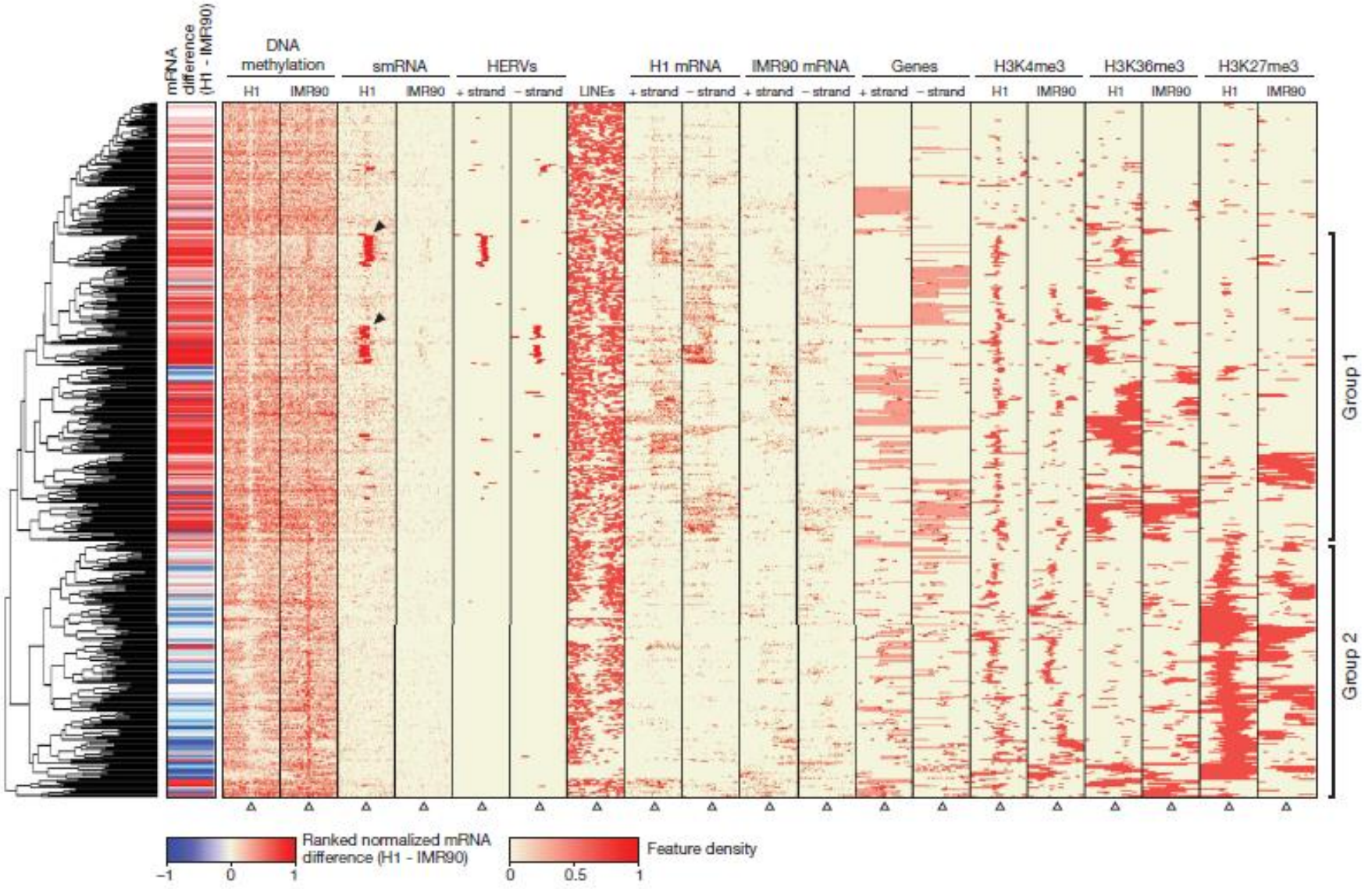
Lister R et al, 2009. Nature. 462(7271): 315-322.

Cell-type variation in DNA methylation



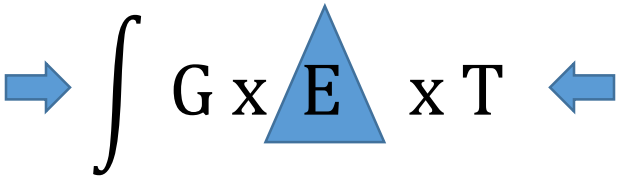
Lister R et al, 2009. Nature. 462(7271): 315-322.

Clustering of genomic, epigenetic and transcriptional features at differentially methylated regions



Lister R et al, 2009. Nature. 462(7271): 315-322.

Risks and Susceptibility



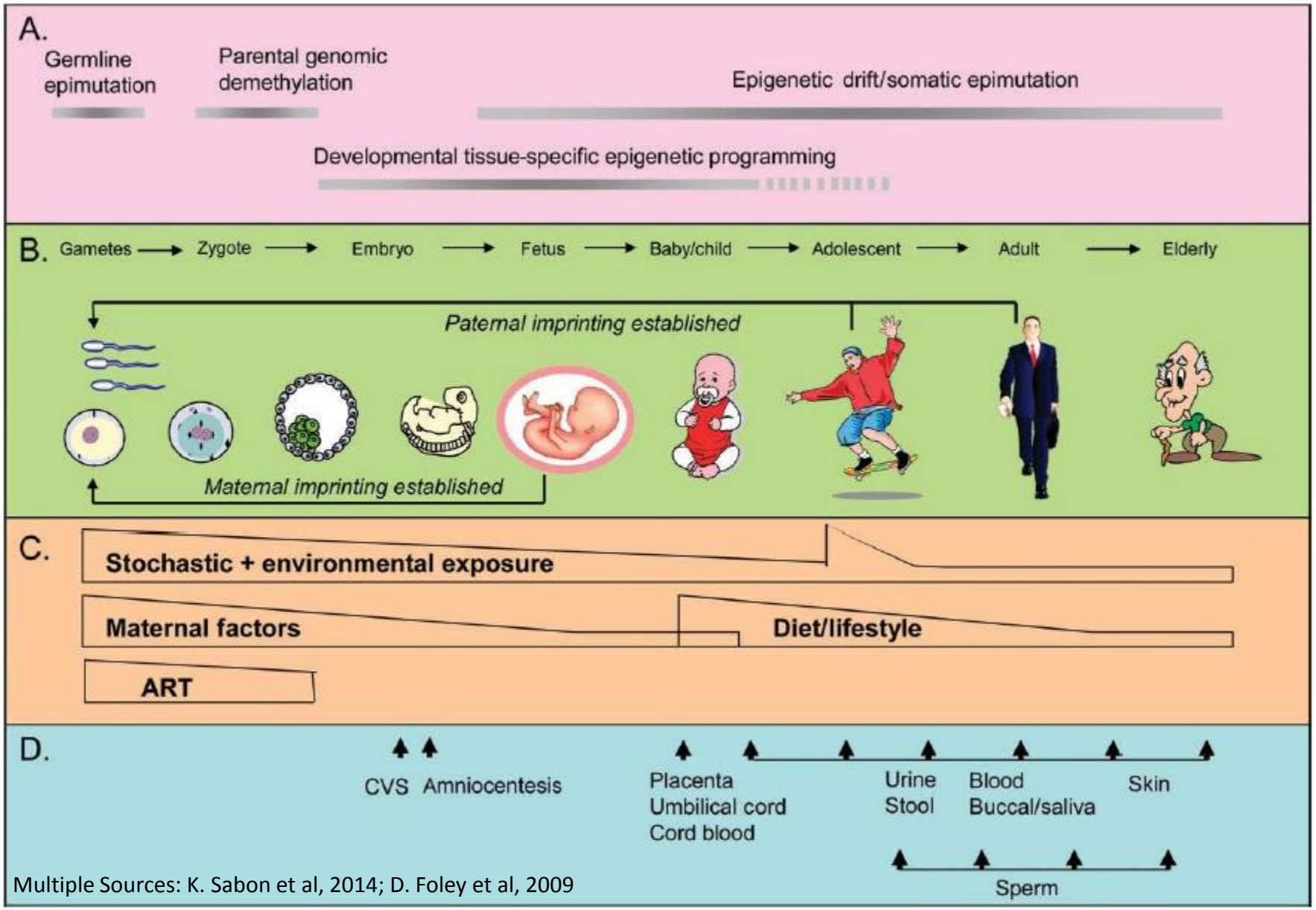
Well Being and Sustainability

GEAS=Genomic and Epigenomic Association Studies

EWAS=Environmental Wide Association Studies

TSEAS=Temporal Social Ecology Association Studies

Life Course Considerations for Epigenetics



Multiple Sources: K. Sabon et al, 2014; D. Foley et al, 2009

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Underlying Genetic Variation

- Epigenetic methylation of genomic CpG islands has genetic, developmental timing and environmental exposure components that control the state of methylation observed.
- Repeatability of measures of DNA methylation in the same individual in a longitudinal sense are necessary to define the variance of the CpG islands and to be able to distinguish genetic versus environmental cues that affect the methylation state.
- As an example of the influence of underlying genetic variation was seen in the Brisbane Systems Genomics Study family cohort. This cohort was queried to determine the genetic versus environmental impacts on DNAm. The genetic contribution to methylation state of the CpG probes was highly variable and was dependent on degree of heritability.
- The effect size of such highly heritable cis-acting SNPs explained 50 to 85% of the variation in methylation at these sites.

Contributions to Variability—Genetics versus Environmental Influences

- As example of the environmental and genetic variability of 37 smoking methylation responsive CpGs was queried in the Lothian Birth Cohort of 1936. Significant association to single nucleotide variation was observed in 12/37 (32%) as a modifier of the methylation state that was comparable to the ~10% effect size of smoking. (Shah et al 2014).

Conclusions on variability

- This paper provides an excellent example of how to examine variability in DNAm and demonstrates the importance of the incorporation of both genetic and environment in longitudinal study design and provides a path for going forward in our analysis of CHC cohorts. We feel that the estimates of genetic contribution and controlling for SNP variation contributed significantly to the ability of this analysis to identify example environmental impacts such as smoking and even to begin to identify different types of smoking exposures across the cohorts (for example, never smoking, versus smoking but quit, versus continued smoking.

- We have cited both general as well as CHC specific examples to support this type of analyses and to make suggestions to move forward our epigenetic analyses.

Exome sequencing identifies somatic mutations of DNA methyltransferase gene *DNMT3A* in acute monocytic leukemia

Xiao-Jing Yan[1,2,4], Jie Xu[1,4], Zhao-Hui Gu[3,4], Chun-Ming Pan[1,4], Gang Lu[1,4], Yang Shen[1], Jing-Yi Shi[1], Yong-Mei Zhu[1], Lin Tang[1], Xiao-Wei Zhang[1], Wen-Xue Liang[1], Jian-Qing Mi[1], Huai-Dong Song[1], Ke-Qin Li[1], Zhu Chen[1,3] & Sai-Juan Chen[1,3]

Yan KJ et al, 2011. Nature Genetics. 43: 309-315.

A framework for the use of genomics data at the EPA

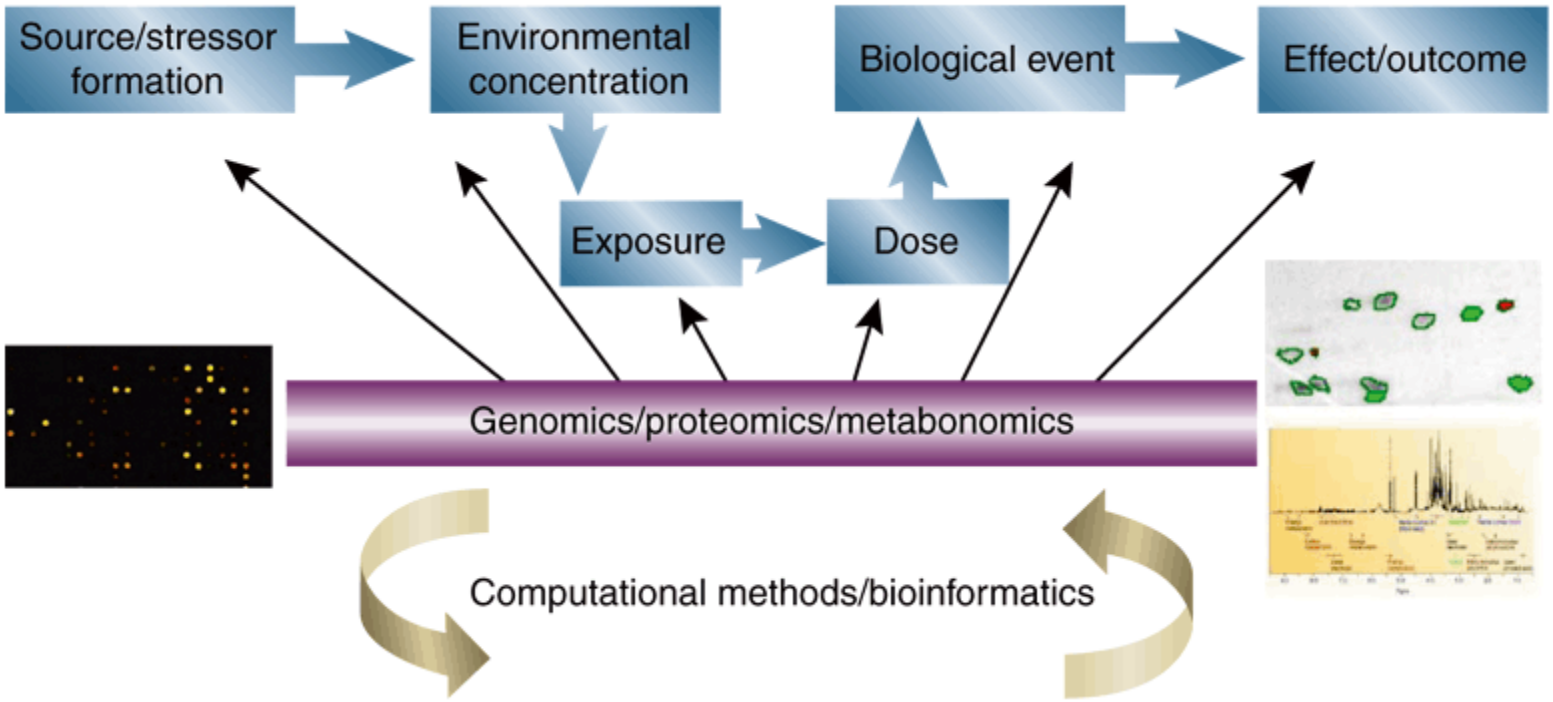


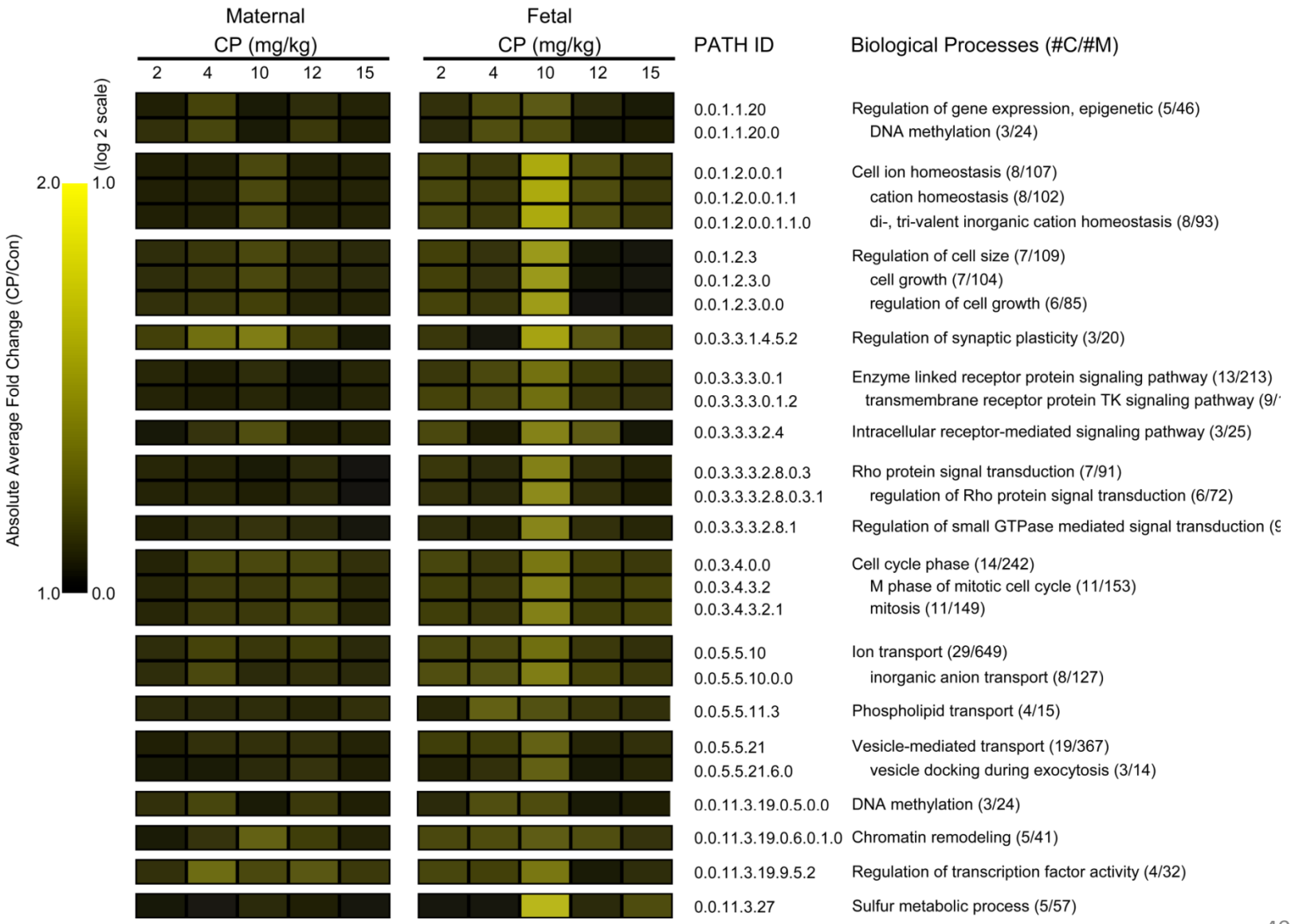
Figure 1. Genomics, proteomics and metabonomics/metabolomics can provide useful weight-of-evidence data along the source-to-outcome continuum when appropriate bioinformatic and computational methods are applied toward integrating molecular, chemical and toxicological information.

The source-to-outcome continuum captures the entire paradigm from the source of environmental contaminants and stressors, through to exposure, effects and ultimate outcomes on human health and ecological populations.

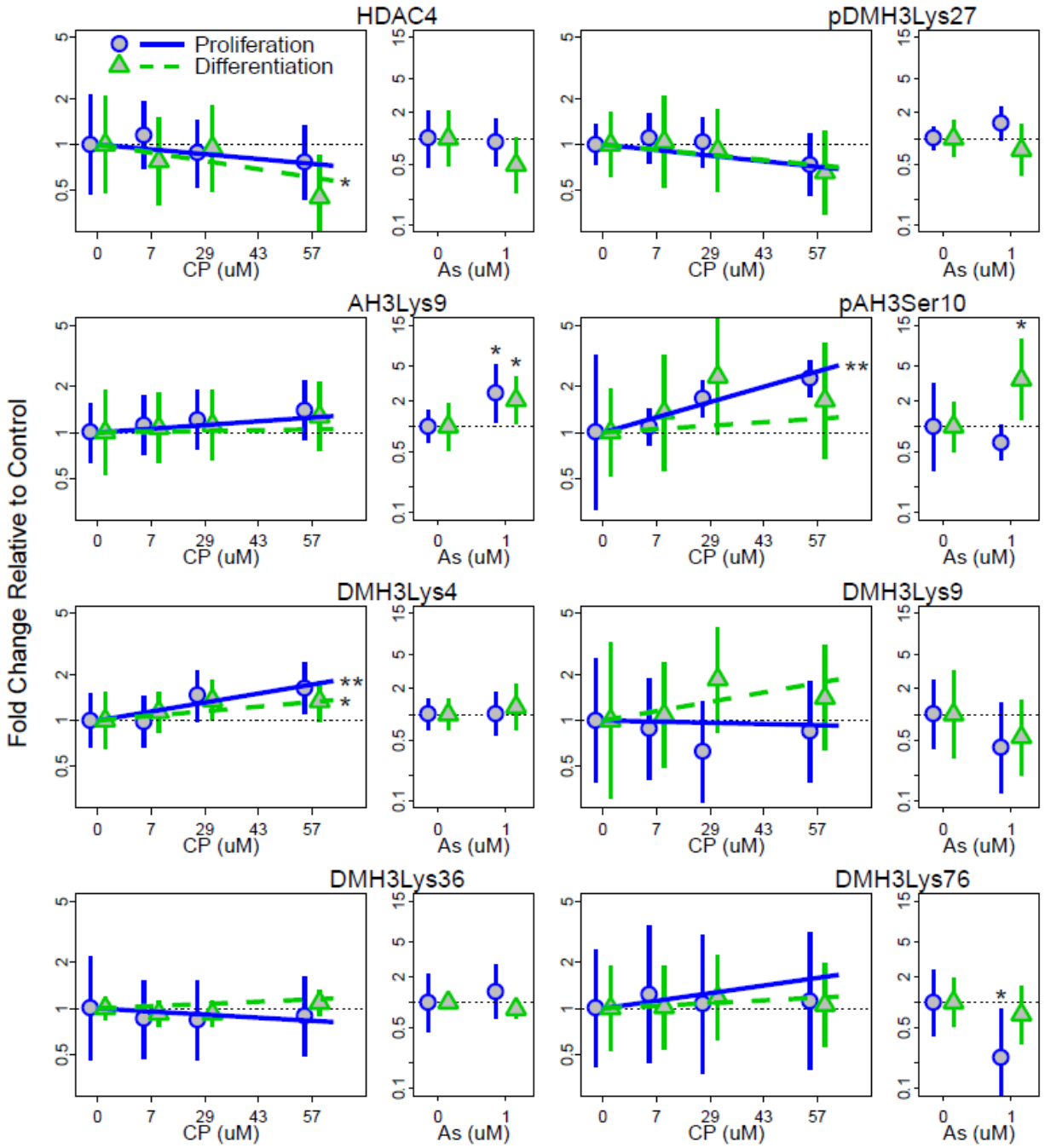
Dix et al, 2006

Risk = Hazard x Exposure

Toxicogenomics profiling in maternal and fetal rodent brains following gestational exposure to chlorpyrifos reveals epigenomic changes

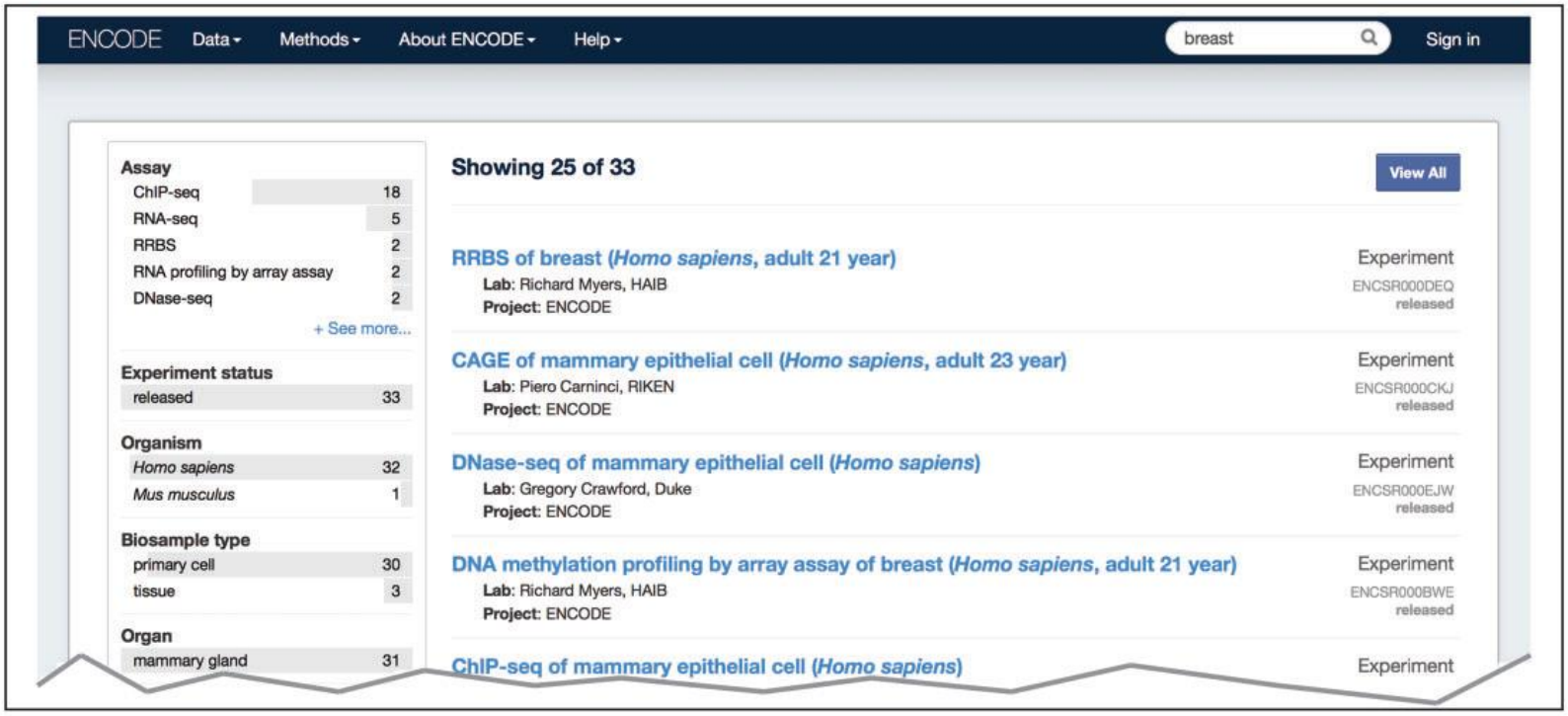


Moreira et al. 2010



Epigenomic Changes in Human Embryonic Stem Cells: Histone Changes Following CP and As exposure are dependent upon proliferation versus differentiation status

Search at the ENCODE portal (https://www.encodeproject.org/). In this example, a free text search is done for ‘breast’



Malladi VS et al. 2015

Outline

- Add text