Epigenetics in Risk Assessment: Clarity or Confusion?

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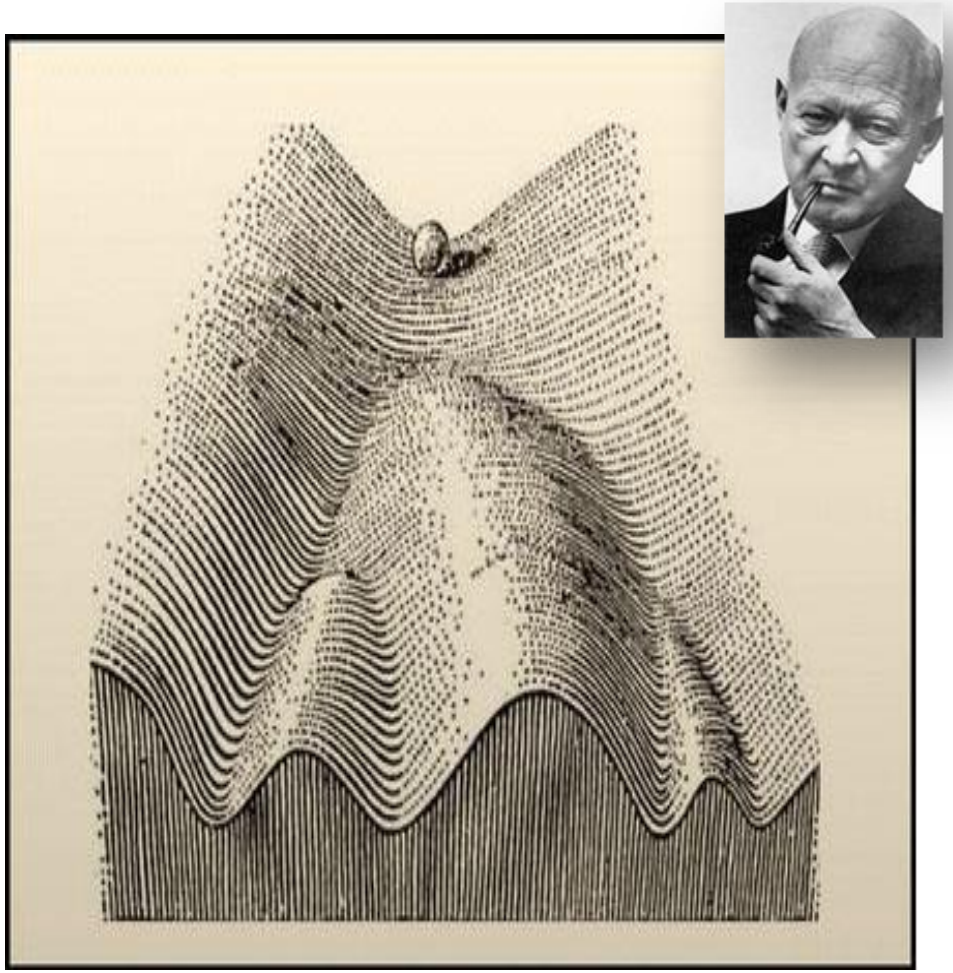
Epigenetic mechanisms

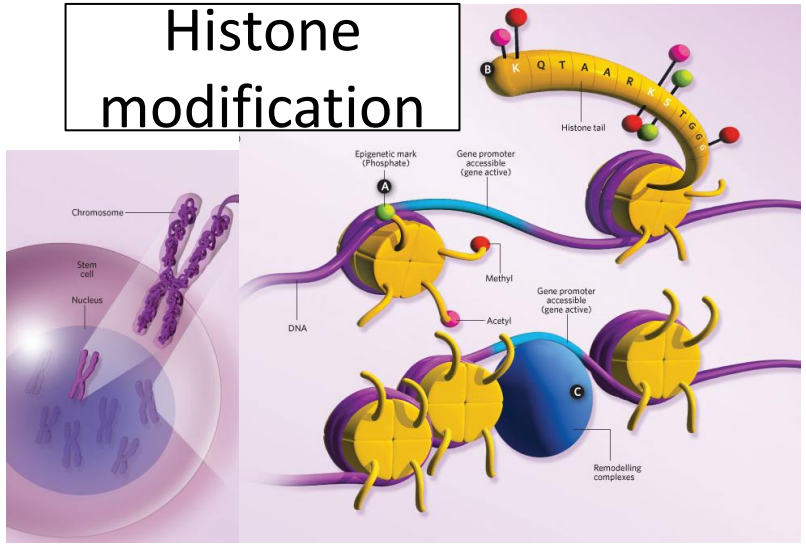


Waddington's epigenetic landscape

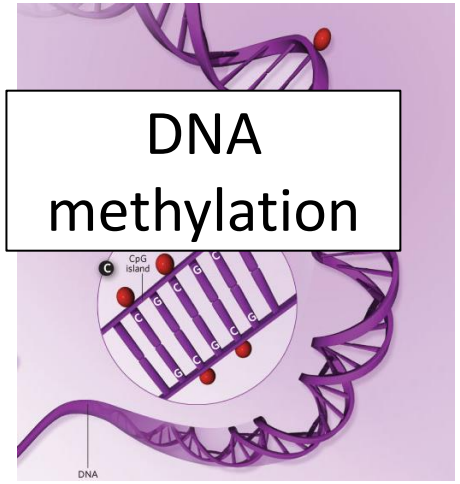
The strategy of genes: a discussion of some aspects of theoretical biology (Allen & Unwin, 1957)

"An interface between the genome and the environment, providing partial mechanistic explanations for the sensitivity of organisms to environmental factors." Mirbahai and Chipman Mutation Res. (2014)

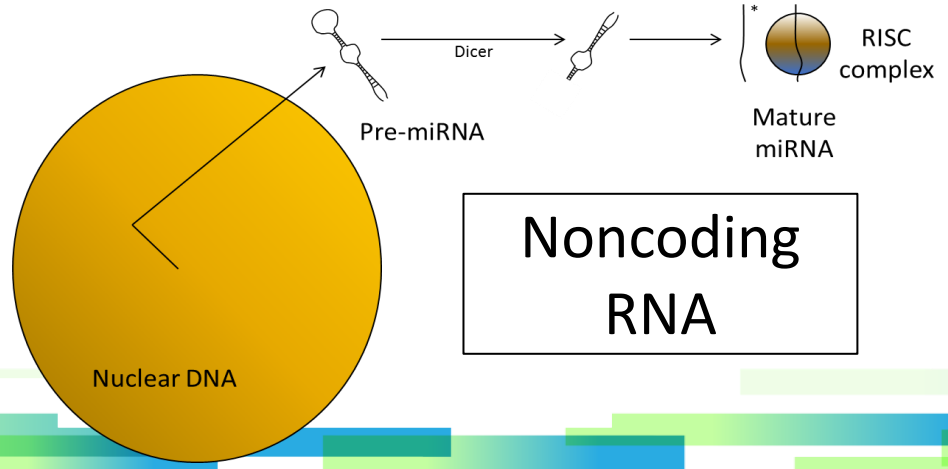
Histone modification



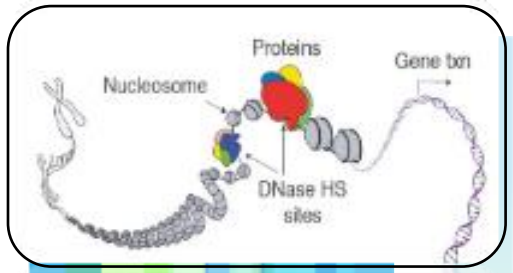
DNA methylation



Kubicek *The Scientist March 1,* 2011



Noncoding RNA

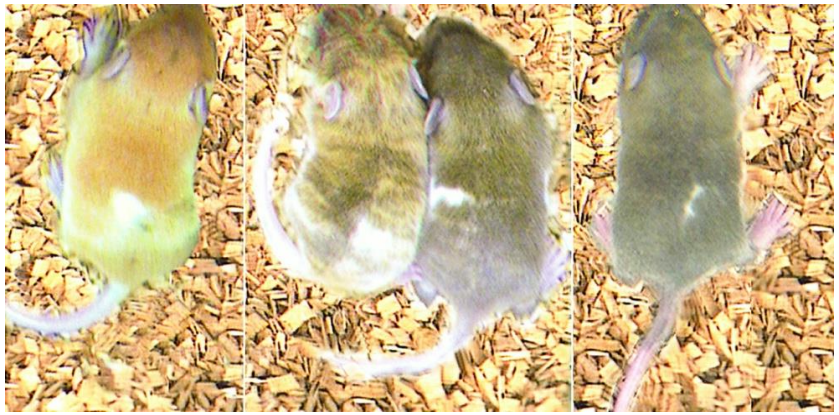


The great promise of epigenetics for toxicology research and risk assessment

- Environmental exposures (developmental environment, chemicals/food, living conditions, SES etc.) can alter the epigenome
- Alterations occur early after exposure and persistent epigenetic marks may serve as a “footprint” of environmental exposure
- The alterations may be mechanistically linked to adverse outcomes, susceptibility, or even transgenerational effects
- Epigenetic measurements may therefore be amenable to chemical safety screening, biomarker development, and risk assessment

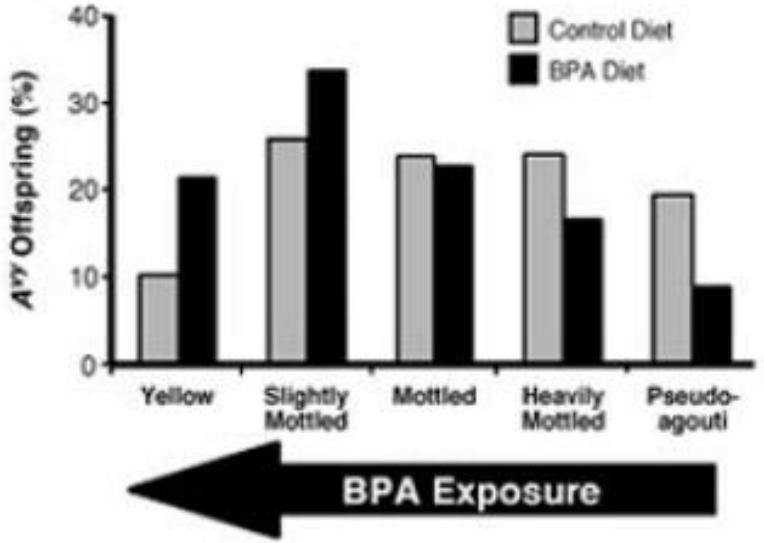
Epigenetic alterations can be caused by environmental exposure

Gradient of
A^{vy}*/a* coat phenotypes



% IAP methylation

Wolff et al. *FASEB* 1998



Dolinoy et al. *PNAS* 2007

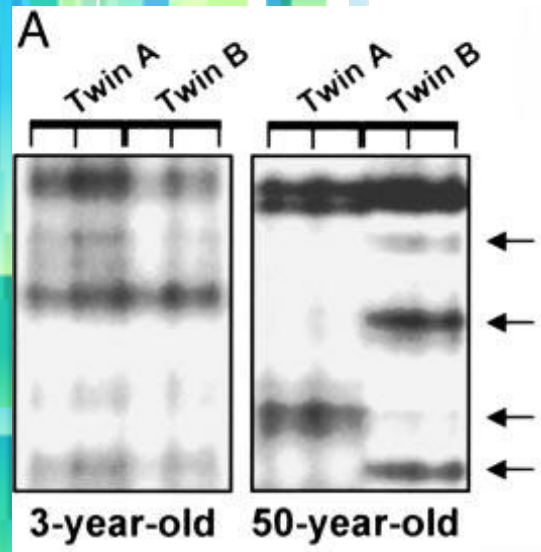
Epigenetic alterations can be caused by environmental exposure

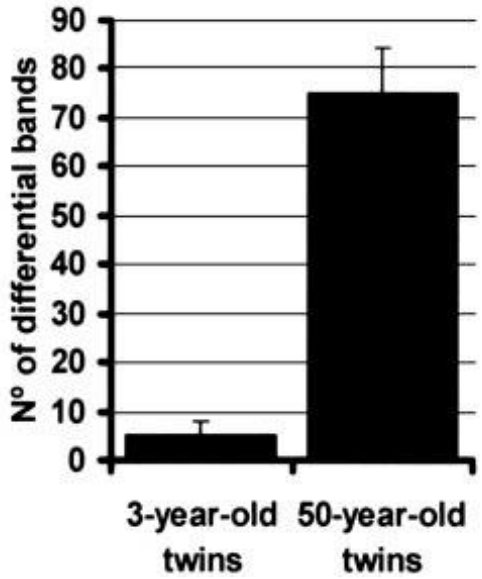
- Non-genotoxic chemicals (phenobarbital, peroxisome proliferators)
- Metals (arsenic, chromium, cadmium, lead)
- Organic pollutants (tobacco smoke, benzene, BPA, BPA substitutes, endosulfan, glyphosate, hexachlorobenze, methoxychlor, butylparaben, flame retardants, phenols, phthalates, polyhalogenated biphenyls, DDE, dioxin)
- Pharmaceuticals
- Dietary compounds
- Mixtures

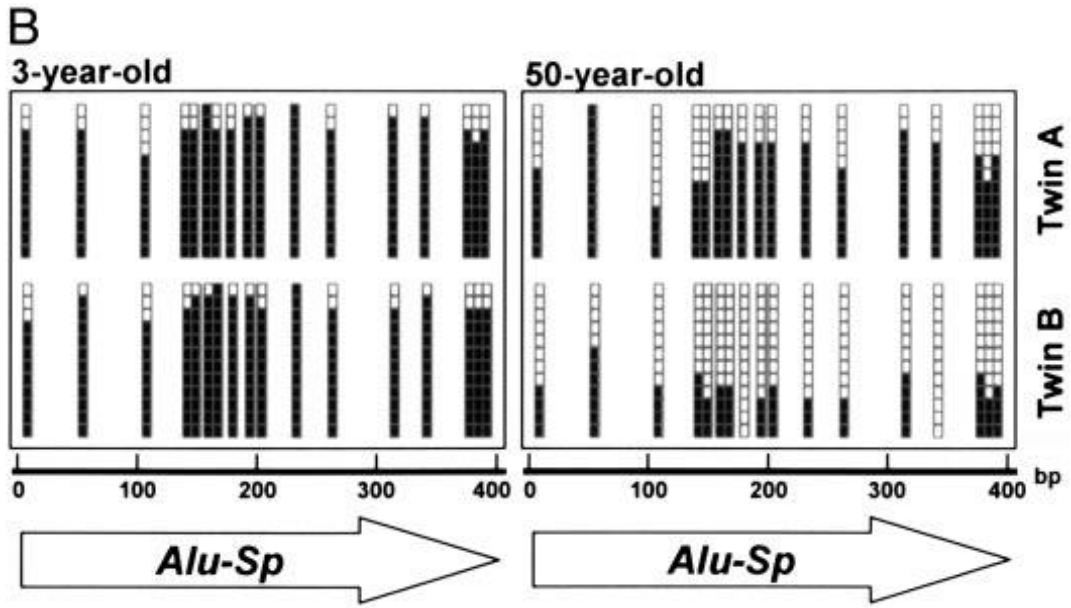
Baccarelli and Bollati, *Curr Opin Pediatr* **21**, 2009; Kotrubash et al., *Tox Mech Meth* **21**, 2011; Chung and Herceg *EHP* 2020

Epigenetics, age, exposure, and phenotype

- Correlation of phenotype, DNA methylation, and aging in humans
  - Some evidence from monozygotic twins study (Fraga et al. *PNAS* 2005)
  - Genetically the same, but phenotypically different; disease states, for example
  - Twins are epigenetically indistinguishable in early life, but robustly different later in life.

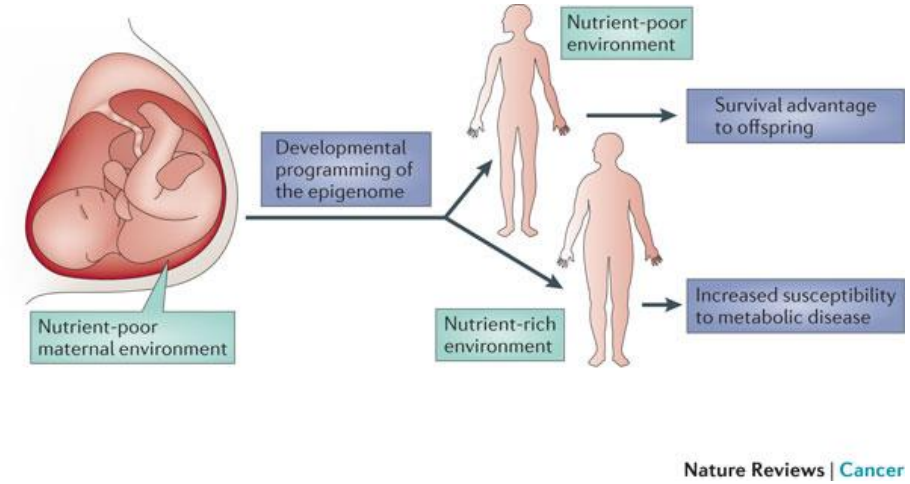




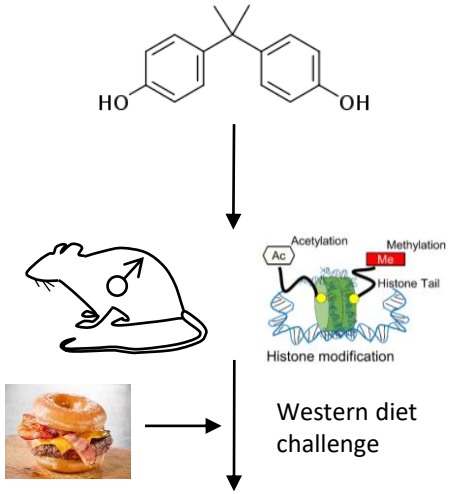


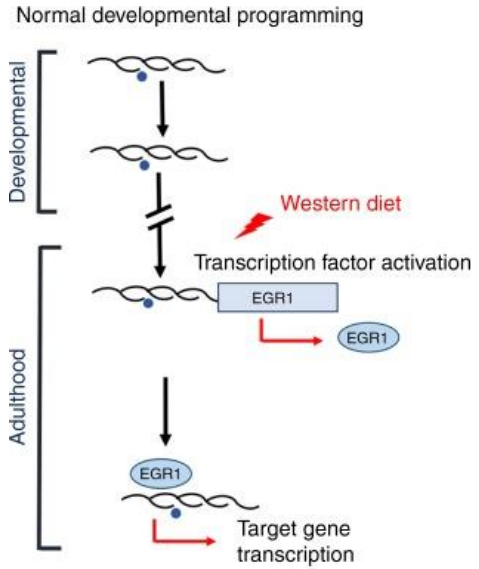
Delayed toxicity/adverse outcome due to short-term exposure

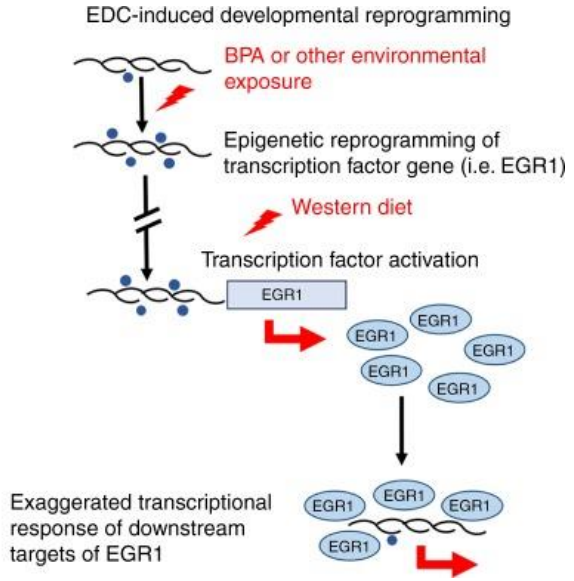
- "Thrifty" hypothesis
  - Poor intrauterine environment leads to an adaptive response that optimizes growth of critical organs at the detriment of others and leads to altered postnatal metabolism (Hales and Barker *British Medical Bulletin* 2001)
    - Links to Type 2 diabetes, obesity, cardiovascular disease



Walker and Ho, *Nat Rev Cancer* 2012





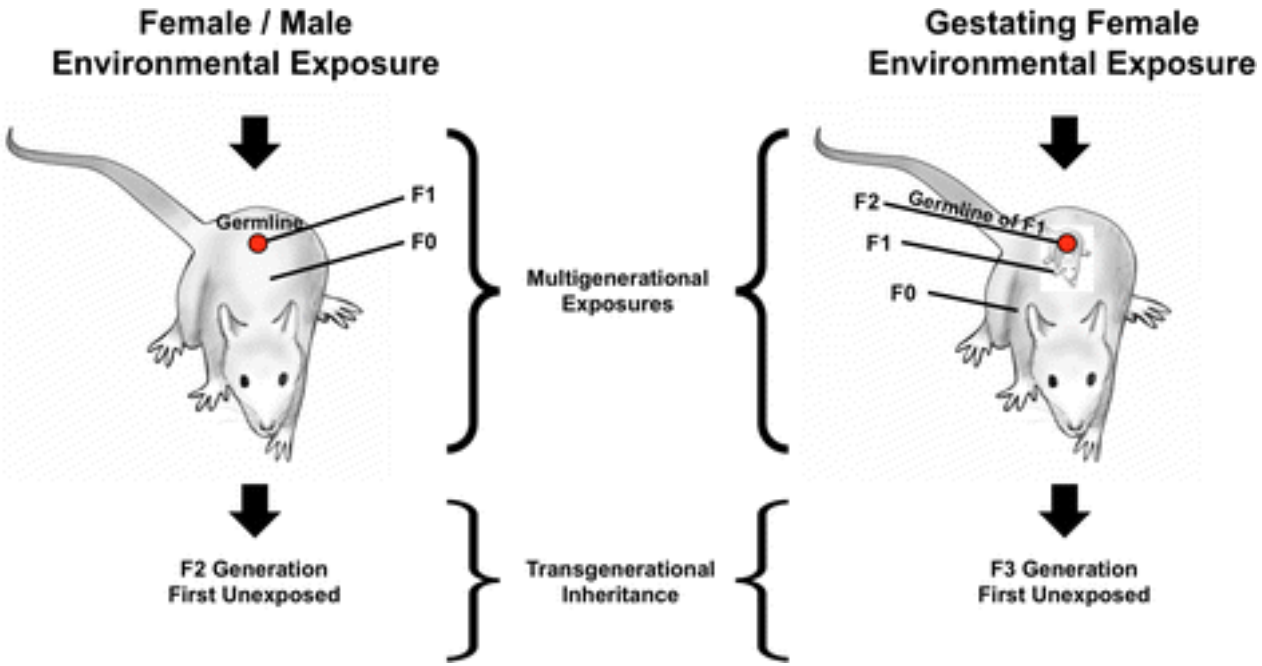


- In rat model, early exposure to EDC at critical window of development led to epigenetic reprogramming which negatively impacted later adulthood challenge to Western diet.
- This resulted in significant changes in liver metabolic function.

Trevino *et al. Nat Comm* 2020

Multi- and transgenerational epigenetic inheritance

- Inherited traits given rise from environmental and developmental cues ~ common in plants
  - Botanist Jean-Baptise Lamarck



Skinner BMC Medicine 2014

Transgenerational inheritance and environmental stress

Skinner BMC Medicine 2014

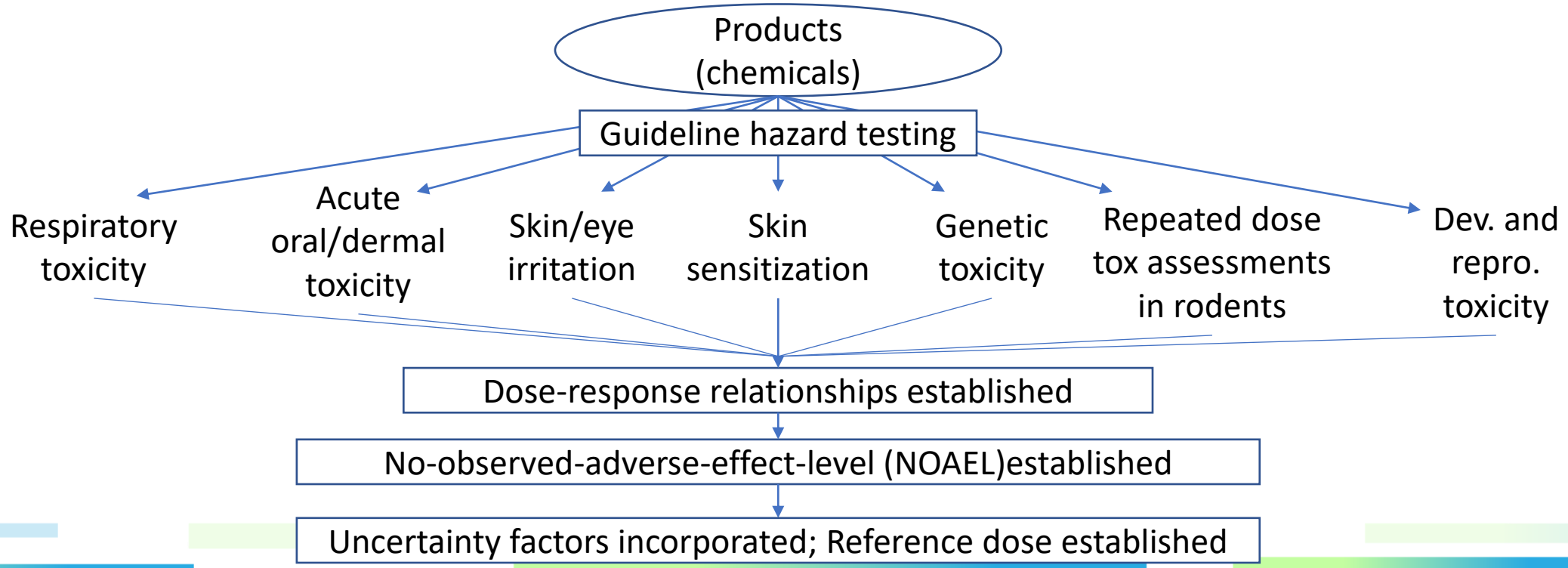
Table 1
Examples of transgenerational inheritance studies

Exposure	Pathology	Reference
Toxicants		
Vinclozolin	Testis, prostate, kidney disease, tumors, immune	Anway *et al.*, 2005 [3]; 2006 [12]
	Gender-specific changes in anxiety-like behavior	Skinner *et al.*, 2008 [13]
	Immune and reproductive	Nilsson *et al.*, 2008 [14]
Methoxychlor	Testis, kidney, ovary, obesity	Anway *et al.*, 2005 [3], Manikkam *et al.* 2014 [15]
Permethrin/DEET	Prostate, kidney disease	Manikkam *et al.* 2012 [16]
Dioxin	Prostate, kidney, fertility, pregnancy	Manikkam *et al.* 2012 [17] Bruner-Tran *et al.* 2011 [18]
BPA/phthalates	Prostate, kidney, obesity	Manikkam *et al.* 2013 [19]
Hydrocarbon mixture (jet fuel)	Prostate, kidney, obesity, immune and reproduction	Tracey *et al.* 2013 [20]
Vinclozolin, permethrin/DEET, plastics, dioxin, jet fuel	Polycystic ovaries, reduced primordial follicle pool	Nilsson *et al.* 2012 [21]
DDT	Obesity, kidney, testis	Skinner *et al.* 2013 [5]
Phthalate	Testis and spermatogonial stem cell	Doyle *et al.* 2013 [22]
Tributyltin	Obesity and adipose cell	Chamorro-Garcia *et al.* 2013 [23]
BPA	Social behavior, implantation, litter size, sperm	Wolstenholme *et al.* 2012 [24]; Salian *et al.* 2009 [25]
Others		
Caloric restriction	Cardiovascular mortality	Bygren *et al.* 2014 [26]
High fat diet	Growth and insulin sensitivity	Dunn and Bale 2011 [6]
Folate	Congenital malformations	Padmanabhan *et al.* 2013 [27]
Drought	DNA methylation changes	Zheng *et al.* 2013 [7]
Heat/salt	Flowering and salt tolerance	Suter and Widmer 2013 [28]
Prediabetes	Glucose tolerance and insulin sensitivity	Wei *et al.* 2014 [29]
Smoking	Abnormal pulmonary function	Rehan *et al.* 2013 [30]
Alcohol	Endocrine and neuronal function	Govorko *et al.* 2012 [31]
Heat stress	Increased Hsp70 production and tolerance to heat stress	Norouzitallab *et al.* 2014 [8]

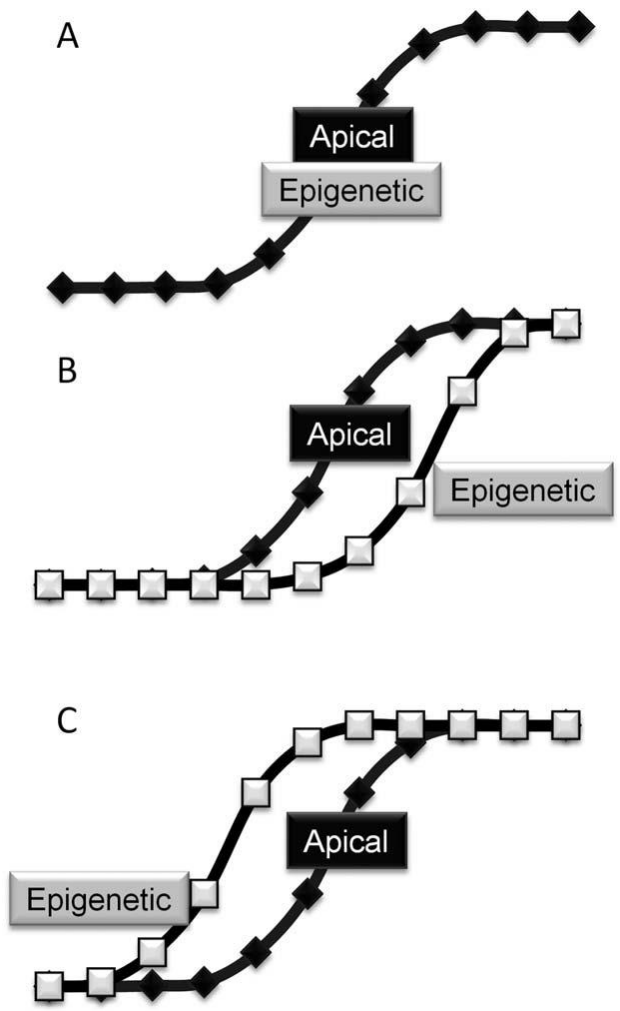
BPA, Bisphenol A; DEET, N,N-diethyl-m-toluamide.

Epigenetic data – added value for risk assessment? Case study comparisons

- What can we glean from published data? Example from Alyea et al. *J Pharm Tox Methods* 2012.
- Comparison of classical apical endpoints (NOAEL) vs. epigenetic effects
- Simplistic overview of a product safety assessment paradigm (e.g., OECD)

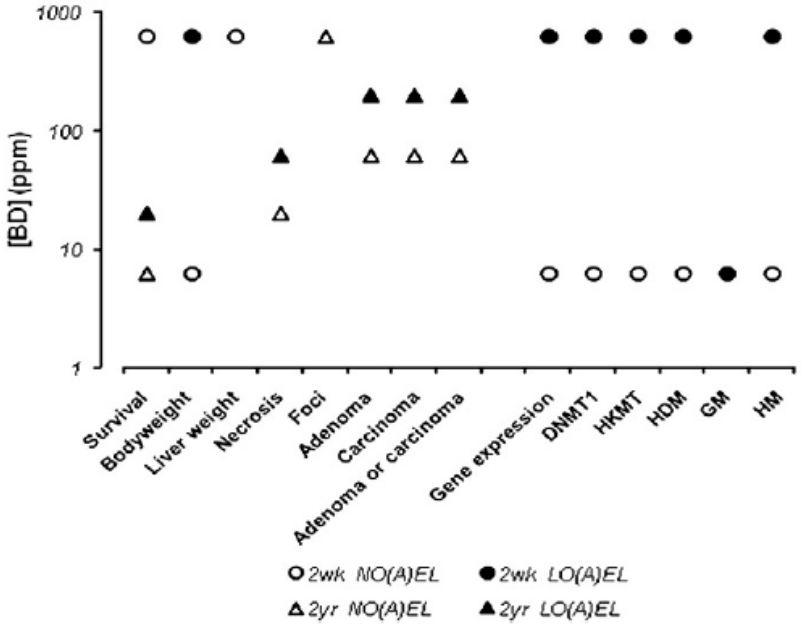


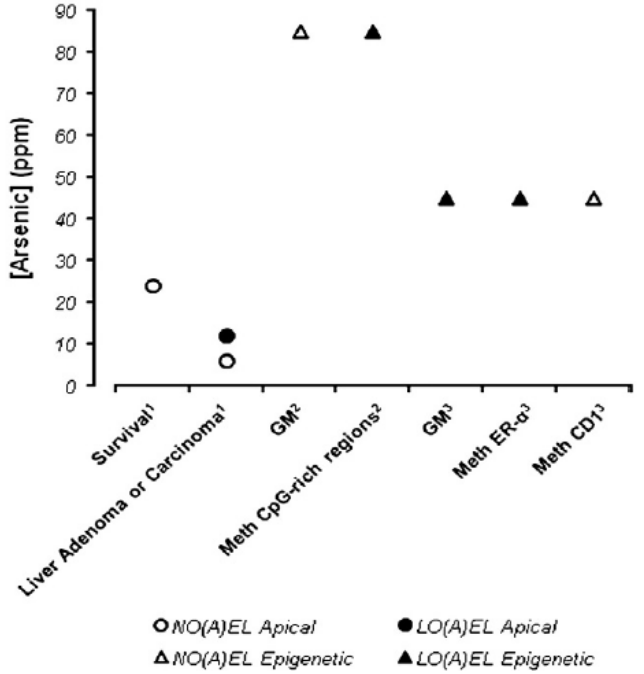
Where do the epigenetic dose response values lie in context of apical endpoints?

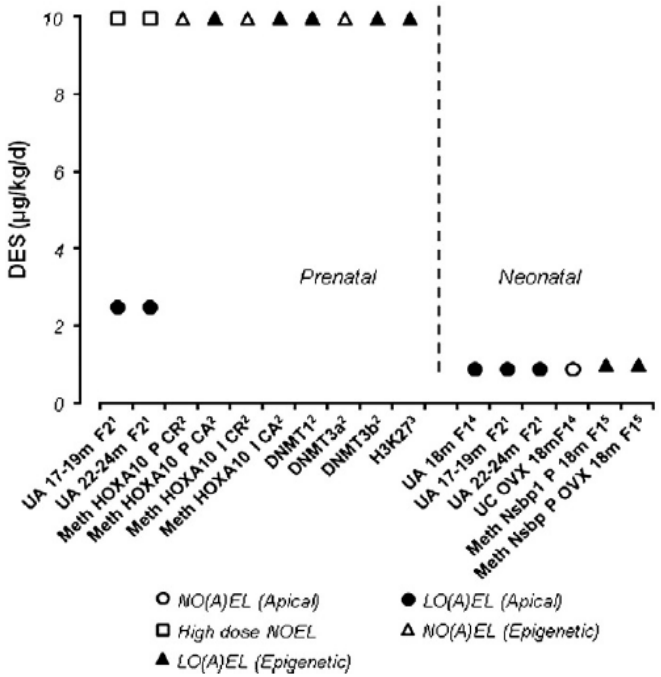


Alyea et al. *Env Mol Mutagenesis* 2014

- Exposure examples: 1,3-Butadiene (BD); Arsenic; Diethylstilbestrol (DES)

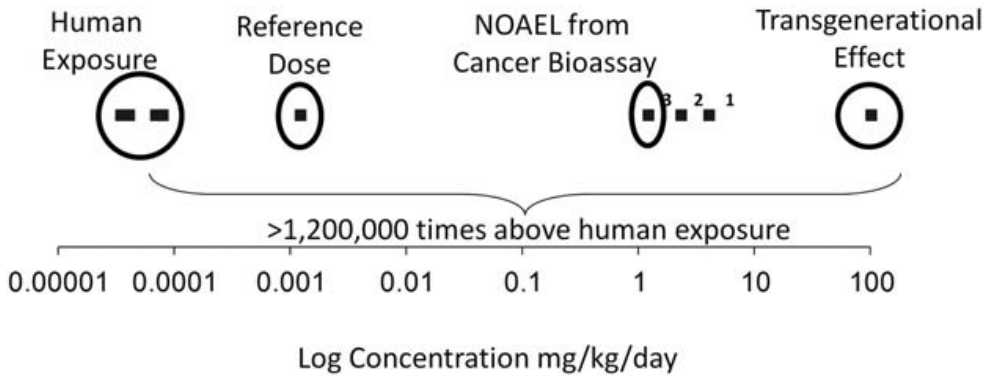






- Epigenetic changes seen at a lower dose that NOAEL; implication of this is unknown
- Epigenetic effects occur at higher dose than apical endpoint that drive liver adenoma/carcinoma NOAEL
- NOAEL of both apical and epigenetic endpoints occur at the same level

- The epigenetic data fails to influence point of departure based on apical endpoints (Alyea et al. *J Pharm Tox Methods* 2012)
  - Apical NOE(A)L or LOE(A)L are protective of epigenetic changes or it is unclear the impact of the epigenetic alterations
  - Alterations need to occur at relevant doses (real world exposure levels, doses below the apical endpoint) ~ vinclozolin example



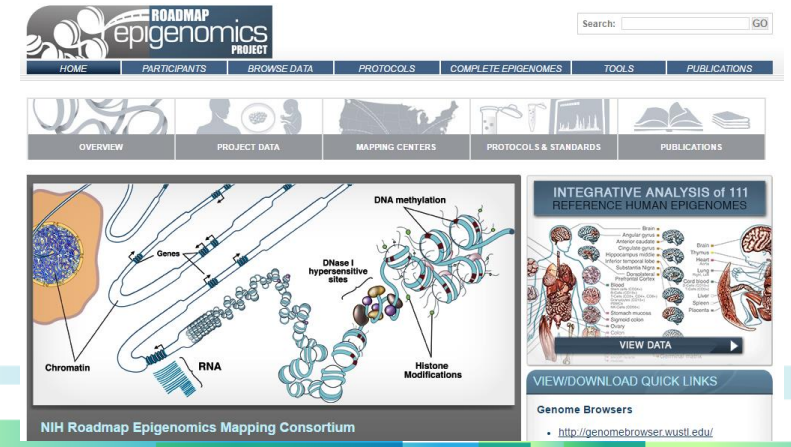
Alyea et al. *Env Mol Mutagenesis* 2014

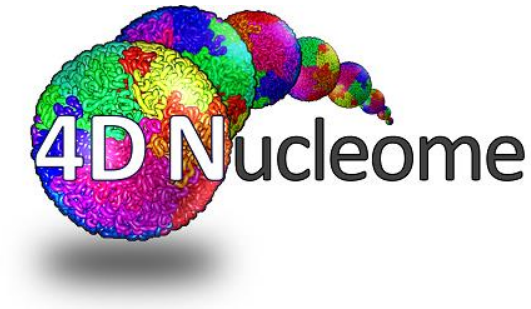
  - Dose and time course studies need to be performed with causal epigenetic measurements – are they indicative of an adverse outcome?

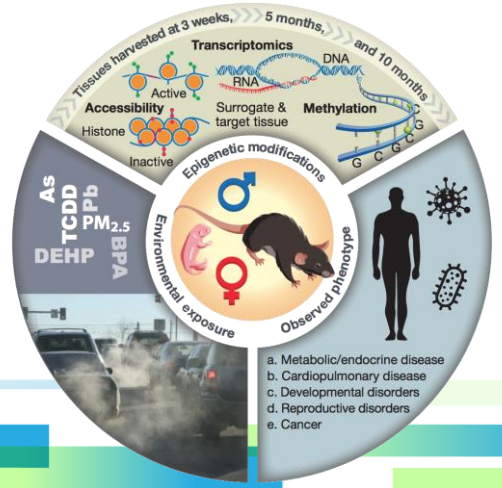
The path forward

Establish what is “normal”

- Epigenetic variability due to cell types, tissues, age, subpopulations
- Many efforts are assisting with this endeavor
  - NIH Roadmap Epigenomics Mapping Consortium (Roadmap Epigenomics Consortium 2015)
  - Encyclopedia of DNA Elements (ENCODE)
  - BLUEPRINT projects (Fernandez et al. 2016)
  - 4D Nucleosome Project (Dekker et al. 2017)
  - Toxicant Exposures and Responses by Genomic and Epigenomic Regulators of Transcription (TaRGET) I and II programs (Wang et al. 2018)



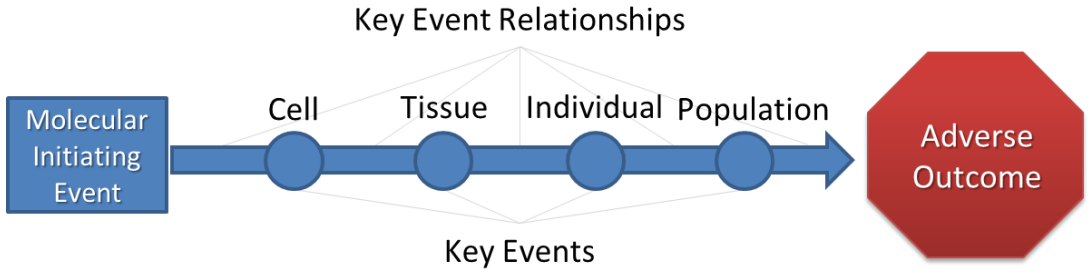


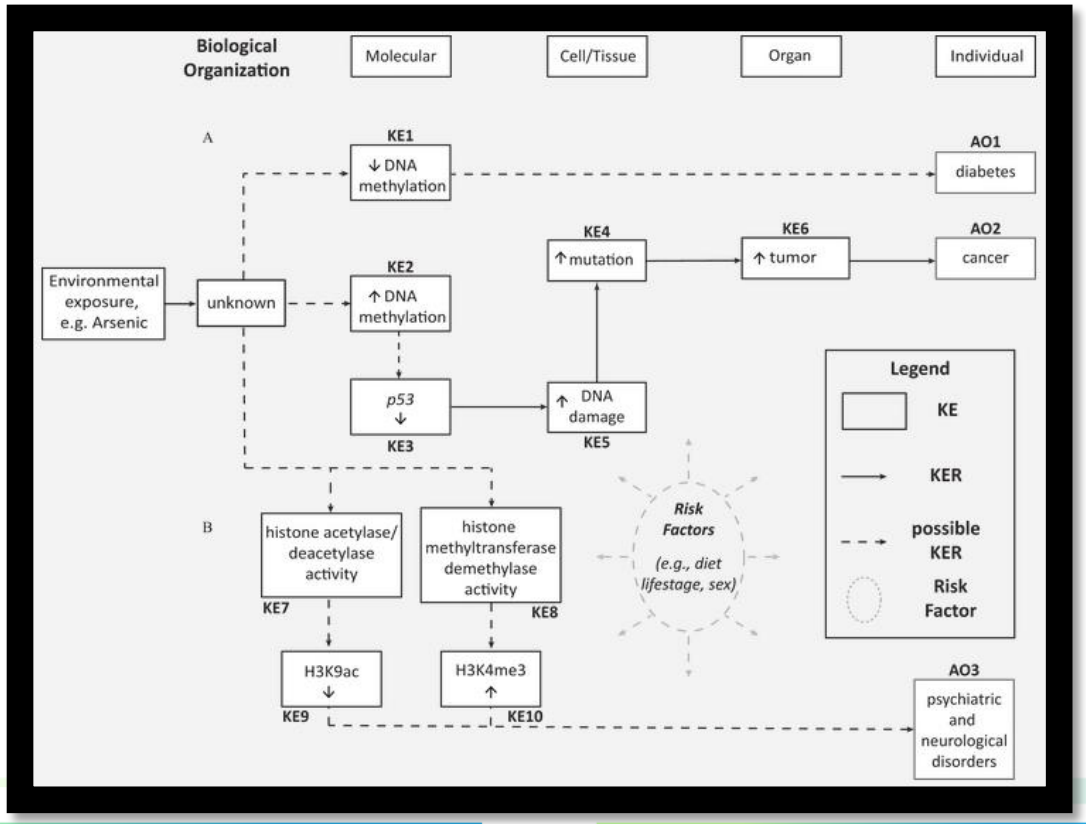


The path forward

Link epigenetic alteration to adverse outcome

- Correlation and causation are often not clear with epigenetic data
- Utilize the Adverse Outcome framework to identify gaps and leverage existing knowledge



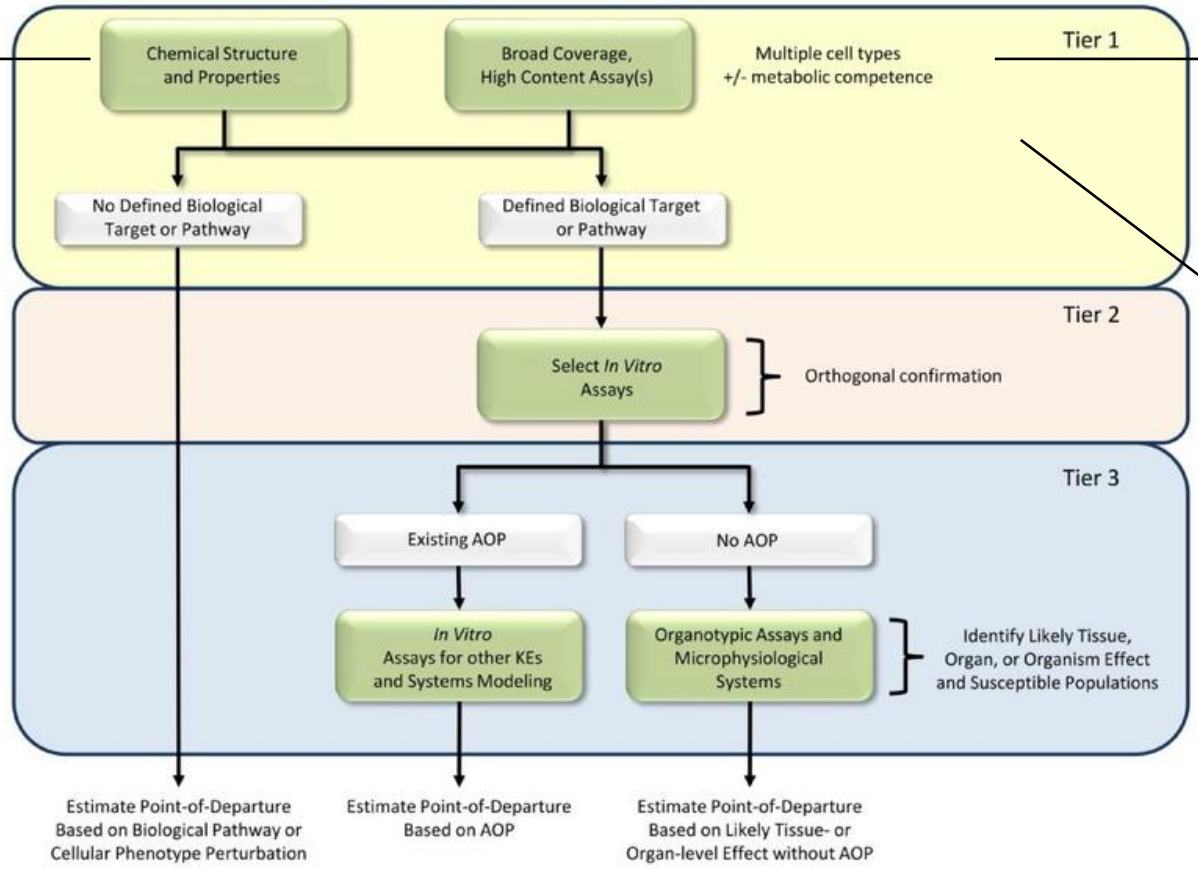


Angrish, Allard, McCullough, Druwe, Chadwick, Hines, and Chorley *EHP* 2018

The path forward

Incorporate into tiered testing strategy

Structural-activity relationships to discover epigenotoxicants (Romero and Medina-Franco *ACS Omega* 2021)



Thomas et al. *Tox Sci* 2019

Microscopic Imaging of the Epigenetic Landscape (MIEL; Farhy et al. eLife 2019)

Demethylation potential by EGFP reporter (TDQ; Qian et al. *BMC Biotech* 2015)

The path forward

Noncoding RNA – Linking AO to early epigenetic changes

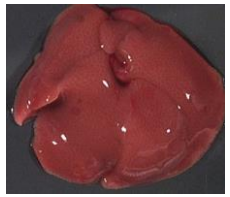
Use microRNA profiling after short-term exposure of liver tumorigen

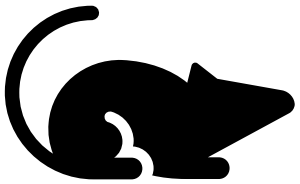
tumorigenic

di(2-ethylhexyl) phthalate (DEHP)

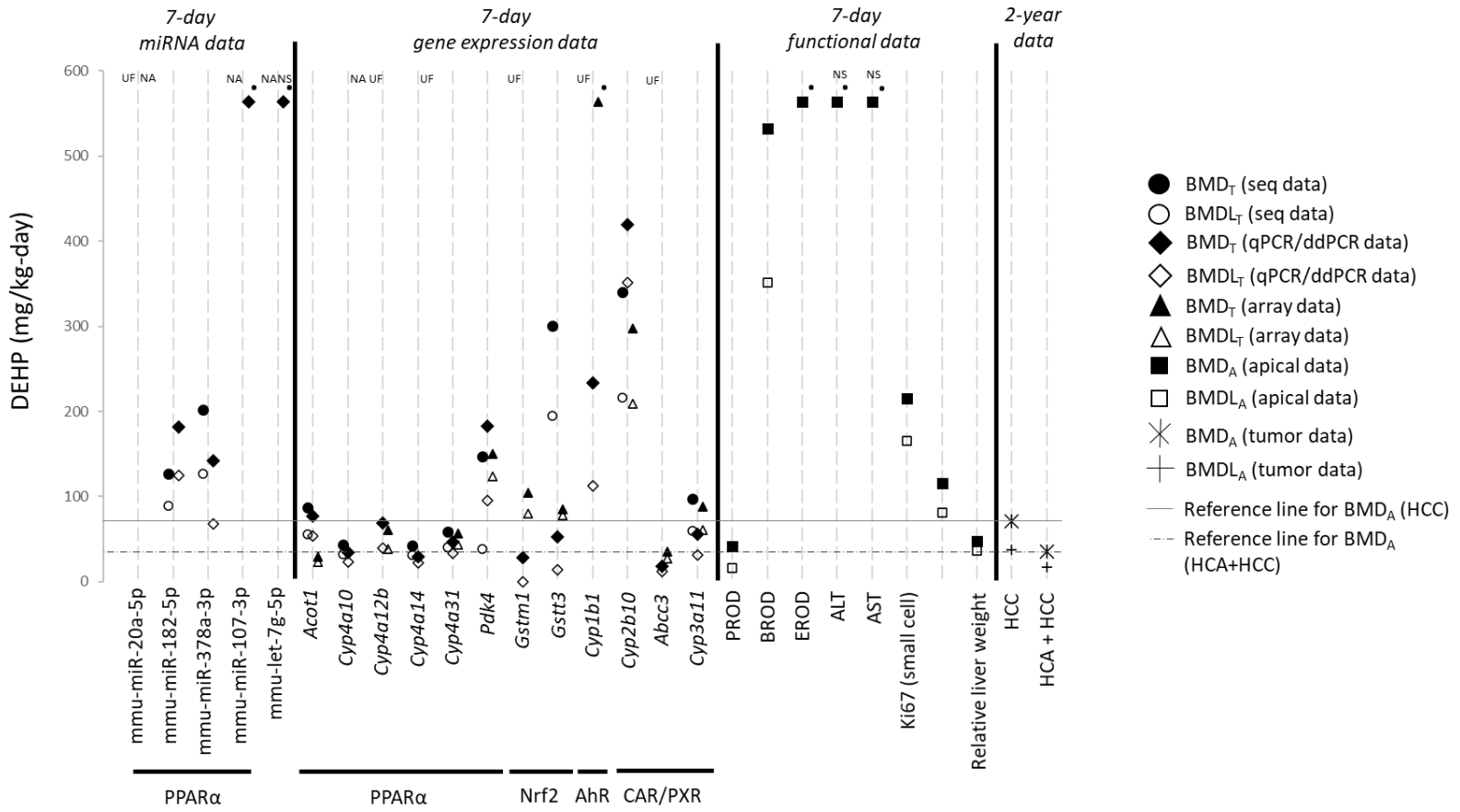
non-tumorigenic

di-n-octyl phthalate (DNOP)
n-butyl benzyl phthalate (BBP)





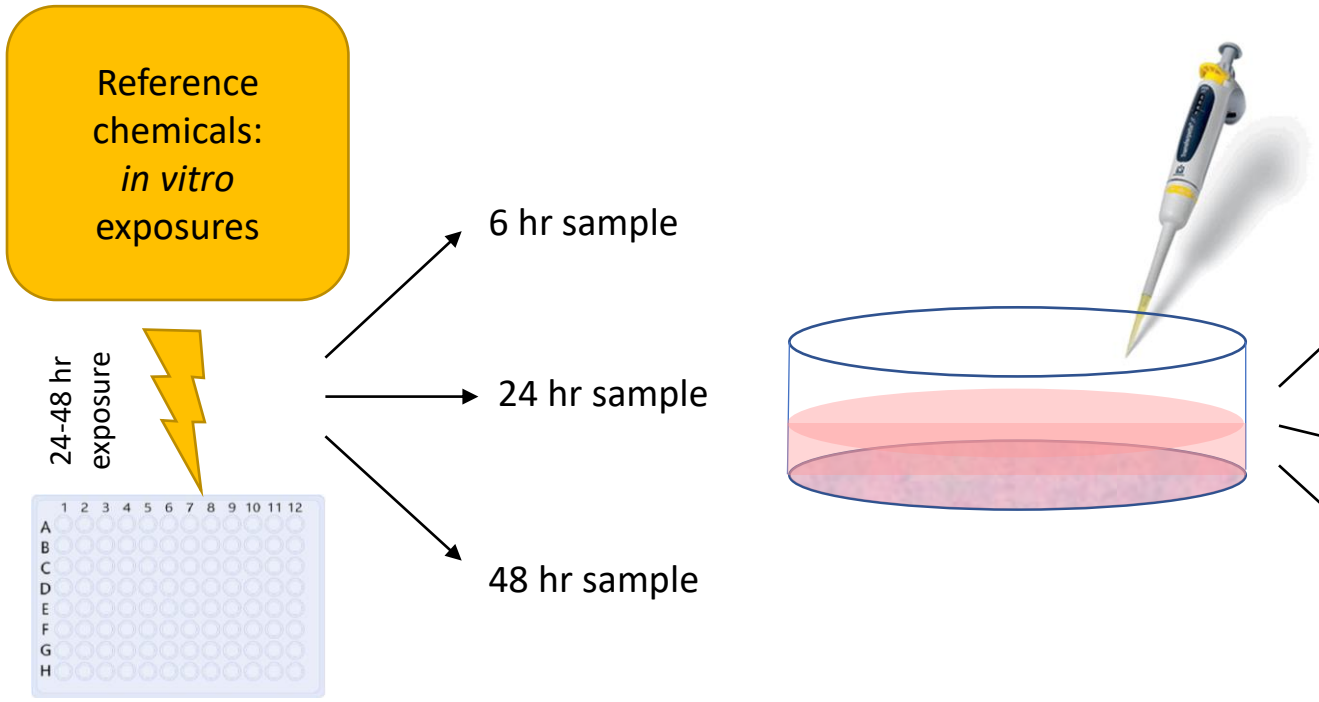
7 days (4 doses)
and
28 days (1 high dose)



Chorley et al. *Tox Letters* 2020

The path forward

- ***Non-destructive measurement of extracellular microRNA to define chemical mode-of-action***



- Confirm KE and AO perturbation
- Link to gene expression networks and link cellular microRNA
- Distinguish active and passive microRNA leakage
- Establish extracellular microRNA patterns linked to KE activation and cellular AO

Exposure | **Sampling** | **Measurements** | **Analyses**

The take home

- Use of epigenetic measurements as a marker environmental exposure and disease susceptibility is of great promise for risk assessment
- Important to identify where epigenetics will add value
  - Add value to traditional apical endpoints; other 'omic endpoints?
  - Uniquely informative? Persistent and causative; generational?
- Correlations need to be solidified. Confidence in "normal" and gaps identified in AOPs – build weight-of evidence
- Tools and methods are available to incorporate high-throughput chemical screening
- Flags for "epigenotoxic" chemicals to support follow-up studies