Transcriptome-Based Estimation of an *In Vivo* POD: Current and Future Utility

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OCTOBER 19, 2020 || SECOND ANNUAL EPA NAM CONFERENCE

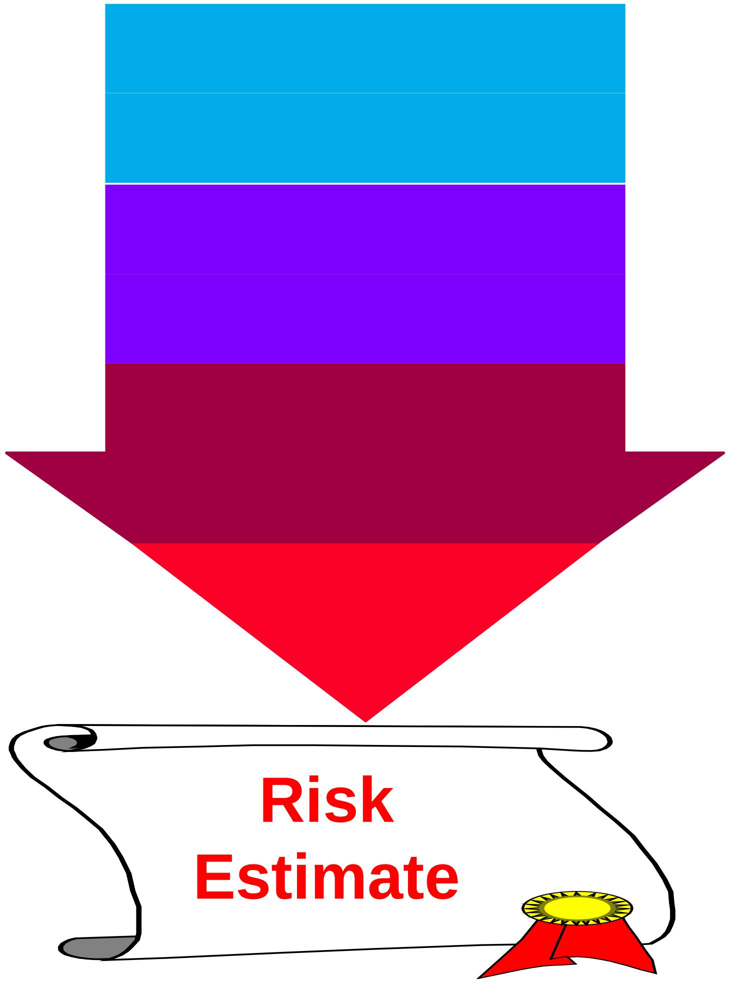
Presentation Outline

1. Scientific basis of using a molecular POD in the regulatory space

2. Data comparing traditional (apical) and transcriptome PODs

3. Utility of a transcriptome-based POD: Now and in the Future

Safety Assessment Process



1. Apical Effect and Hazard Identification
2. Dose Response and Point of Departure (POD) Derivation
3. Exposure Assessment
4. Risk Characterization

Mammalian toxicology testing requirements for agrochemicals

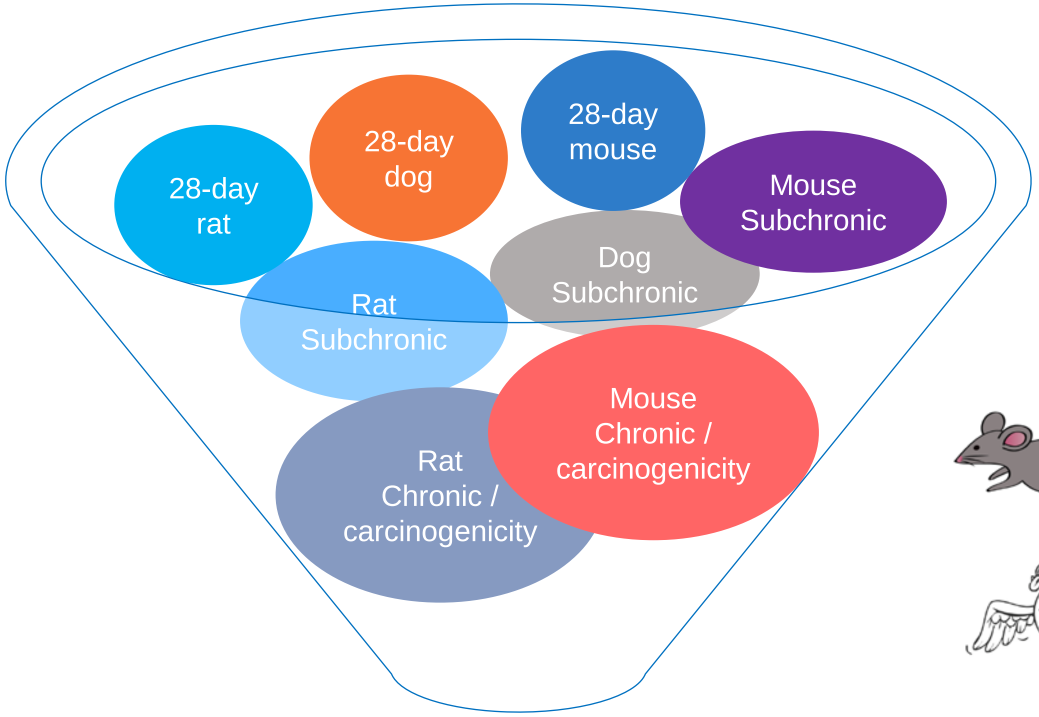
Agrochemicals
- most comprehensive data requirements of any chemical sector

Product registration (per molecule)

- >100 rigorous studies
- Toxicology data package: $29,000,000
- Animal usage: 7400 mammals

Rate-limiting study for mammalian toxicity assessment is the rodent carcinogenicity study

Mammalian General Toxicology Studies



***1500* animals**

4 years

A stew of apical POD values

↓

Final Toxicity Point of Departure Value

All Apical Effects Result From A Prior Change At The Molecular Level

Generic Adverse Outcome Pathway

Exposure → Molecular Change → Apical Effect

Overarching Hypothesis:

A POD based upon *comprehensive* molecular data will be *protective* of *any* downstream apical effect POD.

What about the data?

Questions addressed

1. How stable is a transcriptome POD over exposure time?

2. Is a transcriptome POD similar to a traditional apical endpoint POD?

3. Can a shorter-term study transcriptome POD estimate a carcinogenicity study apical endpoint POD?

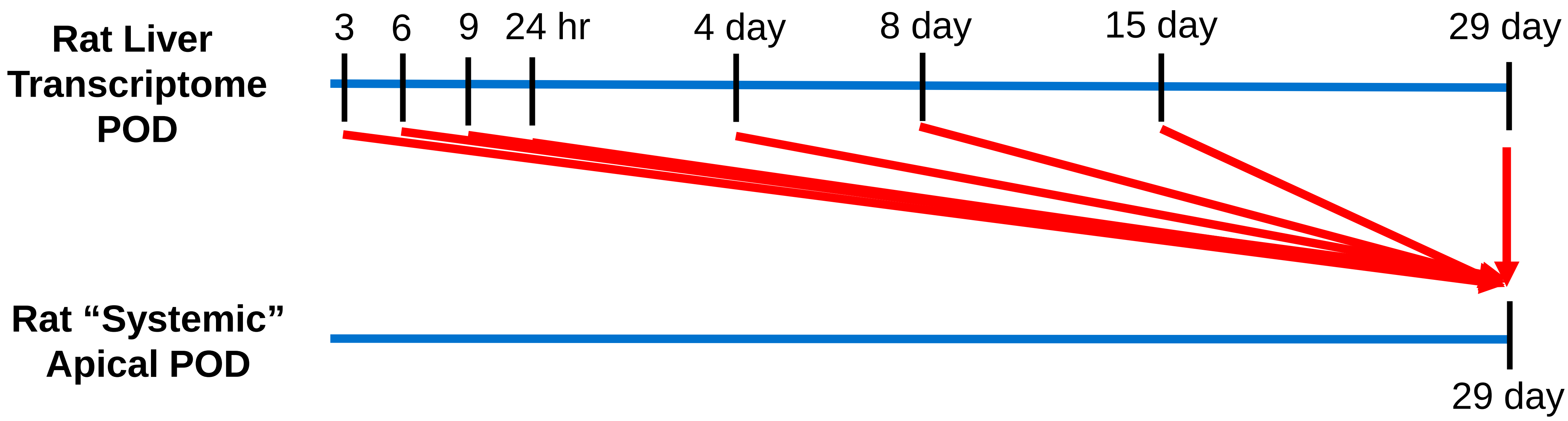
Transcriptome POD Derivation Method

Based upon a published workflow (Johnson, et al, ToxSci, 176, 2020)

1. Generate whole-transcriptome data
2. Using BMDExpress software
   1. Identify genes with treatment-related change
   2. Generate benchmark dose value for each gene
   3. Map all benchmark dose values to GO-BP gene set
   4. Filter GO-BP terms based upon threshold values
   5. Final transcriptome POD is the GO-BP term with the lowest BMD/L value

How stable is a rat transcriptome POD?
Does it predict a longer-term rat apical POD?

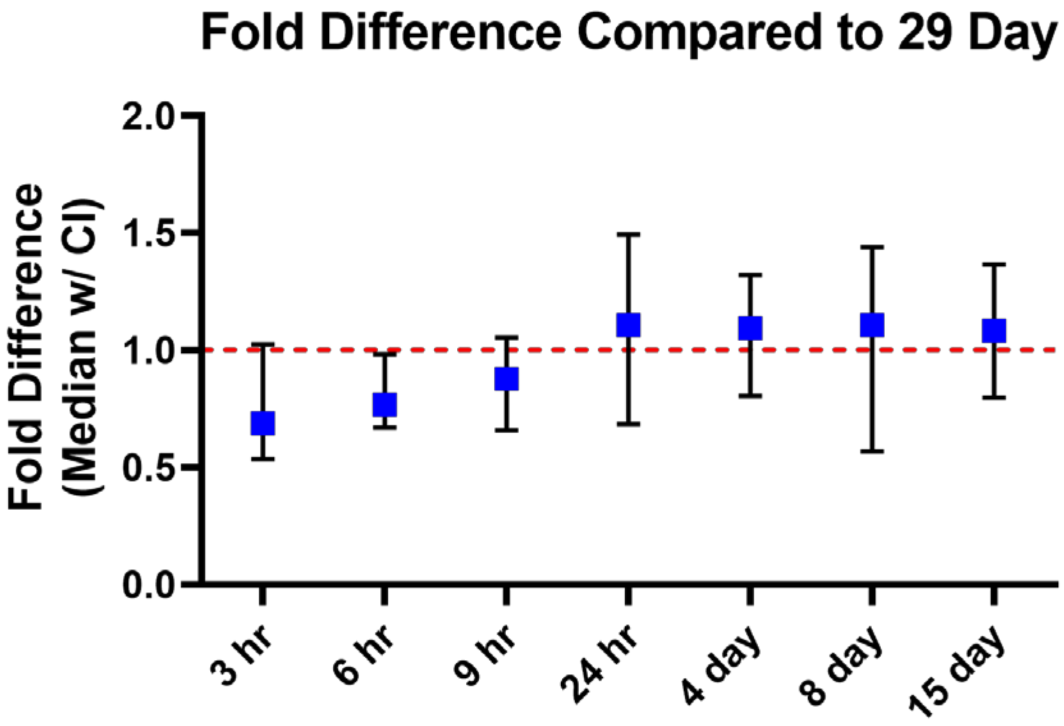
Data across 79 molecules (TG-GATES) were used to address these questions

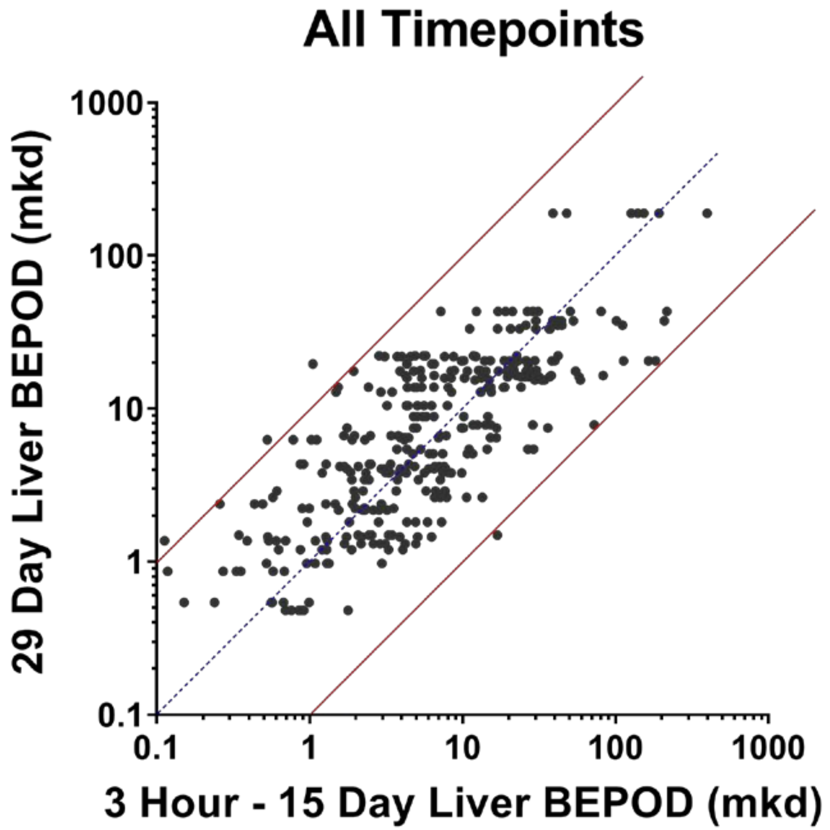


Johnson, et al, ToxSci, 176, 2020

Rat liver transcriptome PODs are stable within 24 hours

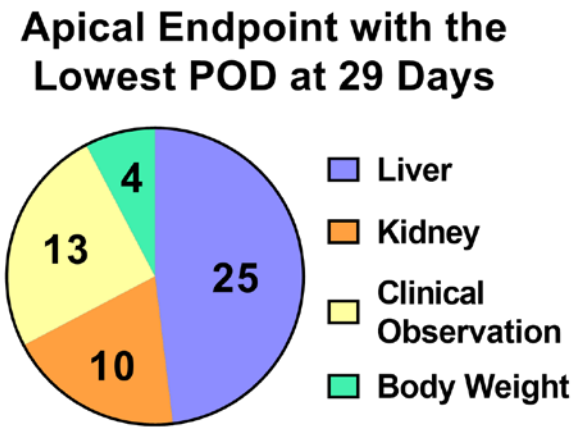
Data across 51 molecules (TG-GATES)

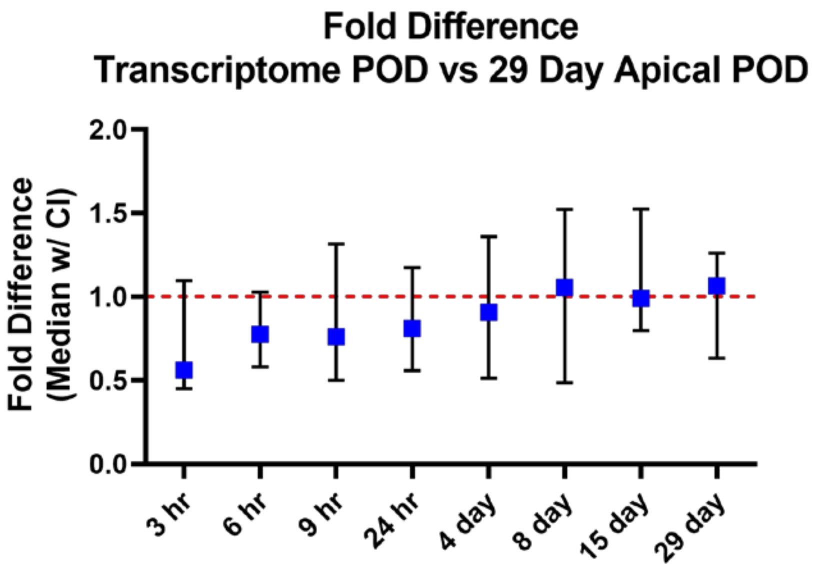


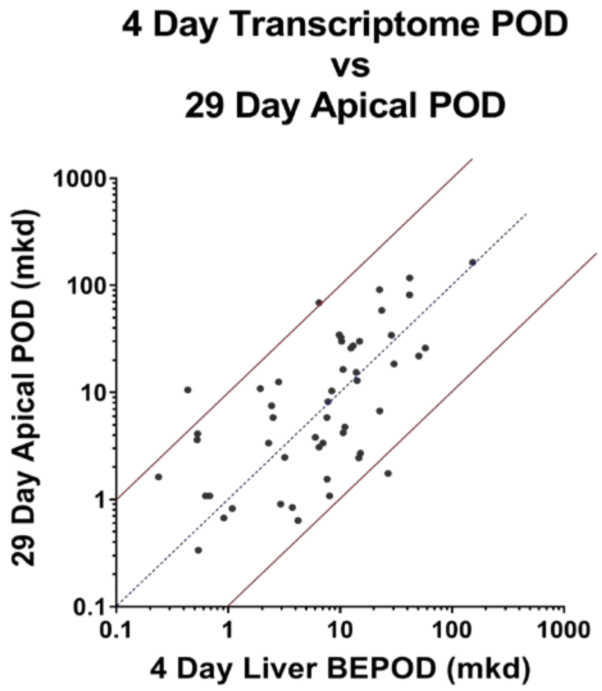


Rat liver transcriptome PODs estimate a future “systemic” apical POD

Data across 51 molecules (TG-GATES)







Does a shorter-term study transcriptome POD estimate a carcinogenicity study apical endpoint POD?

Data across 5 Corteva agrochemicals

Transcriptome Studies

Myclobutanil

- Male rat 14 day oral gavage exposure
- RNAseq analysis of liver and testis

Four additional pesticide chemistries

- Male rat 90 day dietary exposure
- RNAseq analysis of liver and kidney

Carcinogenicity Studies

All chemistries

- Study design followed EPA/OECD guidelines

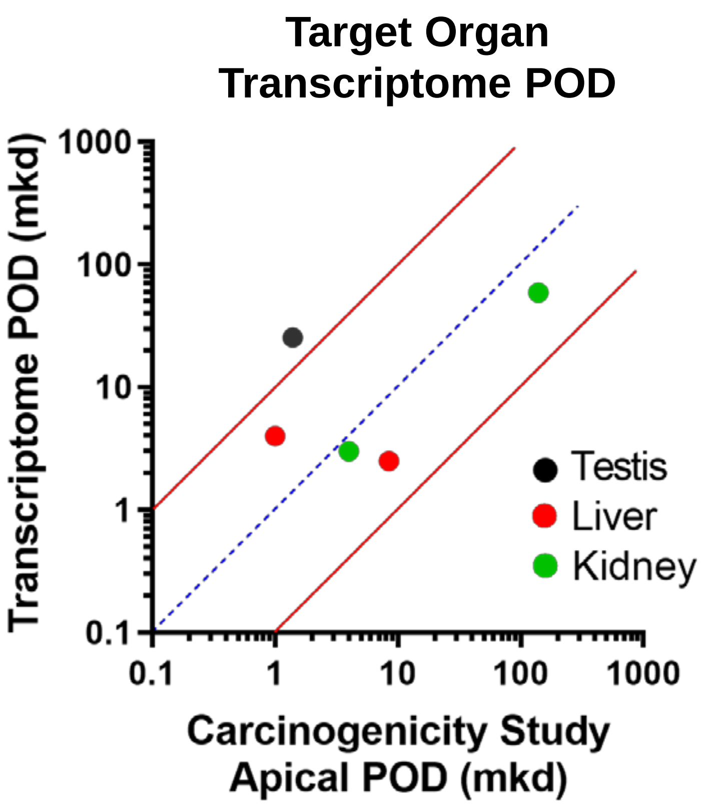
BMDS used to generate apical POD using EPA-developed method

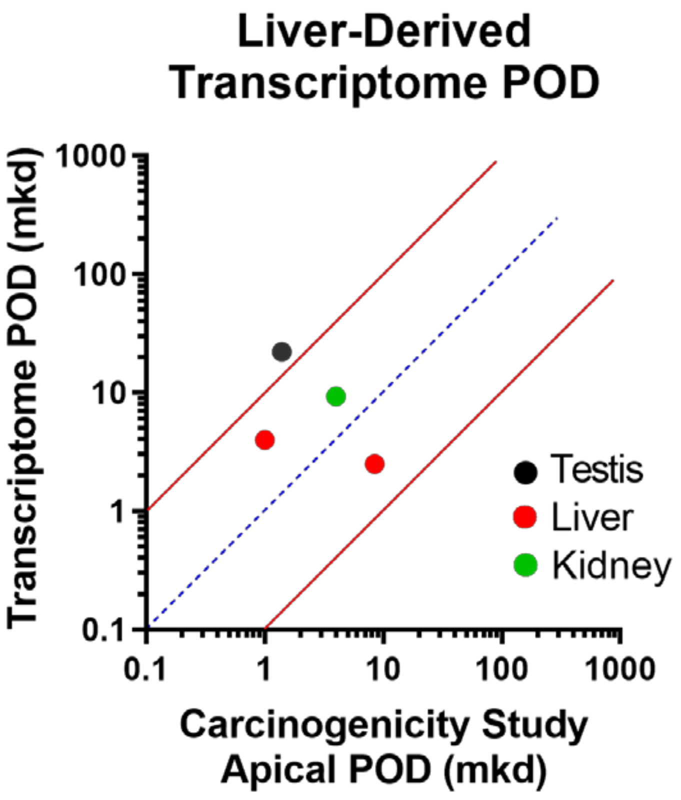
LaRocca, et al, RTP, 113, 2020

Bianchi, et al, submitted

A shorter-term study transcriptome POD estimates a carcinogenicity study apical POD for agrochemicals.

Estimation is within approximately an order of magnitude





Growing consensus that a transcriptome POD estimates an apical POD

Temporal Concordance Between Apical and Transcriptional Points of Departure for Chemical Risk Assessment

Russell S. Thomas,*,[1] Scott C. Wesselkamper,† Nina Ching Y. Wang,† Q. Jay Zhao,† Dan D. Petersen,† Jason C. Lambert,† Ila Cote,‡ Longlong Yang,* Eric Healy,* Michael B. Black,* Harvey J. Clewell III,* Bruce C. Allen,§ and Melvin E. Andersen*

Cross-Species Transcriptomic Analysis of Mouse and Rat Lung Exposed to Chloroprene

Russell S. Thomas,*,[1] Matthew W. Himmelstein,† Harvey J. Clewell III,* Yuching Yang,* Eric Healy,* Michael B. Black,* and Melvin E. Andersen*

Genomic Signatures and Dose-Dependent Transitions in Nasal Epithelial Responses to Inhaled Formaldehyde in the Rat

Melvin E. Andersen,[1] Harvey J. Clewell, III, Edilberto Bermudez, Gabrielle A. Willson, and Russell S. Thomas

Integrating pathway-based transcriptomic data into quantitative chemical risk assessment: A five chemical case study

Russell S. Thomas[a,*], Harvey J. Clewell III[a], Bruce C. Allen[b], Longlong Yang[a], Eric Healy[a], Melvin E. Andersen[a]

Case study on the utility of hepatic global gene expression profiling in the risk assessment of the carcinogen furan

Anna Francina Jackson[a,b], Andrew Williams[a], Leslie Recio[c], Michael D. Waters[c], Iain B. Lambert[b], Carole L. Yauk[a,*]

Evaluation of 5-day *In Vivo* Rat Liver and Kidney With High-throughput Transcriptomics for Estimating Benchmark Doses of Apical Outcomes

William M. Gwinn,*,[1] Scott S. Auerbach,* Fred Parham,* Matthew D. Stout,* Suramya Waidyanatha,* Esra Mutlu,* Brad Collins,* Richard S. Paules,* Bruce Alex Merrick,* Stephen Ferguson,* Sreenivasa Ramaiahgari,* John R. Bucher,* Barney Sparrow,† Heather Toy,† Jenni Gorospe,† Nick Machesky,† Ruchir R. Shah,‡ Michele R. Balik-Meisner,‡ Deepak Mav,‡ Dhiral P. Phadke,‡ Georgia Roberts,* and Michael J. DeVito*

Integrating toxicogenomics into human health risk assessment: Lessons learned from the benzo[*a*]pyrene case study

Nikolai L. Chepelev, Ivy D. Moffat, Sarah Labib, Julie Bourdon-Lacombe, Byron Kuo, Julie K. Buick, France Lemieux, Amal I. Malik, Sabina Halappanavar, Andrew Williams & Carole L. Yauk

Comparison of toxicogenomics and traditional approaches to inform mode of action and points of departure in human health risk assessment of benzo[*a*]pyrene in drinking water

Ivy Moffat, Nikolai L. Chepelev, Sarah Labib, Julie Bourdon-Lacombe, Byron Kuo, Julie K. Buick, France Lemieux, Andrew Williams, Sabina Halappanavar, Amal I Malik, Mirjam Luijten, Jiri Aubrecht, Daniel R. Hyduke, Albert J. Fornace Jr, Carol D. Swartz, Leslie Recio & Carole L. Yauk

Presentation Outline

1. Scientific basis of using a molecular POD in the regulatory space

2. Data comparing traditional (apical) and transcriptome PODs

3. **Utility of a transcriptome-based POD: Now and in the Future**

Proposed current utility of an in vivo transcriptome POD

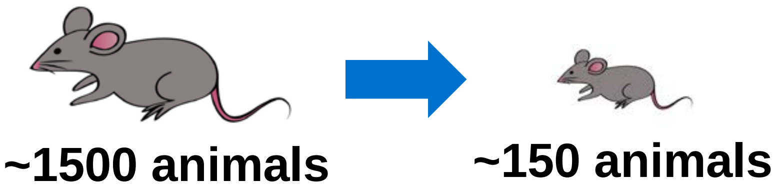
1. **Add to weight of evidence supporting a human health-protective POD in a registration dossier**

2. **Add to weight of evidence supporting waiving of a cancer bioassay**
   – Estimation of an apical POD from a shorter-term study

3. **Internal decision making**
   – Discovery analog selection
   – Study design

Potential utility of a transcriptome POD in regulatory toxicology

Potential animal use reduction for agrochemical general toxicity studies

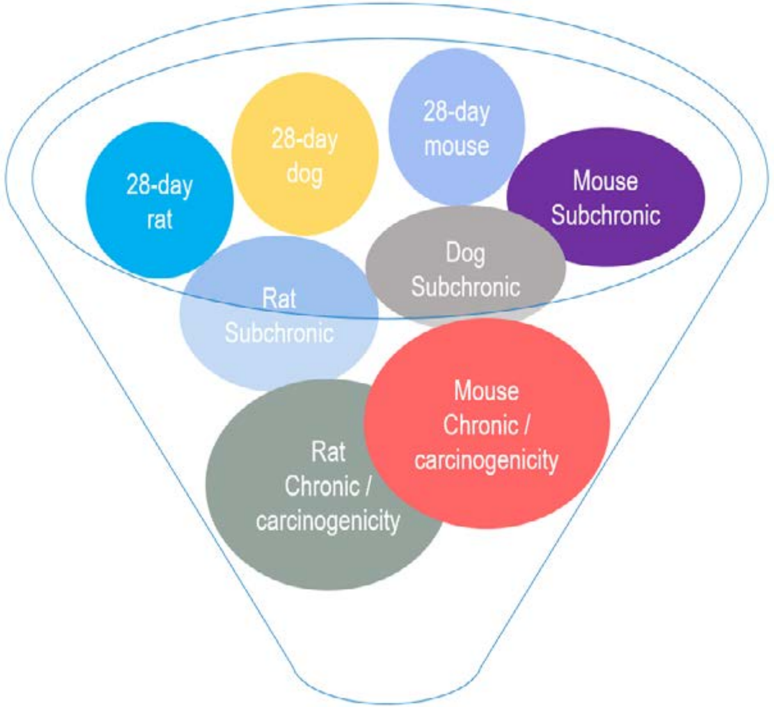


1. **Feasible in the nearer term (use 1)**
   - **Reduction in animal-based studies**
     - Rank-order chemicals (non-pesticides) for animal testing
     - Replacement of currently required study designs with study designs of lower animal usage
   - **Needs are many…**
     - More case studies; harmonization of study methods; acceptance by stakeholders

2. **Feasible in the longer term (use 2)**
   - **Replacing animal models with in vitro models**
   - Needs here are many… including development of appropriate *in vitro* models and IVIVE methods

What if… dramatic reduction in animal use

Current Testing Paradigm

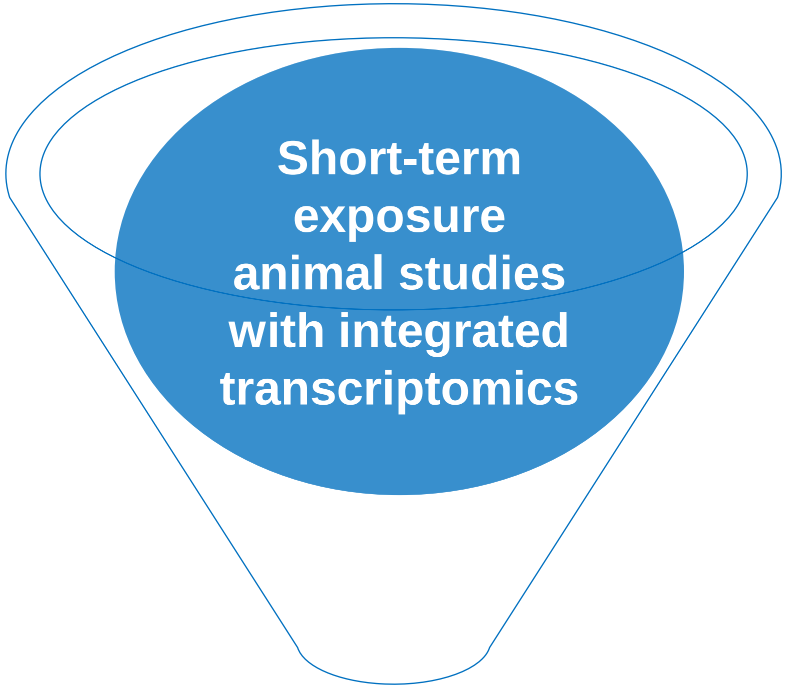


Point of Departure

~*1500* animals

~ 4 years

Future Testing Paradigm



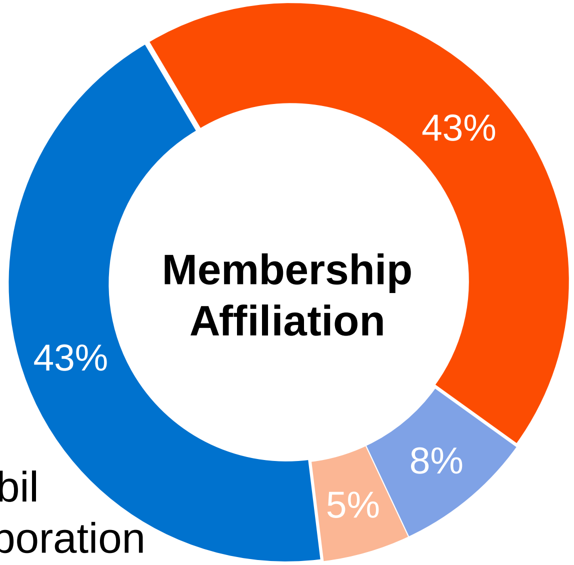
Point of Departure

~*150* animals

<6 months

How to realize change… it takes a village.

HESI eSTAR: Molecular POD Project Team



Regulatory/Government
- Health Canada
- EPA
- European Commission
- NIEHS
- NTP

Industry
- Bayer
- Corteva
- ExxonMobil
- FMC Corporation
- GlaxoSmithKline
- Janssen
- Syngenta

Academic
- Indiana University
- McGill University
- University of North Carolina

Other/Consulting
- Juberg Toxicology Consulting
- JLS Paradox Found Consulting

Draft Project Problem Statement

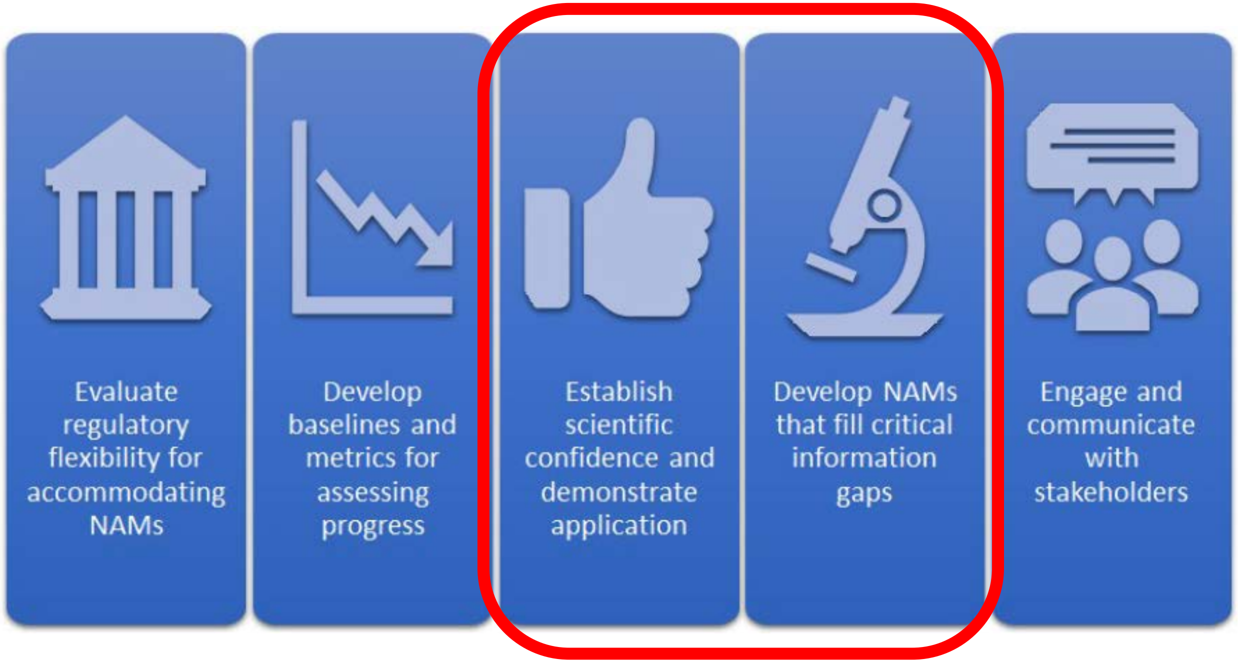
Develop a framework to derive an *in vivo* transcriptome POD for use in chemical risk assessment that will produce a human health-protective POD based upon concerted molecular change.

HESI eSTAR Annual Meeting

- October 28 – 30
- https://register.gotowebinar.com/register/466234021796755980

Transcriptome POD fit into the EPA NAM Workplan objectives

The Five Objectives



Establish Scientific Confidence

- A transcriptome POD estimates an apical POD

Demonstrate Application

- A shorter-term transcriptome POD estimates a longer-term apical POD
  - Potential to waive or replace current rodent studies

Fill Critical Information Gaps

- A *comprehensive* molecular analysis will cover all possible apical effects
  - Baked into the AOP concept of toxicity

A special thanks to…

Corteva Colleagues

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Anand Venkatraman
Jessica Howell
Lynea Murphy
Push Mukerji
Donna Andersen
Bethany Hannas
Reza Rasoulpour
Claire Terry
Zhongyu (June) Yan

External Colleagues

Scott Auerbach (NIEHS)

HESI eSTAR Committee

- Syril Pettit
- Alison Harrill
- Carolina Morell-Perez
- Connie Mitchell

HESI eSTAR Molecular POD Team

- ~30 members in various sectors