Benchmark Dose Modeling of Transcriptomic Data to Inform the Mode-of-Action of Toxicity and Establish a Point of Departure for Risk Assessment

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Gene Expression in Risk Assessment

***Gene expression* is the most fundamental change at the cell level that can result in a change in biological function or a change in the development of a cell**

- Whenever cells are exposed to a substance, cellular proteins, metabolites and genes respond in an integrated way, leaving a toxicological fingerprint
- This fingerprint permits conclusions about the mode of action and the dose-response for early cellular effects (obligatory precursors).
- Short-term *in vivo* transcriptomic dose-response studies are currently being considered by the USEPA as a replacement for long-term animal studies
  - To identify potentially dangerous substances more quickly and with fewer animal experiments than has been needed up to now

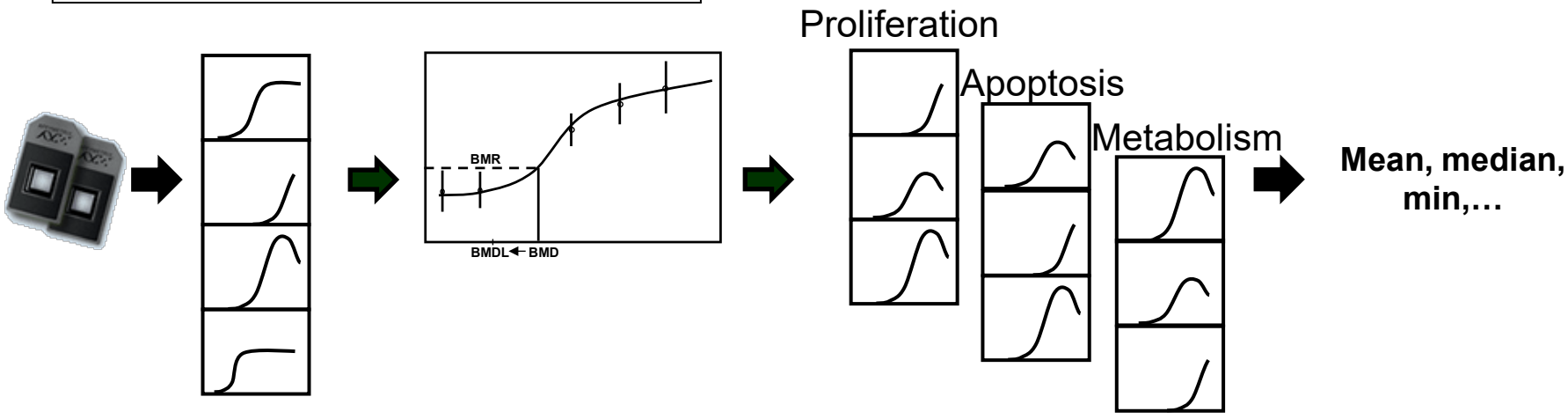
Application of Benchmark Dose Methods to Transcriptomic Data

Fit Each Gene with Statistical Models

- **All genes fit to power, linear, 2° polynomial, 3° polynomial, and hill models**
- **Least complex model that best fits the data selected (i.e., nested likelihood ratio test and AIC)**
- **BMD and BMDL calculated**

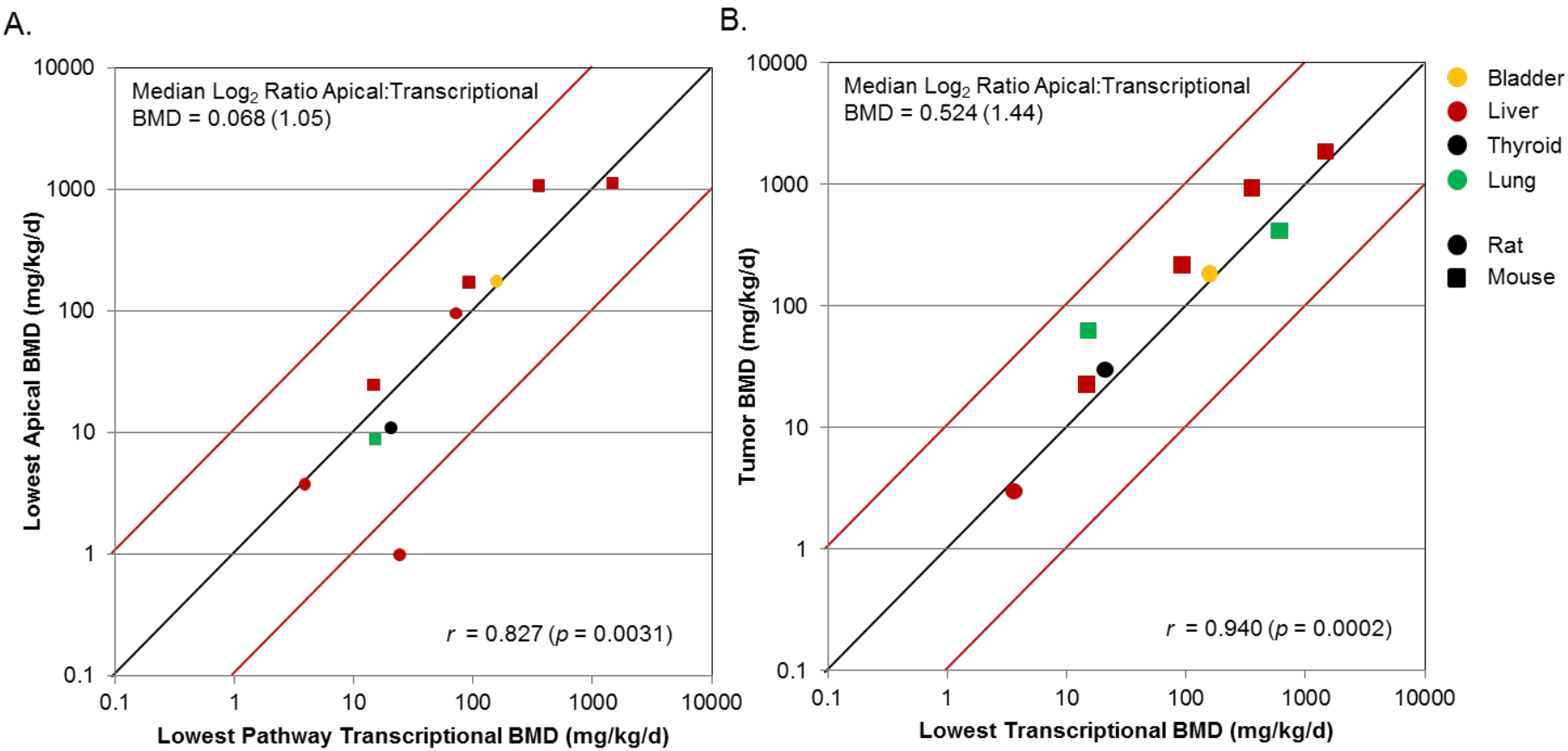
Group Genes by Function

Calculate Summary Values for each Function



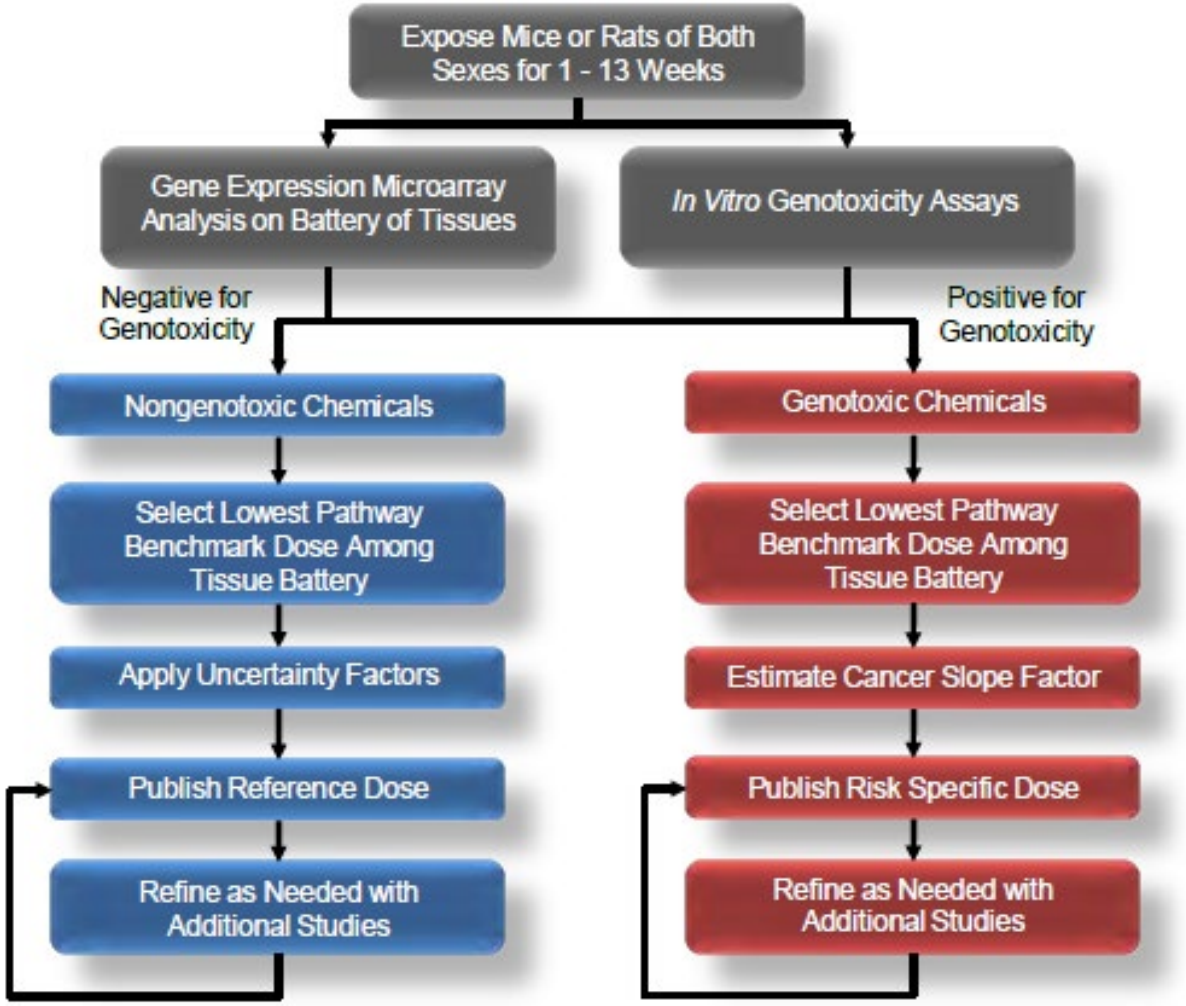
Thomas et al. 2007

Short-Term Genomic Studies Can Predict Safe Chronic Doses



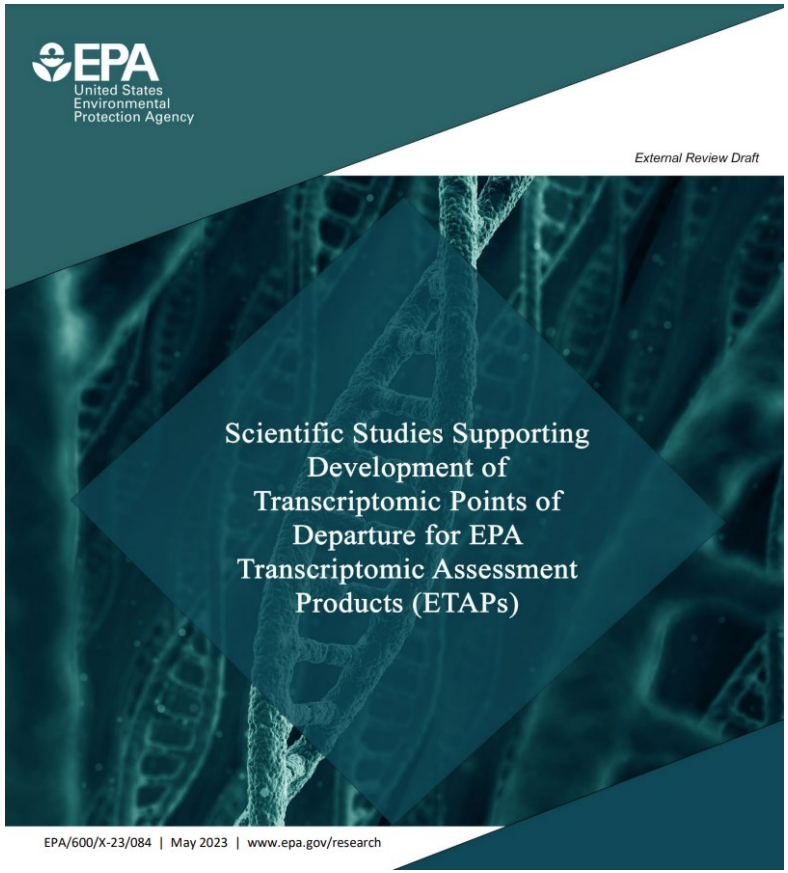
Thomas et al. 2012, 2013a

Transcriptional Risk Assessment Approach



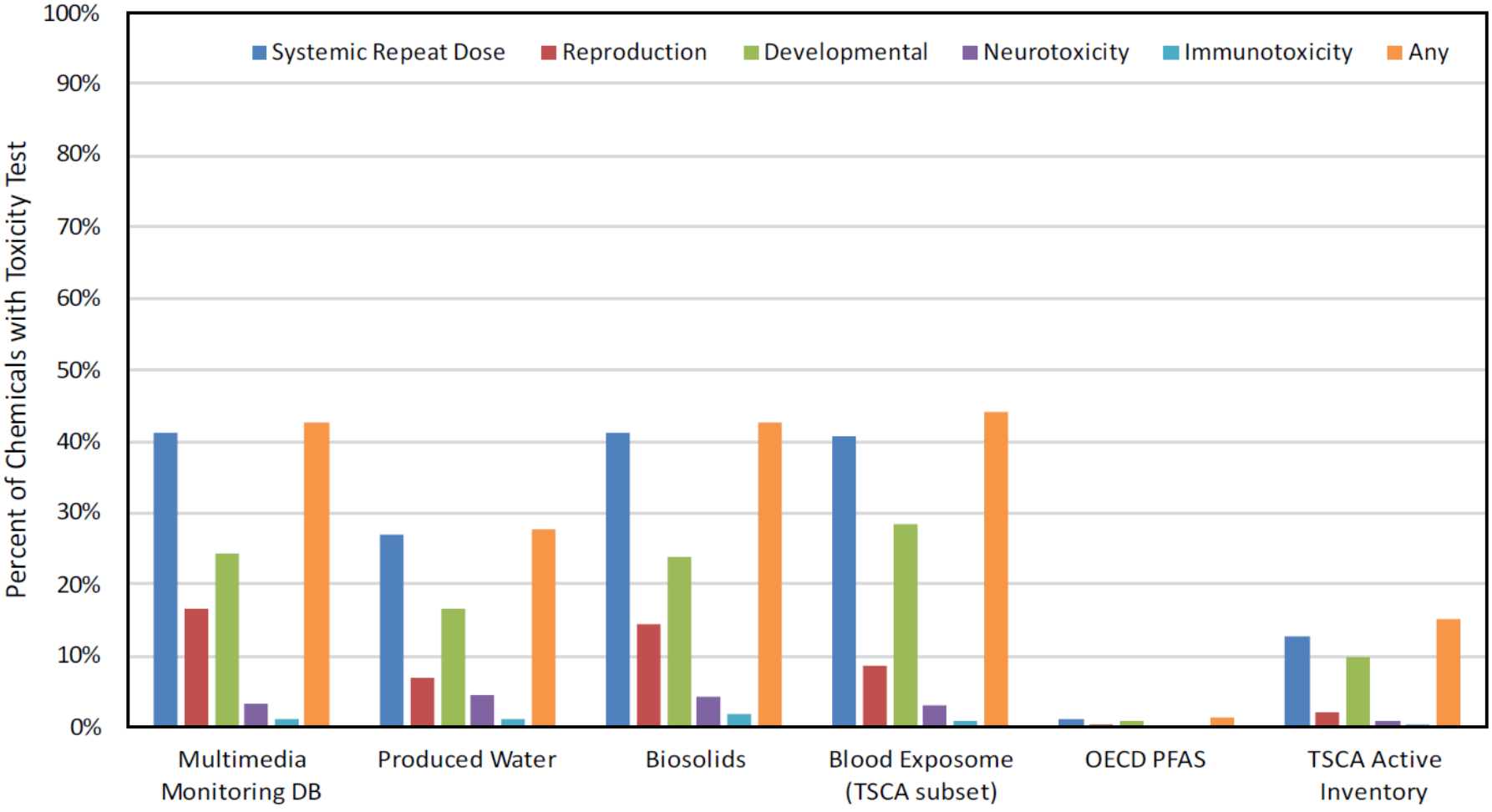
Thomas et al. 2011, 2013b

USEPA Transcriptomic Assessment Products (ETAPs)



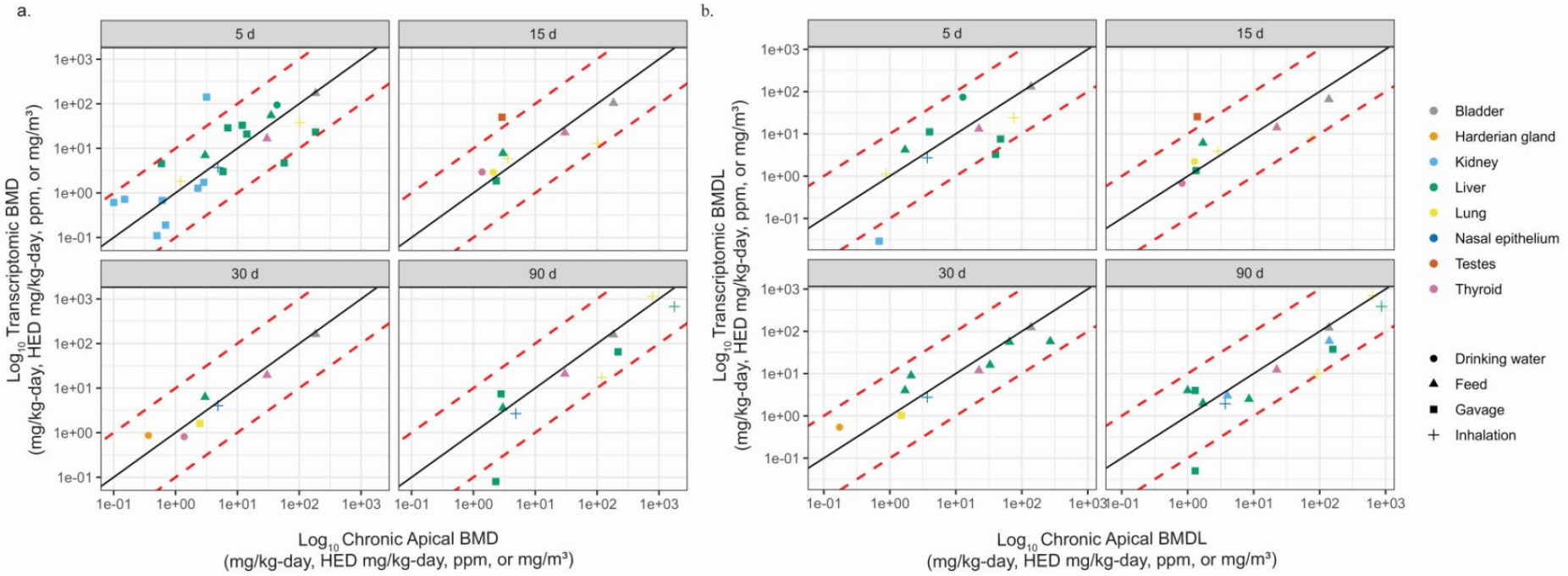
USEPA 2023a,b

Why is the ETAP needed?



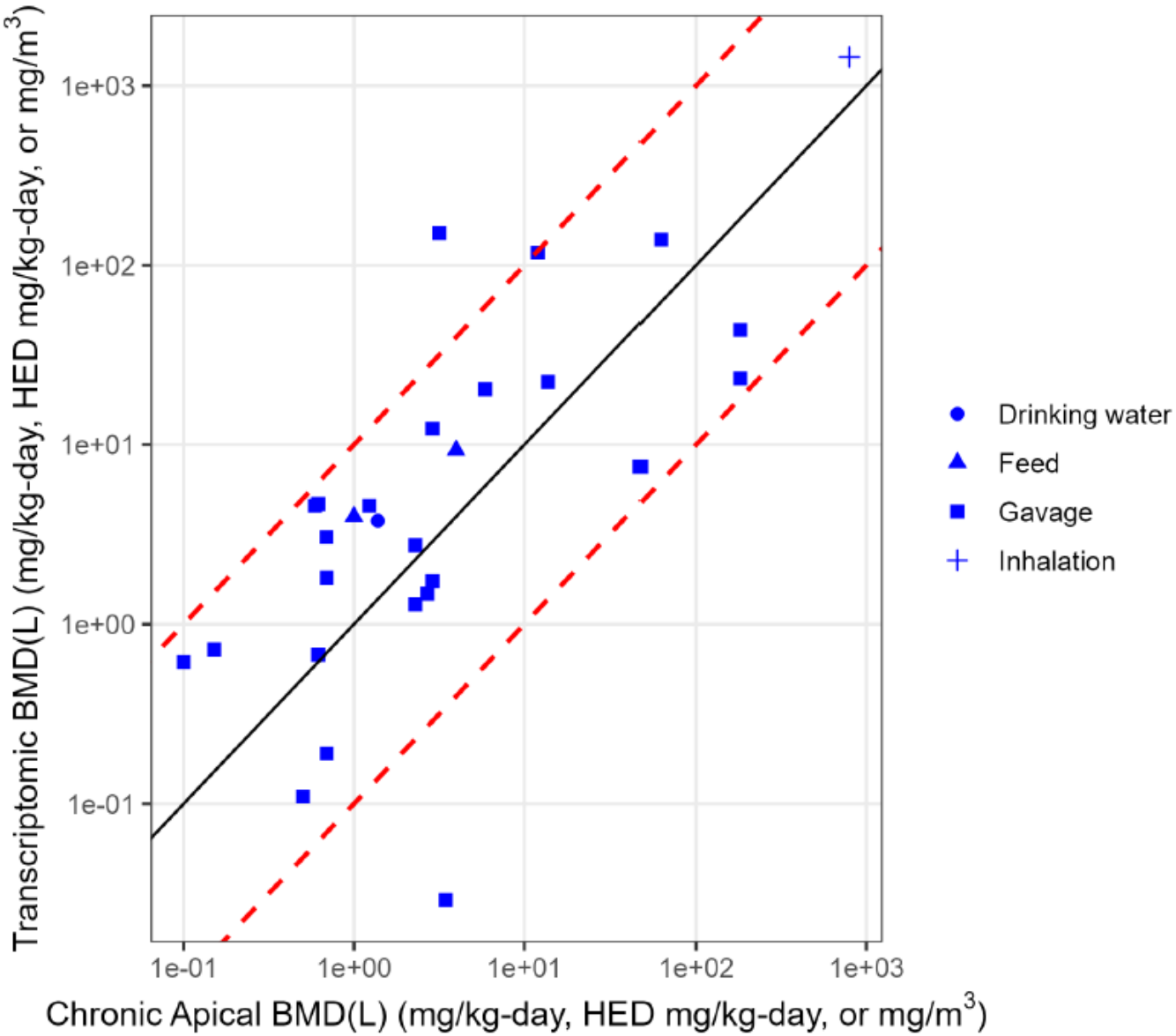
USEPA 2023a

Can the ETAP predict chronic bioassays?



USEPA 2023a

What if we just looked at liver and kidney?



USEPA 2023a

USEPA Conclusions Regarding the Reliability of the ETAP

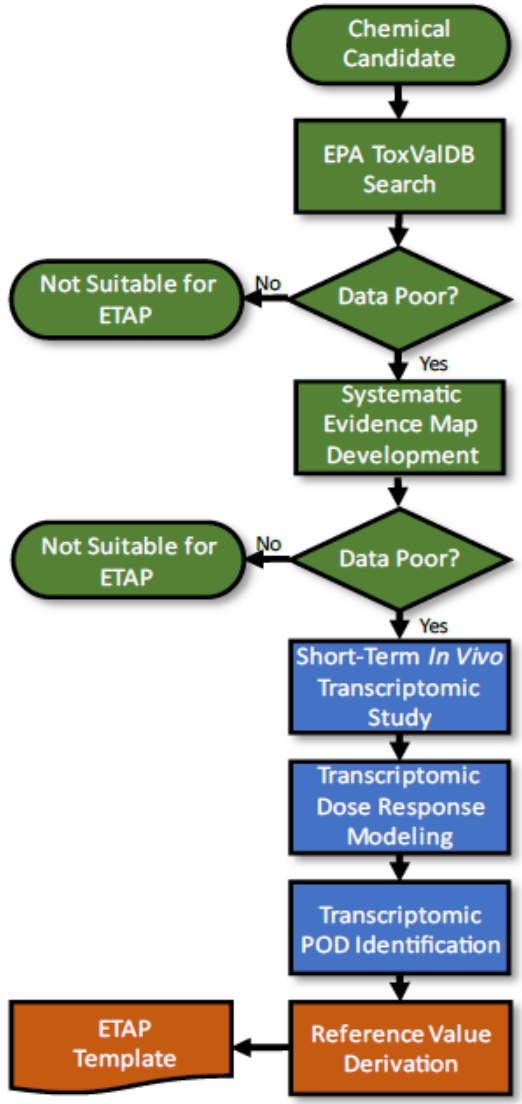
- "For the 38 chemicals with both short-term *in vitro* transcriptomic dose-response data and reported chronic rodent bioassay results, the Pearson's correlation coefficient was 0.825 with a log10 root-mean-square difference (RMSD) of 0.561 (log10 mg/kg-day) and a median absolute ratio of 1.9 ± 0.7 (Median Absolute Deviation; MAD)."

- "The RMSD value is similar to the range of inter-study standard deviation estimates for the lowest observable adverse effect levels (LOAELs) for systemic toxicity in repeated dose studies."

- "The results suggest that the error associated with the concordance between the transcriptomic BMD values versus non-cancer and cancer apical BMD values is approximately equivalent to the inter-study variability in the repeated dose toxicity study itself."

- "The overall conclusions from the literature survey, evaluation of the transcriptomic dose response analysis methods, and the statistical comparison of the concordance with inter-study variances support the use of transcriptomic PODs from 5-day, repeated dose in vivo rodent studies in quantitative human health assessments."

ETAP Development Process



USEPA 2023b

What would be needed to develop an *in vitro* ETAP?

- The full transcriptomic data from the ETAPS will be publicly available.
  - The ETAP only uses the data for dose-response evaluation
  - However, the data could also be mined to determine whether it contains information relevant for mode of action evaluation and potential apical outcome/target tissue identification

- A possible path forward for an in vitro alternative to the ETAP to predict the outcome of a 2-year bioassay would require:
  - conducting 5-day in vitro assays in one or more tissue cultures (e.g., liver, kidney, lung, brain, circulating immune cells, IPSCs*) using chemicals included in the analyses described in the ETAP documentation
  - transcriptomic pathway analysis on the ETAP studies to provide evidence to support AOP identification, and
  - development of an agreed battery of in vitro genotoxicity assays that could be used to determine whether the POD from the in vitro transcriptomic study could represent a threshold for nongenotoxic carcinogenicity.

* McMullen et al. 2018

Use of in vitro studies to inform the dose response for epidemiological associations:

Cancer Risk Assessment for Inorganic Arsenic

Epidemiological data in human populations exposed to inorganic arsenic for multiple generations provides clear evidence of cancer and noncancer effects at drinking water exposures on the order of 100 ppb and above

- There is uncertainty regarding potential for effects at lower concentrations
- Rodent bioassays provide only equivocal evidence of carcinogenicity

In vitro data support an MOA driven by binding to thiols in cellular proteins (Clewell et al. 2018)

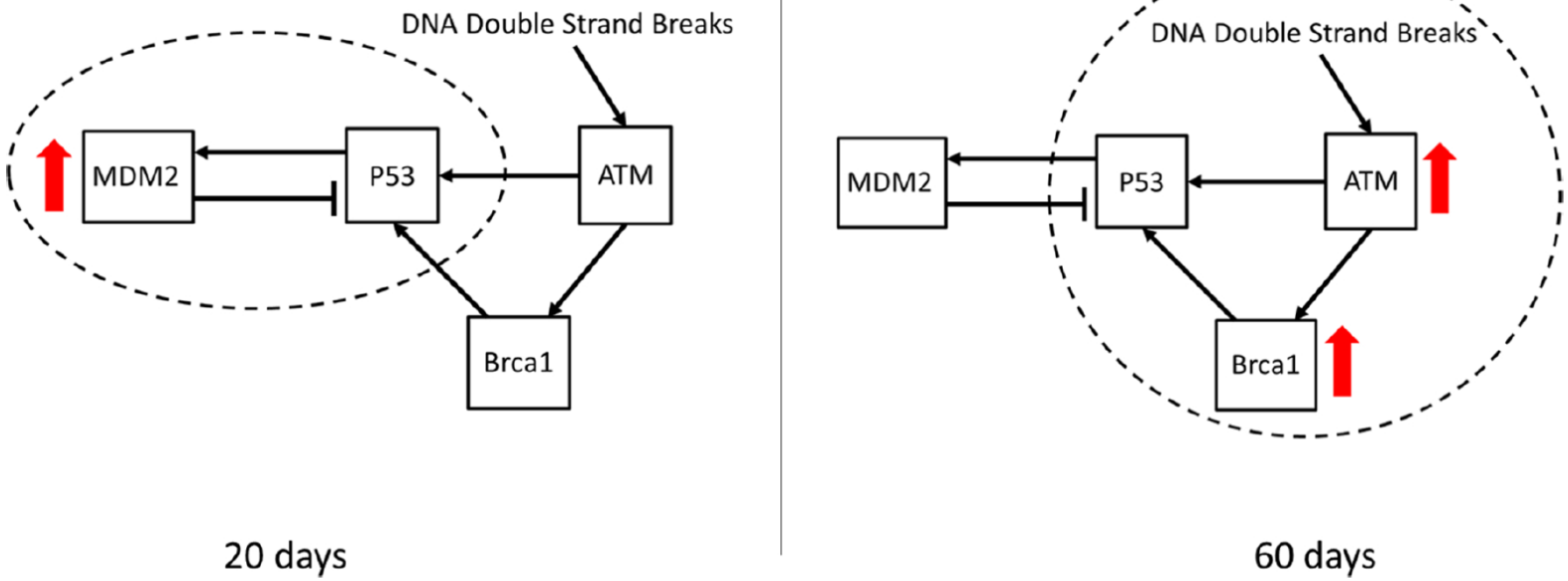
- Co-mutagenicity (J. Yager), co-carcinogenicity (T. Rossman)
- "Fundamentally threshold MOA" (J. Preston, S. Cohen, T. Rossman, EPA Panel on MOA for inorganic arsenic, 1997)
- "Protein level effect, not stochastic effect" (L. Snow)

In vitro exposures of primary human epithelial cells to arsenite for 60-days
(Efremenko et al. 2021)

- In this study we demonstrated the ability to expose primary uroepithelial cells to a low dose of arsenite over a period of up to 60 days.
- Consistent with previous reports that arsenite treatment can partially immortalize cells, the treated cells in this study continued to proliferate over the full 60 day exposure period, in contrast to the untreated controls, which ceased proliferating after approximately 30 days of culturing.
- Key elements leading to the co-mutagenicity of arsenic result from binding to vicinal dithiols in these proteins:
  - Binding to Keap-1 activates the NRF2 oxidative stress response
  - Binding to IKBα / IKB kinase suppresses the NFKB inflammatory response
  - Binding to PARP-1 inactivates a critical enzyme in the cellular DNA repair response

In vitro exposures of primary human epithelial cells to arsenite for 60-days (Efremenko et al. 2021)

- The key response of the cells to arsenite over prolonged exposures involves up-regulation of MDM2 by inflammatory signaling through AP-1 and NFKB, leading to inhibition of P53 DNA damage repair function (co-mutagenicity).



At 20 days (left), MDM2 is upregulated, inhibiting P53 function. At 30 days (right), ATM is upregulated in response to increased DNA double strand breaks.

Benchmark dose ranges for genes with a statistically significant dose-response trend in primary uroepithelial cells from most subjects after treatment with arsenite, MMA^{III} and DMA^{III} (trivalent) mixtures.

Gene Name	Description	Number of Subjects Expressing the Gene/Total Subjects	BMD Range (μM)	BMDL Range (μM)
HMOX1	Oxidative stress response	10/10	0.13-0.50	0.09-0.33
FKBP5	Protein folding	9/10	0.36-0.92	0.24-0.58
TXNRD1	Thioredoxin reductase	9/10	0.32-0.75	0.21-0.48
MT1E	Metallothionine regulation	8/10	0.24-0.77	0.16-0.49
DDB2	DNA damage sensing	8/10	0.30-0.88	0.20-0.56
TXN	Thioredoxin	8/10	0.26-0.76	0.17-0.48
LGALS8	Cell adhesion, growth regulation	8/10	0.16-0.92	0.11-0.58
THBD	Immune response	8/10	0.32-0.90	0.20-0.57

Green: adaptive response; Red: potentially adverse effect

(Tsuji et al. 2019)

Estimation of a Point of Departure for Inorganic Arsenic Carcinogenicity

***In vitro* exposures of human primary cells to trivalent As mixtures:**	**0.01 – 1 µM total As**
⬇	
Lowest *in vitro* BMDL for potentially adverse effects of trivalent mixtures:	**0.2 µM**
⬇	
Equivalent POD for total arsenic in urine (23% trivalent arsenic in urine):	**65 µg/L**
⬇	
Safe drinking water concentration (dividing by 3 = 1/2 of observed 6-fold range for urine:drinking water ratios):	**22 µg/L**
⬇	
Acceptable *daily intake*:	**(~ 0.6 µg/kg/d)**

(Tsuji et al. 2019)

Conclusions: Inorganic Arsenic

- *In vitro* data from a large number of studies are consistent with a threshold for adverse interactions of trivalent arsenic with cells at media concentrations above 0.2 uM
- *In vitro* data from human primary uroepithelial cells suggest that pharmacodynamic variability is within default expectations
- Based on in vitro data from human primary cells, the population threshold for effects of inorganic arsenic is on the order of 22 µg/L (0.6 µg/kg/d)
- This threshold is consistent with arsenic exposures in populations showing no effects of arsenic, as well as in control groups from epidemiological studies that provide evidence of effects in higher exposed groups

(Tsuji et al. 2019)

Summary
Applications of Transcriptomic Dose-Response Studies

- Currently, short-term *in vivo* transcriptomic dose-response studies are being used to predict the quantitative results of chronic animal bioassays for both noncancer and cancer endpoints.

- In the future, short-term *in vitro* transcriptomic dose-response studies can be used to estimate conservative reference doses or points-of-departure for both non-cancer and cancer risk assessments.

- Pathway-based transcriptomic dose-response analysis of *in vitro* exposures can provide key insights into toxic mode-of-action.

- Transcriptomic dose-response studies with human cells exposed *in vitro* can inform the shape of the dose-response curve in the low-dose region for effects observed in epidemiology studies.

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