EPA United States Environmental Protection Agency

Evaluation of Pesticides with Established Liver Tumor Modes of Action

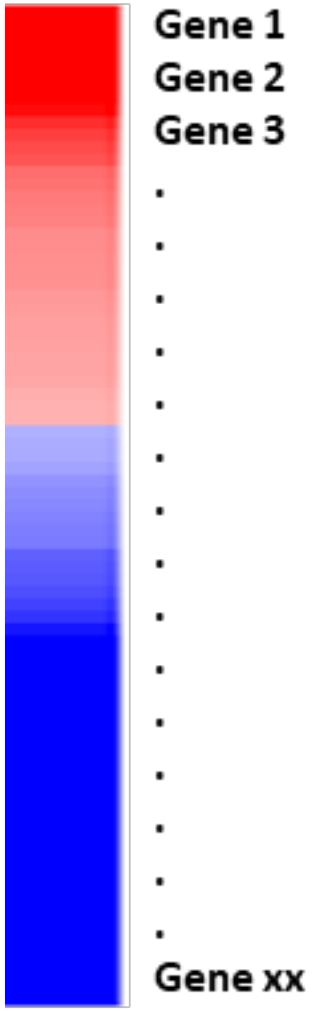
Chris Corton

Center for Computational Toxicology and Exposure
US-Environmental Protection Agency
Research Triangle Park, NC

Disclaimer

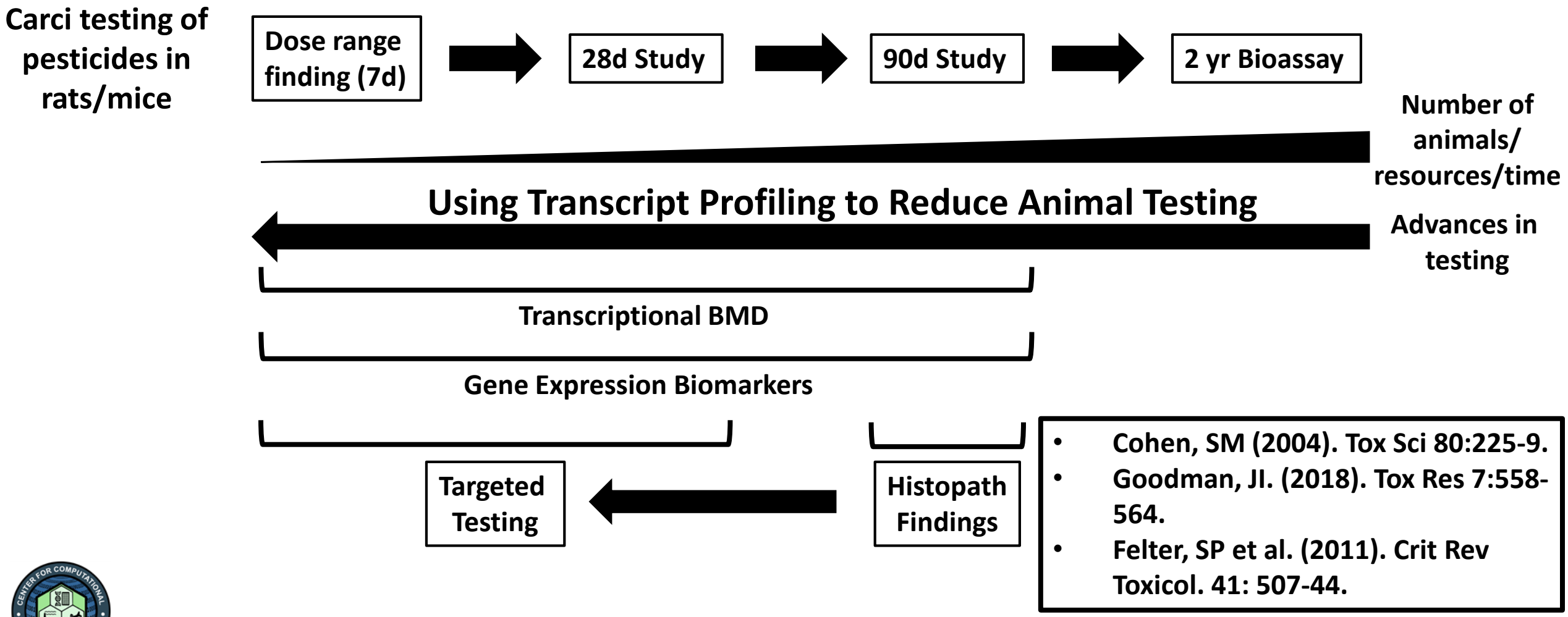
- The views expressed are those of Dr. Chris Corton and do not reflect US-EPA policy or product endorsement by the US-EPA.

Gene Expression Biomarkers



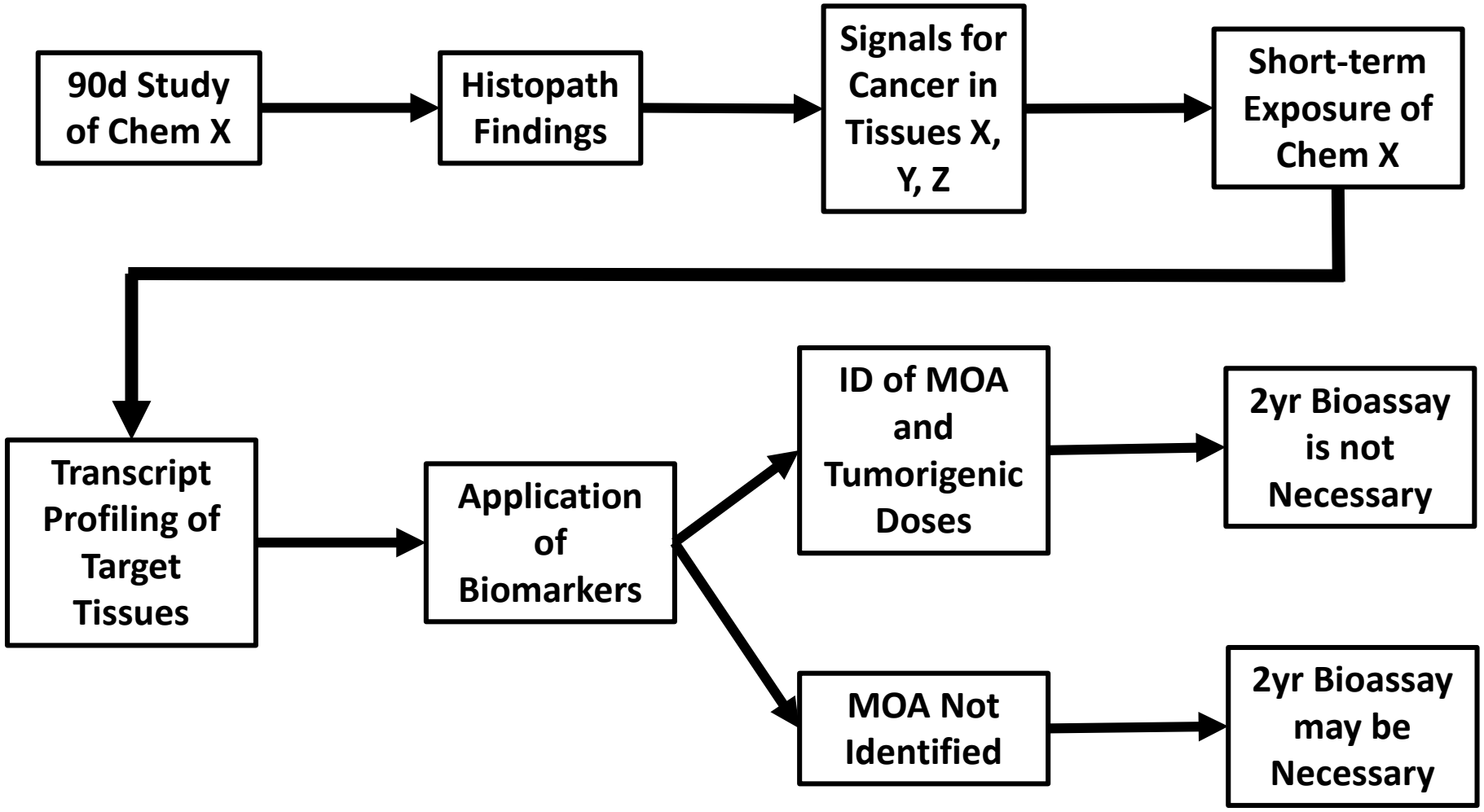
- List of genes and associated fold-change values or ranks
- Measures a molecular initiating event or key event in an adverse outcome pathway using transcript profiling
- Can be used to identify the mechanism of toxicity of a chemical
- Biomarkers that predict MIEs in mouse liver: AhR, CAR, PPARα, Nrf2, Stat5b, SREBP (multiple publications)
- Biomarkers that predict MIEs in rat liver: DNA damage, AhR, CAR, ER, PPARα, Cytotoxicity (Corton et al. (2020). Tox Sci. In press.)
- Levels of biomarker activation are associated with liver tumor incidence (Hill et al. (2020). Tox Sci. In press.

Applications of Genomic Tools to Chemical Testing

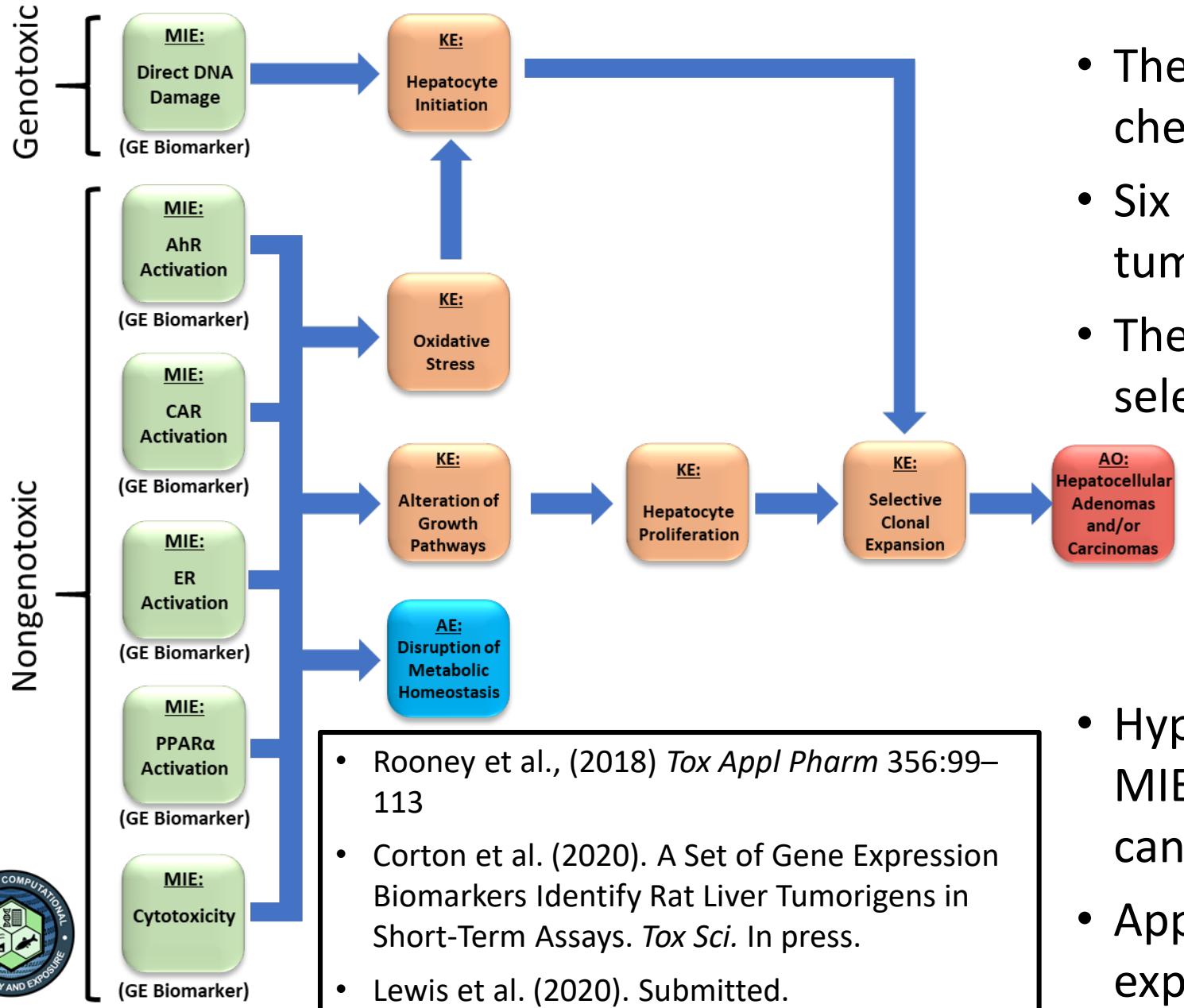


- Cohen, SM (2004). Tox Sci 80:225-9.
- Goodman, JI. (2018). Tox Res 7:558-564.
- Felter, SP et al. (2011). Crit Rev Toxicol. 41: 507-44.

Applications of Genomic Tools to Chemical Testing



Major Adverse Outcome Pathways That Lead to Rodent Liver Tumors



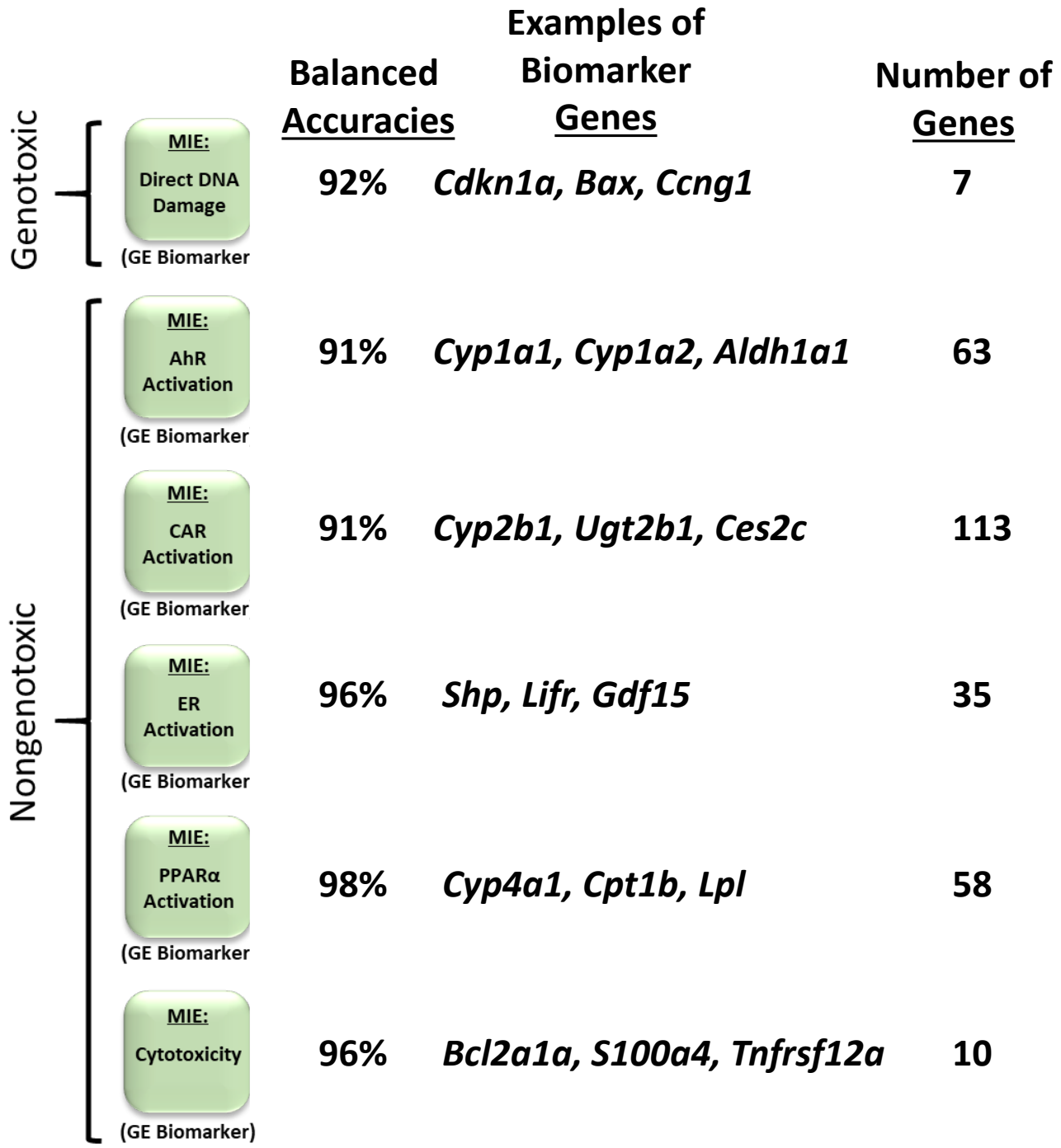
- The liver is the most frequent target of chemical tumorigens
- Six major AOPs lead to rodent liver tumors
- The AOPs converge on the key event of selective clonal expansion

- Rooney et al., (2018) *Tox Appl Pharm* 356:99–113
- Corton et al. (2020). A Set of Gene Expression Biomarkers Identify Rat Liver Tumorigens in Short-Term Assays. *Tox Sci.* In press.
- Lewis et al. (2020). Submitted.

- Hypothesis: measurement of the six MIEs will be sufficient to predict liver cancer
- Approach: measure MIEs with gene expression biomarkers

Predictive Accuracies of Six Gene Expression Biomarkers

- All biomarkers have balanced accuracies above 90%
- Genes identified are known to be regulated by the MIE

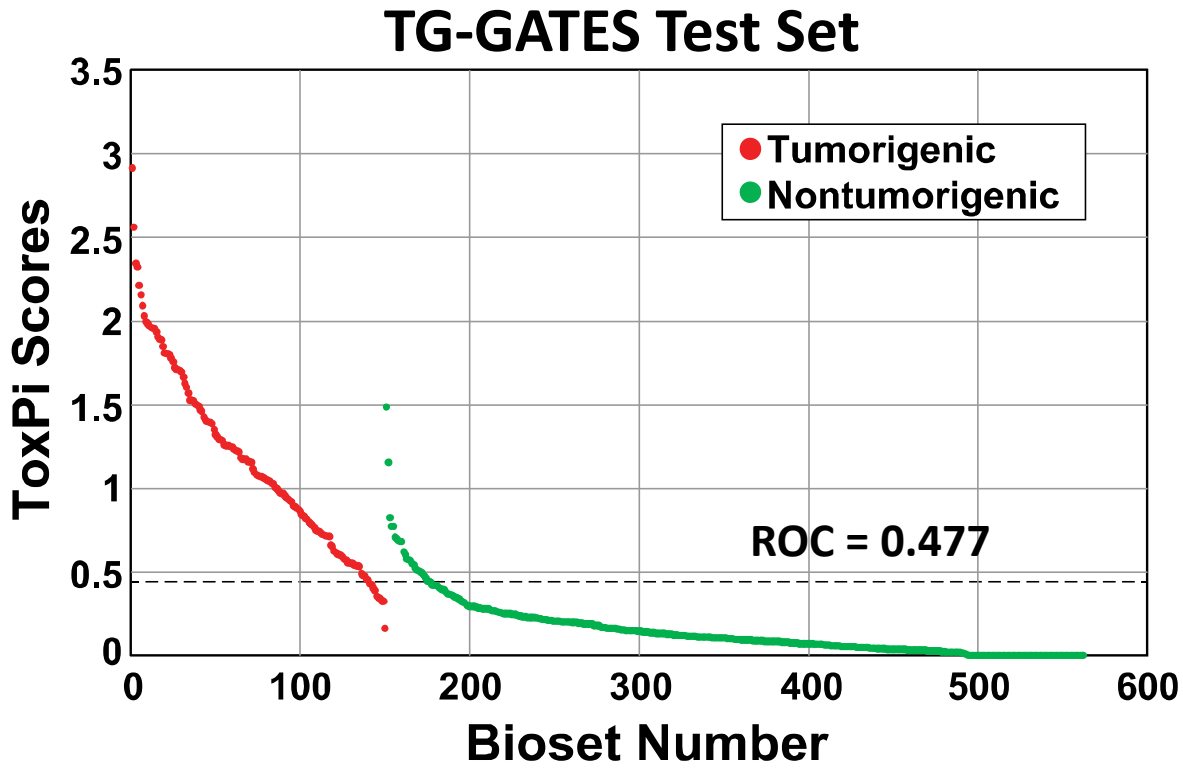


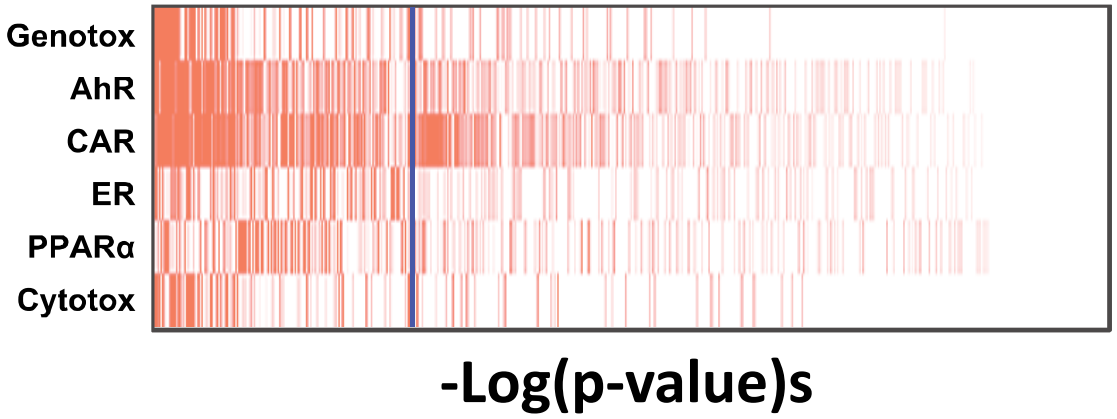
- Rooney et al., (2018) *Tox Appl Pharm* 356:99–113
- Corton et al. (2020). A Set of Gene Expression Biomarkers Identify Rat Liver Tumorigens in Short-Term Assays. *Tox Sci.* In press.

Predictions of Six MIEs Identifies Liver Tumorigens

- Used a combination of ToxPi and Receiver Operating Curves to examine a test set of chemicals
- 90% sensitivity, 97% specificity, and a **balanced accuracy of 93%**

- Out of 38 rat liver tumorigens, only two (5%) were not predicted (acetamide, ethionine)
  - These chemicals may work through different AOPs
  - Allows a better understanding of the weaknesses of the approach

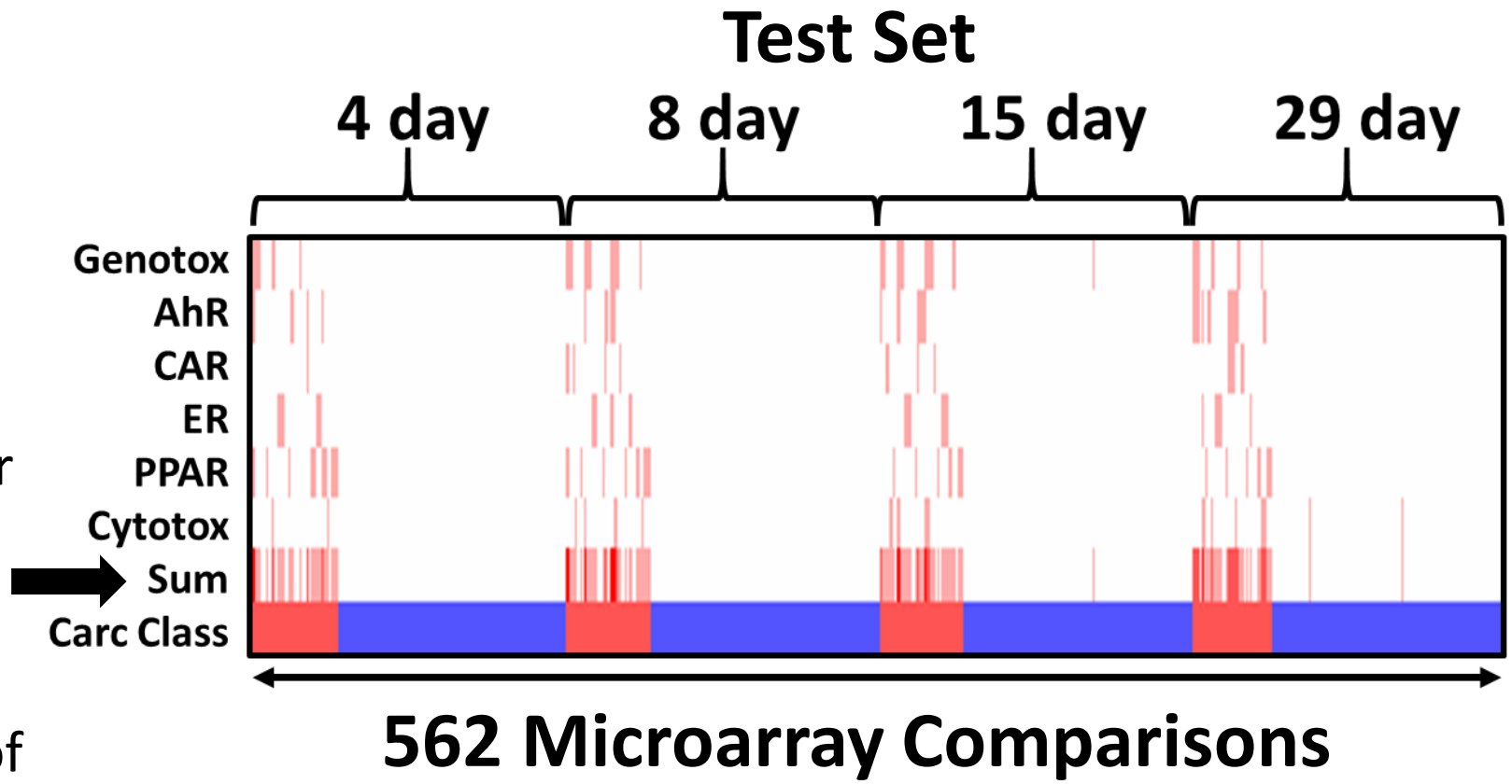




- From Corton et al. (2020). A Set of Gene Expression Biomarkers Identify Rat Liver Tumorigens in Short-Term Assays. *Tox Sci.* In press.

Biomarker Activation Levels Accurately Predict Liver Cancer

- Derived activation levels associated with tumor induction from a training set and then applied to a test set
- Each red line is a chem-dose condition in which the biomarker tumorigenic level is surpassed
- Most of the tumorigenic conditions exceed one or more of the 6 activation levels
- Activation levels rarely exceeded in any of the nontumorigenic conditions



- **<u>Test set</u>: 100% sensitivity, 94% specificity, and a balanced accuracy of 97%**

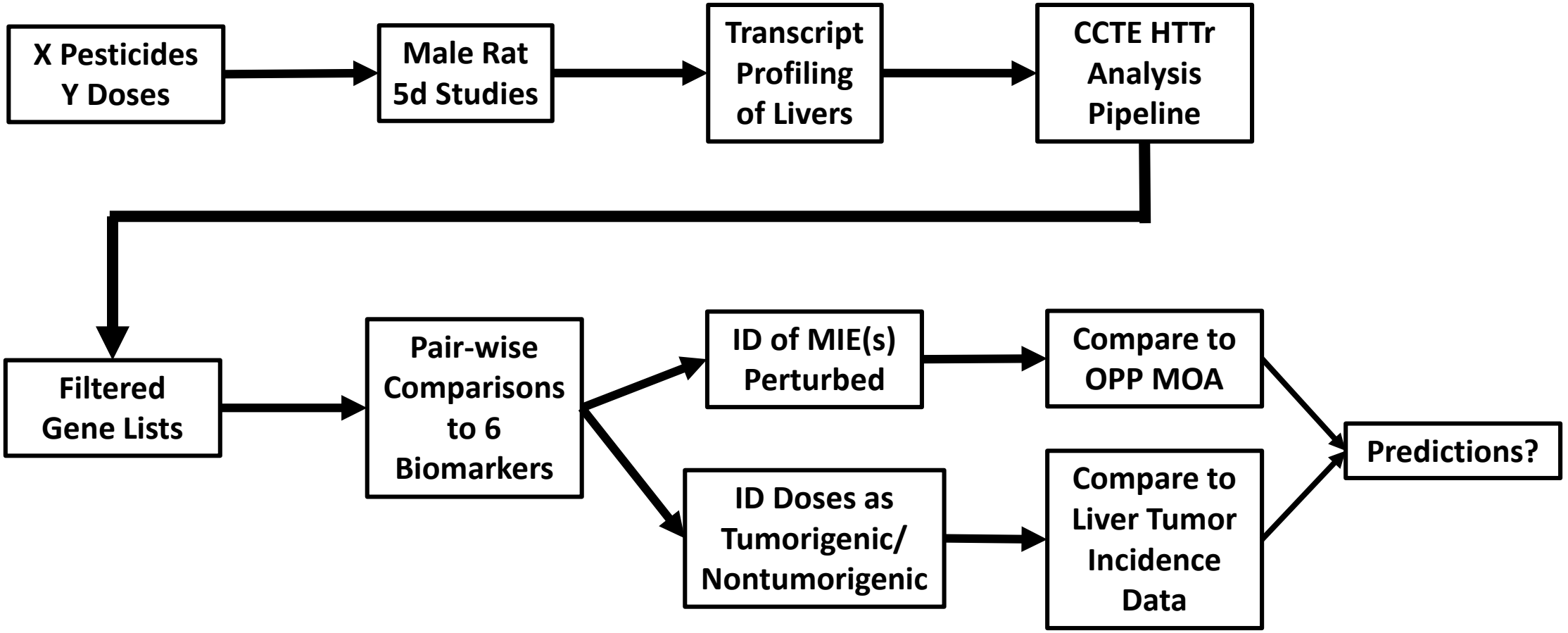
Tumorigenic
Nontumorigenic

From Hill et al. Tox Sci In press

OPP-CCTE Project

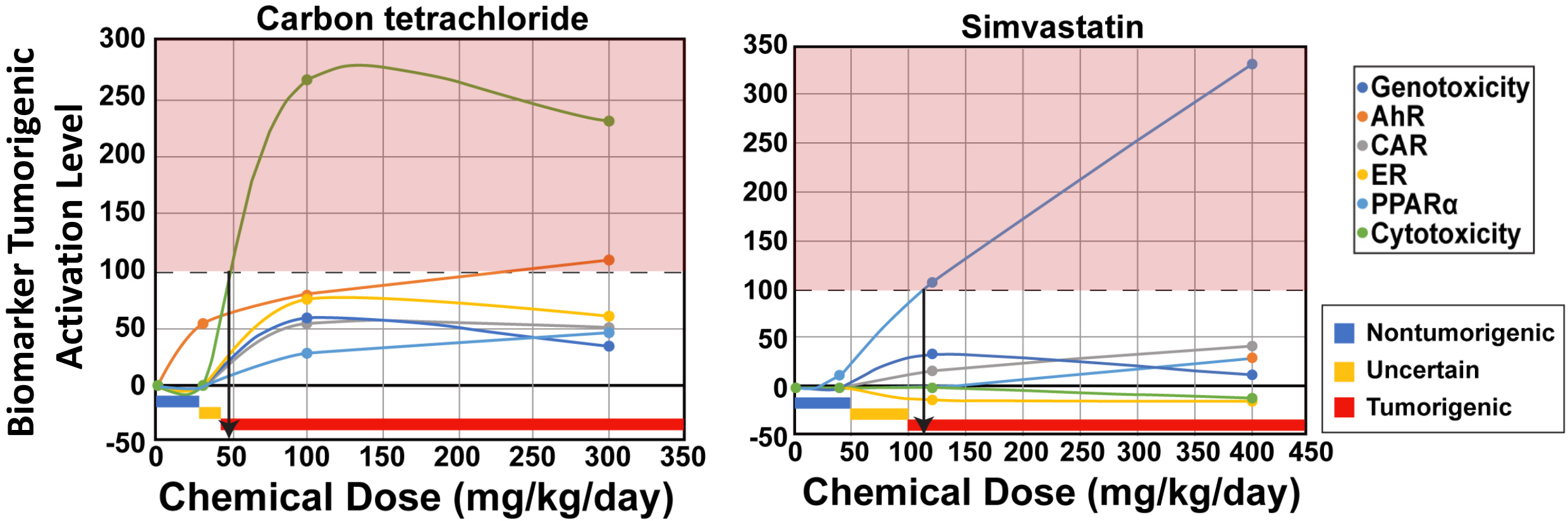
Using biomarkers can we predict from short-term studies of pesticides:

- **Mode of action by which the tumors would arise?**
- **Chemical-dose combinations that will cause tumors?**



Application of Biomarkers and Activation Levels to Model Liver Tumorigens

- Chemicals examined in the TG-GATES study in male rats for 15d at 3 doses



- Approach identifies the MOA and the lowest tumorigenic dose

From Hill et al. Tox Sci. In press

Summary

- An AOP-guided computational approach can be used to identify liver tumorigens in prospective studies
  - Two sets of tools to apply to toxicogenomic studies
    - Gene expression biomarkers
    - Activation levels associated with tumor induction

Emerging Systems Toxicology for the Assessment of Risk Committee (eSTAR)
Carcinogenomics Subcommittee
Co-chairs: Chris Corton, EPA
Keith Tanis, Merck

- The 6 biomarkers could identify chemical-dose pairs from tumorigenic treatments (balanced accuracy = 93%).

- Biomarker activation levels could identify chemical-dose pairs from tumorigenic treatments (balanced accuracy = 97%).

- Will perform a case study on pesticides with known MOA to evaluate the application of the approach.

Acknowledgements

Environmental Protection Agency

John Rooney
Natalia Ryan
Brian Chorley
Thomas Hill
Keith Houck
Grace Patlewicz
Mark Nelms
Jerry Liu
Beena Vallanat
Carlton Jones
Hisham El-Masri
Jaleh Abedini
Jason Brown
Katie Paul-Friedman
Charles Wood

Health Canada

Carole Yauk
Andrew Williams

University of Leiden

Bob van de Water
Steve Hiemstra

PamGene

Rinie van Beuningen
Rene Houtman

City of Hope Medical Center, Duarte

Shiuan Chen
Gregory Chang

Merck

Frank Sistare
Chunhua Qin

NIEHS

Nicole Kleinstreuer
Ying Liu
Cari Martini
Shihan He
Jui-Hua Hsieh

Duke University

Abigail Jackson
Katharine Korunes

National Center for Advancing Translational Sciences

Ruili Huang
Menghang Xia

Indiana Biosciences Research Institute

Jeff Sutherland
Jim Stevens