# A New Approach Methodology for the Prediction of Tumorigenesis in the Rat

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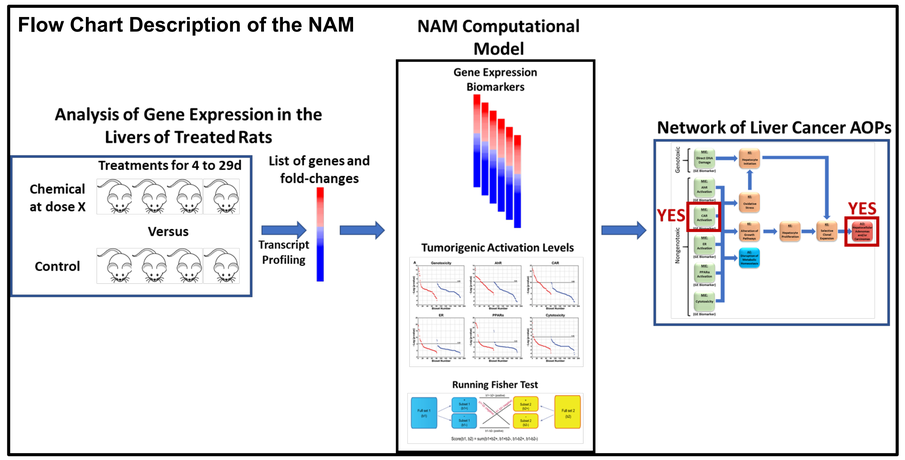
## Abstract

Cancer is the 2nd leading cause of death in the U.S., putting tremendous pressure on the economy. Tens of thousands of chemicals on the market have not been adequately tested for cancer hazard. Testing using the "gold standard" 2-year cancer bioassay is not feasible to fill this data gap. Thus, new approach methodologies (NAMs) are necessary to assess potential carcinogenicity of uncharacterized chemicals. The present study shows how a NAM can be used to identify chemicals and their doses that could cause liver cancer in rats without having to conduct a 2-year bioassay. The NAM uses 6 previously established gene expression biomarkers and tumorigenic activation levels (TALs) to interpret transcript profiles derived from the livers of treated rats. The NAM can identify the chemical mode of action and (non)tumorigenic doses. While this NAM cannot completely replace rodent bioassays, this approach can be used to determine if the 2-year bioassay is necessary potentially reducing cost, time, and resources needed.




## Methods

***Rat Short-term Exposure Studies***

- Male SD rats exposed once daily by oral gavage; liver, blood collected; liver RNA analyzed
  - 4-Day Study: 22 chemicals, 1 dose; Affymetrix platform
  - 5-Day Study: 16 chemicals, up to 10 doses; full genome TempO-Seq platform
  - RNA-Seq Comparison Study: 27 chemicals, 1 dose for 3, 5 or 7 days; Affymetrix and Illumina RNA-Seq platforms

***Determination of Chemical Hepatocarcinogenicity***

- Lhasa Carcinogenicity Potency Database and ToxRef database were used to annotate liver effect incidences of 2-year cancer bioassays: hepatocellular carcinomas, adenomas; multiple liver tumor types; neoplastic nodules; trabecular hepatocellular carcinomas; hepatocellular cholangiocarcinomas
- Doses used in transcript profile studies were annotated for highest nontumorigenic dose and lowest tumorigenic dose (5% over baseline considered tumorigenic)

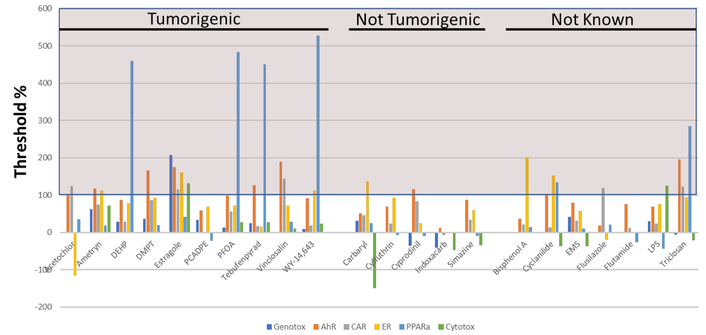
***Comparison of Biomarkers to Gene Lists***

- Gene lists created during rat studies uploaded into BaseSpace Correlation Engine (BSCE) with 6 previously established biomarkers: AhR, CAR, PPARα, ER, cytotoxicity, genotoxicity
- BSCE ranked genes by absolute fold-change; Running-Fisher test compared biomarker gene ranks against gene ranks from the rat studies; correlations were made on the overlapping genes to obtain p-values converted to -Log(p-values)

***Application of Biomarker TALs***

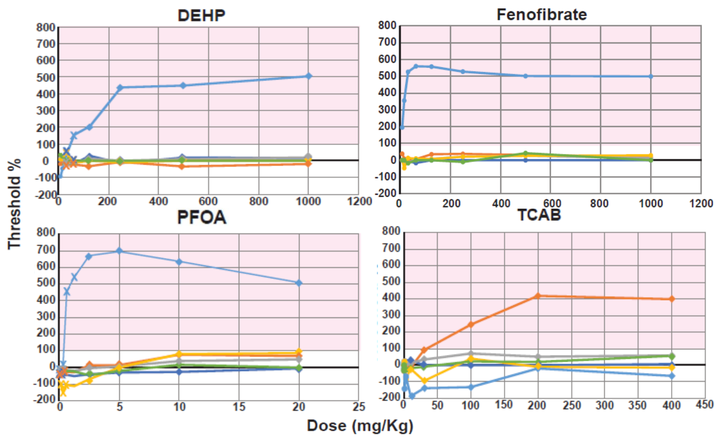
- Defined as highest -Log(p-values) that did not induce tumors derived in previous studies using a large genomic database derived from the livers of rats treated with ~130 chemicals at 3 dose levels for 8 time points (TG-GATES)
- Biomarker -Log(p-values) from the 3 studies were evaluated relative to the TALs for the 6 biomarkers to determine if short-term exposure to a chemical-dose pair exceeded the biomarker activation level.

## Biomarker tumorigenic activation levels identify tumorigenicity of chemical-dose pairs in 4-day short-term exposure study




The 6 biomarkers were compared to the transcript profiles from the short-term rat exposure studies. The -Log(p-value)s – the correlation between the chemicals and the biomarkers – were compared to the tumorigenic activation levels. The y axis represents the (biomarker -Log(p-value)/the tumorigenic threshold) x 100. Any -Log(p-value) >100% for any of the biomarkers is predicted to increase liver tumorigenicity in a chronic exposure.

## Prediction of chemical doses that would increase liver tumors in rats in 5-day short-term exposure studies




Gene expression was evaluated using targeted RNA-seq (TempO-Seq), and the derived gene lists were compared to the 6 biomarkers using the Running Fisher test. The -Log(p-value)s – the correlation between the chemical-dose pair and the biomarkers – were compared to the tumorigenic activation levels. The y axis represents the (biomarker -Log(p-value)/the tumorigenic threshold) x 100. Any -Log(p-value) >100% for any of the biomarkers is predicted to increase liver tumorigenicity in a chronic exposure. Symbols: diamond (tumorigenic), filled circle (non-tumorigenic), X (unknown tumorigenicity). Note: only 4 of the 16 chemicals are shown.

## Predictive accuracies derived using this NAM

| Study | Unit of prediction | Tumorigenic activation level | Total Number of Biosets or Chemicals Examined | TP | TN | FP | FN | Sensitivity | Specificity | PPV | NPV | Balanced Accuracy |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rat 4-day study | Chemical-Dose | TG-GATES | 13 | 8 | 3 | 1 | 1 | 0.889 | 0.75 | 0.889 | 0.75 | 0.819 |
| Rat 4-day study | Chemical-Dose | DrugMatrix | 13 | 7 | 4 | 0 | 2 | 0.778 | 1 | 1 | 0.667 | 0.889 |
| Rat 5-day Study | Chemical-Dose | TG-GATES | 100 | 31 | 51 | 7 | 11 | 0.738 | 0.879 | 0.816 | 0.823 | 0.809 |
| Rat 5-day Study | Chemical-Dose | DrugMatrix | 100 | 22 | 56 | 2 | 20 | 0.524 | 0.966 | 0.917 | 0.737 | 0.745 |
| Rat 5-day Study | Chemical | TG-GATES | 16 | 11 | 3 | 2 | 0 | 1 | 0.6 | 0.846 | 1 | 0.8 |
| Rat 5-day Study | Chemical | DrugMatrix | 16 | 9 | 5 | 0 | 2 | 0.818 | 1 | 1 | 0.714 | 0.909 |

The TALs of the 6 biomarkers (derived using the TG-GATES and DrugMatrix studies) were used to predict hepatocellular adenomas and/or carcinomas. The balanced accuracy is up to 91%.

## Summary

The NAM can be used in a number of testing conditions to accurately identify chemicals and their doses that would induce tumors in the livers of rats

- The NAM can predict tumorigenicity (82% or 89% accuracy) using Affymetrix transcript profiling of the livers of rats exposed to chemicals at one dose for 4 days
- Transcript profiles of Affymetrix and RNA-seq have comparable results in biomarker TAL ranges of -Log(p-values) which means the TALs can be used with RNA-seq data (data not shown)
- Using TempO-Seq transcript profiles, the NAM can predict tumorigenicity of chemicals and their dose levels (80-91% accuracy)

The NAM can identify

- Doses that would induce tumors in the livers of rats
- The mode of action of the chemical
- By inference from the identified mode of action, the human relevance of the predictions would be based on current understanding

This information (along with histopathology, genetic toxicity, and hormonal perturbation) could be used to determine whether a 2-year bioassay is warranted or if the information supports a waiver from requiring a 2-year bioassay

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## Disclaimer