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National Toxicology Program

Overview of the NTP Approach to Genomic Dose-Response (GDR) Modeling Report

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NTP and DTT

National Toxicology Program

(Headquartered administratively at NIEHS)

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NTP Director

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- FDA/NCTR
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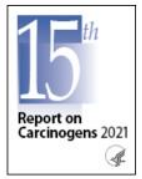
NTP/DTT Products



Report on Carcinogens
A congressionally mandated public-health document about agents that cause cancer. Go »

Technical Reports
Long-term studies of the toxicologic potential of agents in test animals. Go »

Developmental & Reproductive Toxicity Reports
Reports about the potential of agents to affect test animal development and reproduction. Go »

Immunotoxicity Reports
Reports about the potential of agents to damage the immune systems of test animals. Go »

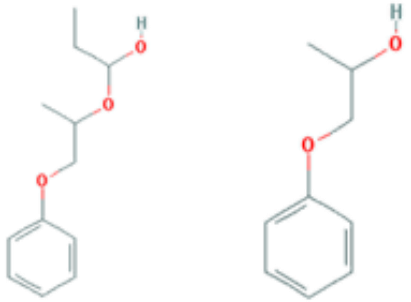
Monographs
Completed assessments of the cancer and noncancer health effects of various agents. Go »

Research Reports
NTP reports that fall outside the scope of other report series. Go »

Toxicity Reports
Short-term studies of the toxicologic potential of agents in test animals. Go »

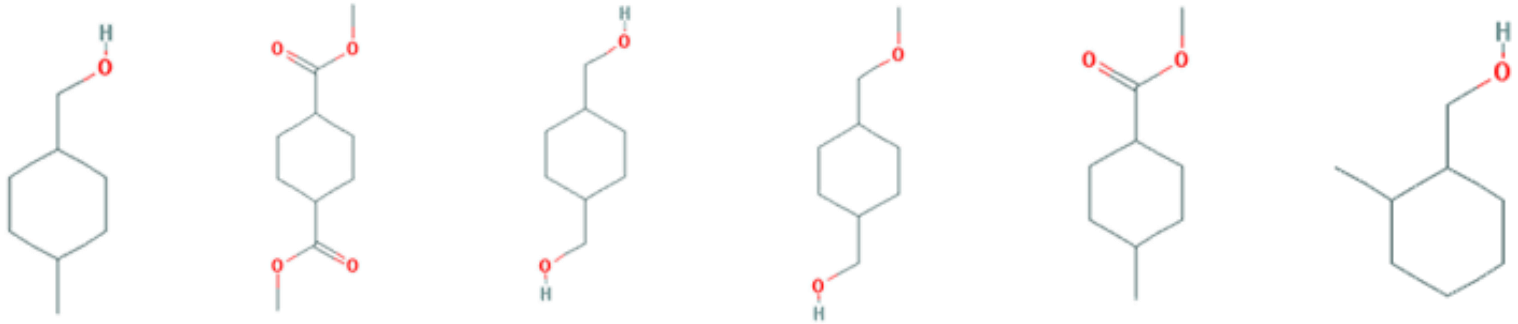
NTP's first attempt at using GDR – Elk River Spill

Stripped PPH



Class: Glycol ethers

Crude MCHM



Class: Alicyclic alcohols

NTP nomination

- Evaluate developmental toxicity
- Determine accuracy of the **point of departure (100 mg/kg/day)** used for drinking water advisory level
- Do it in **one year**

Opportunity to test out the 5-day genomic dose response study

Need for standardized approach to

- Study design
- Analysis
- Reporting

Expert Panel Review of the NTP GDR Analysis Pipeline

Peer Review of Draft NTP Approach to Genomic Dose-Response Modeling

NTP has developed a draft approach using BMDExpress software to perform gene and pathway-level genomic dose-response modeling as part of Tox21 Phase 3 and in vivo screening level studies. NTP's approach considers a number of factors including methods accepted in the peer-review literature, ease of translation to risk assessment, and ease of understanding for the variety of potential end users that may not necessarily be experts in mathematical and systems modeling.

An external panel will provide NTP scientific input on its proposed approach at a public meeting. Prior to the meeting, NTP will host four webinars that present different approaches to genomic dose-response modeling.

Peer Review Meeting

Dates: October 23-25, 2017
Location: Rodbell Auditorium, David P. Rall Building, NIEHS, Research Triangle Park, North Carolina

- Agenda
- Registration
  - Attend in-person
  - View the webcast
- Panel roster
- Charge
- Draft NTP Approach to Genomic Dose-Response Modeling
- Public comments and related information
  - Guidelines for public comments
- Presentations
- Federal Register notice (PDF HTML)

Meeting Webpage:
https://ntp.niehs.nih.gov/about/org/ntpexpertpanel/index.html

Expert Panel

- Carole Yauk (Chair; Health Canada)
- Lyle Burgoon (US Army)
- Ruili Huang (NCATS)
- Kamin Johnson (Dow)
- Rebecca Clewell (Scitovation)
- Jorge Naciff (P&G)
- Setia Pramana (Institute of Statistics)
- James Stevens (Eli Lilly)
- Fred Wright (NC State)

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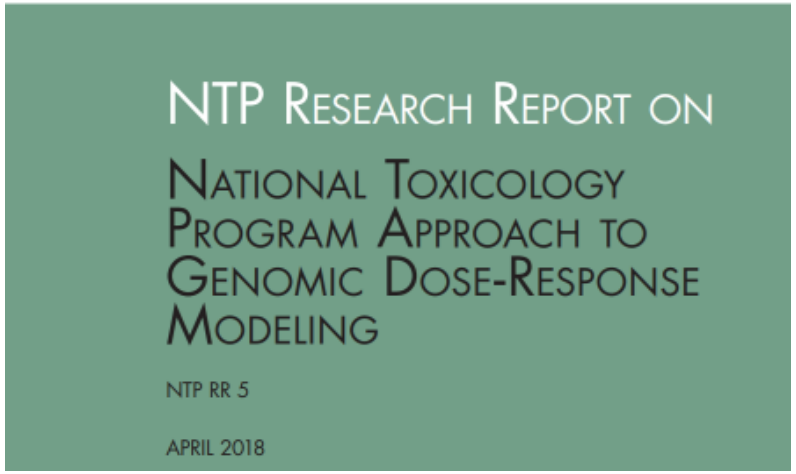
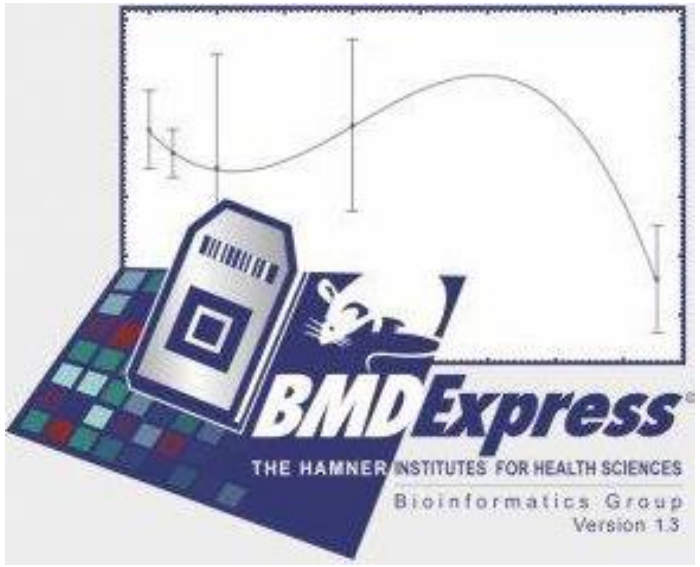


https://ntp.niehs.nih.gov/ntp/results/pubs/rr/reports/rr05_508.pdf

Software to support analysis

2007



2015

Hamner to NTP/DTT

2018

Bioinformatics, 35(10), 2019, 1780–1782
doi: 10.1093/bioinformatics/bty878
Advance Access Publication Date: 17 October 2018
Applications Note

OXFORD

BMC Genomics

BioMed Central

Software

Open Access

BMDExpress: a software tool for the benchmark dose analyses of genomic data

Longlong Yang[1], Bruce C Allen[2] and Russell S Thomas*[1]

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* Corresponding author

Gene expression

BMDExpress 2: enhanced transcriptomic dose-response analysis workflow

Jason R. Phillips[1], Daniel L. Svoboda[1], Arpit Tandon[1], Shyam Patel[1], Alex Sedykh[1], Deepak Mav[1], Byron Kuo[2], Carole L. Yauk[2], Longlong Yang[3], Russell S. Thomas[4], Jeff S. Gift[5], J. Allen Davis[6], Louis Olszyk[7], B. Alex Merrick[8], Richard S. Paules[8], Fred Parham[8], Trey Saddler[8], Ruchir R. Shah[1] and Scott S. Auerbach[8,*]

Overview of approach to GDR studies

Study Design

Many dose levels, limited biological replication, select target organs

BMDExpress 2.0 (now 3.1)

Filtering Measured Features

Statistical and effect size filter

Fitting Features to Dose-Response Models

Fit multiple models, determine potency using best fit model

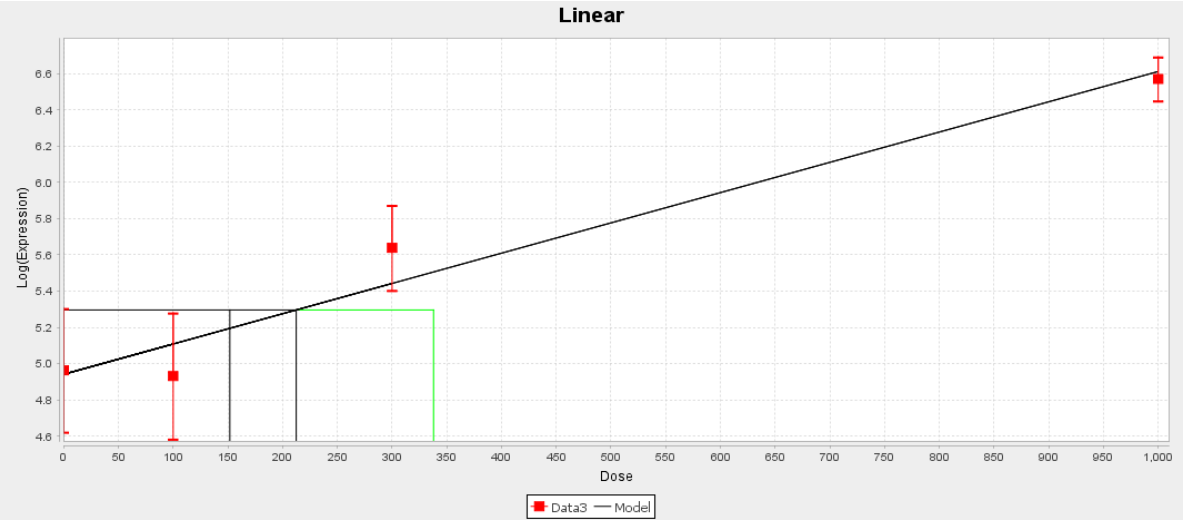
Determination of Gene Set Level Potencies

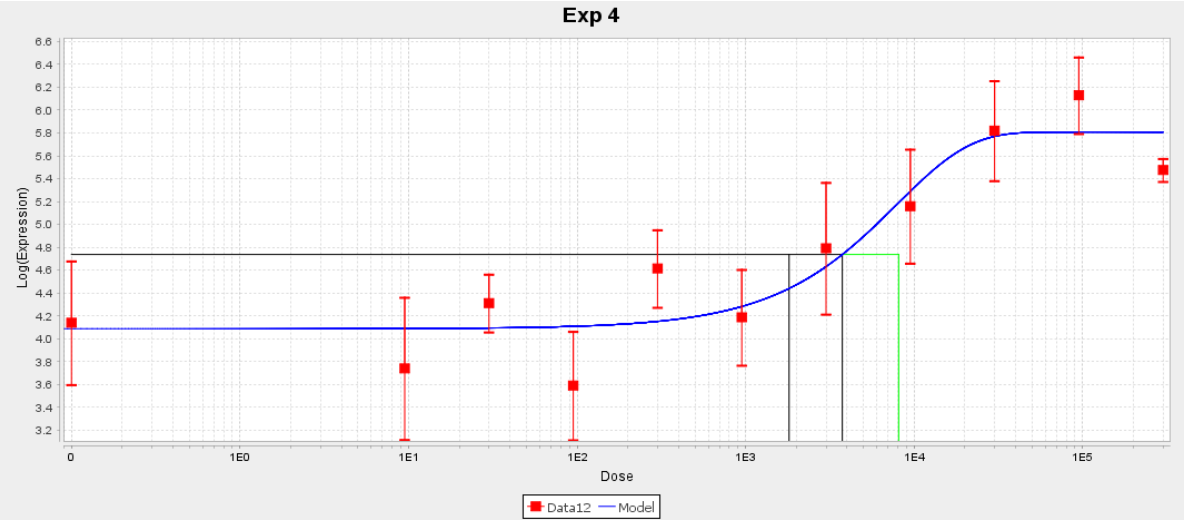
Identify “active” gene sets, report potency for active gene set

Determine a Transcriptomic Point of Departure

Identify the most potent gene set based on median BMD, no bio or tox interpretation

NTP's Proposed Approach to Study Design for Genomic Dose-Response





Study design

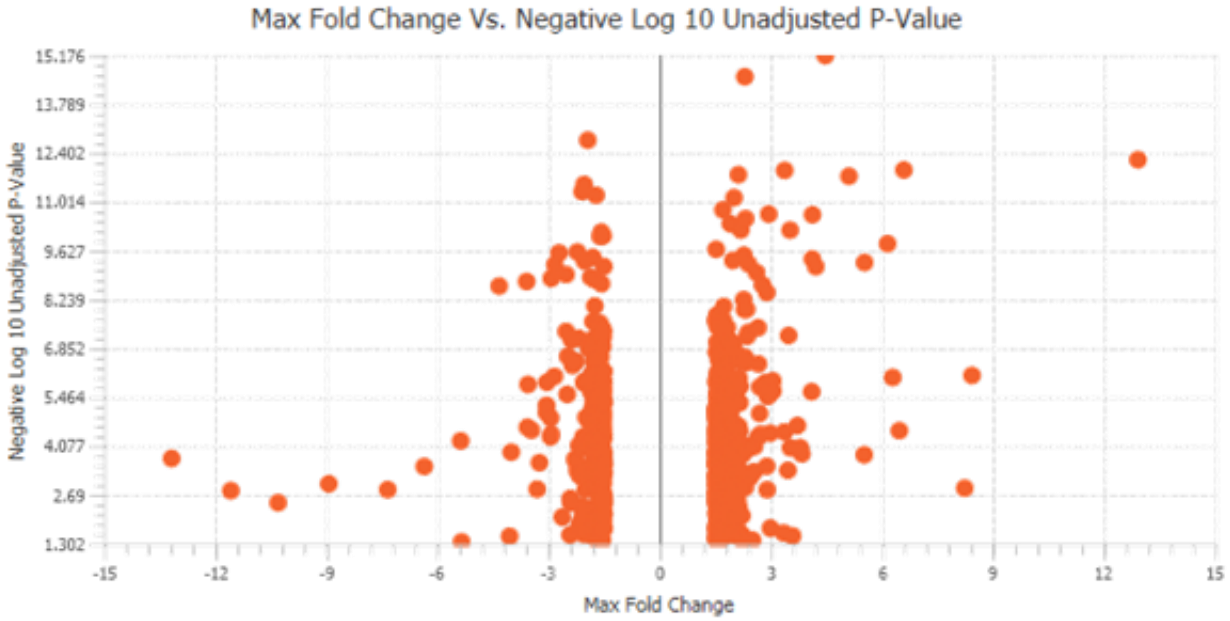
- Traditional guideline study
  - Example design: 3 dose levels and control, 10 biological replicates/dose group
  - Powered for pair-wise analysis
  - Not ideal for a dose-response modeling of high-dimensional data
- NTP GDR study
  - More dose levels fewer biological replicates
  - Will allow for better coverage of the numerous dose-response relationships in each study, more confident fits of the data and greater certainty in the BMD estimates for the features
  - Empirical demonstration of the proposed study design for BMD modeling

- **Guideline studies**: Slob W, Moerbeek M, Rauniomaa E, Piersma AH. A statistical evaluation of toxicity study designs for the estimation of the benchmark dose in continuous endpoints. Toxicol Sci. 2005;84:167–185.
- **Transcriptomic studies**: Ewald JD, Basu N, Crump D, Boulanger E, Head J. Characterizing Variability and Uncertainty Associated with Transcriptomic Dose-Response Modeling. Environ Sci Technol. 2022 Nov 15;56(22):15960-15968

In vivo study design parameters

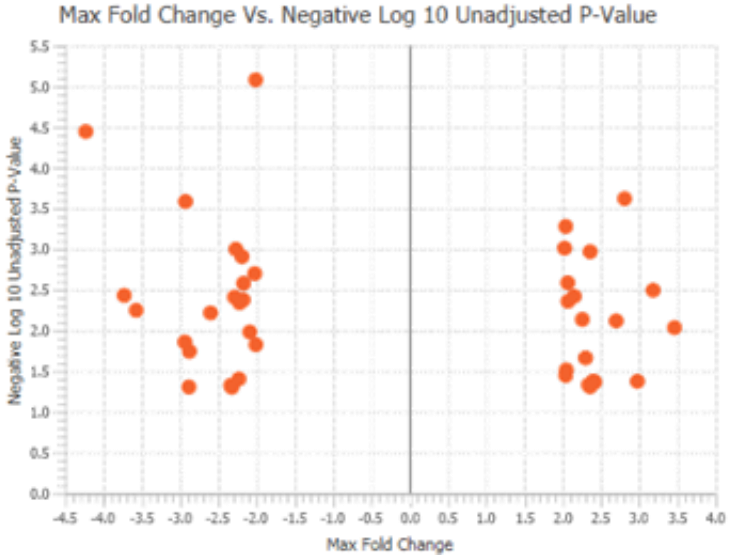
- **Sex/Strain/Species:** Male and/or female Sprague Dawley Rat
  - Historical precedent, legacy data that will help with interpretation
- **Duration:** 5 Days (5 doses, 1 per day, Euthanize 24 hours after last dose)
  - Thomas *et. al,* 2013, showed transcriptional POD from 5 days approximated PODs from apical endpoints including cancer
- **Dose levels**: ≥5 dose levels, 3 animals per dose level
  - >5 dose levels assuming no limited knowledge of toxicological potency
  - 3 animals to ensure there is representation of the variance of response at each dose level
- **Target organ selection:** Liver and expert selected targets
  - Most studies will be done by the oral route
  - Liver is common target organ and often responds to effects in other organs/tissues (i.e., sentinel organ)
  - Other organs selected based on expert review of available data
- **Top dose selection:** 5-day Maximum Tolerated Dose
  - To ensure clear response at the top dose level and ensure the identification of responsive features and improved model fitting

NTP's Proposed Approach to Filtering Unresponsive Genes (Prefilter)



Step 1: Is there any signal?

- One way ANOVA with Benajmini-Hochberg Correction p<0.05
  - If one feature passes, then it is deemed that there is signal in the data
  - Done to avoid modelling noise
  - Not intended to for identifying probes/genes for modeling
    - Use of a trend test in more appropriate to identifying dose-responsive features



Step 2: Finding the dose-responsive features

- Combine a trend test statistical filter with an effect size filter
  - When parameters are integrated into the full analysis pipeline it should….
    - Minimizes the false discovery at a gene set level
    - Maximizes true discovery and repeatability of findings
  - Consistent with MAQC recommendations for maximizing repeatability of differentially expressed genes across laboratories
- Optimal prefilter parameters may differ with technology, sample source and study design
  - Essential to perform empirical characterization identify optimal study parameters
    - Modelling “null” / “sham” data to determine rates of false discovery
    - Using repeat studies to characterize repeatability and maximizing true signal
  - Can involve modification of other parameters in other steps in the analysis pipeline (e.g., active gene set thresholds)

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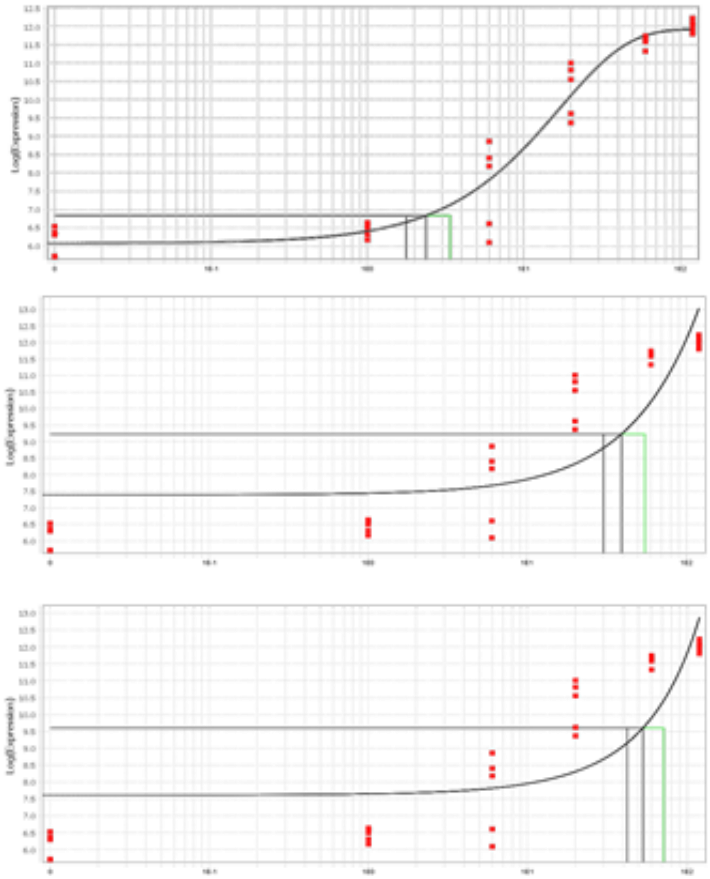
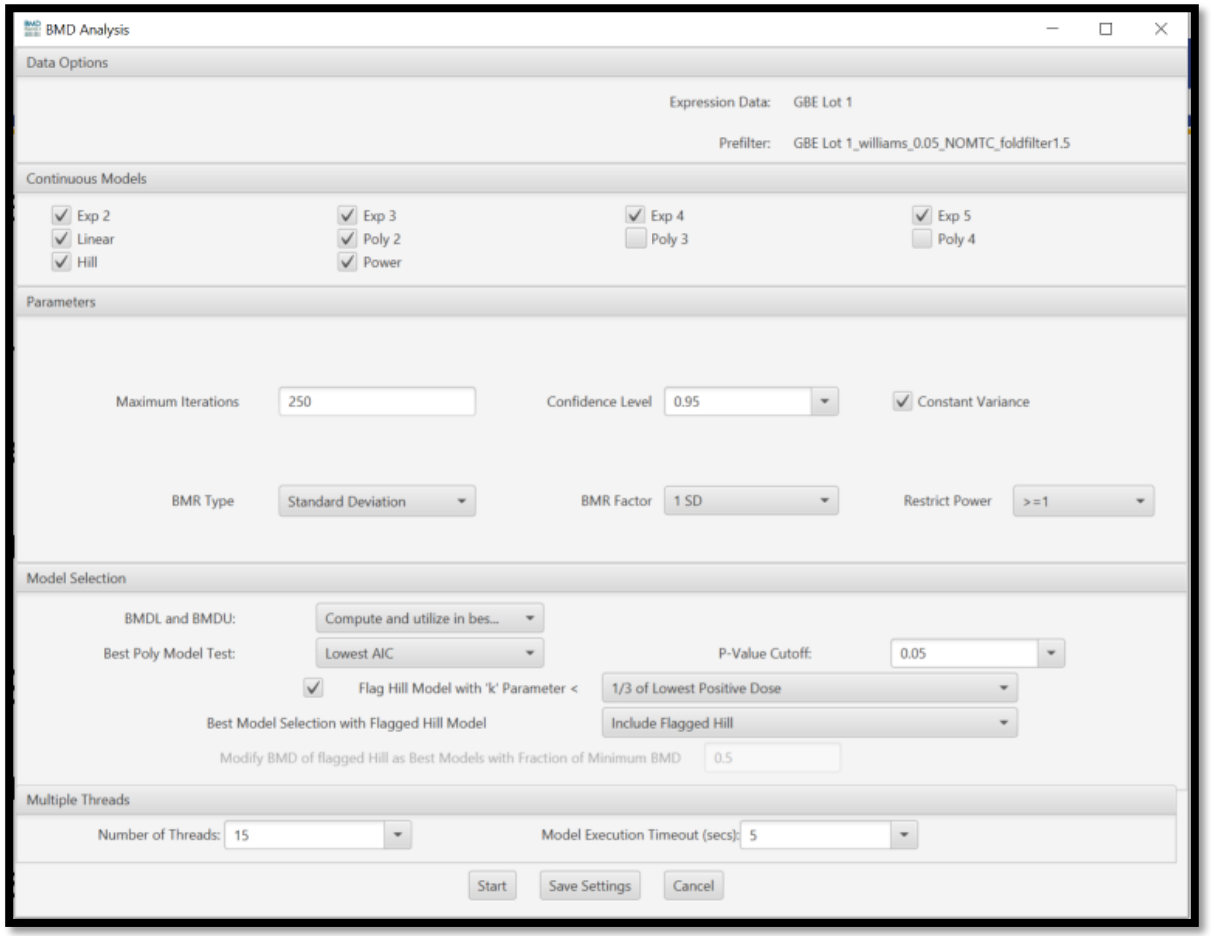
NTP's Proposed Approach to Curve Fitting and Determination of Feature Potency

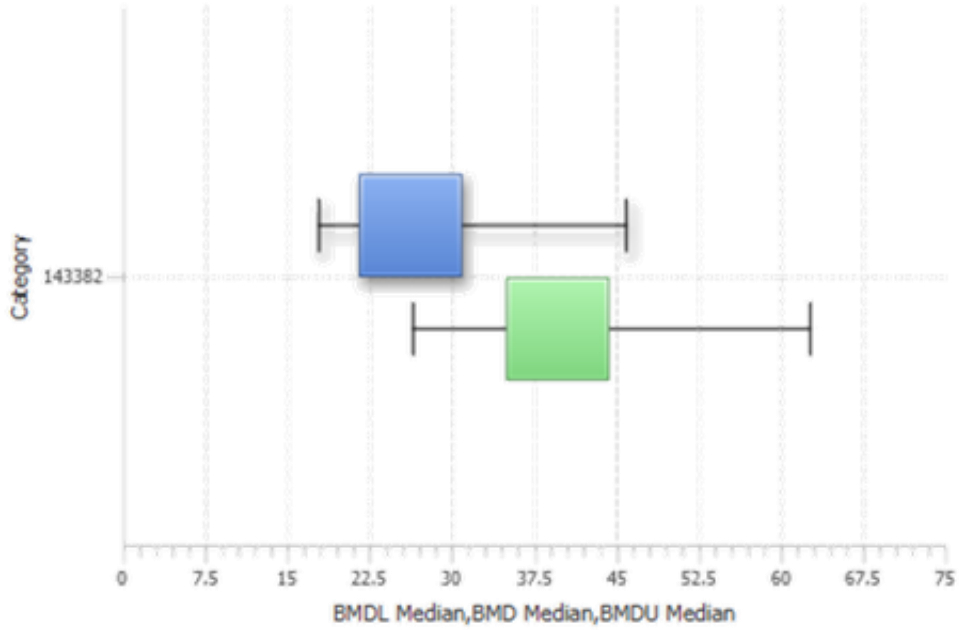


Fitting features to dose-response curves

- Features are fit to 8 parametric continuous models
  - Directly from US EPA's BMD software
  - Hill, Power, Linear, Poly2, Exp2,3,4,5
  - Use of variety of models to fit the data is consistent with EPA guidance
- BMR is based on a 10% change in tail distribution
  - Single tail = 1 SD; Two tail = 1.349 SD
  - Consistent with EPA BMD analysis guidance
- Best model selection
  - Lowest AIC
    - Based on EPA BMD analysis guidance
- From the best fit model a BMD, BMD_L and BMD_U is determined

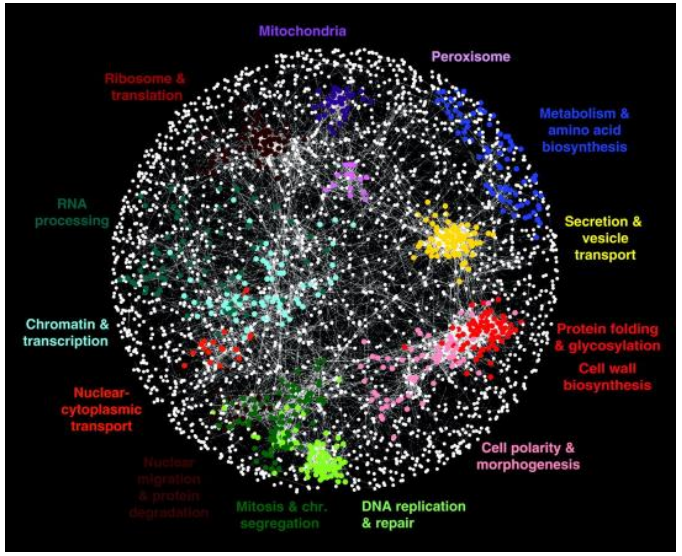


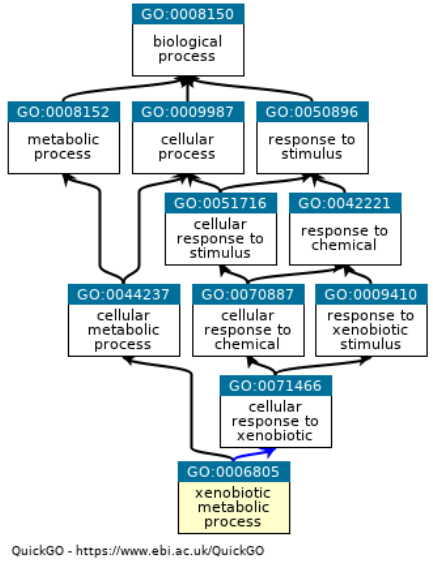
NTP's Proposed Approach to Estimating Gene Set Level Potencies



What gene set and why?

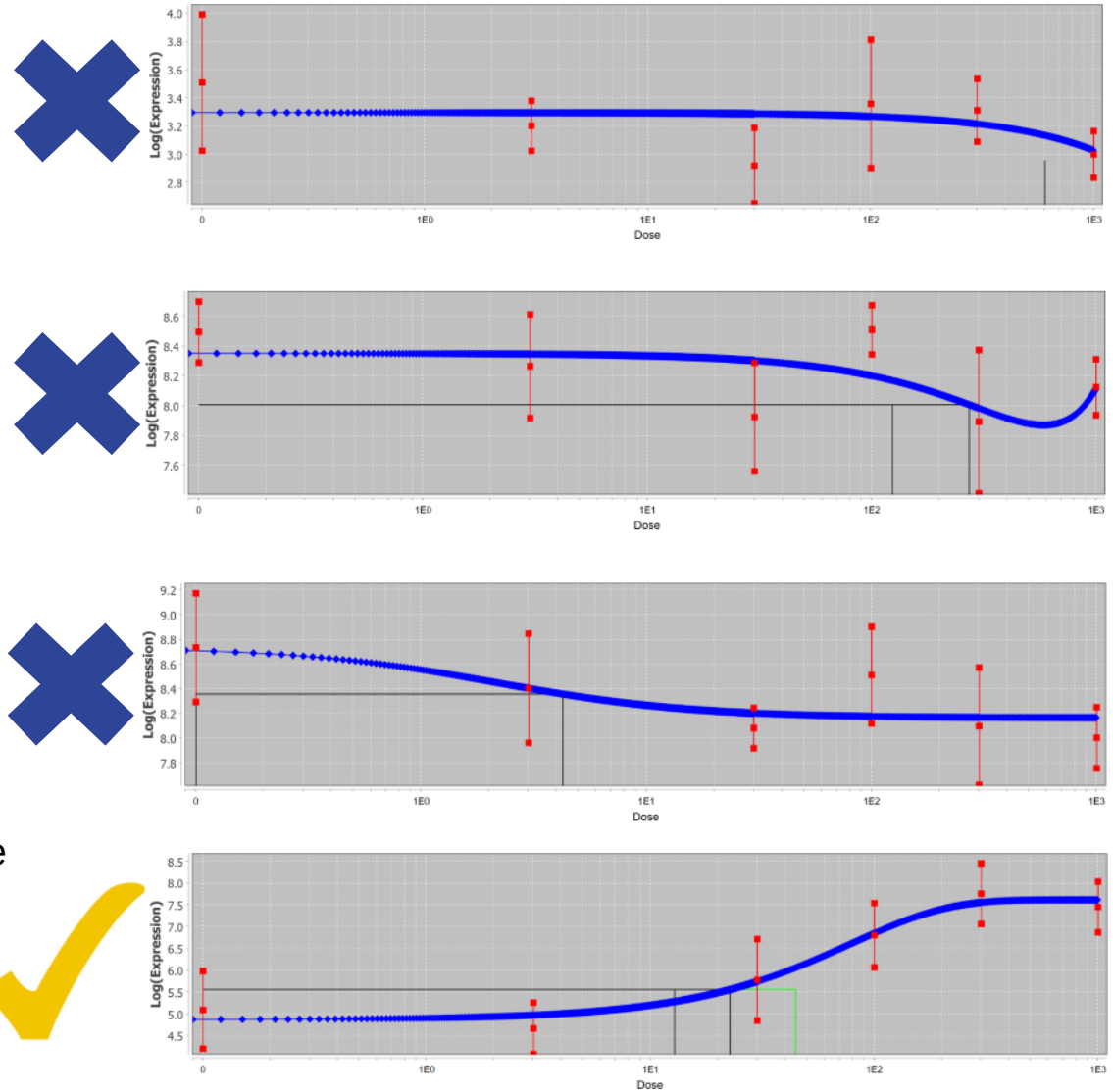
- Gene Ontology Biological Processes
  - Largest of the gene ontologies
  - Offers the most comprehensive coverage of biological space of all annotated gene sets
  - Biological processes are place in a hierarchy to allow multiple granularity levels of biological processes
  - Genes are grouped by biological function hence changes in genes in the gene sets are suggest of biological change





Filtering fitted features before populating gene sets

- For a feature to be considered its best model must…
  - Have convergent BMD, BMD_L and BMD_U values
    - Indicates model parameters are optimized
    - Ensures complete representation of the uncertainty around the BMD
  - Not map to more than one gene
    - Removes features with uncertain gene association
  - Not have a BMD> highest dose
    - Avoids model extrapolation
  - Have a global goodness of fit p-value >0.1
    - Higher values indicate better fit
    - Ensures fit of the model to the data and is consistent w/ EPA guidance
  - BMD_U/BMD_L < 40
    - Removes features with highly uncertain BMDs
    - Accounts for entire confidence interval vs BMD/BMDL



Identifying active gene sets and potency

Gene Set 1 (15 genes)

Gene Name	BMD	BMD_L	BMD_U
Gene 1	10	5	25
Gene 2	50	25	70
Gene 3	100	75	120
Gene 4	150	100	175
Gene 5	200	100	210
Gene 6	Failed fit filter	Failed fit filter	Failed fit filter
Gene 7	Failed fit filter	Failed fit filter	Failed fit filter
Gene 8	Failed fit filter	Failed fit filter	Failed fit filter
Gene 9	Failed fit filter	Failed fit filter	Failed fit filter
Gene 10	Failed fit filter	Failed fit filter	Failed fit filter
Gene 11	Failed fit filter	Failed fit filter	Failed fit filter
Gene 12	Failed fit filter	Failed fit filter	Failed fit filter
Gene 13	Failed fit filter	Failed fit filter	Failed fit filter
Gene 14	Failed fit filter	Failed fit filter	Failed fit filter
Gene 15	Failed fit filter	Failed fit filter	Failed fit filter

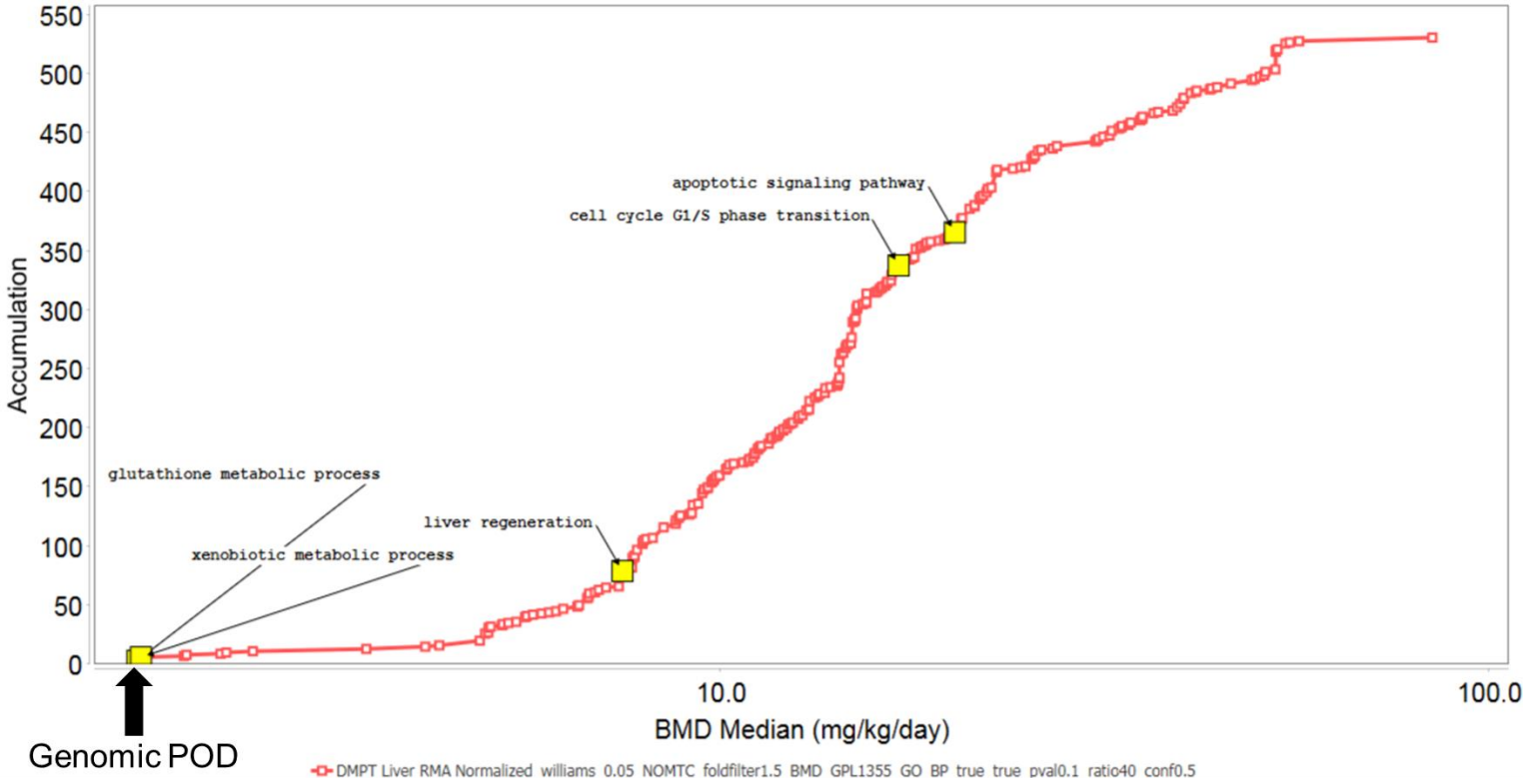
( ) = Median value = Gene Set BMD, $BMD_{L,U}$

- At least 3 genes
  - Ensure that small gene sets are minimally populated
  - Minimum number of genes to identify a median value
- At least 5% populated
  - Ensure larger gene sets require more than 3 genes
- Active gene set parameters may be platform specific
- Gene set potency = median BMD
  - Buffers effects of extreme BMD values in estimating potency

NTP's Proposed Approach to Selecting a Point of Departure and Biological Interpretation

Selecting the genomic/transcriptomic point of departure

- Identify the active gene set with lowest median BMD
  - Empirical findings show the most sensitive gene set as determined by the BMD Median generally agrees with apical points of departure
  - Precautionary approach is applied due to short duration of the study



Biological interpretation

- Until a formalized method for biological interpretation is available refrain from providing potentially misleading biological/toxicological interpretation
- Gene set names can be misleading and mischaracterized as to be associated with formal toxicological hazards
- Focus on reporting genomic/transcriptomic potency

liver regeneration

DNA damage response

cell death

chronic inflammatory response

cell dedifferentiation

Summary

- NTP held an expert panel meeting to peer review its approach to GDR studies, modelling and interpretation
- Design studies for dose response modeling not pairwise analysis
- ANOVA pretest for signal detection and a combine a statistical and effect size in prefilter analysis
- Empirically identify optimal prefilter parameters for the data being analyzed
- Due to the scale and complexity of genomic data use models that accommodate a variety of shapes
- Use a benchmark response that approximates a 10% change per EPA recommendation
- Filter fitted genes for accuracy of fit and uncertainty before gene set analysis
- In gene set analysis we use gene sets that have a broad biological coverage (GO Biological Processes)
- Use a median of the active gene BMDs when reporting the potency of an active gene set
- The gene set with the lowest median BMD is reported as genomic/transcriptomic point of departure
- Avoid biological interpretation until there is more formalized process in place
- This is an “ever green” study design and pipeline; hence substantial improvements will be incorporated in future iterations of the analysis pipeline