NTP
National Toxicology Program

NTP Research Project: West Virginia Chemical Spill

Scott S. Auerbach, Ph.D., DABT
National Institute of Environmental Health Sciences

NTP Board of Scientific Counselors Meeting
December 10, 2014

Outline

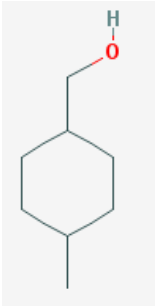
- Background on chemical spill
- Timeline of early activities
- Overview of proposed NTP studies and results to date
- Timeline for future NTP activities
- NTP communications

Background

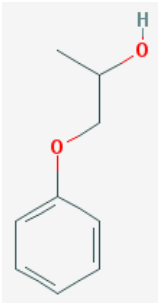
- January 2014
  - 10,000 gallons of a liquid used to wash coal and remove impurities that contribute to pollution during combustion were spilled from a leaking tank into the West Virginia Elk River
  - Water supply of nearly 300,000 people within nine counties in the Charleston, West Virginia metropolitan area was contaminated
  - Reports of licorice odors at homeowner taps and hospital admittances indicated the population was exposed to the contaminated tap water
  - Health effects primarily involved rashes and skin irritation; however, respiratory illnesses, nausea, and diarrhea were also reported

Chemicals in the spill

- Crude MCHM
  - 4-Methylcyclohexanemethanol (MCHM; 34885-03-5)
  - 1,4-Cyclohexanedimethanol (CHDM; 105-08-8)
  - 2-Methylcyclohexanemethanol (2MCHM; 2105-40-0)
  - 4-(Methoxymethyl)cyclohexanemethanol (MMCHM; 98955-27-2)
  - Methyl 4-methylcyclohexanecarboxylate (MMCHC; 51181-40-9)
  - Dimethyl 1,4-cyclohexanedicarboxylate (DMCHDC; 94-60-0)
  - Methanol
- Others
  - Dipropylene glycol phenyl ether (DiPPH; 51730-94-0)
  - Propylene glycol phenyl ether (PPH; 770-35-4)



MCHM



PPH

Timeline of early activities

- January 2014
  - NTP performs preliminary SAR analysis of chemicals identified in spill
  - Analysis suggest chemicals are of limited toxicological concern
  - CDC uses Eastman 28-day study to establish a drinking water advisory level (DWAL) of 1 ppm (0.1 mg/kg/day for a child) for MCHM
  - CDC uses manufacturer teratology study to establish a DWAL of 1.2 ppm for PPH
    - Suggestion that DiPPH should be similar
- July 2014
  - Spilled chemicals nominated by CDC/ATSDR
    - "A research effort aimed at providing meaningful information to public health decision-makers ***over the coming year*** would be most useful."
  - Drs. Tom Frieden and John Bucher met with Senator Manchin and West VA health officials to discuss NTP research plans

Issues considered in formulating study plan

- **Issue 1**: Is the (MCHM) NOEL appropriate?
- **Issue 2**: Hazards following acute exposure
  - Longer-term effects
    - Mutagenicity
    - Developmental effects
    - Hypersensitivity
  - Short-term effects
    - Overt toxicity (Clinical signs)
    - Irritancy
- **Issue 3**: 1 year timeframe

Proposed NTP Studies

Test Article [Abbreviation, CAS Number]	Studies							
	Rat Prenatal Toxicity	Mouse Dermal Irritation and Hypersensitivity	5-Day Rat Toxicogenomic	Bacterial Mutagenicity	Zebrafish Developmental	Nematode Toxicity	High Throughput Screening	Structure Activity Relationship (SAR) Analysis
4-Methylcyclohexanemethanol [MCHM, 34885-03-5]	X	X	X	X	X	X	X	X
Dipropylene glycol phenyl ether [DiPPH, 51730-94-0]			X	X	X	X		X
Propylene glycol phenyl ether [PPH, 770-35-4]			X	X	X	X	X	X
1,4-Cyclohexanedimethanol (CHDM; 105-08-8)				X	X	X	X	X
2-Methylcyclohexanemethanol [2MCHM, 2105-40-0]				X	X	X		X
4-(Methoxymethyl)cyclohexanemethanol [MMCHM, 98955-27-2]				X	X	X		X
4-Methylcyclohexanecarboxylic acid [4331-54-8]					X	X		X
Cyclohexanemethanol, 4-[(ethenyloxy)methyl]- [114651-37-5]					X	X	X	X
Cyclohexanemethanol, alpha,alpha,4-trimethyl- [498-81-7]					X	X		X
Dimethyl 1,4-cyclohexanedicarboxylate [DMCHDC, 94-60-0]				X	X	X	X	X
Methyl 4-methylcyclohexanecarboxylate [MMCHC, 51181-40-9]				X	X	X		X
Phenoxyisopropanol [4169-04-4]					X	X	X	X
Technical product [“crude MCHM”]		X	X	X	X	X		

Guideline

Non-guideline

SAR

HTS

Nematode Toxicity

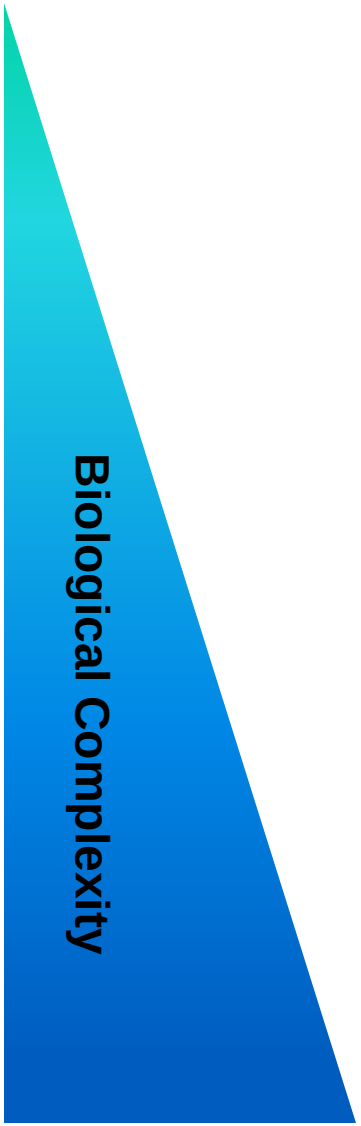
Zebrafish Toxicity

Genotoxicity

Dermal Irritancy/Hypersensitivity

5 Day Toxicogenomics

Prenatal Developmental Toxicity



Structure-Activity Relationship (SAR)

- A structure–activity relationship (**SAR**) is the relationship between a chemical's molecular structure and its biological activity. The relationships are estimated using computational (in silico) approaches.
- SAR provides a **probabilistic forecast** of a chemical's potential hazards
- As with any forecast there is **uncertainty**
  - It can be wrong and, therefore, **requires empirical validation**
- SAR does not:
  - Provide empirical chemical hazard calls
  - Provide a dose at which the forecasted hazard may occur
- SAR does:
  - Facilitate prioritization of research resources by highlighting potential hazards
  - Help in our understanding how a chemical may interact with a biological system

NTP SAR Approach

- **Step 1**: Chemist identifies correct chemical structure (SMILES) of all project chemicals
- **Step 2**: Chemical structure is evaluated across 6 software packages (Software: Leadscope, Case Ultra, Vega, Toxtree, MetaDrug, ADMETPredictor)
  - Approximately 200 models
- **Step 3**: Scientist reviews SAR model results deemed "**positive**" by the software (**ongoing**)
  - Considerations of reviewing scientist
    - Model probability score or confidence in call
    - Biological plausibility of the features driving the positive call
    - Domain of the model (structural similarity of the test chemical to model training data)
- **Step 4**: Report positive results of models deemed to be of adequate reliability

MCHM SAR Results

- A number of models across 6 platforms were identified by the software as “positive”
- Many lacked an explanation and, therefore, were not considered further
- Others lacked plausibility with respect to the structural features driving the “positive” call
- After review only 4 positive model calls were deemed of moderate reliability
  - Developmental toxicity in mammals (2)
  - Irritation of skin and eye (2)
- Results were taken under consideration when formulating the project plan

SAR

HTS

Nematode Toxicity

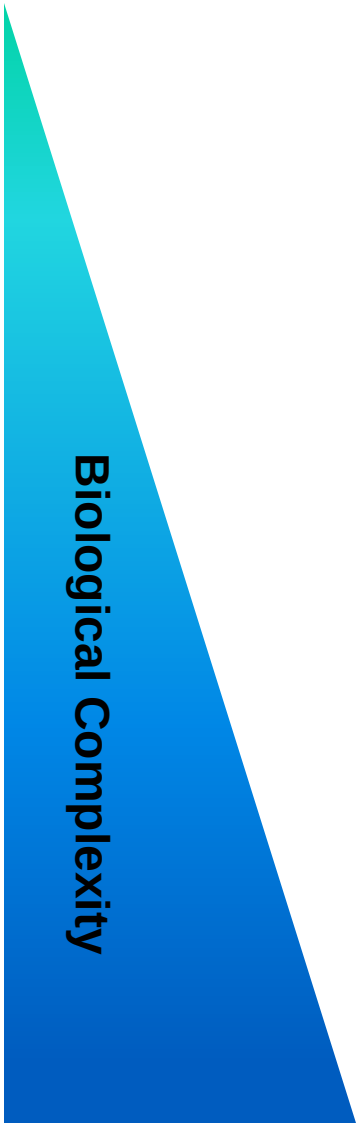
Zebrafish Toxicity

Genotoxicity

Dermal Irritancy/Hypersensitivity

5 Day Toxicogenomics

Prenatal Developmental Toxicity



High Throughput Screening (Tox21)

- High throughput screening (HTS) is the massive parallel *in vitro* screening of chemicals in biological assays
- We have focused on the assays from Tox21
  - Measure biological processes of toxicological relevance
- 3 classes of assays
  - Nuclear receptors; Stress response; Cytotoxicity
- Spilled chemicals in the Tox21 library (# of copies in chemical library)
  - **Spill chemicals**: 4-methylcyclohexanemethanol (1); 1,4-Cyclohexanedimethanol (1); Dimethyl 1,4-cyclohexanedicarboxylate (1); Propylene glycol phenyl ether (2)
  - **Structural analogs**: Phenoxyisopropanol (1); Cyclohexanemethanol, 4-((ethenyloxy)methyl)- (1)

Challenges and Limitations of HTS

- Biological
  - Limited bioactivation capacity of cell systems used
  - Endpoints measure are proximal biological effects not complex outcomes
  - Assays cover a limited number of biological endpoints
- Technical
  - Replicability
  - Chemical stability, identity and purity
    - Analytic characterization is ongoing

HTS Results

- None of the 6 chemicals were active in any of the assays
- **Important**: Analytic evaluation of the library is ongoing
  - Verified chemicals: 1,4-Cyclohexanedimethanol (CHDM); Dimethyl 1,4-cyclohexanedicarboxylate (DMCHDC); Phenoxyisopropanol
  - Unverified chemicals: **4-methylcyclohexanemethanol (MCHM)**; Propylene glycol phenyl ether (PPH); Cyclohexanemethanol, 4-((ethenyloxy)methyl)-

SAR

HTS

Nematode Toxicity

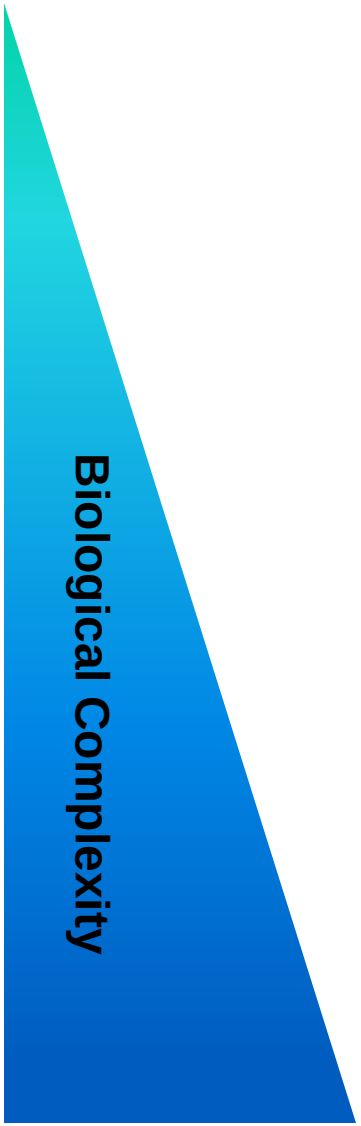
Zebrafish Toxicity

Genotoxicity

Dermal Irritancy/Hypersensitivity

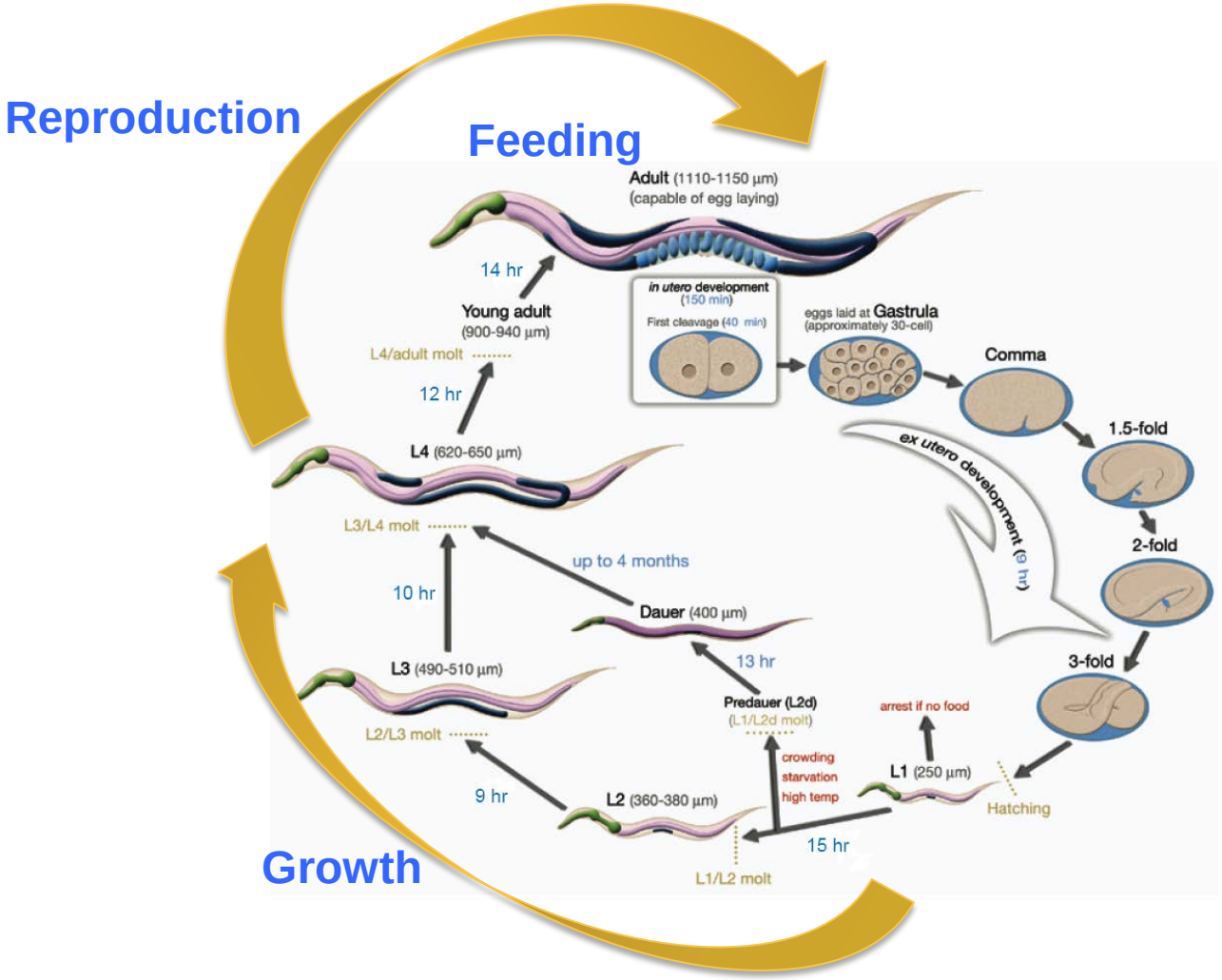
5 Day Toxicogenomics

Prenatal Developmental Toxicity



Nematode Toxicity

Goal: Characterize toxicity over different life stages
Chemicals: All spill chemicals, Crude MCHM, and structural analogs



Status: Studies are nearly complete

SAR

HTS

Nematode Toxicity

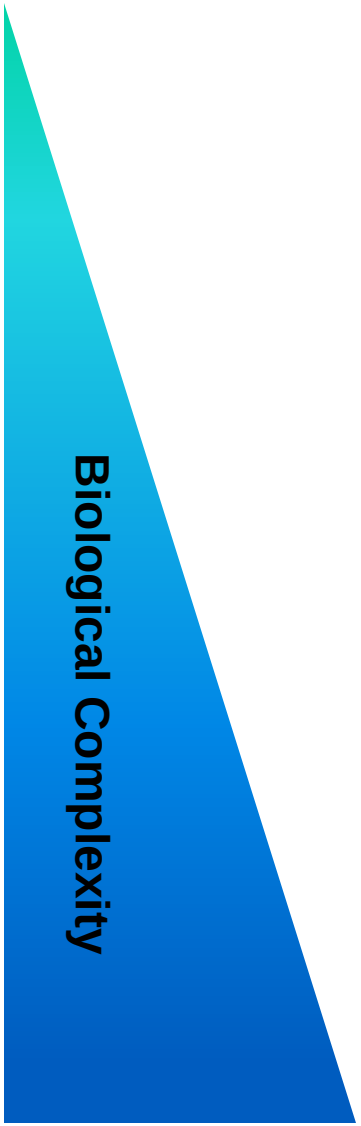
Zebrafish Toxicity

Genotoxicity

Dermal Irritancy/Hypersensitivity

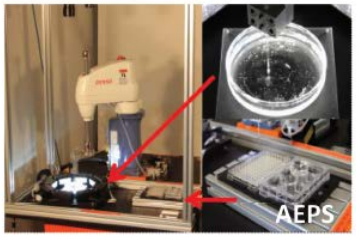
5 Day Toxicogenomics

Prenatal Developmental Toxicity



Zebrafish Developmental Toxicity

Goal: Characterize developmental toxicity
Chemicals: All spill chemicals and structural analogs



24 hours

End Point
Mortality
Developmental Delay
Spontaneous Movement
Notochord

120 hours

End Point
Mortality
Yolk Sac Edema
Body Axis
Eye Defect
Snout
Jaw
Otic Vesicle
Pericardial Edema
Brain
Somite
Pectoral Fin
Caudal Fin
Pigment
Circulation
Truncated Body
Swim Bladder
Notochord & Bent Tail
Touch Response

Status: Chemicals are at the lab

SAR

HTS

Nematode Toxicity

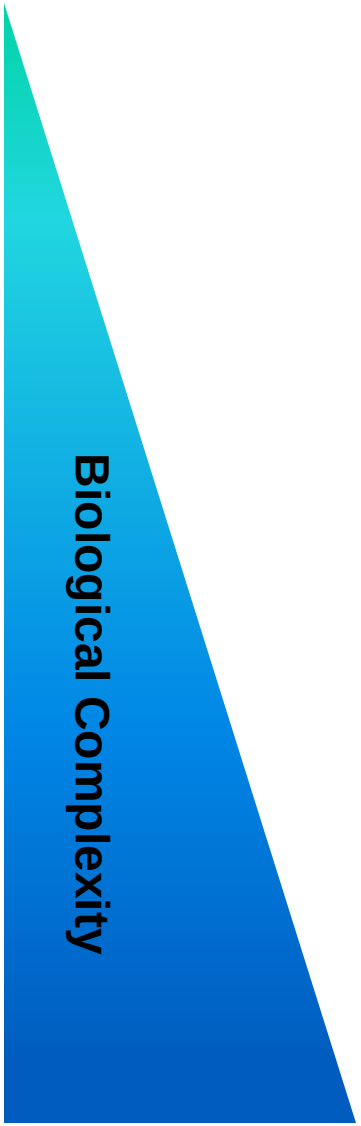
Zebrafish Toxicity

Genotoxicity

Dermal Irritancy/Hypersensitivity

5 Day Toxicogenomics

Prenatal Developmental Toxicity



Guideline Genotoxicity

Goal: Determine if components of the spill can damage DNA

- Bacterial mutagenesis
  - Salmonella/E. coli reverse mutation
  - **Chemicals:** All spill chemicals, Crude MCHM
- *In vivo* rodent micronucleus test
  - **Chemicals**: MCHM, PPH, DiPPH, Crude MCHM

Status: Micronucleus has been completed for 3 of 4 chemicals and is under review. Bacterial mutagenesis will start in the next month.

SAR

HTS

Nematode Toxicity

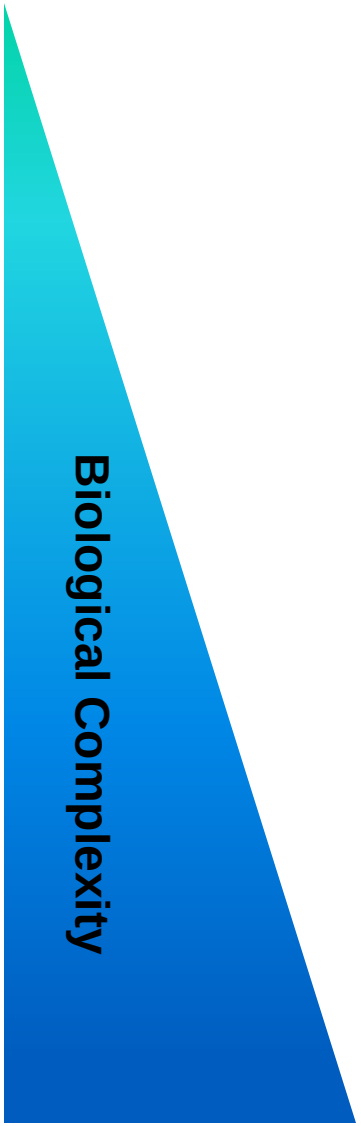
Zebrafish Toxicity

Genotoxicity

5 Day Toxicogenomics

Dermal Irritancy/Hypersensitivity

Prenatal Developmental Toxicity



Five-Day Rat Toxicogenomics

Goal: (1) Rapidly identify a biological pathway-based and gene-based POD (2) Predict toxicological properties of the chemicals through comparison to compendium gene expression data

Chemicals: MCHM, PPH, DiPPH, Crude MCHM

- Male rats (8-10 weeks old)
- 5 repeat doses, 24 hrs apart, euthanize 24 hrs after last dose
- 6 dose groups and a control (wide dose-range)
- Endpoints
  - Liver and kidney gene expression
  - Hematology/clinical chemistry
  - Clinical observations
  - Organ weights
  - *In vivo* micronucleus

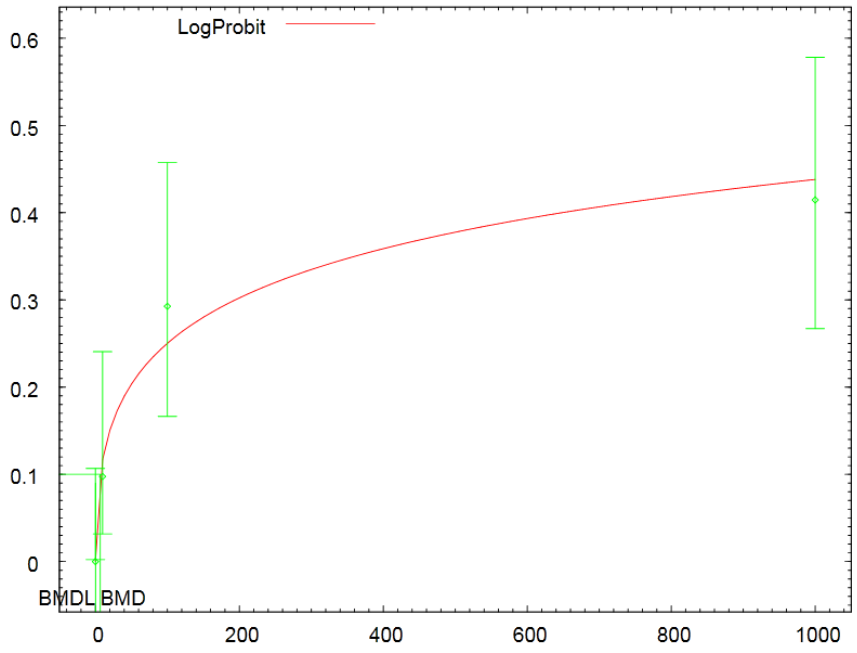
Status: 3 chemicals have gone through in-life. Waiting for results.

Five Day Rat Toxicogenomics

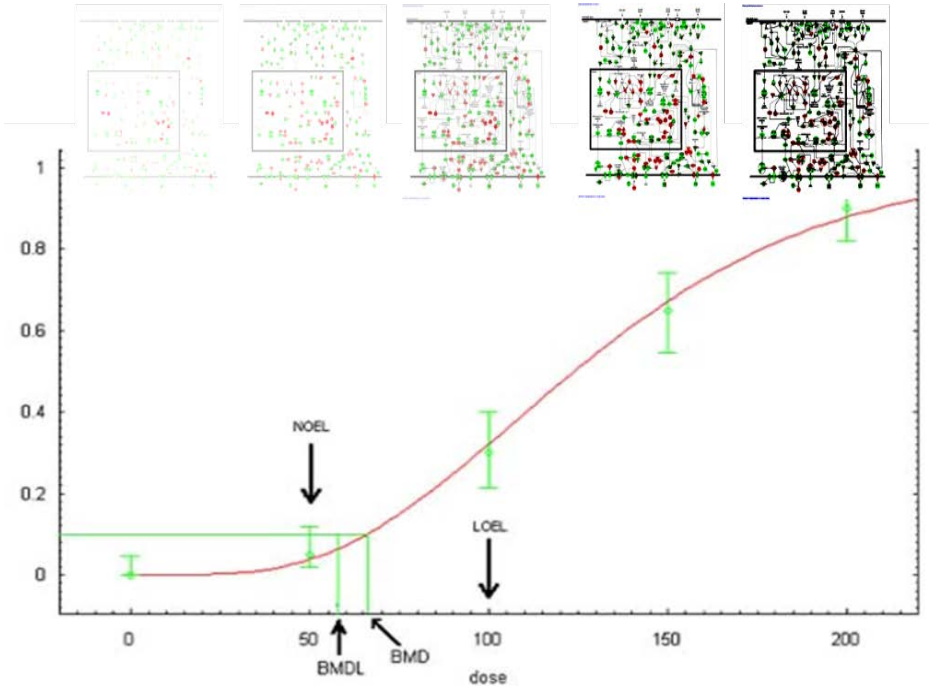
Quantitative Analysis Toxicogenomics Data

Fit a dose-response curve to the gene and biological pathway response data to identify gene and pathway points of departure

Gene

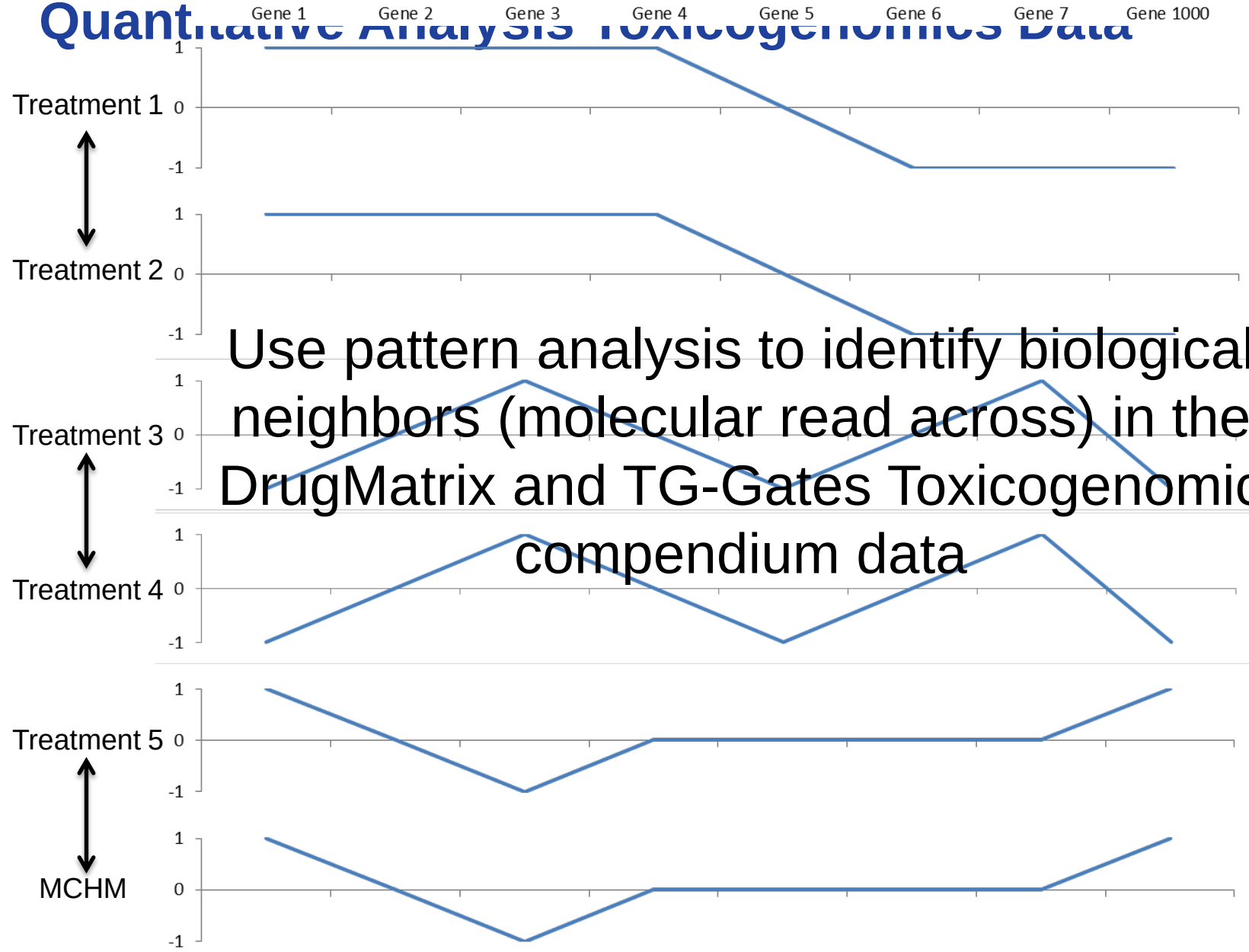


Pathway

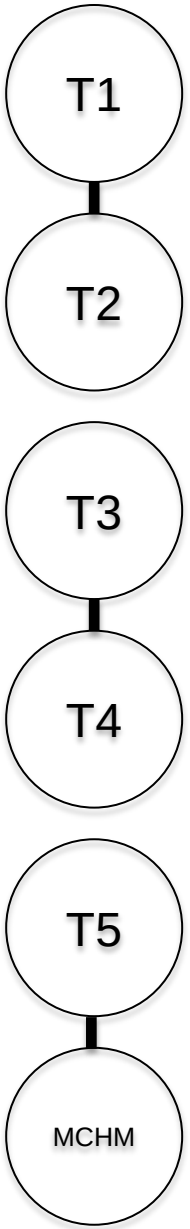


Five Day Rat Toxicogenomics

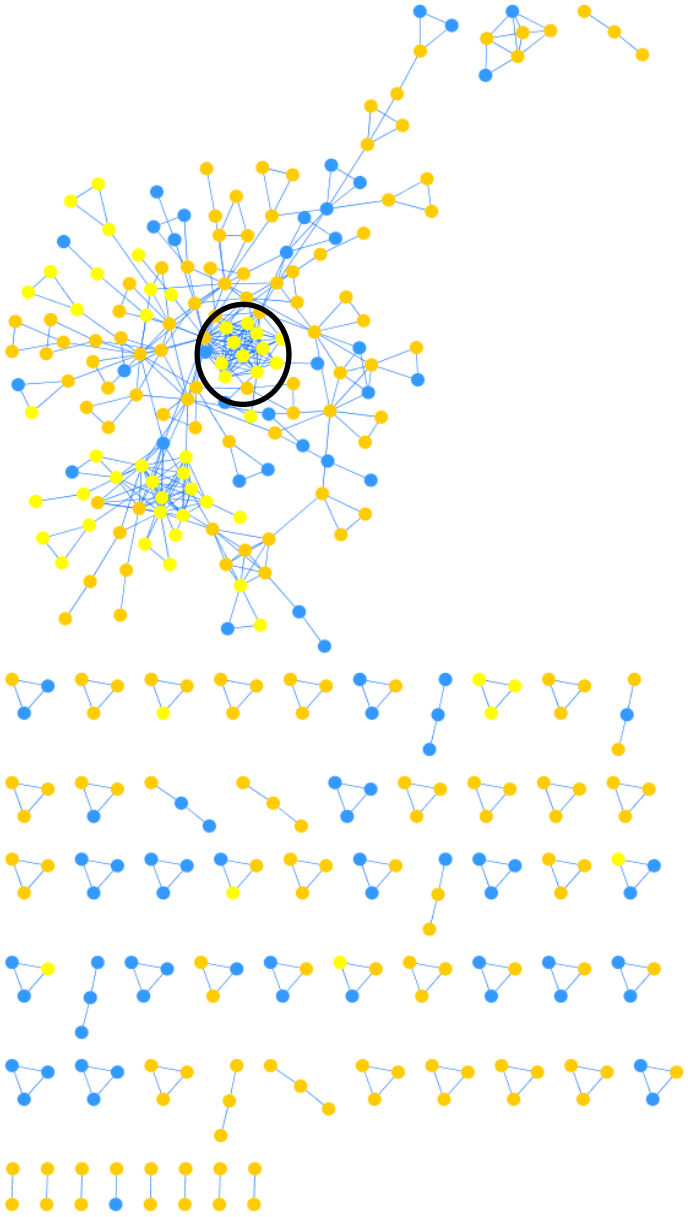
Quantitative Analysis Toxicogenomics Data

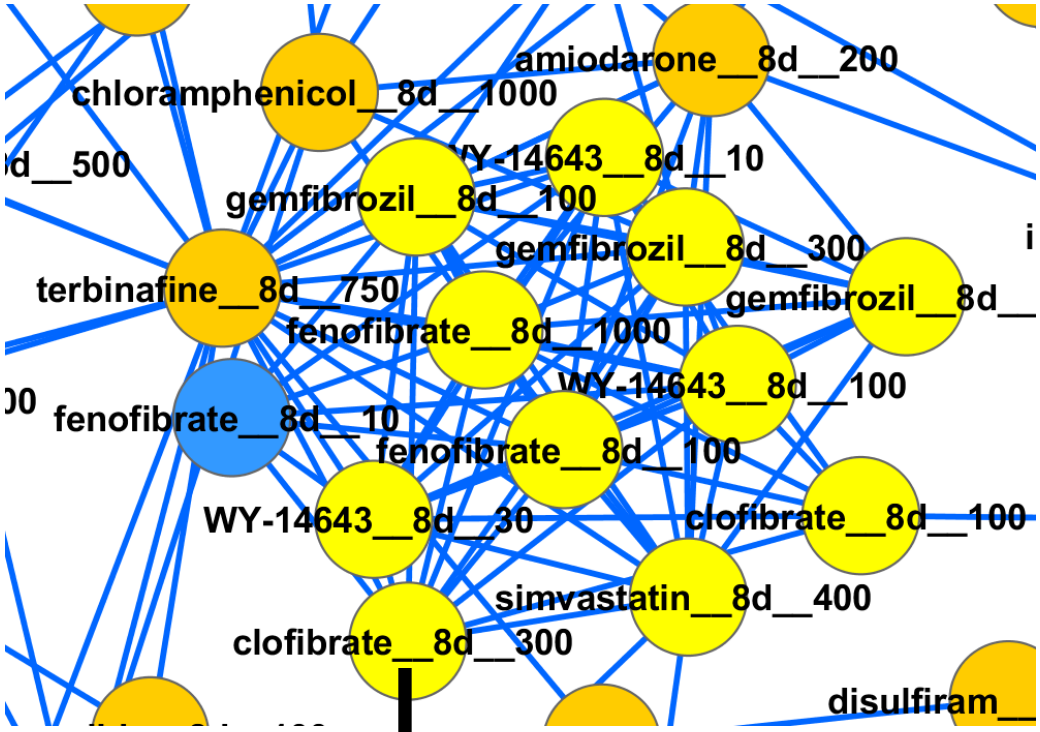


Use pattern analysis to identify biological neighbors (molecular read across) in the DrugMatrix and TG-Gates Toxicogenomic compendium data



Five Day Rat Toxicogenomics (Qualitative)





100 mg/kg MCHM?

SAR

HTS

Nematode Toxicity

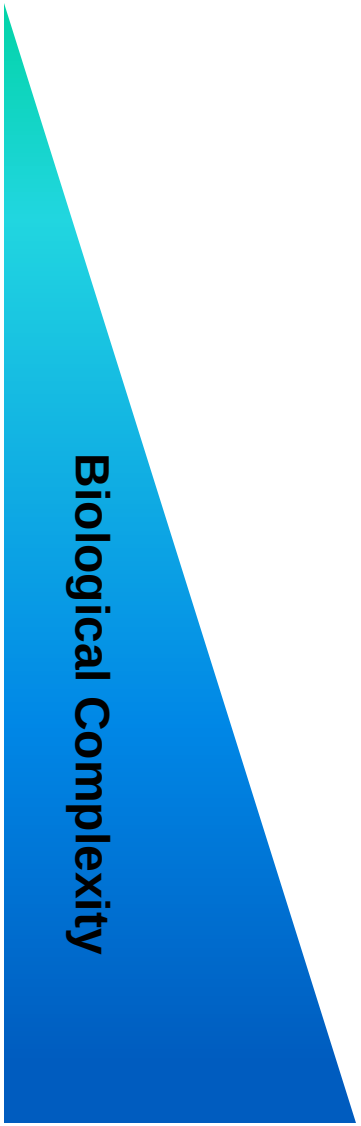
Zebrafish Toxicity

Genotoxicity

5 Day Toxicogenomics

Dermal Irritancy/Hypersensitivity

Prenatal Developmental Toxicity

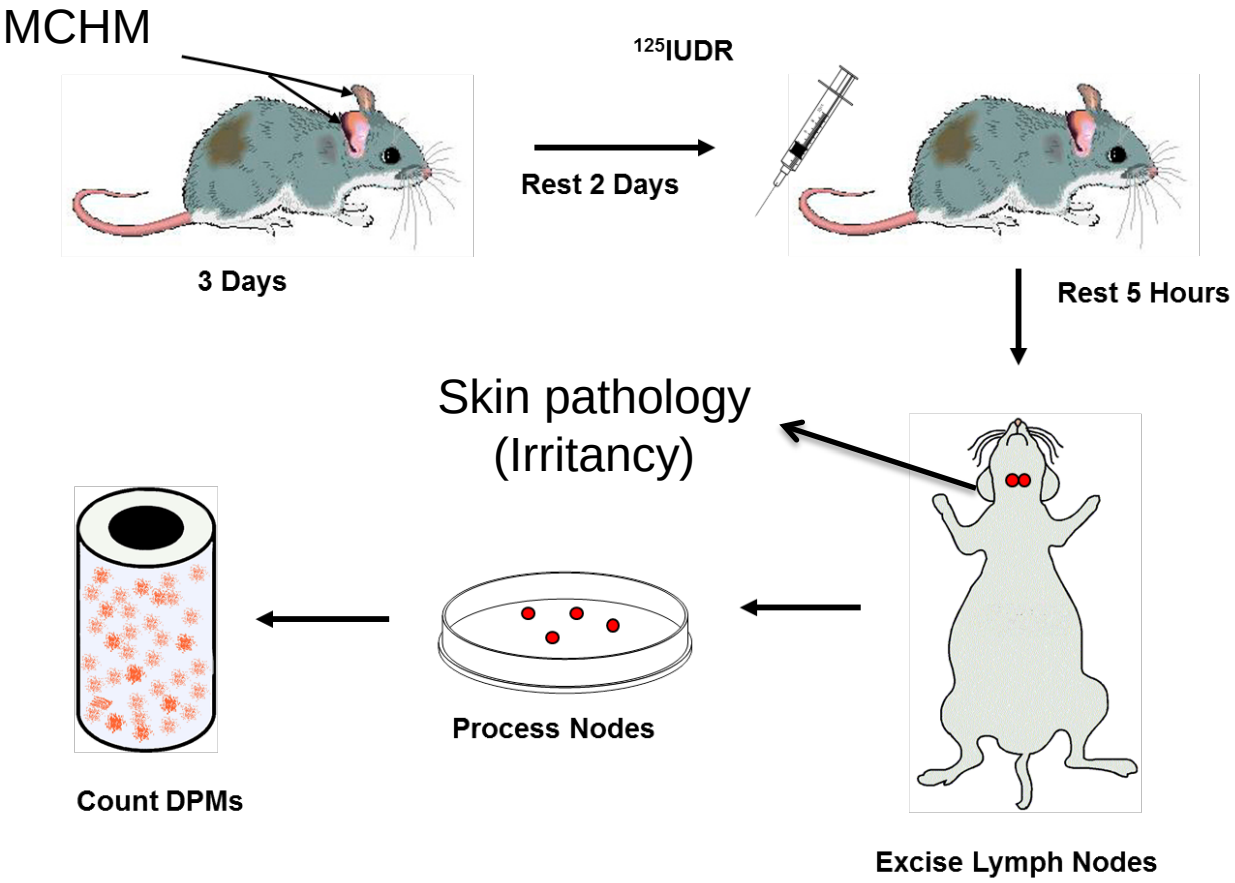


Mouse Dermal Irritation and Hypersensitivity Assay

Goal: Rapidly identify a POD for irritancy and determine if the chemicals can cause sensitization

Chemicals: MCHM, Crude MCHM

Local lymph node assay (LLNA)



Status: Studies start this week

SAR

HTS

Nematode Toxicity

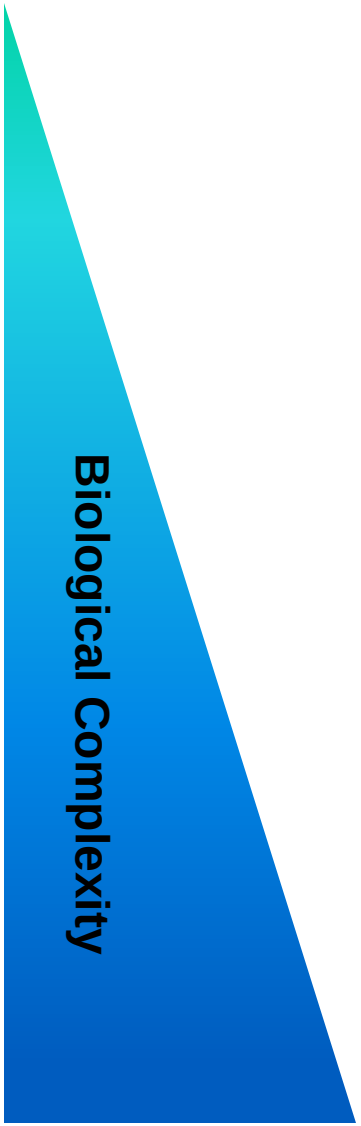
Zebrafish Toxicity

Genotoxicity

5 Day Toxicogenomics

Dermal Irritancy/Hypersensitivity

Prenatal Developmental Toxicity



Guideline Rat Prenatal Developmental Toxicity

Goal: Identify prenatal toxicity hazard and a point of departure
Chemicals: MCHM

- **Dose Range-Finding Study**
  - **Goal**: Identify a dose that produces **minimal evidence of maternal toxicity**
  - n = 10/dose level; 4 dose levels
  - Doses: 0, 150, 300, 600, 900 mg/kg/day
  - Maternal toxicity
  - Fetal endpoints
    - Fetal weight/sex
    - Number of fetuses, resorptions and corpora lutea (pre/post implantation loss)
    - External examination including cleft palate

- **Main study**
  - **Goal**: Identify **teratogenic** or other **developmental** effects that occur at doses **where there is minimal or no evidence of maternal toxicity**
  - n = ~20/dose level; 4 dose levels
  - Fetal endpoints
    - Fetal weight/sex
    - Number of fetuses, resorptions and corpora lutea (pre/post implantation loss)
    - External examinations, visceral examinations, and skeletal examinations
      - Classified as variations or malformations

Prenatal Rat Dose Range-Finding Study Results

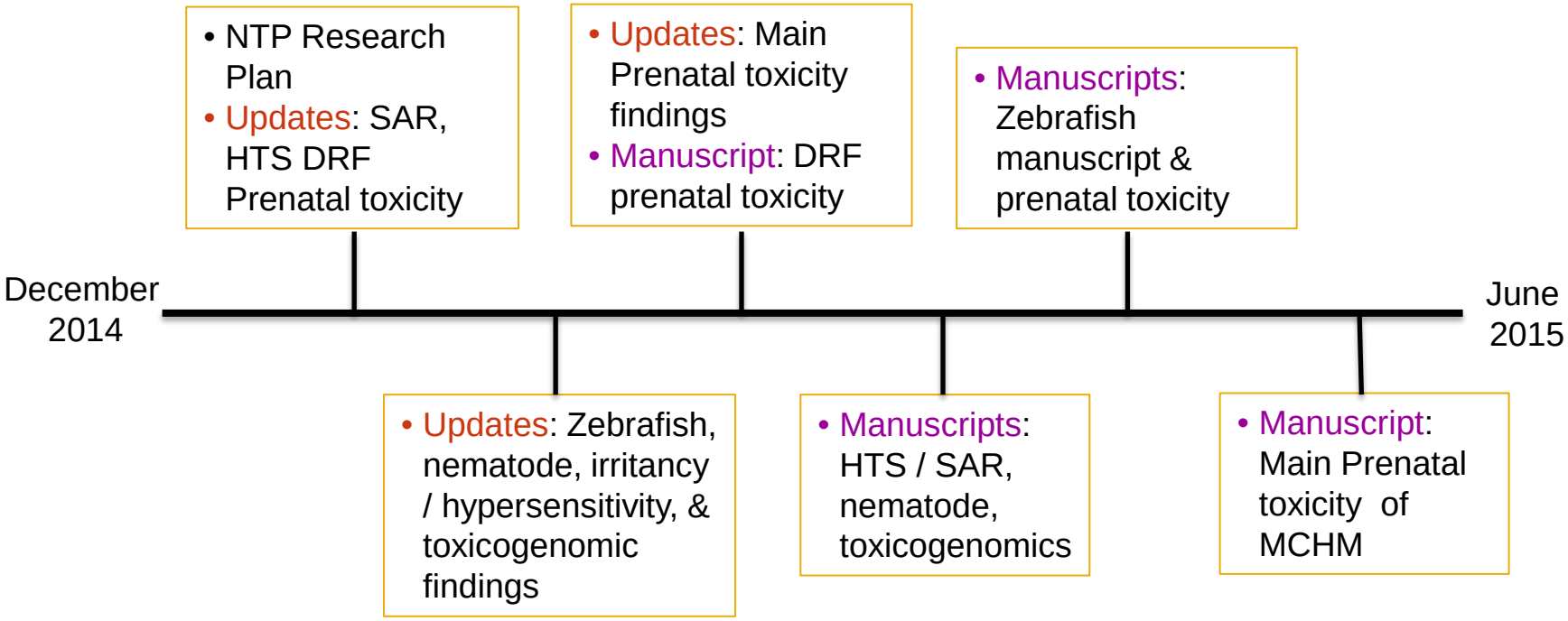
- Doses: 0, 150, 300, 600, 900 mg/kg/day in corn oil
- The top dose group of 900 mg/kg/day and three 600 mg/kg/day dams were terminated early due to excessive maternal toxicity
- 600 mg/kg/day group (those not terminated):
  - Fetal weight decreased and increased post-implantation loss
    - **Likely related to maternal toxicity**
- 300 mg/kg/day group:
  - Fetal weight decreased
- No increase in gross external observations noted among the dose groups
- Results **similar (effect dose) to 28-day Eastman study** (M/F non-pregnant) used to establish drinking water advisory level**:**
  - **Minimal effects seen at 150 mg/kg (100 mg/kg in Eastman Study)**
  - **Maternal toxicity and mortality/moribundity at 900 mg/kg (800 mg/kg in Eastman Study)**

Main Prenatal Developmental Toxicity Study

- Doses of 0, 50, 100, 200, 400 mg/kg/day selected (n = 20 dams/group)
- Additional evaluation of potential maternal toxicity includes:
  - Kidney histology
  - Clinical chemistry
  - Hematology

Status: In life component of the main study is complete

Timeline for Reporting NTP Studies



NTP Communications

- Website - ntp.niehs.nih.gov/results/areas/wvspill
- Newsletters and fact sheet
- Research project plan
- Updates on studies and results (living documents)
  - Rapid communications
  - “To the best of our knowledge at this time”
- Manuscripts (anticipate 3-5)
- NTP Toxicity Report on prenatal developmental toxicity study

Website

Home | Testing Information | Study Results & Research Projects | Public Health | About NTP

Home » Study Results & Research Projects » Areas of Research » West Virginia Chemical Spill

- West Virginia Chemical Spill
- NTP Research Project
- NTP Studies & Results
- Newsletters & Fact Sheets
- Stay Informed & Contact Us

West Virginia Chemical Spill

0

http://ntp.niehs.nih.gov/go/wvspill

In January 2014, approximately 10,000 gallons of chemicals used to process coal spilled from a storage tank into the Elk River in West Virginia. The Elk River is a municipal water source that serves about 300,000 people in the Charleston area.

In July 2014, NTP received a nomination from the Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry to conduct toxicity studies on the predominant chemicals known to be involved in the West Virginia chemical spill. The primary spilled agent was 4-methylcyclohexanemethanol (MCHM). Other chemicals were also present in lower amounts in the tank. Limited data are available to address concerns for potential human health effects for the compounds in the spilled liquid so NTP will study a number of chemicals (see the Table of Chemicals for NTP Studies).

Photo by Raymond Thompson – West Virginia University researchers collect water samples at the confluence of the Elk and Kanawha Rivers.

See the NTP research project and studies and results for more information on NTP studies being conducted to provide information relevant to the potential exposures of the Charleston residents.

Work at Other Federal and State Agencies

- U.S. Department of Health and Human Services
- Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry
- National Library of Medicine
- West Virginia Department of Health and Human Resources

Acknowledgements

- **Chemistry**: Brad Collins (lead), Suramya Waidyanatha
- **SAR**: Scott Masten (lead), Neepa Choksi (ILS Inc), Stephen Ferguson
- **HTS**: Tox21 Consortium
- **Nematode Toxicity**: Windy Boyd (lead)
- **Zebrafish Toxicity**: Ray Tice (lead), Robert Tanguay (Oregon State U)
- **Genotoxicity**: Kristine Witt (lead), Les Recio (ILS Inc)
- **Dermal Irritancy/Hypersensitivity**: Dori Germolec (lead), Burleson Research Labs (Contractor)
- **5 Day Toxicogenomics**: Scott Auerbach (lead), Molly Vallant, Battelle (Contractor)
- **Prenatal Developmental Toxicity**: Chad Blystone (lead), Helen Cunny, Paul Foster, Barry McIntyre, Vicki Sutherland, Southern Research (Contractor)
- **Communications**: Mary Wolfe, Yun Xie, Robin Mackar
- **Wisdom**: John Bucher, Nigel Walker and Scott Masten

Questions?