

# Report on NTP Response to the Elk River Chemical Spill

Scott S. Auerbach, Ph.D., DABT  
Biomolecular Screening Branch  
Division of the National Toxicology Program  
National Institute of Environmental Health Sciences

**NTP Board of Scientific Counselors Meeting  
June 16, 2015**





- Background on Spill
- NTP Study Goals
- Study Results
- How studies addressed NTP goals



# January 9, 2014 (Morning)



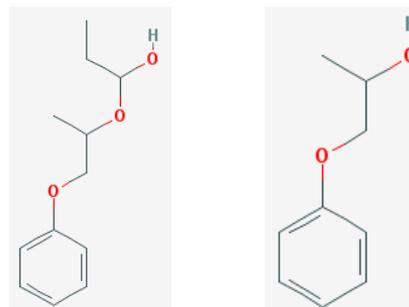
Residents of Charleston, West Virginia began to notice a “sweet smell” (like licorice) in the air and reported it to the WV Department of Environmental Protection.



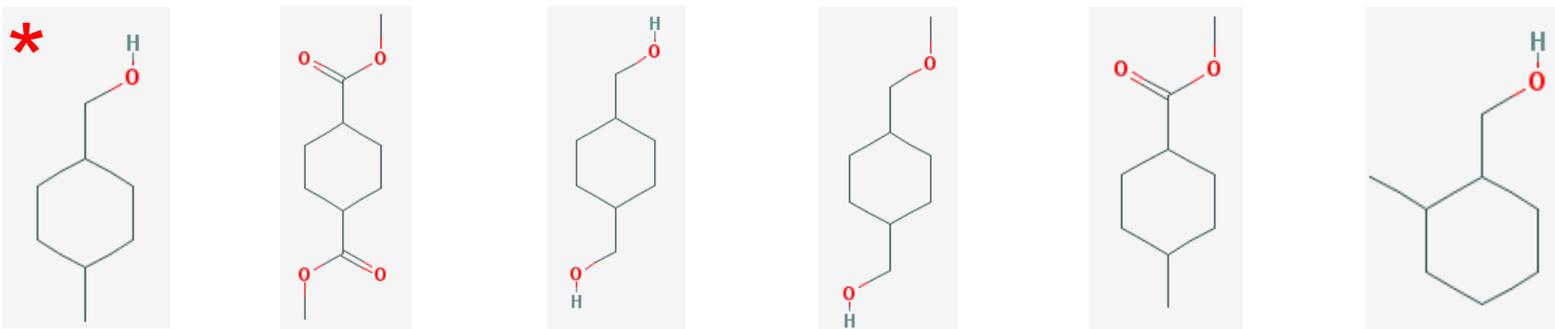
# January 9, 2014 (Morning)



## Phenyl Ethers



## Crude MCHM



Freedom Industries reported a liquid (crude MCHM) used to wash coal was spilled from a leaking tank into the West Virginia Elk River. The spill occurred 1.5 miles upstream of the water intake facility serving 300,000 people across 9 counties.



January 9, 2014

## Initial Drinking Water Advisory

- CDC issues a 1 ppm screening level based for MCHM on limited information on MSDS from Eastman Chemical (the maker of crude MCHM)
- Peak levels in the treated water were approximately 3.5 ppm, but quickly went below 1 ppm
- It is unknown what the concentration in tap water was when peak levels in finished water was in the range of 3.5 ppm





January 16, 2014

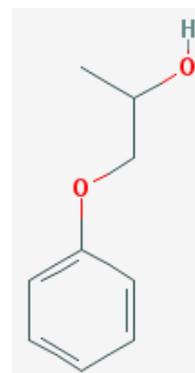
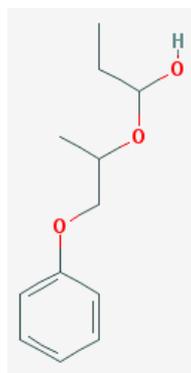
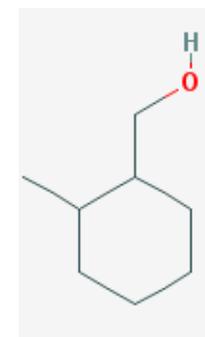
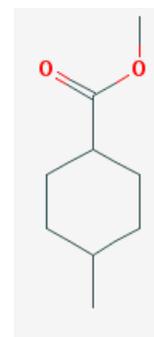
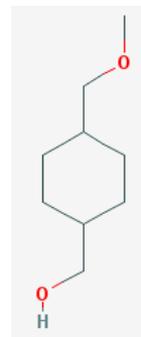
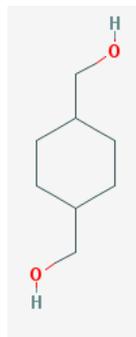
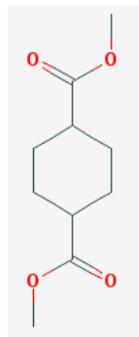
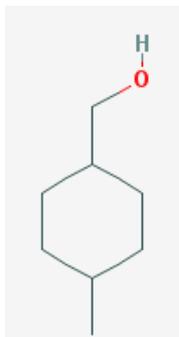
## Reevaluation of Drinking Water Advisory Level (DWAL)



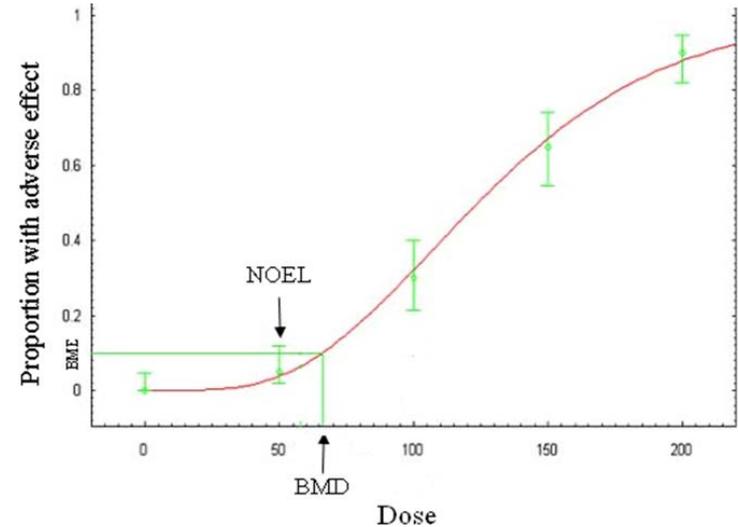
Eastman Chemical releases results of toxicity studies. CDC uses results from a 28-day repeat dose study to calculate a drinking water advisory level in water.

NTP reviews the CDC calculations.

- Point of departure
  - 100 mg/kg/day
- Safety factors
  - Limited database (10)
  - Rodent to human (10)
  - Sensitive individuals (10)
- Dose not anticipated to cause adverse effects
  - 0.1 mg/kg/day
- DWAL (10 kg child)
  - 1 ppm



NTP performs an internal assessment of the chemical structures of spilled chemicals using computer models to predict their toxicological properties. NTP determines the chemicals are of limited toxicological concern; however, a great degree of uncertainty remained.



NCEH/ATSDR (CDC) request the NTP undertake research to address lingering uncertainties



Dr. Bucher



Sen. Manchin



Dr. Frieden

“A research effort aimed at providing meaningful information to public health decision-makers **over the coming year** would be most useful.”

-CDC Nomination letter to NTP



# Goals of NTP Studies

- Reduce uncertainty around the point of departure and safety factors used to develop the drinking water advisory levels
  - NOEL/NOAEL
    - MCHM: 100 mg/kg/day – kidney and liver effects
    - PPH: 40 mg/kg/day – maternal toxicity
  - Drinking Water Advisory Level
    - MCHM: 1 ppm, which equals an intake 0.1 mg/kg/day for a child
    - PPH: 1.2 ppm, which equals 0.04 mg/kg/day for a pregnant woman
- Determine if there are life-stage specific hazards
- Screen minor components of the mixture to determine if there are significant deviations in potency or toxicological properties



# NTP Studies on Elk River Chemicals

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
Dimethyl 1,4-cyclohexanedicarboxylate [DMCHDC, 94-60-0]				X	X	X	X	X
Methyl 4-methylcyclohexanecarboxylate [MMCHC, 51181-40-9]				X	X	X		X
Technical product ["crude MCHM"]		X	X	X	X	X		

Proposed study plan underwent cross-agency review

Guideline studies  
Non-guideline studies



# Structure Activity Relationship (SAR)

- **Description:** Six software platforms containing 199 SAR models that cover many toxicological endpoints were used to predict chemical toxicity of the spilled chemicals based on their structure
- **Purpose:** Rapidly identify potential toxicological hazards
- **Findings**
  - MCHM Class
    - Positive predictions of moderate to high confidence
      - **Developmental toxicity and irritancy**
  - PPH Class
    - Positive predictions of moderate to high confidence
      - **None**



# High Throughput Screening (Tox21)

- **Description:** 27 different cell-based screening studies that evaluate chemical effects on signaling pathways of toxicological concern
- **Findings**
  - None of the spilled chemicals (including MCHM and PPH) were active up to 92  $\mu\text{M}$  (approximately 10 to 20 ppm) in any of the assays run to date (including cytotoxicity, which is run in every assay)
- **Note**
  - Chemical quality control is incomplete

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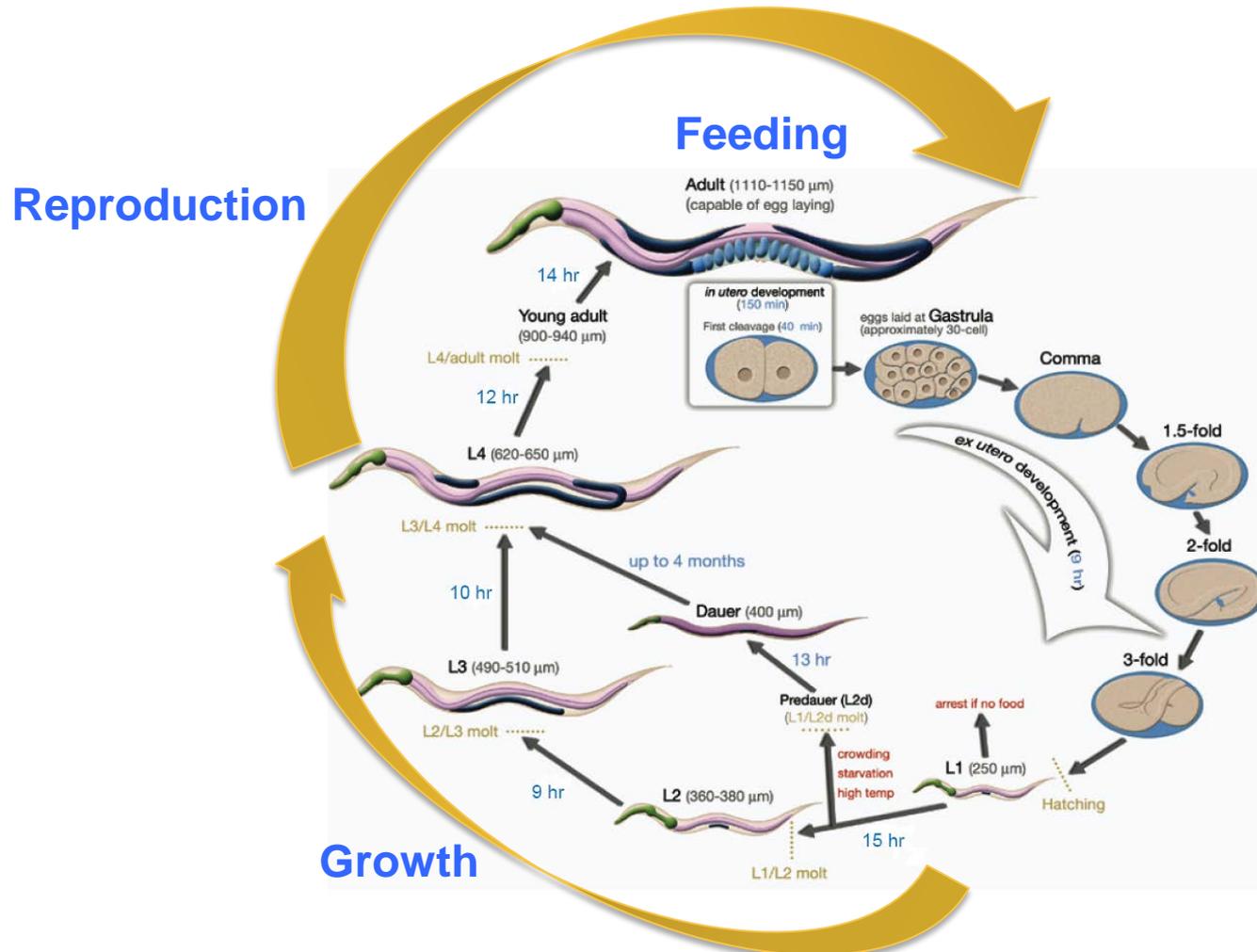
Chemical	Call
4-Methylcyclohexanemethanol [MCHM]	Inactive
Propylene glycol phenyl ether [PPH]	Inactive
1,4-Cyclohexanedimethanol [CHDM]	Inactive
Dimethyl 1,4-cyclohexanedicarboxylate [DMCHDC]	Inactive

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# Nematode Toxicity

**Description:** Growth, feeding, and reproduction are measured in the nematode following chemical treatment





- **Findings**

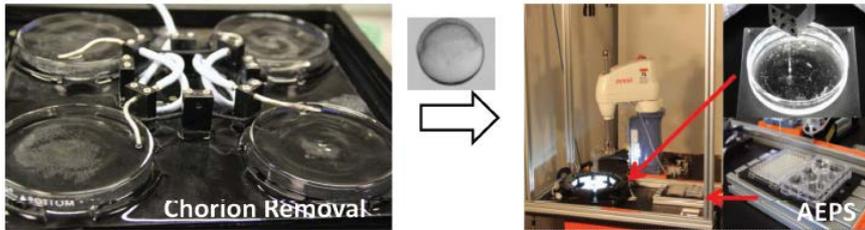
- All chemicals from the spill tested to date (includes MCHM, crude MCHM, and PPH) were inactive up to 200  $\mu\text{M}$  (approximately 20 to 40 ppm)

<b>Chemical</b>	<b>Call</b>
4-Methylcyclohexanemethanol [MCHM]	Inactive
Technical product [crude MCHM]	Inactive
Dipropylene glycol phenyl ether [DiPPH]	Inactive
Propylene glycol phenyl ether [PPH]	Inactive
1,4-Cyclohexanedimethanol [CHDM]	Inactive
4-(Methoxymethyl)cyclohexanemethanol [MMCHM]	Inactive
Dimethyl 1,4-cyclohexanedicarboxylate [DMCHDC]	Inactive
Commercial product [Dowanol DiPPH glycol ether]	Inactive



# Zebrafish Developmental Toxicity

**Description:** Development and behavior are evaluated in zebrafish embryos and larva following chemical exposure



**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



# Zebrafish Developmental Toxicity

## • Findings

- Dimethyl 1,4-cyclohexanedicarboxylate (DMCHDC, less than 1% of spilled material) was toxic to developing fish at a dose of 67.3  $\mu\text{M}$  (approximately 13 ppm)
  - **Specific effects:** curved or bent axis, pericardial edema, yolk sack edema, and mortality (at the highest dose)
- All other chemicals (including MCHM, crude MCHM, and PPH) tested to date were not active up to 100  $\mu\text{M}$  (approximately 10 to 20 ppm)

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Chemical	Call
4-Methylcyclohexanemethanol [MCHM]	Inactive
Technical product [crude MCHM]	Inactive
Propylene glycol phenyl ether [PPH]	Inactive
1,4-Cyclohexanedimethanol [CHDM]	Inactive
Dimethyl 1,4-cyclohexanedicarboxylate [DMCHDC]	Active



# Bacterial Mutagenesis

- **Description:** Mutant colonies are counted after treatment of bacteria with chemical
- **Findings**
  - None of the chemicals from the spill tested to date (including MCHM, crude MCHM and PPH) were mutagenic in 3 different strains of bacteria in tests conducted with and without metabolic activation

<b>Chemical Name</b>	<b>Call</b>
4-Methylcyclohexanemethanol [MCHM]	Inactive
Technical product [crude MCHM]	Inactive
Propylene glycol phenyl ether [PPH]	Inactive
Dipropylene glycol phenyl ether [DiPPH]	Inactive
Commercial Product [Dowanol DiPPH glycol ether]	Inactive
Methyl 4-methylcyclohexanecarboxylate [MMCHC]	Inactive
4-(Methoxymethyl)cyclohexanemethanol [MMCHM]	Inactive
2-Methylcyclohexanemethanol [2MCHM]	Inactive



# 5-Day Rat Toxicogenomics

- **Description:**

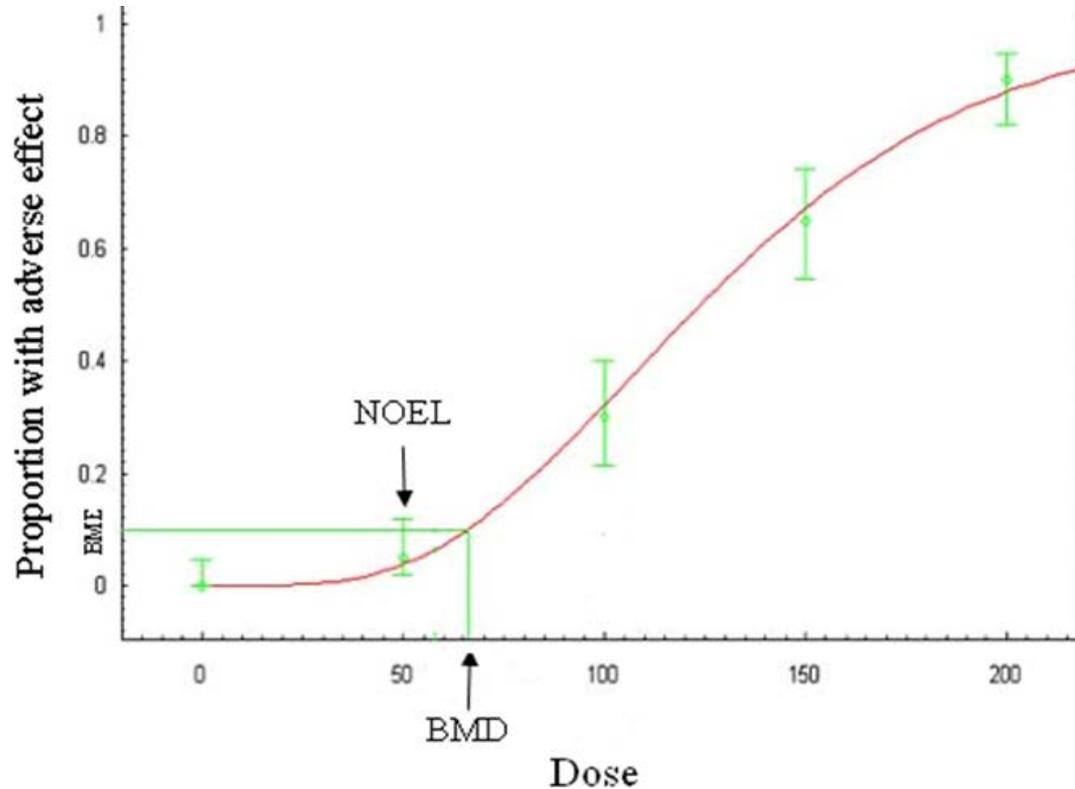
- Male rats (8-10 weeks old)
- 5 repeat doses, 24 hours apart, euthanize 24 hours after last dose
- 6 dose groups and a vehicle control (wide dose range)
- Endpoints
  - Liver and kidney gene expression
  - Hematology/clinical chemistry
  - Clinical observations
  - Organ weights
  - Erythrocyte micronucleus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

- **Purpose:** Screening level study to identify the lowest dose level (benchmark dose (BMD)) that produces an integrated biological response (**not toxicity**) as measured by the response of genes in Molecular Biological Process groups.



## Benchmark Dose (BMD)

- The dose of a test article that corresponds to a specified level of response above or below that observed in a control or background population

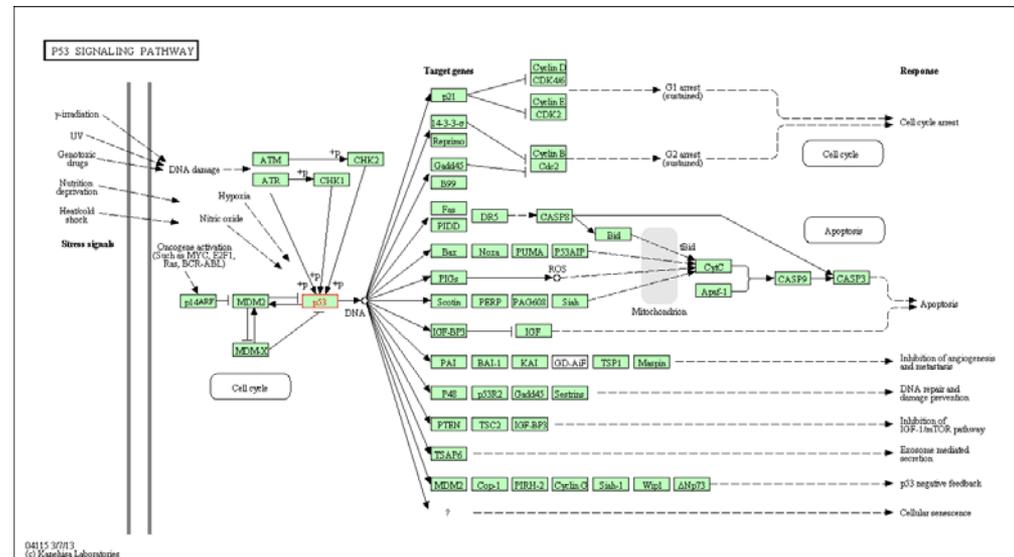




# 5-Day Rat Toxicogenomics

## Molecular Biological Process

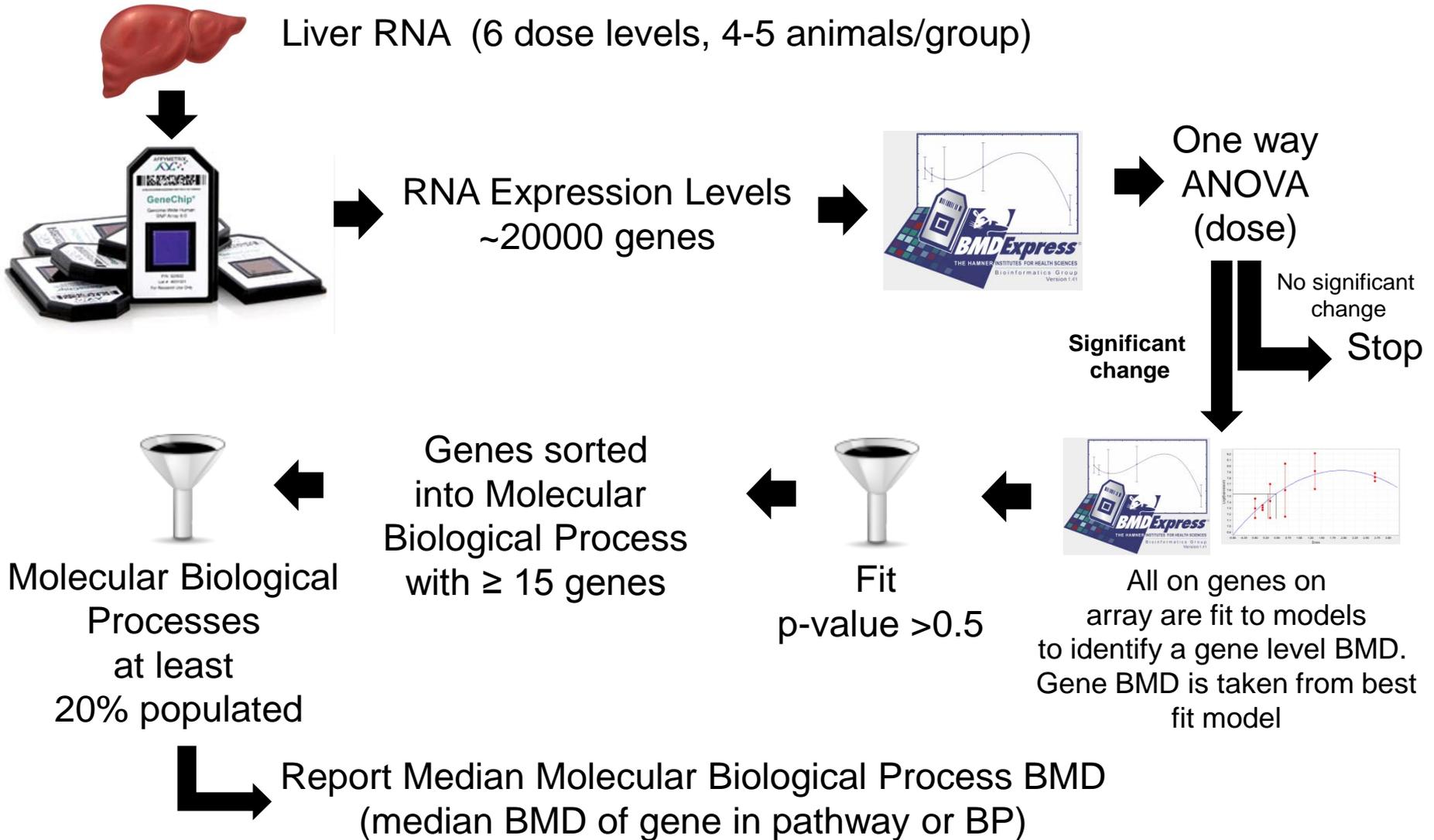
- A group of genes that function together control a cellular process (e.g., P53 signaling pathway, Lipid metabolism)
  - Different types of Molecular Biological Processes
    - KEGG Pathways
    - GO Biological Processes





# 5-Day Rat Toxicogenomics

## Molecular Biological Process BMD





# 5-Day Rat Toxicogenomics

## Determining a Molecular Biological Process (MBP) BMD

Gene Name	BMD (mg/kg/day)
Gene 1	10
Gene 2	50
Gene 3	100
Gene 4	150
Gene 5	200
Gene 6	Failed fit filter
Gene 7	Failed fit filter
Gene 8	Failed fit filter
Gene 9	Failed fit filter
Gene 10	Failed fit filter
Gene 11	Failed fit filter
Gene 12	Failed fit filter
Gene 13	Failed fit filter
Gene 14	Failed fit filter
Gene 15	Failed fit filter

MBP BMD  
(median gene)



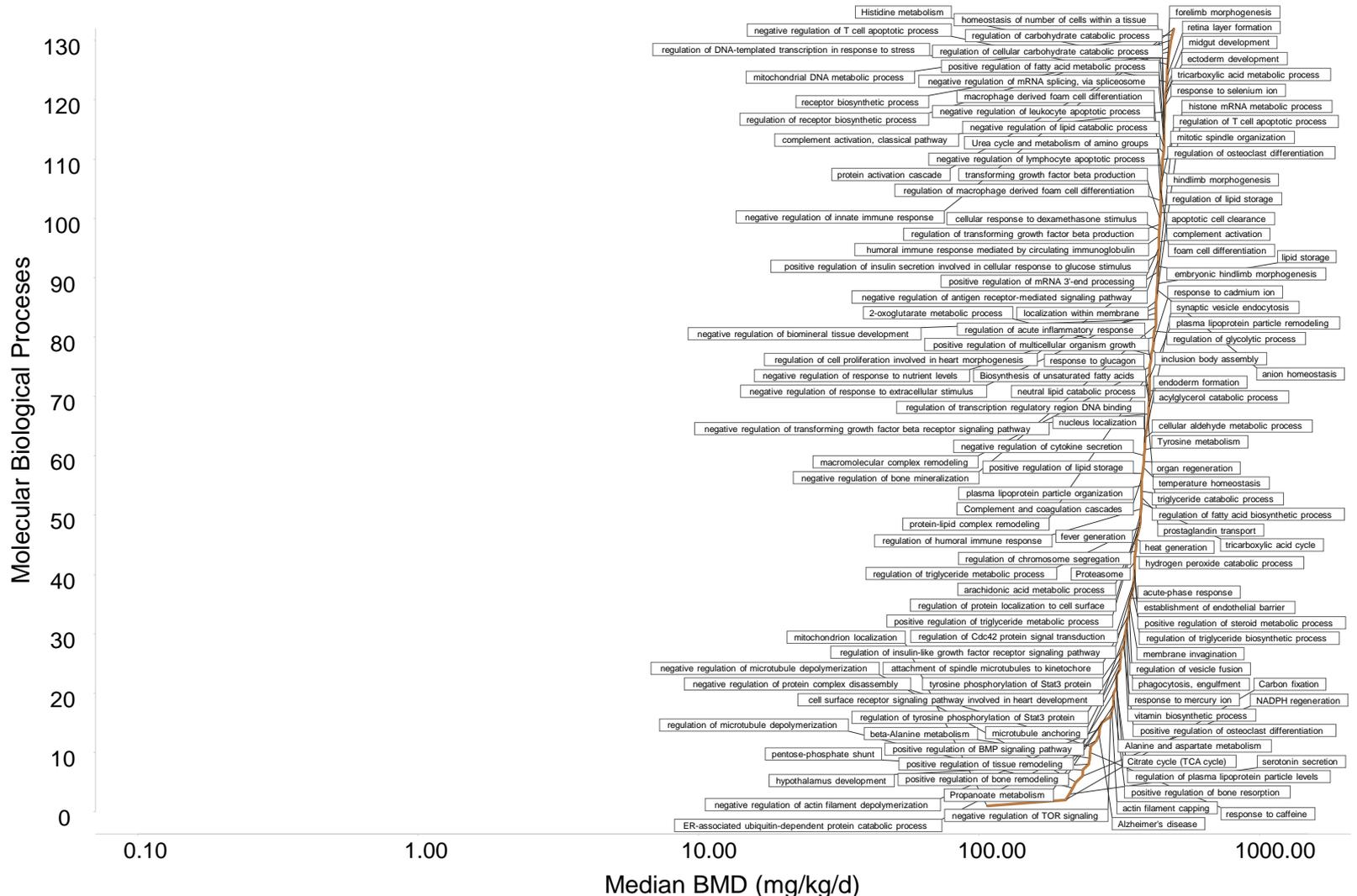
## Findings

- MCHM
  - Dose range: 0.1 to 500 mg/kg/day; 6 dose levels
  - Weak effect on gene expression in liver; no effect in kidney
  - 132 Molecular Biological Processes were considered active and had reported BMD values
  - Minimum biological effect benchmark dose: 107 mg/kg/day
  - Other findings
    - Increased triglycerides at high dose
    - No increase in micronuclei in immature erythrocyte population in blood



# 5-Day Rat Toxicogenomics

## MCHM Molecular Biological Process Accumulation Plot





## Findings

- Crude MCHM
  - Dose range: 0.1 to 500 mg/kg/day; 6 dose levels
  - Weak effect on gene expression in liver; no effect in kidney
  - 132 Molecular Biological Processes were considered active and had reported BMD values
  - Minimum biological effect benchmark dose: 63 mg/kg/day
  - Other findings
    - Increased triglycerides at the top 2 doses
    - No increase in micronuclei in immature erythrocyte population in blood



## Findings

- PPH
  - Dose range: 1 to 2000 mg/kg/day; 6 dose levels
  - Weak effect on gene expression in liver and kidney
  - 144 and 104 Molecular Biological Processes were considered active and had reported BMD values in liver and kidney, respectively
  - Minimum biological effect benchmark dose: 0.6 mg/kg/day
  - Other findings
    - 2000 mg/kg/day was overtly toxic
    - Increased in ALT levels at 500 mg/kg/day and higher
    - No increase in micronuclei in immature erythrocyte population in blood



# Dermal Irritancy and Hypersensitivity

- **Description:** Proliferation of lymph node cells and localized skin swelling are measured following repeated application of chemical to mouse skin to determine if the chemicals cause irritation or allergic reaction
- **Findings**
  - MCHM
    - Caused mild irritation at 20% (approximately 200,000 ppm) and higher
    - Did NOT cause dermal sensitization up to 50% (approximately 500,000 ppm)
  - Crude MCHM
    - Caused mild irritation at 75% (approximately 750,000 ppm)
    - Caused a dermal sensitization response at 40% (approximately 400,000 ppm) and higher



# MCHM Rat Prenatal Developmental Toxicity

- **Description:** Maternal and prenatal developmental parameters are measured in pregnant rats and their fetuses following chemical exposure during gestation (approximately two weeks exposure)
- **Findings:** Dose Range Finding Study
  - Doses: 150, 300, 600, 900 mg/kg/day
  - Days of dosing: GD 6 to GD 21
  - Overtly toxic to dams at dose levels of 600 and 900 mg/kg/day
  - Increased fetal loss at doses where maternal toxicity was observed
  - Dose-related decrease in fetal weight starting at 150 mg/kg/day



# MCHM Rat Prenatal Developmental Toxicity

- **Findings: Main Study**

- Doses: 0, 50, 100, 200, 400 mg/kg/day
- Days of dosing: GD 6 to GD 21
- No maternal toxicity observed
- No effects on fetal survival
- Fetal weight decreased at 200 (-3%) and 400 (-15%) mg/kg/day
- Increase in malformations observed at 400 mg/kg/day
  - Increased incidence of supernumerary ribs (extra rib)
  - Decrease fusion of the cartilage to the sternum
- Findings indicate that MCHM produces prenatal toxicity in the absence of maternal toxicity
  - No effect level between either 50 or 100 mg/kg/day



# Study Results Summary

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]	A	A	A	X	X	X	X	A
Dipropylene glycol phenyl ether [DiPPH, 51730-94-0]				X	O	X		X
Propylene glycol phenyl ether [PPH, 770-35-4]			A	X	X	X	X	X
1,4-Cyclohexanedimethanol (CHDM, 105-08-8)				O	X	X	X	A
2-Methylcyclohexanemethanol [2MCHM, 2105-40-0]				X	O	*		A
4-(Methoxymethyl)cyclohexanemethanol [MMCHM, 98955-27-2]				X	O	X		A
Dimethyl 1,4-cyclohexanedicarboxylate [DMCHDC, 94-60-0]				O	A	X	X	A
Methyl 4-methylcyclohexanecarboxylate [MMCHC, 51181-40-9]				X	O	*		A
Technical product ["crude MCHM"]		A	A	X	X	X		
Commercial Product (Dowanol DiPPH glycol ether)				X		X		
Cyclohexanemethanol, 4-((ethenyloxy)methyl)- [114651-37-5]					O	X	X	A
4-Methylcyclohexanecarboxylic acid [4331-54-8]					O	X		A
Cyclohexanemethanol, alpha,alpha,4-trimethyl- [498-81-7]					X	X	X	A
Phenoxyisopropanol [4169-04-4]					X	X	X	X

X= done and are inactive/negative; A= done and are active/positive; O = data will be available by early to mid June

\*Were not available at the time of testing, are currently available, and may still be tested; structural analogs are shown in yellow (not found in the spilled material)



# Summary of Findings

- SAR indicated that the MCHM class of chemicals may be irritating to the skin and sensory organs, and toxic to developing animals.
- None of the chemicals from the spill that were tested in HTS and Nematode Toxicity Studies were active.
- None of the chemicals from the spill except DMCHDC (very minor spill component) that were tested in the Zebrafish Developmental Toxicity Studies were active.
- None of the chemicals from the spill that were tested in Bacterial Mutagenesis and In vivo Micronucleus Studies were positive.
- MCHM and crude MCHM produced changes in biological activity at doses of approximately 100 mg/kg/day (approximates 1000 ppm in drinking water). PPH produced changes in biological activity at doses in the range of 1 mg/kg/day (approximates 30 ppm in drinking water).
- MCHM was a mild irritant but not a sensitizer and crude MCHM was a mild irritant and weak sensitizer.
- At doses well in excess of the drinking water advisory level MCHM was toxic to developing rats. Toxicity in the developing rats was observed at dose levels where there was no maternal toxicity. The most sensitive effect in the Rat Developmental Toxicity Study of MCHM was decreased fetal weight.



# Results in Context of NTP Study Goals

- Reduce uncertainty around the point of departure and safety factors used to develop the drinking water advisory levels
  - Results from the Rat Developmental Toxicity Studies and 5-Day Toxicogenomics Studies confirm a NOEL of approximately 100 mg/kg/day for MCHM (approximates 1000 ppm in drinking water for an infant). The NOEL is consistent with the 28-day study used to develop the drinking water advisory level.
  - PPH produced changes in biological activity starting at approximately 1 mg/kg/day (approximates 30 ppm in drinking water for a pregnant woman); however, the toxicological implications of these findings require further investigation. Many guideline studies are available for PPH that support the point of departure used by CDC.
  - Confirmed lack of genotoxic potential of the spilled chemicals, therefore reducing the concerns related to long-term effects such as carcinogenicity.



# Results in Context of NTP Study Goals

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- Determine if there are life-stage specific hazards
  - In rats, the fetus is more sensitive to toxicity from MCHM than the adult; however, toxicity was only observed at doses well in excess of the drinking water advisory level that was derived by CDC.
- Evaluate minor components of the spilled material to determine if there are differences in potency or toxicological properties compared to MCHM
  - There is minimal difference in potency or toxicity between most of the minor constituent chemicals and MCHM.
  - There are minimal differences between MCHM and crude MCHM in potency or toxicity.

Data produced by NTP to date supports a focus on MCHM in determining the health risks associated with the spill and the selection of 100 mg/kg/day as a point of departure on which to base a drinking water advisory level.



# Acknowledgements

- **Chemistry:** Brad Collins (lead), Suramyia Waidyanatha, MPI (Contractor)
- **SAR:** Scott Masten (lead), Neepa Choksi (ILS, contractor), Stephen Ferguson
- **HTS:** Tox21 Consortium
- **Nematode Toxicity:** Windy Boyd (lead)
- **Zebrafish Toxicity:** Ray Tice (lead), Robert Tanguay and Lisa Truong (Oregon State U, contractor)
- **Genotoxicity:** Kristine Witt (lead), Les Recio (ILS, contractor)
- **Dermal Irritancy/ Immune Toxicity:** Dori Germolec (lead), Burleson Research Technologies, Inc (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)
- **Analysis:** Jennifer Fostel and Vistrionix team; Grace Kissling; Laura Betz (SSS, contractor)
- **Communications:** Mary Wolfe, Yun Xie, Robin Macker
- **Wisdom:** John Bucher, Nigel Walker, Scott Masten, Ray Tice