NTP
National Toxicology Program

4-Methylcyclohexanemethanol (MCHM)

NTP Technical Report on the Prenatal Developmental Toxicity Studies of 4-Methylcyclohexanemethanol in Sprague Dawley (Hsd:Sprague Dawley SD) Rats (Gavage Studies)

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National Institute of Environmental Health Sciences

Peer-Review Meeting
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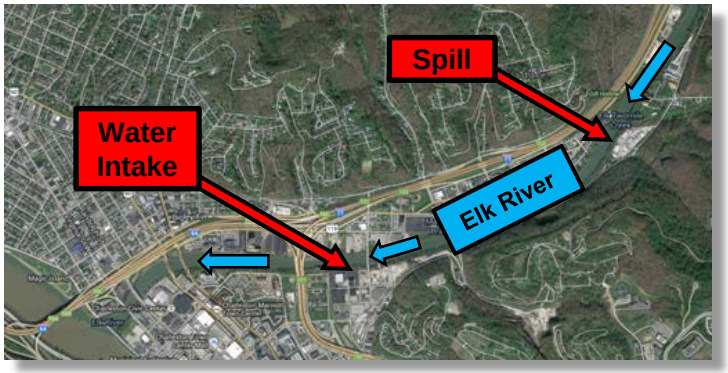
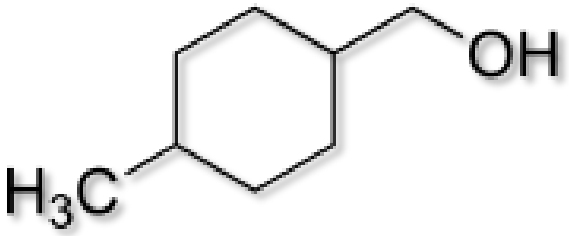
Elk River Chemical Spill

- Approximately 10,000 gallons of crude MCHM leaked into the Elk River (Charleston, WV) in January 2014
  - 68-89% of the spilled liquid was 4-methylcyclohexanemethanol (MCHM)
- Residents served by the municipal water supply were likely exposed to MCHM
  - Noted a “licorice” smell from tap water
  - Reported incidents of nausea, headaches, and rashes following the spill (Thomasson et al, 2017)



Background

- NTP partnered with the Centers for Disease Control and Prevention (CDC) and the Agency for Toxic Substances and Disease Registry (ATSDR) to evaluate toxicity of MCHM and other chemicals associated with the Elk River spill.

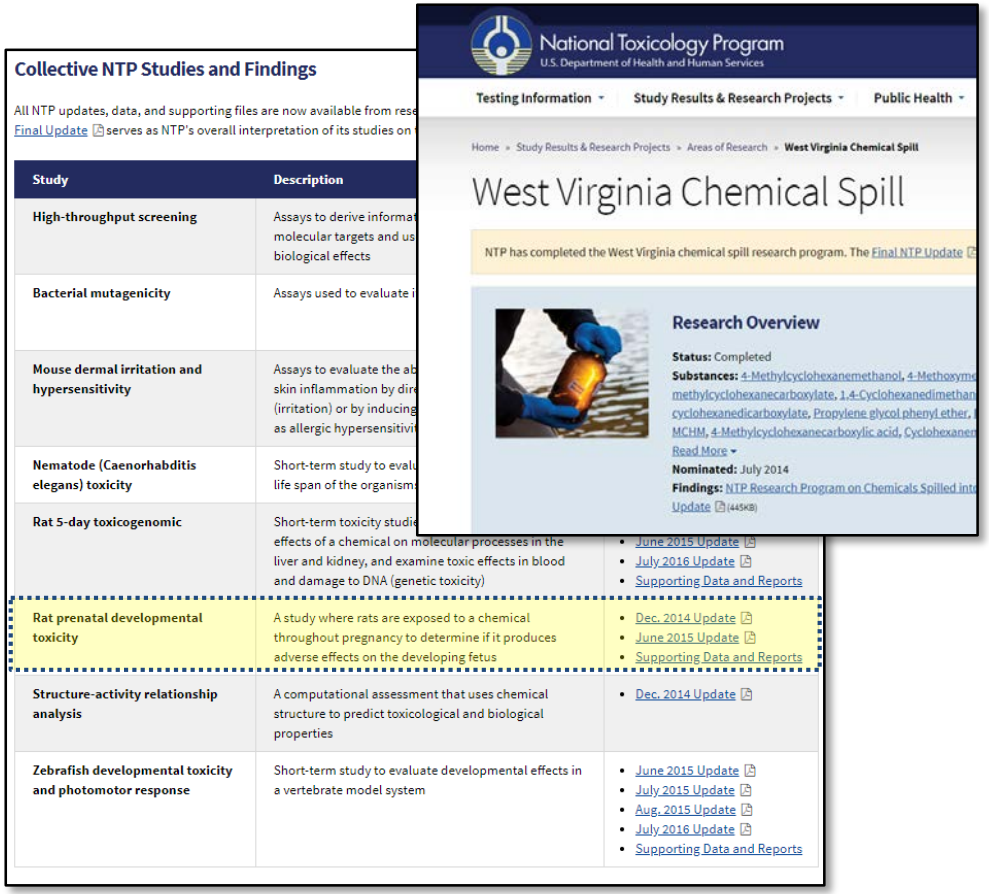


4-methylcyclohexanemethanol

- **Prenatal Developmental Toxicity Study Rationale:**
  - Concern for women of child-bearing potential and the developing embryo/fetus.
  - Evaluate the adequacy of the 1 part per million (ppm) advisory level for MCHM in drinking water

NTP West Virginia Chemical Spill Program

- Part of a broader NTP response to provide data to federal, state, and local public health agencies
- Data were made available and posted to the NTP public website (beginning December 2014)
- The data support the adequacy of the 1 ppm drinking water advisory level for MCHM at the time of the spill
- This draft report incorporates these findings into the NTP DART Report format
  - Level of Evidence Conclusion provided



Collective NTP Studies and Findings

All NTP updates, data, and supporting files are now available from rese
Final Update serves as NTP's overall interpretation of its studies on

Study	Description	
High-throughput screening	Assays to derive informat molecular targets and us biological effects	
Bacterial mutagenicity	Assays used to evaluate i	
Mouse dermal irritation and hypersensitivity	Assays to evaluate the ab skin inflammation by dire (irritation) or by inducing as allergic hypersensitivi	
Nematode (Caenorhabditis elegans) toxicity	Short-term study to evalu life span of the organism	
Rat 5-day toxicogenomic	Short-term toxicity studi effects of a chemical on molecular processes in the liver and kidney, and examine toxic effects in blood and damage to DNA (genetic toxicity)	• June 2015 Update • July 2016 Update • Supporting Data and Reports
Rat prenatal developmental toxicity	A study where rats are exposed to a chemical throughout pregnancy to determine if it produces adverse effects on the developing fetus	• Dec. 2014 Update • June 2015 Update • Supporting Data and Reports
Structure-activity relationship analysis	A computational assessment that uses chemical structure to predict toxicological and biological properties	• Dec. 2014 Update
Zebrafish developmental toxicity and photomotor response	Short-term study to evaluate developmental effects in a vertebrate model system	• June 2015 Update • July 2015 Update • Aug. 2015 Update • July 2016 Update • Supporting Data and Reports

National Toxicology Program
U.S. Department of Health and Human Services

Testing Information | Study Results & Research Projects | Public Health

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

West Virginia Chemical Spill

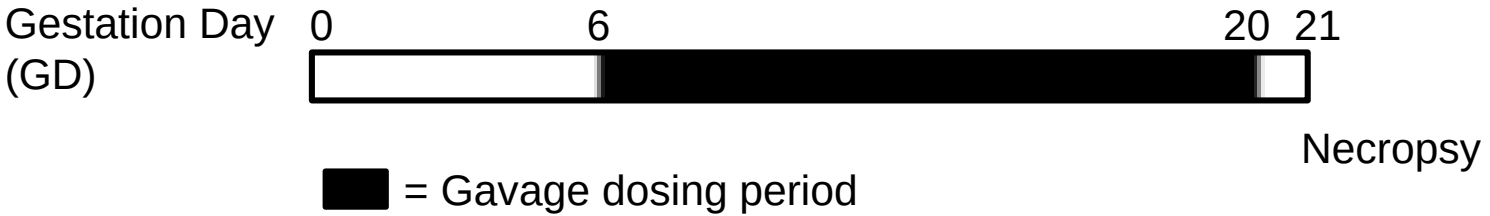
NTP has completed the West Virginia chemical spill research program. The Final NTP Update

Research Overview

Status: Completed
Substances: 4-Methylcyclohexanemethanol, 4-Methoxyme
methylcyclohexanecarboxylate, 1,4-Cyclohexanedimethan
cyclohexanedicarboxylate, Propylene glycol phenyl ether,
MCHM, 4-Methylcyclohexanecarboxylic acid, Cyclohexaner
Read More
Nominated: July 2014
Findings: NTP Research Program on Chemicals Spilled int
Update (445KB)

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

Dose Range-Finding Study Design



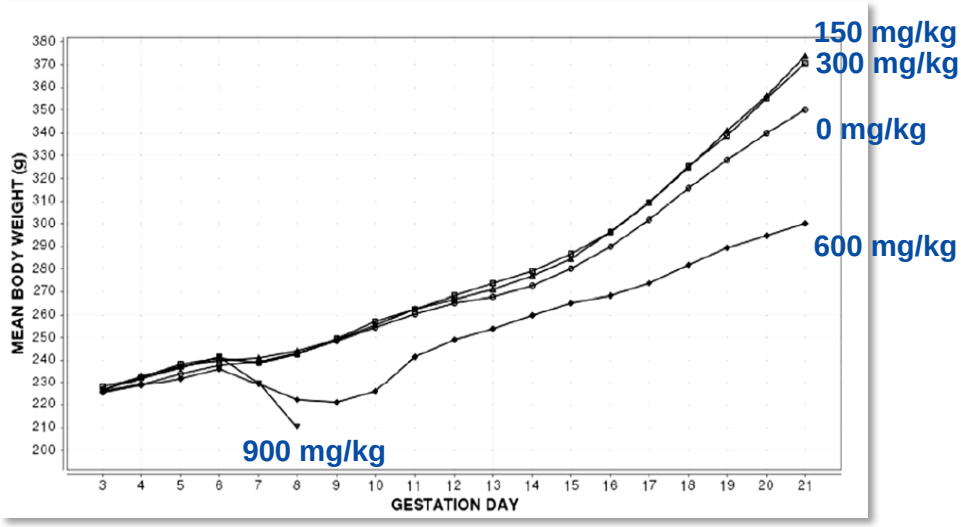
- **Doses: 0, 150, 300, 600, 900 mg/kg/day (gavage)**
  - Based on a range of doses identified from acute and four-week repeat-dose toxicity studies in adult rats (Eastman Chemical, 1990)
- N=10 time-mated, female rats per group
- Maternal endpoints: Clinical observations, body weights, body weight gain, feed consumption, and uterine parameters.
- Fetal endpoints: External examination and litter parameters including number of live/dead fetuses, sex ratio, fetal weight

Dose Range-Finding Study: Maternal Findings

- All dams in 900 mg/kg group were removed from study by GD 8 due to clinical signs of overt toxicity
  - Ataxia, cold to touch, clear ocular discharge, excessive salivation, lethargy, and/or piloerection
- Three (3) dams in the 600 mg/kg group displayed similar clinical observations, euthanized on GD 9 or 10
- No clinical signs of maternal toxicity was observed at ≤300 mg/kg

Dose Range-Finding Study: Maternal Findings

- At 600 mg/kg:
  - 44% lower overall weight gain from GD 6-21 compared to controls
  - 14% lower terminal body weight on GD 21
- Body weight gains and terminal body weights were similar to controls at ≤300 mg/kg



Dose Range-Finding Study: Uterine and Litter Findings

Endpoint	0 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
Maternal Terminal Body Weight (g)	349.9 ± 11.9	373.9 ± 11.5	370.5 ± 8.1	**300.0 ± 16.1***
Gravid Uterine Weight (g)	75.9 ± 10.1	88.8 ± 7.2	92.8 ± 4.7	**40.6 ± 14.3***
No. Litters	9	9	8	**5**
No. Live Fetuses	94	117	114	**47**
No. Live Fetuses per Litter	10.4 ± 1.5	13.0 ± 1.2	14.3 ± 0.9	**6.7 ± 2.5**
No. Resorptions (Early + Late)	11	5	2	**56**
No. Whole Litter Resorptions	0*	0	0	**2**
Post-implantation Loss (%)	9.8 ± 2.7%	5.7 ± 3.4%	2.1 ± 1.4%	**53.3 ± 17.5%**
Fetal Weight per Litter (g)	5.34 ± 0.16**	5.11 ± 0.11	**4.72 ± 0.07***	**3.28 ± 0.37****
	--	-4%	**-12%**	**-39%**

Values are reported as counts or mean ± standard error; (g)=grams
Statistically significant trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column); *P≤0.05, ** P≤0.01.

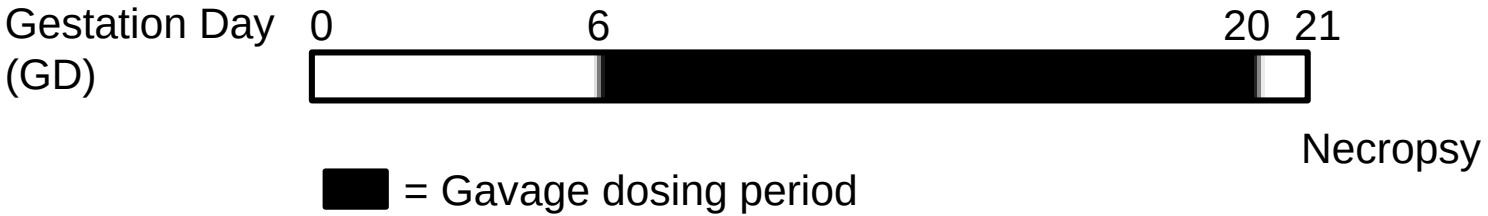
- Fewer number of live fetuses/litter and higher post-implantation loss at 600 mg/kg
- Lower fetal weights at 300 and 600 mg/kg relative to controls

Dose Range-Finding Study Summary

- Treatment-related maternal findings at doses ≥600 mg/kg
  - Overt toxicity at 900 mg/kg
  - Lower body weight gain (44%) from GD 6-21 at 600 mg/kg relative to controls
  - Increased post-implantation loss (53%) at 600 mg/kg
- Exposure-related decrease in fetal body weights
  - 12% and 39% lower at 300 and 600 mg/kg, respectively

- Based on these findings, doses of **0, 50, 100, 200, and 400 mg/kg** were selected for the main study

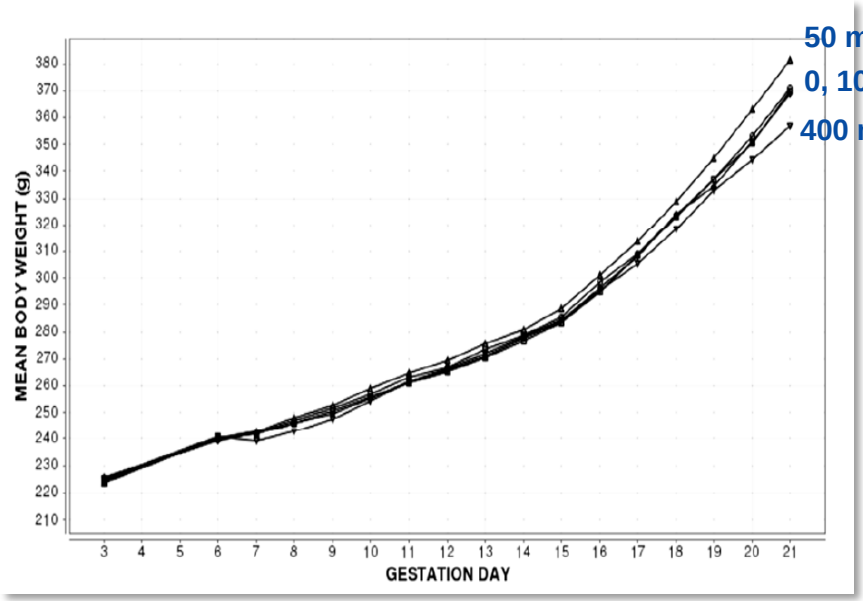
Main Study Design



- **Doses: 0, 50, 100, 200, 400 mg/kg/day (gavage)**
  - A fourth dose was included to better characterize the dose-response, if present
- N=25 time-mated, female rats per group
- Endpoints (in addition to those assessed in the Dose Range-Finding Study):
  - Maternal: Clinical chemistry on GD 21
  - Fetal: Visceral, head, and skeletal examinations

Main Study: Maternal Findings

- No dams were removed due to moribundity/mortality
- No treatment-related clinical observations were noted in any dose group
- Maternal body weight:
  - Decrease (11%) in body weight gain from GD 6-21 at 400 mg/kg
  - No effects on terminal body weights at necropsy on GD 21



Main Study: Maternal Clinical Pathology

Endpoint	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Urea nitrogen (mg/dL)	17.9 ± 0.6**	17.7 ± 0.5	17.5 ± 0.5	18.7 ± 1.0	**20.8 ± 0.6****
Glucose (mg/dL)	181.2 ± 10.3**	153.9 ± 7.4	176.0 ± 11.1	**150.6 ± 10.6**	**141.3 ± 9.3****
Triglycerides (mg/dL)	145.5 ± 21.0**	114.8 ± 18.0	94.6 ± 12.6	108.4 ± 14.0	**194.6 ± 31.8***
Calcium (mg/dL)	12.2 ± 0.2*	11.7 ± 0.2	11.8 ± 0.2	11.7 ± 0.2	**11.4 ± 0.3***
Alkaline phosphatase (IU/L)	138.4 ± 6.9	142.0 ± 4.7	131.7 ± 4.2	134.9 ± 5.4	**157.0 ± 6.2***
Total protein	5.17 ± 0.11**	4.93 ± 0.1	**4.84 ± 0.1***	**4.91 ± 0.15***	**4.74 ± 0.14****
Albumin (g/dL)	3.07 ± 0.09*	2.93 ± 0.07	2.95 ± 0.1	3.01 ± 0.12	2.85 ± 0.13
Globulin (g/dL)	2.10 ± 0.05**	2.01 ± 0.06	**1.89 ± 0.04****	**1.90 ± 0.04****	**1.89 ± 0.06****

Statistically significant (P≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column); ** P≤0.01
Values are reported as mean ± standard error; (g)= grams.

- Total protein was reduced ≤10% in 100, 200, and 400 mg/kg dams compared to controls
  - Mostly attributed to a ~10% reduction in the globulin fraction

Main Study: Uterine and Litter Parameters

Endpoint	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Maternal Terminal Body Weight (g)	370.9 ± 5.7**	381.5 ± 4.3	370.1 ± 5.8	368.8 ± 5.6	356.9 ± 4.9
Gravid Uterine Weight (g)	91.8 ± 4.1**	96.9 ± 3.6	90.4 ± 4.8	88.6 ± 4.9	**75.6 ± 4.2****
No. Litters	23	21	22	19	21
No. Live Fetuses	296	283	279	247	254
No. Live Fetuses per litter	12.9 ± 0.6	13.5 ± 0.6	12.7 ± 0.7	13.0 ± 0.8	12.1 ± 0.8
No. Resorptions (Early + Late)	22	18	12	14	20
No. Whole Litter Resorptions	0	0	1	0	0
Post-implantation Loss (%)	8.0 ± 2.5%	6.5 ± 3.1%	8.1 ± 4.5%	5.2 ± 2.0%	7.8 ± 2.5%
Fetal Weight per litter (g)	5.14 ± 0.07**	5.16 ± 0.08	5.14 ± 0.08	4.98 ± 0.09	**4.39 ± 0.09****
	--	+0.4%	0%	-3%	**-15%**

Values are reported as counts, or mean ± standard error; (g)= grams
Statistically significant trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column); *P≤0.05, ** P≤0.01

- 18% lower gravid uterine weight, 15% lower fetal weight in the 400 mg/kg dose group compared to controls
- No exposure-related external findings present in fetuses at any dose level.

Main Study: Fetal Visceral Findings

Endpoint		0 mg/kg/d	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	Historical Control
No. fetuses examined		296	283	279	247	254	1,324
No. litters examined		23	21	21	19	21	104
Kidney, discolored (Variation)	Fetuses	0*	1 (0.4%)	0	0	3 (1.2%)	0% (0/1324)
	Litters	0*	1 (4.8%)	0	0	3 (14.3%)	0% (0/104)
Adrenal, discolored (Variation)	Fetuses	0*	1 (0.4%)	0	0	3 (1.2%)	0% (0/1324)
	Litters	0*	1 (4.8%)	0	0	3 (14.3%)	0% (0/104)
Adrenal, misshapen (**Malformation**)	Fetuses	0*	1 (0.4%)	0	0	3 (1.2%)	0% (0/1324)
	Litters	0*	1 (4.8%)	0	0	3 (14.3%)	0% (0/104)

* Statistically significant trend (Cochran-Armitage), $p<0.05$

- There was a positive trend in the incidence of misshapen adrenals (malformation) and discoloration (variations) of the kidney and adrenals
  - All 3 findings occurred in 3 fetuses from 3 different litters in the 400 mg/kg group

Main Study: Fetal Skeletal Findings

Endpoint		0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	Historical Control
No. fetuses examined		296	283	279	247	254	1,324
No. litters examined		23	21	21	19	21	104
7th right costal cartilage, not fused to the sternum (Malformation)	Fetuses	0**	0	0	2 (0.8%)	**4 (1.6%)***	0% - 0.4%
	Litters	0**	0	0	1 (5.3%)	**4 (19.1%)***	0% - 5.6%
Short cervical SNR, total (Malformation)	Fetuses	0**	0	0	0	**6 (2.4%)****	0% - 0.4%
	Litters	0**	0	0	0	3 (14.3%)	0% - 4.4%
Full thoracolumbar SNR, total (Malformation)	Fetuses	2 (0.7%)**##	1 (0.4%)	6 (2.2%)	5 (2.0%)	**26 (10.3%)**##**	0.3% - 3.4%
	Litters	2 (8.7%)**	1 (4.8%)	3 (14.3%)	4 (21.1%)	**7 (33.3%)***	4.8% - 31.6%
Malformations (skeletal), total combined incidences	Fetuses	3 (1.0%)**##	3 (1.1%)	6 (2.2%)	7 (2.8%)	**40 (15.8%)**##**	0.7% - 3.4%
	Litters	3 (13.0%)**	3 (14.3%)	3 (14.3%)	5 (26.3%)	**12 (57.1%)****	9.5% - 31.6%

(SNR) = supernumerary ribs
Statistically significant according to mixed effects logistic regression. ##, P<0.01.
* Statistically significant according to Cochran-Armitage (trend) or Fisher exact (pairwise) test. **P<0.01

- Increase in combined incidences of malformations of the axial skeleton at 400 mg/kg

Main Study Summary

- Reduced maternal serum protein at doses ≥100 mg/kg/day
- No exposure-related fetal findings at doses ≤200 mg/kg/day
- Exposure-related fetal findings at 400 mg/kg/day:
  - 15% lower fetal body weights compared to controls
  - Increased incidences of malformations of the axial skeleton: full thoracolumbar supernumerary ribs (SNR), short cervical SNR, and costal cartilage not fused to the centrum
  - Misshapen adrenal glands (malformations)

Conclusions

Under the conditions of this prenatal study:

- ***Clear evidence*** of developmental toxicity of MCHM in Hsd:Sprague Dawley SD rats based on findings of:
  - Reduced fetal weight
  - Malformations of the axial skeleton
  - Malformations of the adrenal glands
- These findings occurred in fetuses of dams administered 400 mg/kg and in the absence of overt maternal toxicity

Questions?