Maternal and Prenatal Dose Range-Finding Study of 4-Methylcyclohexanemethanol (MCHM) in Harlan Sprague Dawley Rats

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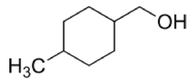
Abstract

4-methylcyclohexanemethanol (MCHM), used during the purification of coal, leaked from storage containers into the Elk River of West Virginia resulting in potential exposure to the residents of the area. The National Toxicology Program is conducting a prenatal toxicity evaluation in the Harlan Sprague Dawley rat. Doses for the range-finding study were 0, 150, 300, 600, and 900 mg/kg/day MCHM administered via gavage (corn oil vehicle) from gestational day (GD) 6-20 to rats (n=10). Parameters evaluated in dams included mortality, clinical observations, body weights, food consumption, gravid uterus weight, and postmortem observations on GD 21 (gross visceral examination, corpora lutea, implantations, embryo/fetal mortality). Parameters evaluated in offspring included fetal sex, weight, external abnormalities, and placental appearance. Due to excessive maternal toxicity, the 900 mg/kg/day group was terminated early as were three dams in the 600 mg/kg/day group. In the 600 mg/kg/day group, fetal weight was decreased and post-implantation loss was increased (53.3% vs. 9.8% in controls) due to increased number of dead fetuses and early/late resorptions. At 300 mg/kg/day, embryo-fetal toxicity consisted of decreased fetal weight (12%). There was no increase in fetal gross external observations among the dose groups. These data provide preliminary maternal and prenatal toxicity information on MCHM and will guide the design of the full prenatal toxicity evaluation.

Introduction



- A chemical spill into the Elk River, WV, estimated at 10,000 gallons, occurred on January 9th, 2014. The predominant chemical contained in the spill was 4-methylcyclohexanemethanol (MCHM)[1].
- MCHM is used to wash coal and remove impurities from coal.
- There is a limited amount of toxicity data available in the literature regarding MCHM:
  - Acute oral and dermal toxicity studies in rodents
  - Acute eye and skin irritation studies
  - 28-day toxicity study in rats
- In the 28-day toxicity study, doses of 0, 25, 100, 400 administered to male and female rats led to[2]:
  - Increased liver and kidney weight
  - Clinical chemistry changes
  - Kidney tubular degeneration
  - Liver Inflammation
- A No Observable Effect Level (NOEL) of 100 mg/kg/d was determined in the 28 day toxicity study. This dose was used to derive a short-term health advisory of 1 ppm (1 µg/ml) for MCHM by the Centers for Disease Control and Prevention (CDC)[1].
- Due to concern of exposure to pregnant women, the National Toxicology Program (NTP) will conduct a prenatal developmental toxicity study in order to assess potential toxicity to the developing embryo and fetus, and maternal toxicity.
- This dose range finding study was conducted in order to select doses for the prenatal developmental toxicity study.

Materials and Methods

- MCHM (CAS# 34885-03-5) was administered at dose levels of 0, 150, 300, 600, 900 mg/kg/d (n = 10) in corn oil vehicle to time-mated female Harlan Sprague Dawley Rats from GD 6 to GD 20.
- Clinical observations, body weight, body weight gain, and food consumption were collected in time-mated animals.
- On GD 21, animals were euthanized and uterine contents were examined. Uteri with no visible implants were placed in aqueous solution of 10% ammonium sulfide to detect early resorptions.
- Number of corpora lutea, implants, and fetuses were counted and fetal weights were collected. Post-implantation loss was calculated as: (number of implantations – number of live fetuses)/number of implantations
- Fetuses were examined for gross external malformations

Results (Preliminary)

- Due to clinical signs of overt toxicity, the 900 mg/kg/d group was euthanized on GD 8, in addition three animals in the 600 mg/kg/d group on were euthanized on GD 9-10 (Table 1).
- Clinical observations in these animals included ataxia, cold to the touch, hypoactivity, lethargy, and hunched posture.
- Food consumption was reduced in the 600 mg/kg/d group by approximately 15% (Table 2).
- Maternal body weight (Figure 1) and body weight gain was reduced in the 600 mg/kg/d group. Adjusting for uterine weight, body weight was reduced by 5% in the 600 mg/kg/d group (Table 2).
- Increased incidence of resorptions and reduced live fetuses/litter were observed in the remaining 600 mg/kg/d animals (Table 3).
- Total fetal weights, male fetal weights, and female fetal weights were reduced in the 300 and 600 mg/kg/d groups (Figure 2). In the 150 mg/kg group, total fetal weights were reduced by 5% (not statistically significant).
- There was no increased incidence of fetal gross external observations.

Table 1: Maternal Disposition

	0 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg	900 mg/kg
Time-Mated Females	10	10	10	10	10
Pregnant Females	9	10	9	10	9
Euthanized Early	0	0	0	3	9
Early Delivery	0	1	1	0	0
GD 21 Examination	9	9	8	7	0

Table 2: Maternal

	0 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
GD 21 Body Weight (g)	350± 12	374± 11	370 ± 8	300 ± 16
GD 21 Adjusted Body Weight (g)[1]	274 ± 3	285 ± 6	280 ± 6	259 ± 6
Food Consumption GD 6 – 21 (g/day)	20 ± 1	21 ± 1	22 ± 1	17 ± 1
Body Weight Gain (g) GD 6 – 21	112 ± 9	132 ± 8	130 ± 5	63 ± 17
Adjusted Body Weight Gain (g)[1] GD 6 - 21	36 ± 2	43 ± 3	39 ± 3	23 ± 4

[1] Adjusted for gravid uterine weight

Mean ± Standard Error

Figure 1: Maternal Body Weights

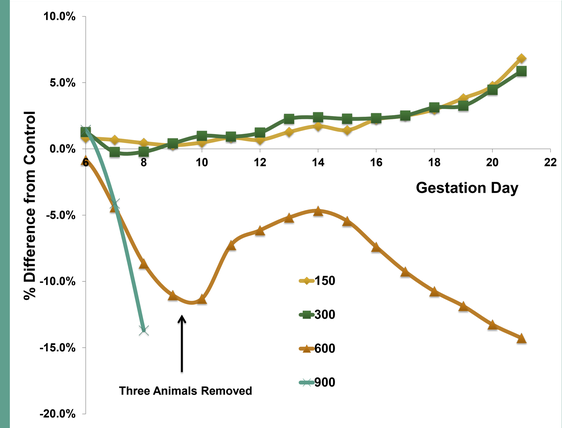
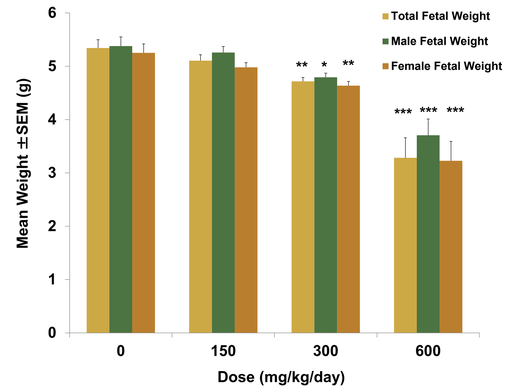


Table 3: Uterine Examinations

Endpoint	0 mg/kg/d	150 mg/kg/d	300 mg/kg/d	600 mg/kg/d
Corpora Lutea	16.7 ± 1.2	16.2 ± 0.5	16.2 ± 0.9	15.7 ± 0.7
Implants	11.7 ± 1.7	13.6 ± 0.9	14.5 ± 0.8	15.0 ± 0.8
Whole Litter Resorptions	0	0	0	2
Total Fetuses/Litter	10.4 ± 1.5	13.0 ± 1.2	14.3 ± 0.9	7.0 ± 2.7
Live Fetuses/Litter	10.4 ± 1.5	13.0 ± 1.2	14.3 ± 0.9	6.7 ± 2.5
Resorptions/ Litter	1.2 ± 0.4	0.6 ± 0.3	0.3 ± 0.2	8.0 ± 2.9
Post-implantation Loss (%)	9.8 ± 2.7	5.7 ± 3.4	2.1 ± 1.4	53.3 ± 17.5

Mean ±Std Error

Figure 2: Fetal Body Weights



Discussion

- The apparent increase in resorptions and decrease in live litter size, indicating potential embryo-fetal toxicity in the 600 mg/kg dose group, occurred with observations consisting of maternal toxicity of early euthanasia of three females, reduced body weight gain, and body weight.
- The reduction in fetal weights (male and/or female) in the 300 and 600 mg/kg/d groups suggests that MCHM has the potential to be fetal toxic. Maternal toxicity was not observed in the 300 mg/kg/d group and below.
- An apparent (non-statistically significant) reduction in total fetal weight of 5% in the 150 mg/kg/d dose group level suggests that MCHM may be fetal toxic at this dose level, which the larger study will evaluate.
- Although external gross observations of fetuses did not reveal a chemical related effect, the follow up study will evaluate more endpoints (e.g. visceral and skeletal malformations and variations) in a larger number of animals providing a better assessment of embryo-fetal toxicity in this animal model.

Future Directions

- These data were used to select doses for a full prenatal developmental toxicity study.
- Doses of 0, 50, 100, 200, 400 mg/kg were selected; four doses were used to capture the dose response of potential toxicity.
- These data will useful for the risk assessment of MCHM and health assessment of the Elk River Spill

References

[1] CDC Screening Level Assessment: http://emergency.cdc.gov/chemical/MCHM/westvirginia2014/mchm.asp

[2] Four-Week Oral Toxicity Study of 4-Methylcyclohexane Methanol in the Rat. R. Hosenfeld, Eastman Kodak Company, 1990.