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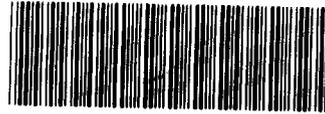
Richmond, CA
April 12, 1996

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Chevron Research and
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Toxicology & Health Risk Assessment

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Attention: TSCA 8(e) Coordinator
Office of Pollution Prevention and Toxics
U. S. Environmental Protection Agency
401 M Street S. W.
Washington, DC 20460



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ORIGINAL

RE: TSCA 8(e) Submission for Tertiary Amyl Methyl Ether (TAME)

Dear Sir or Madam:

This notice is submitted pursuant to Section 8(e) of the Toxic Substances Control Act on behalf of Amerada Hess Corporation, Chevron Products Company, CITGO Petroleum, Exxon Company USA, Marathon Oil Company, Sun Refining and Marketing, and Texaco Refining and Marketing. This notice is based on test results obtained under the Enforceable Consent Agreement (54 FR 14910 - March 21, 1995) for TAME (CAS No. 994-05-8). The required studies are being coordinated by staff from the American Petroleum Institute.

We are advising the EPA of results from a range-finding study for developmental toxicity in mice and rats conducted at 7000, 4000, and 1000 ppm TAME (OPPTS Guidelines 870.3700). An increased incidence of cleft palate was found in the pups of pregnant mice exposed to 4000 ppm TAME. Considering previous Agency guidance for reporting of developmental effects, we are notifying the EPA of these statistically significant findings. A copy of the available data is enclosed.

In mice,

- At 7000 ppm (one-half the of the lower explosive limit) TAME caused excessive toxicity including mortality. Three dams died and the rest were euthanized after one or two exposures.
- At 4000 ppm, dams displayed clinical signs of central nervous system depression including ataxia, hunched and prone posture, sedation (comatose), and forced respiration. One dam at this dose level died on gestation day 15. An external malformation (cleft palate) was found in three of six litters for a total of 10 affected out of 69 pups examined. No pups from the control group had this malformation. Fetal body weights were also significantly reduced by maternal exposure to TAME.

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- At 1000 ppm, no clinical signs of central nervous system depression were observed in the dams. No maternal or fetal toxicity was observed. The no-observed-adverse-effect-level for mice in this study was determined to be 1000 ppm.

The elevated incidence of mouse fetal malformations in this range-finding study can be linked to the maternal stress that was obvious at 4000 ppm. Maternal stress per se has been shown to cause cleft palate in mice. For example, transportation, food and water deprivation, low humidity, food restriction, and restraint of pregnant mice, all result in cleft palate in the offspring (references enclosed). A similar finding of cleft palate in mice was observed at maternally toxic doses of methyl tertiary butyl ether (MTBE). The no-observed-adverse-effect-level for maternal and fetal effects of MTBE in mice was also 1000 ppm.

A range-finding developmental study in rats was conducted concurrently with this reported study in mice. However, no fetal malformations were observed the rats. There was evidence of maternal and fetal toxicity (decreased body weight) demonstrating that the exposure concentrations of TAME were sufficient to test for such a response in rats. The results in the rat reinforce the idea that mice are uniquely susceptible to stress during pregnancy that may result in cleft palate in the pups.

The definitive developmental study in mice and rats will begin in April 1996. Results of that study will be forwarded to EPA under the conditions of the Enforceable Consent Agreement, Docket Number OPPTS-4205Q. If you have any questions about this submission, please contact Dr. Richard Rhoden at the American Petroleum Institute (202) 682-8480.

Sincerely,



Richard D. Cavalli
Manager, Toxicology & Health Risk Assessment

Enclosure: 1

cc. Mr. Gary Timm
Office of Pollution Prevention and Toxics
U. S. Environmental Protection Agency
401 M Street S. W.
Washington, DC 20460

REFERENCES

1. K.S. Brown, M.C. Johnston, J.D. Niswander, Isolated cleft palate in mice after transportation during gestation. Teratology 5: 119-124 (1972)
2. K.S. Brown, M.C. Johnston, P.F. Murphy, Isolated cleft palate in A/J mice after transitory exposure to drinking-water deprivation and low humidity in pregnancy. Teratology 9: 151-158 (1974)
3. S. Rosenzweig, F.M. Blaustein, Cleft palate in A/J mice resulting from restraint and deprivation of food and water. Teratology 3: 47-52 (1970)
4. R.D. Hemm, L. Arslanoglou, J.J. Pollock, Cleft Palate following prenatal food restriction in mice: association with elevated maternal corticosteroids. Teratology 15: 243-248 (1977)
5. K.S. Khera, Maternal toxicity - a possible factor in fetal malformations in mice. Teratology 29: 411-416 (1984)

Chemical Industry Institute of Toxicology



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President

March 19, 1996

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Dr. Richard Rhoden
American Petroleum Institute
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RE: CIIT Protocol 95051
RTI Contract No.: 65C-6398-200

Dear Dr. Rhoden:

As mandated by the protocol, enclosed please find the results of the study entitled "Concentration Range-Finding Study for the Developmental Toxicity Evaluation of Inhaled Tertiary Amyl Methyl Ether (TAME) in CD-1® Mice" (RTI-543, CIIT No. 95051) as text and seven draft summary tables.

As you recall, the study design involved three (3) chemical vapor-exposed groups and one (1) air-exposed group (control), each comprised of eight (8) timed-mated female mice. TAME exposure atmospheres were measured with two calibrated infrared spectrophotometers (MIRAN 1A). The mice (concurrently with CD® rats) were exposed to 0, 1000, 4000 or 7000 ppm TAME vapor for six (6) hours per day on gestational day (gd) 6 through 16. Dams were weighed on gd 0, 6, 9, 12, 15, 16 and 17, and feed consumption was measured for gd 0-6, 6-9, 9-12, 12-15, 15-16 and 16-17. Weight change and feed consumption were summarized for gd 0-6 (pre-exposure period), 6-17 (exposure period), and 0-17 (gestation period). Clinical observations were recorded once daily prior to the exposure period, and at least twice daily, pre- and post daily exposures, during the exposure period. At scheduled sacrifice on gd 17, the dams were moved to RTI, euthanized by CO₂ asphyxiation, weighed and necropsied. For each female, the body, liver and gravid uterus were weighed, ovarian corpora lutea were counted, and the number and status of all uterine implantation sites were documented: total, nonlive (resorptions, dead fetuses) and live fetuses. All live fetuses were counted, weighed, sexed externally and examined for external malformations (including cleft palate) and variations, euthanized by decapitation, and discarded.

The dates of performance were as follows: the breeding males and females arrived at CIIT on January 29, 1996; animals were paired 1:1 on February 22, 1996; gd 0 dates were February 23-24, 1996; exposures (gd 6 through 16), were February 29 - March 11, 1996; scheduled sacrifice at RTI (gd 17) was on March 11-12, 1996. The protocol was signed by the Study Director on January 26, 1996. There are also two amendments to this protocol.

RESULTS

The atmospheres of TAME vapor in the chambers were homogeneous during the pre-study analyses. Analytical mean values (grand means of daily means) for exposures beginning on February 29 (group 1) and on March 1, 1996 (group 2) were 0±0 (SD), 0±0 for 0 ppm; 989±8, 989±9 for 1000 ppm; 3948±144, 3986±112 for 4000 ppm; and 6985, 7041 for 7000 ppm. The analytical to target ratio (A/T) was 98.8±0.8 (SD), 98.9±0.9 for 1000 ppm; 98.7±3.6, 99.7±2.8 for 4000 ppm; and 100.0±0.0, 100.0±0.0 for 7000 ppm, respectively. The estimated limit of detection was 89.2 ppm (5.4% of the lowest

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calibration reading) for 4000 and 7000 ppm, and 38.7 ppm (5.9% of the lowest calibration reading) for 1000 ppm. Mean temperature values within the four chambers were 70.80, 70.80°F at 0 ppm; 72.01, 72.01°F at 1000 ppm; 70.48, 70.63°F at 4000 ppm; and 71.83, 69.03°F at 7000 ppm. Relative humidity values were 44.8, 43.8% at 0 ppm; 50.1, 50.1% at 1000 ppm; 50.3, 50.4% at 4000 ppm; and 55.8, 51.9% at 7000 ppm, respectively (Table 1). Please note that due to excessive toxicity at 7000 ppm, including mortality of three dams, this exposure concentration group was terminated (and surviving dams euthanized) with the Sponsor's concurrence on Friday, March 1, 1996 (Amendment No. 2 to protocol).

The distribution and fate of the study females are provided in Table 2. As indicated above, three dams died at 7000 ppm and the rest were euthanized after one or two exposure days. One dam (of 8, 12.5%) died at 4000 ppm. No dams were removed from study or delivered early. A total of five females were not pregnant at necropsy, one at 0 ppm, two at 1000 ppm and two at 7000 ppm. In addition, of the pregnant females, one each at 0 and 4000 ppm carried a fully resorbed litter at scheduled sacrifice on gd 17 (Table 2). All subsequent summarized data include pregnant animals only; table headers use the target TAME concentrations for consistency. Maternal body weights were equivalent across all groups on gd 0 and on gd 6 (immediately prior to the first exposure). All mice at 7000 ppm were terminated prior to gd 9 so all subsequent data, e.g., body weights, feed consumption, etc., after gd 6 do not include dams at 7000 ppm. Maternal body weights on gd 9, 12, 15, 16 and 17 (in-life and at sacrifice) were statistically equivalent across all groups although the mean values at 4000 ppm were lower than the values at 0 or 1000 ppm for all time points. Mean maternal weight change was statistically significantly reduced at 4000 ppm for gd 12-15, 6-17 (exposure period) and 0-17 (gestational period). A significant treatment-related downward trend ($p < 0.05$) was present for weight changes for gd 15-16, but there were no significant pairwise comparisons to the control group value. Maternal gestational weight change, corrected for the weight of the gravid uterus was equivalent across all groups. Gravid uterine weight was significantly reduced at 4000 ppm (most likely due to the reduced fetal body weights at this exposure concentration, see Table 2). Maternal absolute liver weights were equivalent across all groups; maternal liver weight relative to sacrifice weight was significantly increased at 4000 ppm (most likely due to the reduced maternal body weights in this group at scheduled sacrifice) (Table 2).

Maternal clinical observations, prior to the exposure period, immediately prior to and after each daily exposure during the exposure period and at sacrifice, are presented in Table 3. The only clinical observation noted at 0 ppm was clinical weight loss (defined as ≥ 1.0 g in any weigh period) in one dam each on gd 9 and 16. At 1000 ppm, the only clinical observations were rough coat and slight rough coat (and alopecia on nose of one dam throughout the in-life portion of the study). At 4000 ppm, dams were noted as ataxic, comatose and prone (post exposure only), hunched, with respiration shallow, slow, gasping, labored and/or rapid, rough coat, and clinical weight loss in one dam on gd 15 and in two dams on gd 16, with one dam (no. 14) found dead on gd 15. Red/reddish brown vaginal discharge was recorded at 4000 ppm in one dam on gd 12, two dams on gd 13, one dam on gd 14 and one dam on gd 15. At 7000 ppm (for gd 6 and 7 only), dams were observed as comatose, prone (post-exposure), respiration slow, slow and abdominal, irregular and gasping, with rough coat and tremors; two dams were found dead on gd 6 after exposure (nos. 12 and 13) and one was found dead (no. 5) on gd 7 (with the remaining dams, nos. 20, 21 and 29 euthanized on the morning of gd 7). Necropsy findings for unscheduled deaths at 7000 ppm included dark red nails on paws, lungs with anterior/apical portions or all lobes dark red, stomach with feed present. At 4000 ppm, the one female which died presented with dark red nails on all paws, medial lobe and left lung dark red and feed present in stomach. At scheduled necropsy, one dam at 4000 ppm exhibited three 1 mm red areas on left and right lung lobes (Table 3).

Maternal feed consumption, expressed as g/day and g/kg body weight/day, is presented in Table 4. Feed consumption in g/day and g/kg/day was equivalent across all groups prior to exposures, gd 0-6. Feed consumption in g/day and g/kg/day was significantly reduced at 4000 ppm for gd 6-9 (first exposure interval) and gd 15-16. In addition, feed consumption as g/kg/day (but not as g/day) was significantly reduced at 1000 ppm for gd 15-16. A significant dose-related downward trend ($p < 0.05$) was noted for feed consumption as g/day for gd 12-15 with no statistically significant pairwise comparisons; the value at 4000 ppm was clearly reduced. For gd 16-17 (measured from the morning of gd 16 prior to exposure

to the morning of gd 17 at scheduled sacrifice), feed consumption in g/kg/day was clearly increased at 4000 ppm, but the difference was not statistically significant (Table 4).

Gestational parameters are presented in Table 5. The mean numbers of ovarian corpora lutea per dam were equivalent across all groups. The mean number of uterine implantation sites per dam was slightly reduced at 4000 ppm (11.00 ± 1.70 [SEM]) relative to the value at 0 ppm (11.57 ± 1.48) or at 1000 ppm (11.50 ± 0.96). Percent preimplantation loss was therefore slightly, but not statistically significantly increased at 4000 ppm. Resorptions expressed as percent per litter or percent litters with resorptions were equivalent at 0 and 4000 ppm (each group had a dam with a fully resorbed litter) with the incidence lower at 1000 ppm. There were no late fetal deaths in this study. The incidence of nonlive implants (resorbed plus dead) therefore paralleled the values for resorptions. Adversely affected implants (number and percent per litter, number and percent of litters with adversely affected implants), which include nonlive plus malformed, appeared increased at 4000 ppm (due to fetal findings); percent adversely affected implants per litter was significantly reduced at 1000 ppm, due to the low incidence of resorptions at this exposure concentration. The number of live fetuses per litter and the sex ratio (percent male fetuses per litter) were equivalent across all groups. Mean fetal body weight per litter (all fetuses or separately by sex) was significantly reduced at 4000 ppm, with the reduction on the order of 50% (Table 5).

Summary and statistical analyses of fetal external findings (malformations and variations) are provided in Table 6 with listing of findings by defect type in Table 7. The numbers of fetuses (and litters) examined were 69(6), 67(6) and 69(6) at 0, 1000 and 4000 ppm, respectively (Table 6). The only fetal external malformation observed was cleft palate in three of six litters examined at 4000 ppm for a total of 10 affected (out of 69 examined). The finding of fetal cleft palate was not unanticipated since TAME has anesthetic qualities at high concentrations and mice respond by elevated corticosteroid levels which cause cleft palate in the developing offspring. (The exact same response in mice was observed in the inhalation developmental toxicity assessment of MTBE; Bevan *et al.*, 1995.) Therefore, the percent fetuses with malformations per litter and the percent litters with malformed fetuses were significantly increased at 4000 ppm. The only fetal external variation observed was open eye at 4000 ppm, with four fetuses in one litter with bilateral open eye and one fetus in the same litter with unilateral (left) open eye (Table 7). The finding of open eye may be due to the small size of the fetuses at 4000 ppm, since the fetal eye closes late in in utero development and these fetuses were clearly delayed in growth and probably in development. The incidence of fetal variations, while present only at 4000 ppm, was not statistically significantly different by fetuses per litter or by litter (Table 6).

RECOMMENDATIONS FOR TARGET EXPOSURE CONCENTRATIONS FOR THE DEFINITIVE STUDY

Based on the draft results of the range-finding study (with a small number of dams and fetuses), 7000 ppm is too maternally toxic, 4000 ppm results in obvious maternal and developmental toxicity including malformations (cleft palate) and variations (open eye), and 1000 ppm appears to result in little or no maternal or developmental toxicity. The only maternal findings at 1000 ppm were rough coat and significantly reduced feed consumption as g/kg/day for gd 15-16 only; there were no observations indicative of developmental toxicity. Therefore, our recommendations for target exposure concentrations for the definitive study in CD-1® mice are 0, 250, 1750 and 3500 ppm. The rationale is as follows. In the range-finding study, 4000 ppm resulted in profound maternal and developmental toxicity with fetal body weights/litter approximately 50% of the control and 1000 ppm values. In sharp contrast, there were essentially no maternal or developmental effects at 1000 ppm and the shape of the dose-response curve between 1000 and 4000 ppm is not known. Therefore, 3500 ppm should still be an effect level, and appropriate for the high exposure concentration. The recommended mid exposure concentration, 1750 ppm (one-half of the top concentration), should be a minimal effect level. The recommended low

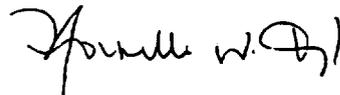
exposure concentration, 250 ppm, should be a "no observable adverse effect level" (NOAEL) for both dams and conceptuses. In our opinion, these exposure concentrations will also be appropriate for the rats (draft letter report under separate cover).

Please let us know at your earliest convenience of your decision on target concentrations so we may perform the appropriate start-up generation and analytical work prior to the arrival of the females for the definitive study, currently scheduled for March 28, 1996 for mice.

Thank you for your assistance on this project. We appreciate the opportunity to assist API in its toxicologic testing needs.

Sincerely,


Frank Welsch, DVM, DABT
Study Director
Chemical Industry Institute of Technology


Rochelle W. Tyl, Ph.D., DABT
Co-Investigator/Research Director
Center for Life Sciences and Toxicology
Research Triangle Institute

RWT:ng

Enclosures

cc: Dr. J. A. Bond, CIIT
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Ms. M. C. Marr/Ms. C. B. Myers, RTI
Ms. S. M. Taulbee, RTI
Dr. R. O. McClellan, CIIT

REFERENCE:

Bevan, C., Tyl, R.W., Neeper-Bradley, T.L., Fisher, L.C., Parson, R.D., Douglas, J.F., and Andrews, L.S. Developmental toxicity evaluation of methyl tertiary-butyl ether (MTBE) by inhalation in mice and rabbits. *J. Appl. Toxicol.*, in press, 1995.

Table 1. TAME Chamber Analyses

	Target TAME Concentrations, ppm			
	0	1000	4000	7000
Actual Chamber Concentration, ppm				
group 1 ^a	0.0 ± 0.0 ^b	988.5 ± 8.4	3947.7 ± 143.5	6984.9 ^c
group 2	0.0 ± 0.0	988.9 ± 8.9	3986.3 ± 112.4	7040.5
Nominal Chamber Concentration, ppm				
group 1	0.0 ± 0.0	1098.1 ± 4.2	4324.6 ± 605.3	7685.1
group 2	0.0 ± 0.0	1099.3 ± 4.5	4142.9 ± 26.1	7295.6
Actual to Normal Concentration, %				
group 1	100.0 ± 0.0	90.0 ± 0.7	92.7 ± 11.2	90.9
group 2	100.0 ± 0.0	90.0 ± 0.7	96.2 ± 2.5	96.5
Actual to Target Concentration, %				
group 1	100.0 ± 0.0	98.8 ± 0.8	98.7 ± 3.6	99.8
group 2	100.0 ± 0.0	98.9 ± 0.9	99.7 ± 2.8	100.6
Temperature (Dry Bulb), °F				
group 1	70.80 ± 2.23	72.01 ± 0.02	70.48 ± 1.37	71.83
group 2	70.80 ± 2.24	72.01 ± 0.02	70.63 ± 1.45	69.03
Relative Humidity, %				
group 1	44.8 ± 4.5	50.1 ± 0.2	50.3 ± 2.0	55.8
group 2	43.8 ± 4.3	50.1 ± 0.2	50.4 ± 1.9	51.9

- a Group 1 exposures began on February 29, 1996.
Group 2 exposures began on March 1, 1996.
- b Grand mean of 11 daily means ± standard deviation.
- c Value for one exposure day.

Table 2. Summary and Statistical Analysis of Maternal Body Weights, Weight Changes, Organ Weights and Relative Organ Weights (page 1 of 3)

	Tertiary Amyl Methyl Ether (ppm, inhaled)			
	0	1000	4000	7000
SUBJECTS (No. Dams)				
No. on Study	8	8	8	8
No. Removed	0	0	0	0
No. Dead or Euthanized	0	0	1 ^a	6 ^b
No. Nonpregnant	1	2	0	2
No. (%) Pregnant at Sacrifice on Gestational Day 17	7 (87.5)	6 (75.0)	7 (100.0)	
No. (%) with 100% Resorptions	1 (14.3)	0 (0.0)	1 (14.3)	
Maternal Body Weight (gd 0) (g)^c				
	27.7 ± 0.8 N=7	28.0 ± 1.2 N=6	27.8 ± 1.0 N=8	27.5 ± 0.9 N=6
Maternal Body Weight (gd 6) (g)^c				
	30.2 ± 0.8 N=7	30.9 ± 1.4 N=6	30.4 ± 0.8 N=8	29.3 ± 0.8 N=6
Maternal Body Weight (gd 9) (g)^c				
	32.2 ± 1.1 N=7	33.2 ± 1.2 N=6	32.4 ± 0.9 N=8	
Maternal Body Weight (gd 12) (g)^c				
	36.3 ± 1.6 N=7	37.3 ± 1.8 N=6	35.9 ± 1.1 N=8	
Maternal Body Weight (gd 15) (g)^c				
	42.2 ± 2.6 N=7	44.2 ± 1.9 N=6	39.3 ± 2.2 N=7	
Maternal Body Weight (gd 16) (g)^c				
	44.0 ± 3.1 N=7	46.4 ± 2.1 N=6	39.3 ± 2.4 N=7	
Maternal Body Weight (gd 17) (g)^c				
	46.8 ± 3.4 N=7	48.4 ± 2.2 N=6	41.9 ± 2.7 N=7	

(continued)

Table 2. Summary and Statistical Analysis of Maternal Body Weights, Weight Changes, Organ Weights and Relative Organ Weights (page 2 of 3)

	Tertiary Amyl Methyl Ether (ppm, inhaled)			
	0	1000	4000	7000
Maternal Body Weight (gd 17 at sacrifice) (g) ^C	46.1 ± 3.4 N=7	48.4 ± 2.0 N=6	41.3 ± 2.8 N=7	
Maternal Body Weight Change (gd 0 to 6) (g) ^C	2.5 ± 0.2 N=7	2.8 ± 0.6 N=6	2.7 ± 0.4 N=8	1.8 ± 0.5 N=6
Maternal Body Weight Change (gd 6 to 9) (g) ^C	1.9 ± 0.6 N=7	2.4 ± 0.5 N=6	1.9 ± 0.5 N=8	
Maternal Body Weight Change (gd 9 to 12) (g) ^C	4.2 ± 0.7 N=7	4.1 ± 0.9 N=6	3.5 ± 0.5 N=8	
Maternal Body Weight Change (gd 12 to 15) (g) ^C	5.9 ¶ ± 1.2 ¶ N=7	6.9 ± 0.6 N=6	3.1 ■ ± 1.0 N=7	
Maternal Body Weight Change (gd 15 to 16) (g) ^C	1.8 ± 0.7 ¶ N=7	2.2 ± 0.2 N=6	0.0 ± 0.9 N=7	
Maternal Body Weight Change (gd 16 to 17) (g) ^C	2.7 ± 0.8 N=7	2.1 ± 0.4 N=6	2.5 ± 0.9 N=7	
Maternal Body Weight Change (gd 6 to 17) (g) ^C	16.5 ¶¶ ± 3.0 ¶¶ N=7	17.6 ± 1.3 N=6	11.3 ■ ± 2.1 N=7	
Maternal Body Weight Change (gestation) (g) ^C	18.3 ¶¶ ± 2.8 ¶¶ N=7	20.4 ± 1.0 N=6	13.4 ■ ± 2.0 N=7	

(continued)

Table 2. Summary and Statistical Analysis of Maternal Body Weights, Weight Changes, Organ Weights and Relative Organ Weights (page 3 of 3)

	Tertiary Amyl Methyl Ether (ppm, inhaled)			
	0	1000	4000	7000
Maternal Body Weight Change (corrected) (g) ^{c,d}	3.84 ± 0.40 N=7	4.68 ± 0.57 N=6	4.33 ± 0.68 N=7	
Gravid Uterine Weight (g) ^c	14.48 ††† ± 2.55 ††† N=7	15.68 ± 1.07 N=6	9.09 [□] ± 1.52 N=7	
Maternal Liver Weight (g) ^c	2.58 ± 0.22 N=7	2.92 ± 0.14 N=6	2.91 ± 0.17 N=7	
Relative Maternal Liver Weight (% sacrifice weight) ^c	5.57 ††† ± 0.11 ††† N=7	6.04 ± 0.20 N=6	7.09 ^{□□} ± 0.18 N=7	

^aDam 14 was found dead on the morning of gestational day 15.

^bDam 5 was found dead on the morning of gestational day 7. Dams 12 and 13 were found dead on the afternoon of gestational day 6. Dams 20, 21, and 29 were euthanized at the sponsor's request on the morning of gestational day 7.

^cIncludes all pregnant dams until they died, were euthanized, or until terminal sacrifice on gestational day 17. Reported as the mean ± S.E.M.; gd=gestational day.

^dWeight change during gestation minus gravid uterine weight.

[†]p<0.05; Kruskal-Wallis Test.

^{†††}p<0.01; Kruskal-Wallis Test.

^{*}p<0.05; Jonckheere's Test.

^{**}p<0.01; Jonckheere's Test.

^{***}p<0.001; Jonckheere's Test.

[□]p<0.05; Mann-Whitney U Test.

^{□□}p<0.01; Mann-Whitney U Test.

Table 3. Summary of Clinical Observations and Necropsy Findings (page 1 of 4)

Day ^a	Clinical Observation ^b	Tertiary Amyl Methyl Ether (ppm, inhaled)			
		0	1000	4000	7000
2	Rough coat, slight		1		
4	Alopecia: nose		1	1	
	Rough coat, slight		1		
5	Alopecia: nose		1	1	
	Rough coat, slight		2		
6	Alopecia: nose		1	1	
	Ataxia ^c			3	
	Ataxia, severe			1	
	Comatose ^c				4
	Found dead				2
	Prone ^c			2	
	Prone, no eye blink reflex			1	
	Prone, will right if turned over			1	
	Respiration labored			1	
	Respiration shallow				3
	Respiration slow			2	
	Respiration slow, abdominal				1
	Rough coat			3	
	Rough coat, slight, improving		1		
7	Alopecia: nose		1	2	
	Ataxia, moderate			1	
	Ataxia, severe			4	
	Comatose			1	
	Comatose, no blink or pain reflex				1
	Euthanized				3
	Extremities reddened			1	
	Found dead				1
	Prone				1
	Prone, not able to right self			1	
	Prone, not able to right self, has eye blink reflex			1	
	Respiration irregular, gasping			1	
	Respiration labored			1	
	Respiration slow				1
	Respiration slow, labored			1	
	Respiration slow, shallow				1
	Rough coat		1	4	1
	Rough coat, slight, improving		1		
Tremors				1	

(continued)

Table 3. Summary of Clinical Observations and Necropsy Findings (page 2 of 4)

Day ^a	Clinical Observation ^b	Tertiary Amyl Methyl Ether (ppm, inhaled)			
		0	1000	4000	7000
8	Alopecia: nose		1	2	
	Comatose, no eye blink reflex			1	
	Comatose, not able to right self			1	
	Gasping			1	
	Hunched			1	
	Prone			1	
	Prone, drawn			1	
	Prone, not able to right self			4	
	Respiration irregular, gasping			1	
	Respiration labored			3	
	Respiration rapid			1	
	Respiration rapid, gasping			1	
	Respiration slow, shallow			1	
	Rough coat		2	2	
Tremors			2		
9	Alopecia: nose			1	
	Ataxia, severe			2	
	Ataxia, severe; rolls from side to side when moving			1	
	Gasping			3	
	Hunched			2	
	Prone, not able to right self			5	
	Respiration labored			1	
	Rough coat		2	6	
	Tremors			2	
	Weight loss ^d : 1.28 g	1			
10	Alopecia: nose		1	2	
	Ataxia, severe			2	
	Gasping			6	
	Hunched			1	
	Prone			5	
	Prone, not able to right self			1	
	Rough coat		1	4	
11	Alopecia: nose		1	2	
	Ataxia, severe			3	
	Gasping			5	
	Hunched			1	
	Prone			2	
	Prone, not able to right self			4	
	Respiration labored			1	
	Rough coat		2	7	
12	Alopecia: nose		1	2	
	Ataxia, severe			4	
	Gasping			5	
	Prone			4	
	Rough coat	1	2	5	
	Vaginal discharge, red			1	

(continued)

Table 3. Summary of Clinical Observations and Necropsy Findings (page 3 of 4)

Day ^a	Clinical Observation ^b	Tertiary Amyl Methyl Ether (ppm, inhaled)			
		0	1000	4000	7000
13	Alopecia: nose		1	2	
	Ataxia, severe			1	
	Fur: yellow on abdomen			1	
	Gasping			3	
	Prone			7	
	Rough coat		1	6	
	Tremors			1	
	Vaginal discharge, red			1	
	Vaginal discharge, reddish brown			1	
14	Alopecia: nose		1	2	
	Ataxia			2	
	Ataxia, severe			2	
	Gasping			5	
	Prone			6	
	Rough coat		1	5	
	Vaginal discharge, red			1	
15	Alopecia: nose		1	1	
	Ataxia			2	
	Ataxia, severe			2	
	Found dead			1	
	Gasping			5	
	Prone			3	
	Rough coat		1	6	
	Tremors			1	
	Vaginal discharge, red			1	
	Weight loss: 2.24 g			1	
	16	Alopecia: nose		1	1
Ataxia, severe				2	
Gasping				3	
Prone				5	
Rough coat			2	6	
Weight loss: 1.66 g		1			
1.01, 4.98 g				2	
17	Alopecia: nose			1	
	Rough coat		1	1	

NECROPSY FINDINGS FOR UNSCHEDULED DEATHS

Day ^a	Finding	Tertiary Amyl Methyl Ether (ppm, inhaled)			
		0	1000	4000	7000
6	Dark red nails on all paws				1
	Lungs: all lobes dark red				1
	anterior portion dark red				1
	Stomach: food mostly digested				1
	full of food				1

(continued)

Table 3. Summary of Clinical Observations and Necropsy Findings (page 4 of 4)

Day ^a	Finding	Tertiary Amyl Methyl Ether (ppm, inhaled)			
		0	1000	4000	7000
7	Dark red nails on all paws				1
	Eye(s): opaque				1
	right, opaque				1
	Lungs: apical portion of left and right lobes dark red				1
	apical portion of right lobe dark red				1
	midline dark red, all lobes, pink at edges				1
	Stomach: food present				1
	full of food				1
	very little food				2
Urinary bladder: full				1	
15	Dark red nails on all paws			1	
	Lungs: medial lobe and left lung dark red			1	
	Stomach: food present			1	

NECROPSY FINDINGS FOR SCHEDULED DEATHS

Day ^a	Finding	Tertiary Amyl Methyl Ether (ppm, inhaled)			
		0	1000	4000	7000
17	Lungs: three 1 mm red areas on left and right lobes			1	

^aGestational day.

^bClinical observations are tabulated once per day per animal.

^cAll ataxia, prone, and comatose observations were post exposure.

^dClinical weight loss is weight loss \geq 1.0 gram in any one weight period.

Table 4. Summary and Statistical Analysis of Maternal Feed Consumption (page 1 of 2)

	Tertiary Amyl Methyl Ether (ppm, inhaled)			
	0	1000	4000	7000
No. Dams Gestational Day 0	7	6	8	6
No. Dams Gestational Day 6	7	6	8	6
No. Dams Gestational Day 9	7	6	8	0
No. Dams Gestational Day 12	7	6	8	0
No. Dams Gestational Day 15	7	6	7	0
Maternal Feed Consumption (gd 0 to 6) (g/day) ^a				
	7.8	8.1	8.5	7.8
	± 0.2	± 0.4	± 0.4	± 0.3
	N=7	N=6	N=8	N=6
Maternal Feed Consumption (gd 6 to 9) (g/day) ^a				
	7.2 ¶¶	8.1	5.6 □	
	± 0.3 ¶	± 0.4	± 0.5	
	N=7	N=5 ^b	N=8	
Maternal Feed Consumption (gd 9 to 12) (g/day) ^a				
	6.8	8.1	7.6	
	± 0.5	± 0.8	± 0.7	
	N=7	N=6	N=8	
Maternal Feed Consumption (gd 12 to 15) (g/day) ^a				
	8.7 ¶	8.4	6.5	
	± 1.0 ¶	± 0.5	± 0.5	
	N=7	N=6	N=7	
Maternal Feed Consumption (gd 15 to 16) (g/day) ^a				
	12.9 ¶	8.2	5.6 □	
	± 2.4 ¶	± 1.3	± 1.1	
	N=7	N=6	N=7	
Maternal Feed Consumption (gd 16 to 17) (g/day) ^a				
	7.8	8.8	8.6	
	± 0.4	± 1.0	± 0.4	
	N=6 ^c	N=6	N=7	
Maternal Feed Consumption (gd 6 to 17) (g/day) ^a				
	7.8	8.2	6.8	
	± 0.6	± 0.6	± 0.3	
	N=6 ^c	N=5 ^b	N=7	
Maternal Feed Consumption (gd 0 to 17) (g/day) ^a				
	7.7	8.1	7.4	
	± 0.4	± 0.6	± 0.3	
	N=6 ^c	N=5 ^b	N=7	

(continued)

Table 4. Summary and Statistical Analysis of Maternal Feed Consumption (page 2 of 2)

	Tertiary Amyl Methyl Ether (ppm, inhaled)			
	0	1000	4000	7000
Maternal Feed Consumption (gd 0 to 6) (g/kg/day) ^a	268.3 ± 7.4 N=7	274.6 ± 9.7 N=6	296.0 ± 16.3 N=8	276.4 ± 16.1 N=6
Maternal Feed Consumption (gd 6 to 9) (g/kg/day) ^a	232.5 ¶ ± 8.9 ✕ N=7	251.1 ± 15.5 N=5 ^b	178.0 □ ± 16.9 N=8	
Maternal Feed Consumption (gd 9 to 12) (g/kg/day) ^a	198.9 ± 14.1 N=7	227.9 ± 19.8 N=6	220.8 ± 18.0 N=8	
Maternal Feed Consumption (gd 12 to 15) (g/kg/day) ^a	220.5 ± 22.7 N=7	208.3 ± 15.2 N=6	174.5 ± 11.7 N=7	
Maternal Feed Consumption (gd 15 to 16) (g/kg/day) ^a	292.7 ¶¶ ± 40.7 ✕ N=7	179.6 □ ± 25.1 N=6	141.4 □□ ± 26.1 N=7	
Maternal Feed Consumption (gd 16 to 17) (g/kg/day) ^a	188.8 ± 29.0 N=6 ^c	185.0 ± 17.0 N=6	214.4 ± 8.3 N=7	
Maternal Feed Consumption (gd 6 to 17) (g/kg/day) ^a	207.7 ± 14.2 N=6 ^c	202.5 ± 11.5 N=5 ^b	188.2 ± 7.9 N=7	
Maternal Feed Consumption (gd 0 to 17) (g/kg/day) ^a	215.1 ± 11.4 N=6 ^c	210.3 ± 10.8 N=5 ^b	209.9 ± 10.1 N=7	

^aIncludes all pregnant dams until they died, were euthanized or until terminal sacrifice on gestational day 17. Reported as the mean ± S.E.M.; gd = gestational day.

^bDecrease in N is due to one dam's feed being wet and some feed found outside of the feed hopper therefore an accurate weight could not be obtained.

^cDecrease in N is due to one dam's feed consumption value being unrealistic (i.e. negative value) and therefore it was excluded.

¶ p<0.05; Kruskal-Wallis Test.

¶¶ p<0.01; Kruskal-Wallis Test.

✕ p<0.05; Jonckheere's Test.

✕✕ p<0.01; Jonckheere's Test.

□ p<0.05; Mann-Whitney U Test.

□□ p<0.01; Mann-Whitney U Test.

Table 5. Summary and Statistical Analysis of Uterine Contents, Live Fetal Sex and Live Fetal Body Weights
(page 1 of 3)

	Tertiary Amyl Methyl Ether (ppm, inhaled)			
	0	1000	4000	7000
ALL LITTERS^a	7	6	7	0
No. Corpora Lutea per Dam^b	12.14 ± 0.96 N=7	12.17 ± 1.01 N=6	12.71 ± 0.78 N=7	
No. Implantation Sites per Litter^b	11.57 ± 1.48 N=7	11.50 ± 0.96 N=6	11.00 ± 1.70 N=7	
Percent Preimplantation Loss per Litter^b	9.43 ± 6.91 N=7	6.62 ± 3.22 N=6	17.17 ± 11.28 N=7	
No. Resorptions per Litter^b	1.71 ± 0.47 N=7	0.33 ± 0.21 N=6	1.14 ± 0.34 N=7	
Percent Resorptions per Litter^b	23.48 ± 12.92 N=7	2.71 ± 1.78 N=6	21.27 ± 13.32 N=7	
No. Litters with Resorptions	6	2	5	
% Litters with Resorptions	85.71	33.33	71.43	
No. Late Fetal Deaths per Litter^b	0.00 ± 0.00 N=7	0.00 ± 0.00 N=6	0.00 ± 0.00 N=7	
Percent Late Fetal Deaths per Litter^b	0.00 ± 0.00 N=7	0.00 ± 0.00 N=6	0.00 ± 0.00 N=7	
No. Litters with Late Fetal Deaths	0	0	0	
% Litters with Late Fetal Deaths	0.00	0.00	0.00	

(continued)

Table 5. Summary and Statistical Analysis of Uterine Contents, Live Fetal Sex and Live Fetal Body Weights
(page 2 of 3)

	Tertiary Amyl Methyl Ether (ppm, inhaled)			
	0	1000	4000	7000
No. Nonlive Implants per Litter ^{b,c}	1.71 ± 0.47 N=7	0.33 ± 0.21 N=6	1.14 ± 0.34 N=7	
Percent Nonlive Implants per Litter ^{b,c}	23.48 ±12.92 N=7	2.71 ± 1.78 N=6	21.27 ±13.32 N=7	
No. Litters with Nonlive Implants ^c	6	2	5	
% Litters with Nonlive Implants ^c	85.71	33.33	71.43	
No. Adversely Affected Implants per Litter ^{b,d}	1.71 ± 0.47 N=7	0.33 ± 0.21 N=6	2.57 ± 0.72 N=7	
Percent Adversely Affected Implants per Litter ^{b,d}	23.48 ¶ ±12.92 N=7	2.71 ▫ ± 1.78 N=6	31.49 ±12.33 N=7	
No. Litters with Adversely Affected Implants ^d	6	2	6	
% Litters with Adversely Affected Implants ^d	85.71	33.33	85.71	
<u>LIVE LITTERS</u> ^e	6	6	6	0
No. Live Fetuses per Litter ^b	11.50 ± 1.02 N=6	11.17 ± 0.87 N=6	11.50 ± 0.99 N=6	
Percent Male Fetuses per Litter ^b	51.99 ± 7.50 N=6	47.26 ± 5.30 N=6	49.17 ± 9.37 N=6	

(continued)

Table 5. Summary and Statistical Analysis of Uterine Contents, Live Fetal Sex and Live Fetal Body Weights (page 3 of 3)

	Tertiary Amyl Methyl Ether (ppm, inhaled)			
	0	1000	4000	7000
No. Male Fetuses per Litter^b	6.00 ± 1.06 N=6	5.17 ± 0.48 N=6	5.33 ± 0.76 N=6	
No. Female Fetuses per Litter^b	5.50 ± 0.92 N=6	6.00 ± 0.89 N=6	6.17 ± 1.35 N=6	
Average Fetal Body Weight (g) per Litter^b	1.020 $\overline{\overline{\overline{\uparrow\uparrow\uparrow}}}$ ± 0.034 $\overline{\overline{\overline{\times\overline{\times}}}}$ N=6	1.008 ± 0.053 N=6	0.497 $\overline{\overline{\overline{\times\overline{\times}}}}$ ± 0.036 N=6	
Average Male Fetal Body Weight (g) per Litter^b	1.043 $\overline{\overline{\overline{\uparrow\uparrow\uparrow}}}$ ± 0.033 $\overline{\overline{\overline{\times\overline{\times}}}}$ N=6	1.031 ± 0.056 N=6	0.500 $\overline{\overline{\overline{\times\overline{\times}}}}$ ± 0.038 N=6	
Average Female Fetal Body Weight (g) per Litter^b	0.994 $\overline{\overline{\overline{\uparrow\uparrow\uparrow}}}$ ± 0.035 $\overline{\overline{\overline{\times\overline{\times}}}}$ N=6	0.987 ± 0.054 N=6	0.484 $\overline{\overline{\overline{\times\overline{\times}}}}$ ± 0.030 N=6	

^aIncludes all dams pregnant at terminal sacrifice on gestational day 17; litter size = no. implantation sites per dam.

^bReported as the mean ± S.E.M.

^cNonlive = late fetal deaths plus resorptions.

^dAdversely affected = nonlive plus malformed.

^eIncludes only dams with live fetuses; litter size = no. live fetuses per dam.

$\overline{\overline{\overline{\uparrow\uparrow\uparrow}}}$ p<0.05; Kruskal-Wallis Test.

$\overline{\overline{\overline{\uparrow\uparrow\uparrow}}}$ p<0.01; Kruskal-Wallis Test.

$\overline{\overline{\overline{\times\overline{\times}}}}$ p<0.01; Jonckheere's Test.

$\overline{\overline{\overline{\times\overline{\times}}}}$ p<0.05; Mann-Whitney U Test.

$\overline{\overline{\overline{\times\overline{\times}}}}$ p<0.01; Mann-Whitney U Test.

Table 6. Summary and Statistical Analysis of External Malformations and Variations (page 1 of 2)

	Tertiary Amyl Methyl Ether (ppm, inhaled)			
	0	1000	4000	7000
No. Fetuses Examined ^a	69	67	69	
No. Litters Examined ^b	6	6	6	
No. Fetuses Malformed per Litter ^{c,d}	0.00 ± 0.00 N=6	0.00 ± 0.00 N=6	1.67 ± 0.84 N=6	
Percent Fetuses with Malformations per Litter ^{c,e}	0.00 ¶ ± 0.00 N=6	0.00 ± 0.00 N=6	12.74 ■ ± 5.97 N=6	
No. Fetuses with Malformations ^c	0	0	10	
% Fetuses with Malformations ^c	0.00	0.00	14.49	
No. Litters with Malformations ^e	0	0	3	
% Litters with Malformations ^e	0.00 £	0.00	50.00 Ø	
No. Fetuses with Variations per Litter ^{c,d}	0.00 ± 0.00 N=6	0.00 ± 0.00 N=6	0.83 ± 0.83 N=6	
Percent Fetuses with Variations per Litter ^{c,d}	0.00 ± 0.00 N=6	0.00 ± 0.00 N=6	5.56 ± 5.56 N=6	

(continued)

Table 6. Summary and Statistical Analysis of External Malformations and Variations (page 2 of 2)

	Tertiary Amyl Methyl Ether (ppm, inhaled)			
	0	1000	4000	7000
No. Fetuses with Variations ^c	0	0	5	
% Fetuses with Variations ^c	0.00	0.00	7.25	
No. Litters with Variations ^e	0	0	1	
% Litters with Variations ^e	0.00	0.00	16.67	

^aOnly live fetuses were examined for external malformations and variations.

^bIncludes only litters with live fetuses.

^cFetuses with one or more external malformations or variations.

^dReported as the mean \pm S.E.M.

^eLitters with one or more fetuses with external malformations or variations.

[¶] $p < 0.05$; Kruskal-Wallis Test.

[■] $p < 0.05$; Mann-Whitney U Test.

[£] $p < 0.05$; Chi-Square Test.

[∅] $p < 0.05$; Fishers' Exact Test.

Table 7. External Morphological Abnormalities in CD-1 Mouse Fetuses Following Maternal Exposure to Tertiary Amyl Methyl Ether on Gestational Days 6 through 17: Listing by Defect Type^a (page 1 of 1)

	Tertiary Amyl Methyl Ether (ppm, inhaled)			
	0	1000	4000	7000
EXTERNAL MALFORMATIONS				
Total No. of Fetuses Examined for External Malformations ^b	69	67	69	0
No. of Fetuses with External Malformations ^c	0	0	10	
% Fetuses with External Malformations	0.0%	0.0%	14.5%	
Total No. of Litters Examined for External Malformations ^d	6	6	6	0
No. of Litters with External Malformations ^e	0	0	3	
% Litters with External Malformations	0.0%	0.0%	50.0%	
Cleft Palate				10(3)
EXTERNAL VARIATIONS				
Total No. of Fetuses Examined for External Variations ^b	69	67	69	0
No. of Fetuses with External Variations ^c	0	0	5	
% Fetuses with External Variations	0.0%	0.0%	7.2%	
Total No. of Litters Examined for External Variations ^d	6	6	6	0
No. of Litters with External Variations ^e	0	0	1	
% Litters with External Variations	0.0%	0.0%	16.7%	
Open Eye: Bilateral				4(1)
Left				1(1)

^aA single fetus may be represented more than once in listing individual defects. Data are presented as the number of fetuses (number of litters).

^bOnly live fetuses were examined.

^cFetuses with one or more external malformations/variations.

^dIncludes only litters with live fetuses.

^eLitters with one or more externally malformed/variant fetuses.