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UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

ABERDEEN PROVING GROUND, MD 21010-5422

TOXICOLOGICAL STUDY NO. 75-51-0753-90
ASSESSMENT OF THE DEVELOPMENTAL TOXICITY OF
PROPYLENE GLYCOL MONOMETHYL ETHER ACETATE
(PM ACETATE) IN RATS
DECEMBER 1989

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DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010-5422

REPLY TO
ATTENTION OF

HSHB-MO-T (40)

19 APR 1990

MEMORANDUM FOR HQDA(SGPS-PSP), 5109 Leesburg Pike, Falls Church, VA 22041-3258

SUBJECT: Toxicological Study No. 75-51-0753-90, Assessment of the Developmental Toxicity of Propylene Glycol Monomethyl Ether Acetate (PM Acetate) in Rats, December 1989

Copies of this report with Executive Summary are enclosed.

FOR THE COMMANDER:

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19. ABSTRACT (Continue on reverse if necessary and identify by block number):

This study evaluated the potential maternal, embryotoxic and teratogenic parameters of PM Acetate in Sprague-Dawley rats following inhalation of vapors in days 6-15 of gestation. No teratological or other developmental effects were seen in fetuses at air concentrations as high as 4000 ppm, in spite of slight toxic effects in dams at concentrations as low as 500 ppm. It is recommended that (a. an interim Workplace Exposure Limit be established at 100 ppm with a Short Term Exposure Limit of 150 ppm, (b. the beta isomer constitutes no more than 3 percent of the total PM Acetate in all military specifications, (c. an inhalation developmental study be performed in the rabbit, and (d. workers be monitored for the central nervous system and irritation effects.

parts per million

(propylene glycol monomethyl ether → acetate)

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EXECUTIVE SUMMARY
TOXICOLOGICAL STUDY NO. 75-51-0753-90
ASSESSMENT OF THE DEVELOPMENTAL TOXICITY
OF PROPYLENE GLYCOL MONOMETHYL ETHER
ACETATE (PM ACETATE) IN RATS
DECEMBER 1989

1. PURPOSE. We performed this study to evaluate the potential maternal, embryotoxic and teratogenic parameters of PM Acetate in Sprague-Dawley rats following inhalation of vapors on days 6 through 15 of gestation.

2. CONCLUSIONS. No teratological or other developmental effects were seen in fetuses at concentrations as high as 4160 parts per million (ppm), in spite of slight toxic effects in dams at all concentrations tested. An interim workplace exposure limit for PM Acetate established at 100 ppm with a Short Term Exposure Limit (STEL) of 150 ppm can be expected to provide protection for workers exposed to vapors provided the ß isomer is limited to 3 percent of the total PM Acetate. An inhalation developmental study using 95 percent α isomer of PM Acetate has not been conducted in the rabbit. Routine monitoring of exposed workers should be performed as a precautionary measure to protect worker health.

3. RECOMMENDATIONS. Establish an interim workplace exposure limit for PM Acetate at 100 ppm with a STEL of 150 ppm. Require all military specifications relating to PM Acetate limit the ß isomer to 3 percent or less of the total PM Acetate. Conduct a developmental study of the teratogenic effects of PM Acetate in rabbits. Monitor all workers with a potential exposure to PM Acetate.

DEPARTMENT OF THE ARMY
U.S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010-5422

REPLY TO ATTENTION OF

HSHB-MO-T

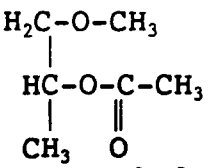
TOXICOLOGICAL STUDY NO. 75-51-0753-90 ASSESSMENT OF THE DEVELOPMENTAL TOXICITY OF PROPYLENE GLYCOL MONOMETHYL ETHER ACETATE (PM ACETATE) IN RATS DECEMBER 1989

I. REFERENCES. See the Appendix A for a list of references.

II. AUTHORITY. 1st End, HQDA (DASG-PSP-O), dated 22 October 1987, to letter, HSC, HSCL-P, dated 25 September 1987, subject: Threshold Limit Value (TLV) for PM Acetate.

III. PURPOSE. We performed this study to evaluate the potential maternal, embryotoxic and teratogenic parameters of PM Acetate in Sprague-Dawley rats following inhalation of vapors on Days 6 through 15 of gestation.

IV. TEST MATERIAL. Synonyms for propylene glycol monomethyl ether acetate (CAS No. 108-65-6) include but are not limited to PM Acetate, propylene glycol methyl ether acetate, PGMEA, 1-methoxy-2-propanol acetate and 1-methoxy-2-acetoxypropane. PM Acetate, currently used in the U.S., is a mixture which contains at least 95 percent α isomer and at most 3 percent ß isomer. The molecular formula is $C_6H_{12}O_3$ with a molecular weight of 132.18. The chemical structure is shown below. See Appendix B for chemical analysis, isomeric mixture of the compound used in the study and structure of the ß isomer.



Propylene glycol monomethyl ether acetate (α isomer)

V. BACKGROUND.

A. Uses and Exposure. PM Acetate is a paint thinner used in the Chemical Agent Resistant Coating (CARC) paint operations as stipulated by Military Specification MIL-T-81772B, Type I. Within the Army, exposure is expected during painting, spray painting and drying operations. Both men and women are employed in the operations or in adjacent working areas.

B. Lack of Existing Exposure Limits. Currently, there are no exposure limits for PM Acetate. There is no Permissible Exposure Limit (PEL) published by the Occupational Safety and Health Administration (OSHA), no Recommended Exposure Limit (REL) published by the National Institute for Occupational Safety and

Health (NIOSH) and no Threshold Limit Value (TLV®) published by the American Conference of Governmental Industrial Hygienists (ACGIH).

C. Prospects for Published Exposure Limits. The OSHA has published an "Advance Notice of Proposed Rulemaking" for occupational exposure to four similar solvents which have been implicated in reproductive and developmental effects (reference 1). The final rule is expected to be published in late 1990 and probably will not include PM Acetate. The OSHA may expand the scope of its rulemaking to include other glycol ethers-either on a substance by substance basis or on a generic basis. To the ACGIH, establishing a TLV for PM Acetate is not a high priority (reference 2). The American Industrial Hygiene Association (AIHA) will recommend a workplace exposure limit in May 1990.

D. Literature review. A review of the available literature on PM Acetate revealed limited toxicity data and no developmental toxicity studies. A summary of this data follows:

1. PM Acetate did not irritate intact or abraded skin of rabbits or guinea pigs. It caused moderate irritation in rabbit eyes. The oral Lethal Dose (50 percent) (LD_{50}) for male and female rats is 10,000 and 8,500 milligrams per kilogram (mg/kg) respectively (reference 3).

2. PM Acetate vapor caused slight to moderate degeneration of olfactory epithelium in a 9-day vapor inhalation study. This effect was seen at concentrations of 3000 parts per million (ppm) for rats and 300 ppm for mice (reference 4). Damage was presumed to be caused by acetic acid formed from the hydrolysis of PM Acetate in the nasal epithelium by carboxylesterase (reference 5). This effect may be more enhanced in rodents, which are obligate nasal breathers than it would be in species which can also breath through the mouth. Unpublished reports of dizziness in humans at unknown air concentrations during transfer operations indicate that there is potential for systemic effects in humans.

3. PM Acetate is reported to be metabolized to propylene glycol monomethyl ether (PGME, CAS No. 107-98-2) and acetic acid when administered to rats and mice by both the oral and inhalation routes (reference 4).

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E. Study Performed at the U.S. Army Environmental Hygiene Agency (USAEHA). The subject study was performed at USAEHA to evaluate the developmental toxicity of PM Acetate directly in a mammalian species. The laboratory rat was selected as the species of choice for this study which was conducted in accordance with guidelines set forth by the U.S. Environmental Protection Agency (EPA) (reference 6).

VI. METHODS.

A. Pilot Study.

1. The pilot study was performed as a range-finding experiment to determine the exposure concentrations for the main study.

2. Virgin female and naive male sexually mature Sprague-Dawley rats 9 to 12 weeks of age were used to produce litters. These rats were obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts and were identified as CRL:COBS-CD-(SD)BR colony animals.

3. All rats were maintained in a temperature-, humidity-, light-controlled room. The conditions were 70-76 °F; 50 percent ± 10 percent relative humidity; and a 12-hour light, 12-hour dark cycle. Certified pesticide-free rodent chow from Zeigler Brothers, Inc., Gardners, Pennsylvania and in-house tap water were available ad libitum (reference 7).

4. During the mating period, female rats were paired with male rats in a 21 cm high x 21 cm wide x 31 cm deep hanging wire cage until positive mating occurred. Positive mating was determined to have occurred when a sperm plug was observed on the pad under the suspended wire cage or when sperm were detected by a vaginal wash. That day was set as Day 0 of gestation. Seven Pregnancy Groups, designated A through G, consisted of all positively mated females discovered on the same calendar day. After positive mating, each female was identified by toe clip and housed singly in a 20 cm high x 20 cm wide x 45 cm deep polycarbonate box with sawdust bedding. Rats from each Pregnancy Group were divided as evenly as possible among the 400, 1500, 3000 ppm PM Acetate exposure groups and the control group. The concentrations were based on the developmental study of the ether, PGME (reference 8). Each group contained six pregnant rats except the 3000 ppm exposure group, which contained seven. Males may have mated with several females. After the mating period, males were removed from the study and euthanized by carbon dioxide inhalation.

5. From Days 6 through 15 for 6 hours per day, pregnant rats were exposed either to PM Acetate vapor or room air. Vapors were generated by piping hot air through PM Acetate coated glass beads thus evaporating the liquid (reference 9). Chamber concentrations were measured three times a day at Hours 1, 3 and 5 by pumping chamber air to a MIRAN 80 Infrared Analyzer. The mean time weighted average concentrations were 375, 1480 and 2680 ppm, which were within 6, 1 and 11 percent of the target concentrations, respectively.

6. All females were observed daily for changes in appearance and behavior; including time of onset, degree, and duration. Those signs of toxicity were recorded in a laboratory notebook.

7. Individual body weights for mated females were recorded on gestation Days 0, 6, 10, 13, 16 and 20. Food was weighed each morning to measure the previous day's consumption. Daily food consumption as a percentage of body weight was calculated by using the average body weight for each trimester of pregnancy.

8. On Day 20 of gestation, each female was euthanized in turn by carbon dioxide inhalation. To avoid a large time differential between groups, one dam from each exposure or control group was euthanized in the following order: Control, low, medium, high, high, medium, low, low, medium, etc. The gravid uterus was removed and weighed, after which counts and location of corpora lutea, total implantations, resorptions, viable fetuses and nonviable fetuses were recorded. Fetuses were numbered in order starting at the upper right uterine horn and continuing to the upper left horn. After opening the uterus, each viable fetus was removed, examined externally, sexed and weighed. The dams were examined grossly for any structural abnormalities or pathological changes which may have influenced the pregnancy. The liver and brain were weighed, but no tissues were saved. All data were recorded on HSHB-MO-T Form 40, 28 October 1987, Prenatal Toxicity Record.

9. Each fetus was weighed, sexed and examined for external malformations. These fetal examinations were conducted to screen for potential fetotoxicity and/or developmental toxicity.

B. Main Developmental Study.

1. The main study was conducted in the same manner as the pilot except as noted. After mating, rats were assigned either to the 500, 2000, 4000 ppm PM Acetate exposure groups or

to the control group. Variable fertility resulted in 23 pregnant rats in all groups except the 4000 ppm exposure group, which contained 20.

2. During exposure, vapor concentrations were measured on a MIRAN® 1A Infrared Analyzer. The mean time weighted average concentrations were 500, 1980, and 4160 ppm, which were within 0, 1, and 4 percent of the target concentrations.

3. At necropsy, both dams and fetuses were examined. Each fetus was weighed, sexed and examined for external anomalies. Following decapitation, fetuses were examined for visceral abnormalities using Staples' technique (reference 10). Fetuses were eviscerated, skinned and placed in denatured ethanol to be fixed for 10 days. The skeletons were stained (reference 11, Appendix C) and examined for abnormalities. After fixing in Bouin's fluid (Appendix C), heads were examined by Wilson's technique (reference 12).

4. Experimental data were collected on specialized forms, large tabular sheets or in laboratory notebooks 127, 130, 132 and 133. Statistical analyses were performed on maternal, litter and fetal data. Only those differences between exposure and control group values that were significant at $P < 0.05$ were reported. Analyses of fetal data were performed based on the litter as the experimental unit.

a. Group data. The following group parameters were calculated or counted without statistical analysis using the accompanying definitions:

(1) Parameters

(a) Fertility Index = $\frac{\text{pregnant animals}}{\text{positively mated animals}} \times 100$

(b) Gestation Index = $\frac{\text{viable litters}}{\text{pregnant animals}} \times 100$

(c) Index of alive fetuses = $\frac{\text{alive fetuses}}{\text{total fetuses}} \times 100$

(d) Resorption Index = $\frac{\text{total number of resorptions}}{\text{total number of implantations}} \times 100$

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(e) Index of malformations =

$$\frac{\text{total number of fetuses with malformations}}{\text{total number of fetuses}} \times 100$$

(f) Index of variations =

$$\frac{\text{total number of fetuses with variations}}{\text{total number of fetuses}} \times 100$$

(g) Number of runts.

(2) Definitions.

(a) Early resorption - Reabsorption of the conceptus by the dam in the early stages of pregnancy. Deciduoma or placental remains without embryonic remains are the evidence that is observed in the uterus.

(b) Late resorption - Reabsorption of the conceptus by the dam in the late stages of pregnancy. Placental and embryonic remains are the evidence that is observed in the uterus.

(c) Malformation - A life threatening or debilitating defect, e.g., exencephaly, gastroschisis or cleft palate.

(d) Variation - An anomaly that is a minor deviation from the norm, e.g., supernumerary ribs or slight hydronephrosis.

(e) Normal - No malformations or variations.

(f) Runt - A fetus weighing 70 percent or less than the mean weight of its litter.

b. Maternal data. Maternal food consumption as a percentage of body weight was analyzed using a one-way analysis of variance followed by the Student-Newman-Keuls test. Maternal body weight, body weight gain, organ-to-body weight ratios and organ-to-brain weight ratios were analyzed using a one-way analysis of variance followed by Duncan's test.

c. Litter data.

(1) Number per litter. The number of corpora lutea, implantations and live fetuses per litter were analyzed using the t-test.

(2) Percent per litter. Percentage data, which were percent female (sex ratio), resorptions, malformations, variations and normal fetuses per litter, were transformed by the angular transformation and analyzed with a t-test.

(3) Percent of litters with an effect. The percent of litters which contained a runt, resorption, dead fetus, malformation or variation was analyzed using chi-square and the square root of chi-square. The percent of litters which contained all normal fetuses was analyzed in the same way.

d. Fetal data. Fetal body weights were analyzed by a nested one-way analysis of variance (reference 13).

5. Quality Assurance was performed as described in Appendix E.

VII. FINDINGS.

A. Pilot Study.

1. Ataxia was observed intermittently in several rats only in the 3000 ppm exposure group during exposure, but subsided soon after the rats were returned to their boxes.

2. Food consumption by dams as a percentage of body weight was lower in the 3000 ppm exposure group when compared to dams in the control group. This reduction coincided with the exposure period (Days 6 through 15) with the exception of the second and third exposure (Days 7 and 8). Food consumption returned to normal upon cessation of exposure. In the 1500 ppm exposure group, food consumption was lower only after the first exposure and in the 400 ppm exposure group it was never lower (data for the pilot study not shown herein).

3. Body weights, liver-to-body weight ratios and liver-to-brain weight ratios were not significantly different in any exposure group. There were no dose-related gross necropsy findings in the dams or fetuses.

B. Main Developmental Toxicity Study.

1. Group parameters. Group indexes, counts and other summary data for the study are presented without analysis in Table 1.

TABLE 1. GROUP PARAMETERS
PM Acetate Rat Inhalation Developmental Study

	Control	500 ppm	2000 ppm	4000 ppm
Females mated	25	25	25	25
Fatalities	0	0	0	0
Females at Sacrifice	25	25	25	25
Females pregnant	23	23	23	20
Fertility Index (%)	92	92	92	80
Litters	23	23	23	20
Gestation Index (%)	100	100	100	100
Implantations, Total	333	330	320	278
Implantations per Dam	14.5	14.3	13.9	13.9
Fetuses, Total	319	304	307	260
Fetuses per Dam	13.9	13.2	13.3	13.0
Index of alive fetuses (%)	100	100	100	100
Dead Fetuses, Total	0	0	0	0
Dead Fetuses per Dam	0.	0.	0.	0.
Resorptions, Total	14	26	13	18
Early Resorptions	14	26	8	18
Late Resorptions	0	0	5	0
Resorptions per Dam	0.6	1.1	0.6	0.9
Resorption Index (%)	4.20	7.88	4.06	6.47
Malformations, Total	0	1	1	0
Litters with Malformations	0	1	1	0
Malformations per Dam	0	0.04	0.04	0
Index of Malformations (%)	0	0.33	0.33	0
Variations, Total	17	19	16	11
Litters with Variations	9	13	7	6
Variations per Dam	0.74	0.83	0.70	0.55
Index of Variations (%)	5.33	6.25	5.21	4.23
Runts	0	0	2	3
Sex Ratio (M/F)	1.10	0.90	0.99	1.08

2. Maternal parameters.

a. Toxic signs. Nearly half of the 20 dams in the 4000 ppm exposure group exhibited dyspnea at various times throughout the exposure period (Days 6 through 15). Breathing returned to normal soon after the dams were returned to their boxes. Half had red to reddish brown discharges from the nose and/or eyes on Days 8 and 10 through 15. Four dams were observed to have yellow staining in the fur of the urogenital area ranging from slight to marked on Days 6, 8, 13 and 14. Reduced muscle tone was observed during handling in 15 dams on two separate occasions. Movement returned to normal within 20 minutes of being returned to their boxes. In the 2000 ppm exposure group, one dam exhibited dyspnea, one had a ruffled pelt and two had red discharges from the eye or mouth. No toxic signs were observed in the 500 ppm exposure group.

b. Food consumption. In the 4000 ppm exposure group, a reduction of food consumption as a percentage of maternal body weight coincided with exposure to PM Acetate. A similar pattern was seen in the 2000 ppm exposure group where food consumption was lower on Days 7, Days 11 through 13 and Day 15. In the 500 ppm exposure group, food consumption was lower on Days 7 and 11 (Table 2).

c. Maternal body weights and body weight gains. Maternal body weights were lower in the 4000 and 2000 ppm exposure group after the last day of exposure only. Maternal body weights in the 500 ppm exposure group were always the same as controls (Table 3). However, when weight gains were analyzed, dams in the 4000 ppm exposure group did not gain as much as controls during the exposure period and gained less overall. Dams in the 2000 ppm exposure group gained less weight during the first two thirds of the exposure period and less overall also. Dams in the 500 ppm exposure group gained weight at the same rate as dams in the control group (Table 4).

d. Maternal organ weight ratios. There were no differences in relative liver or uterus weights between dams in the control group and dams in any exposure group whether ratios were calculated from body weights or brain weights (Table 5).

3. Litter and Fetal Parameters.

a. Corpora lutea, implantation, litter size, resorption and fetal death. The number of corpora lutea, implantation sites and live fetuses per litter was the same in the exposed groups as controls. Both the percent of conceptuses resorbed per litter and the percent of litters which contained a resorption were the same in the exposed groups as the controls. There were no dead fetuses in any litter (Table 6).

TABLE 2. MEAN MATERNAL FOOD CONSUMPTION (g/100 g body wt). PM Acetate Rat Inhalation Developmental Study.

Exposure Group	Day +0	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10	+11	+12	+13	+14	+15	+16	+17	+18	+19
Control	7.0	8.8	9.0	9.5	9.6	9.6	8.0	8.6	8.1	8.3	8.3	9.2	9.6	8.8	9.2	9.6	8.3	8.7	8.3	7.3
500 ppm	7.9	9.4	9.0	9.7	9.7	9.0	8.0	7.2*	8.2	8.0	8.3	8.5*	8.5	8.8	9.0	9.1	8.3	8.7	8.0	8.1
2000 ppm	6.6	8.4	8.8	8.8	9.0	9.0	6.7	6.9*	7.6	7.4*	7.6*	7.9*	7.8*	7.6*	8.2	8.2*	7.7	8.1	8.0	8.1
4000 ppm	7.1	8.6	9.0	9.6	9.2	9.5	5.2*	5.6*	6.4*	6.8*	6.7*	7.1*	7.0*	6.8*	7.1*	7.6*	7.6	8.3	8.2	8.1

* Significant at $P < 0.05$.

TABLE 3. MEAN DAM BODY WEIGHT (g)
PM Acetate Rat Inhalation Developmental Study

Exposure Group	Day +0	+6	+10	+13	+16	+20
Control	231	259	275	294	316	377
500 ppm	229	256	271	288	308	367
2000 ppm	232	256	266	281	300*	360
4000 ppm	233	263	264	277	291*	353

* Significant at P < 0.05.

TABLE 4. MEAN DAM BODY WEIGHT GAIN (g)
PM Acetate Rat Inhalation Developmental Study

Exposure Group	Day +6	+10	+13	+16	+20	Total Gain
Control	28	17	18	22	61	146
500 ppm	27	15	17	20	58	138
2000 ppm	24	10*	15*	18	60	128*
4000 ppm	28	1*	13*	14*	62	118*

* Significant at P < 0.05.

TABLE 5. ACTUAL AND RELATIVE MATERNAL ORGAN WEIGHTS
PM Acetate Rat Developmental Inhalation Developmental Study

Exposure Group	Organ Weights (g)		Organ-to-Body Weight Ratios (g/100 g bw)		Organ-to-Brain Weight Ratios (g/g brain wt)	
	Liver	Uterus	Liver	Uterus	Liver	Uterus
Control	16.2	80.2	4.3	21.2	8.67	42.69
500 ppm	15.6	76.2	4.2	20.8	8.40	41.12
2000 ppm	15.6	73.2	4.3	20.3	8.46	39.72
4000 ppm	15.1	71.1	4.3	20.0	8.01	37.68

No statistically significant differences between groups.

TABLE 6. LITTER AND FETAL PARAMETERS
PM Acetate Rat Inhalation Developmental Study

	Control	500 ppm	2000 ppm	4000 ppm
Corpora Lutea/litter	14.7	14.7	14.4	14.5
Implantation sites/ litter	14.5	14.3	13.9	13.9
Live fetuses/litter	13.9	13.2	13.3	13.0
% of conceptuses Resorbed/litter	4%	8%	4%	6%
% of Litters with Reabsorption	52%	65%	39%	55%
Dead fetuses/litter	0	0	0	0
Fetal Body Weights	3.9	3.9	3.6	3.6
% of Litters w/Runts	0%	0%	9%	15%
% Female/litter	48%	53%	50%	47%
% of fetuses/litter w/Malformations	0%	0.3%	0.3%	0%
% of fetuses/litter w/Variations	5%	6%	5%	4%
% of Normal fetuses/ litter	95%	93%	95%	96%
% of Litters w/ Malformations	0%	4%	4%	0%
% of Litters w/ Variations	39%	57%	30%	30%
% of Litters w/All Normal Fetuses	61%	39%	65%	70%

NOTE: No statistically significant differences between groups.

b. Fetal body weights and runts. There were no dose related differences in fetal body weights. The average fetal body weights in the 4000 and 2000 ppm exposure groups was approximately 5 percent lower than the low and controls. This difference was statistically significant in the 2000 ppm exposure group, however, the 4000 ppm exposure group was marginally non-significant. There was no difference in the percent of litters which contained runts (Table 6).

c. Fetal sex ratio. There was no difference in the male to female sex ratio (Table 6).

d. Malformations and variations. There were no differences in the percent of fetuses per litter that were malformed, had variations or were normal. In addition, there were no differences in the percent of litters which contained a malformation, a variation or contained all normal fetuses (Table 6). A summary of malformations and variations is shown in Table 7.

VIII. DISCUSSION.

A. Most of the effects observed in dams in the subject study were transient in nature. Dyspnea as well as reductions in muscle tone, food consumption and body weight were seen only during the exposure period. Total body weight gain was the only effect in dams which was significant at the end of the study. Observation of normal muscle tone in dams following high peak concentrations of PM Acetate may have been due to rapid recovery of toxic effects during longer chamber clearing times.

B. PM Acetate exhibits more similarity in its toxic effects to its metabolite, PGME, than to its ß isomer. The α isomer of both caused nearly identical effects in vapor inhalation developmental studies in rats (reference 8). Neither compound was teratogenic at similar concentrations. Both compounds caused transient central nervous system effects, decreased food consumption and lesser weight gain in pregnant animals. However, there are no studies that test the teratogenic potential of α PM Acetate in rabbits.

C. In sharp contrast, a reported vapor inhalation developmental study in rabbits using the ß isomer (>95 percent) of PM Acetate caused severe malformations. Anomalies of the fetal sternum, paw, and heart were seen in the absence of maternal toxicity at concentrations of 550 ppm. Vapors had no effect on fetuses or does at 145 ppm. In rat fetuses, vapors of the ß isomer caused skeletal anomalies of the thoracic vertebrae, but only at maternally toxic concentrations of 2700 ppm. In a dermal study in rabbits, the ß isomer had no effect on fetuses or does (reference 14).

TABLE 7. SUMMARY OF MALFORMATIONS AND VARIATIONS - Fetuses*/(Litter†). PM Acetate Rat Inhalation Developmental Study.

	Control	500 ppm	2000 ppm	4000 ppm
Number of fetuses (Number of litters)	319 (23)	304 (23)	307 (23)	260 (20)
Malformations				
Visceral				
Internal hydrocephalus, slight	0 (0)	0 (0)	1 (1)	0 (0)
Skeletal				
Centrum T11 scrambled	0 (0)	1 (1)	0 (0)	0 (0)
Variations				
External				
Small & irregular tail	1 (1)	0 (0)	0 (0)	0 (0)
Short tail	0 (0)	1 (1)	0 (0)	0 (0)
Visceral				
Situs Inversus	0 (0)	0 (0)	1 (1)	0 (0)
No aortic arch	0 (0)	0 (0)	0 (0)	1 (1)
No innominate artery	0 (0)	1 (1)	0 (0)	1 (1)
Innominate artery enlarged	1 (1)	0 (0)	0 (0)	0 (0)
Large ductus arteriosis and small pulmonary artery	1 (1)	0 (0)	0 (0)	0 (0)
Enlarged diameter ureter(s)	0 (0)	3 (2)	0 (0)	0 (0)
Enlarged renal pelvis(es)	0 (0)	2 (1)	0 (0)	0 (0)
Testicle underdeveloped	0 (0)	1 (1)	0 (0)	0 (0)
Testicle not fully descended	0 (0)	0 (0)	0 (0)	1 (1)
Skeletal				
Single ossification point lateral to C7	3 (1)	0 (0)	0 (0)	0 (0)
Rib 13 absent	1 (1)	2 (2)	0 (0)	0 (0)
Rib 13 short	0 (0)	0 (0)	0 (0)	1 (1)
Rib 13 incompletely ossified	0 (0)	0 (0)	1 (1)	0 (0)
Rudimentary 14th rib(s)	7 (4)	2 (2)	4 (3)	4 (2)
Thoracic centrum(s) bifid	7 (2)	10 (6)	11 (4)	4 (2)
Lumbar centrum(s) bipartite	0 (0)	2 (1)	0 (0)	0 (0)

* Number of fetuses with a given malformation or variation. A single fetus may have more than one malformation or variation.

† The number of litters which contained a given malformation or variation.

D. Differences in teratogenic potential of α and ß isomers of PM Acetate may be explained by the formation of different metabolites. Based upon inspection of the chemical structure, Merkle et. al. have suggested that the ß isomer is metabolized to 2-methoxypropionic acid. 2-Methoxypropionic acid is a homolog to methoxyacetic acid, which is accepted as the teratogenic metabolite of methoxyethanol (reference 14).

E. PM Acetate has low toxicity, did not produce teratogenic or other developmental effects in rats and is metabolized to propylene glycol monomethyl ether. This ether has a TLV of 100 ppm with a STEL of 150 ppm. It is reasonable to establish an interim workplace exposure limit for PM Acetate at the same level as the ether. An air concentration of 100 ppm PM Acetate which contains 3 percent of the ß isomer would expose a worker to 3 ppm of the ß isomer. This would provide a calculated safety factor 48 times lower than the no observed adverse effect level in rabbits (reference 14).

F. Transient effects seen in pregnant rats occurred at concentrations 5 to 40 times the recommended workplace exposure limit. However, routine monitoring remains a prudent tool to use in the event that these or other effects are seen in humans at lower concentrations.

IX. CONCLUSIONS.

A. No teratological or other developmental effects were seen in fetuses at concentrations as high as 4160 ppm, in spite of slight toxic effects in dams at all concentrations tested.

B. An interim workplace exposure limit for PM Acetate established at 100 ppm with a Short Term Exposure Limit of 150 ppm can be expected to provide protection for workers exposed to vapors provided that the ß isomer is limited to 3 percent of the total PM Acetate.

C. An inhalation developmental study using 95 percent α isomer of PM Acetate has not been conducted in the rabbit.

D. Routine monitoring of exposed worker should be performed as a precautionary measure to protect worker health.

X. RECOMMENDATIONS.

A. After consideration of the literature, results of the inhalation developmental study and the similarity to its corresponding ether; reported results of the ß isomer of PM Acetate; and due to the potential for central nervous system and irritation effects we recommend the following:

1. Establish an interim workplace exposure for PM Acetate at 100 ppm with a STEL of 150 ppm.

2. Ensure all military specifications relating to PM Acetate require the ß isomer to be limited to 3 percent or less of the total PM acetate.

3. Monitor all workers with a potential exposure to PM Acetate.

B. Due to the lack of teratogenic information on PM Acetate in rabbits, we recommend that an inhalation developmental study be conducted in that species.

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APPENDIX A

REFERENCES

1. Volume 52, No. 63, Federal Register pg. 10586, 2 April 1987.

2. FONECON between Ms. Kim Stewart, Publications Development/Technical Information, American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio and Mr. Arthur Asaki, this Agency, subject: TLV for PM Acetate, 23 July 1987.

3. 2-Propanol, 1-Methoxy-Acetate; Premanufacture Notice, 46 Federal Register 12308, 13 February 1981.

4. Miller, R. R., E. A. Hermann, J. T. Young, L. L. Calhoun and P. E. Kastl, Propylene glycol monomethyl ether acetate (PGMEA) metabolism, disposition, and short-term vapor inhalation toxicity studies, Toxicology and Applied Pharmacology (75) 521-530, 1984.

5. Stott, William T., and M. J. McKenna, Hydrolysis of several glycol ether acetates and acrylate esters by nasal mucosal carboxylesterase <u>in vitro</u>, Fundamental and Applied Toxicology, 5, 399-404, 1985.

6. Title 40, Code of Federal Regulations (CFR), 1987 rev, Toxic Substances Control Act Test Guidelines, Health Effects Testing Guidelines, Inhalation developmental toxicity study, Part 798.4350.

7. Standing Operating Procedure, USAEHA, HSHB-LT/WP, U.S. Army Environmental Hygiene Agency, March 1982, subject: Animal Facilities, Toxicology Division Buildings E2100 and E2101.

8. Hanley, Thomas R., Jr., Linda L. Calhoun, Barry L. Yano and K. S. Rao, Teratologic evaluation of inhaled propylene glycol monomethyl ether in rats and rabbits, Fundamental and Applied Toxicology (4) 784-794, 1984.

9. Miller, R. R., R. L. Letts, W. J. Potts and M. J. McKenna, Improved methodology for generating controlled test atmospheres, American Industrial Hygiene Journal (41) 844-846, 1980.

10. Robert Staples, Detection of visceral alterations in mammalian fetuses, Teratology 9, A37-A38, 1974. (As adopted by the National Center for Toxicological Research, 1984.)

11. A. B. Dawson, "Note on the staining of the Skeleton of Cleared Specimens with Alizarin Red S", Stain Technol, 1:123-124 (1926).

12. James Wilson and J. Warknay, Teratology, Principles and Techniques, The University of Chicago Press, Chicago, IL, 1967.

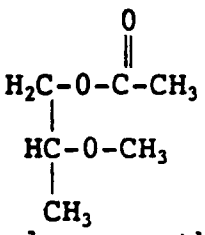
13. Gaylor, David W., Methods and Concepts of Biometrics Applied to Teratology, in Wilson, James G. and F. Clarke Fraser, eds., Handbook of Teratology, Volume 4, Plenum Press, New York, 1978.

14. Merkle, Jutta, Hans-Joachim Klimisch and Rudolf Jackh, Prenatal toxicity of 2-methoxypropylacetate-1 in rats and rabbits, Fundamental and Applied Toxicology, (8), 71-79, 1987.

APPENDIX B

CHEMICAL ANALYSIS AND ISOMERIC MIXTURE

Lot number 870916 of PM Acetate was used in this study and supplied by Dow Chemical USA, Midland, MI 48674. USAEHA quality assurance identified the lot as 100 percent (±2 percent) total PM Acetate. Chemical analyses performed by Dow determined that it contained 99.3 percent total PM Acetate which consisted of 97.3 percent of the α isomer (1-methoxy-2-acetoxypropane) and 2.0 percent of the ß isomer (2-methoxy-1-acetoxypropane, CAS Nos. 28677-93-2 and 70657-70-4). Although the remainder was not analyzed, it can be expected to consist of residual propylene glycol monomethyl ether, acetic acid and water.



Propylene glycol monomethyl ether acetate (ß isomer)

APPENDIX C

METHOD FOR FETAL SKELETAL STAINING AND FORMULA FOR BOUIN'S FLUID

1. METHOD FOR FETAL SKELETAL STAINING

a. Denatured alcohol was drained from bottles containing fetuses and replaced by a 1 percent aqueous potassium hydroxide solution. Fetuses remained in this solution, changed daily, until bones were clearly visible through the soft tissue.

b. Once cleared, the potassium hydroxide solution was replaced with Mall's solution (2,392.5 ml distilled water, 600 ml glycerin, 7.5 ml 4 percent potassium hydroxide and a pinch of thymol crystals) containing alizarin red stain (approximate proportion: 2-3 drops of saturated aqueous alizarin red S for every 100 ml Mall's solution). Fetuses were stained overnight.

c. The staining solution was drained and replaced with clear Mall's solution. To hasten the removal of stain from the soft tissue, the clear Mall's solution was changed twice daily.

d. When the soft tissue was free of stain, fetuses were transferred to a 50 percent glycerin solution. At approximately 24-hour increments, glycerin was changed to 75, 90 and finally 100 percent to remove all residual Mall's solution.

e. Fetuses were read by floating in 100 percent glycerin in a large weight boat placed on an X-ray backlight.

f. Fetuses were stored by litter in 100 percent glycerin with a few thymol crystals added.

2. FORMULA FOR BOUIN'S FLUID

a. Stock Solution

Saturated Picric Acid Solution	750 ml
30 - 40 percent Formaldehyde	250 ml

b. Working Solution

Stock Solution	95 ml
Glacial Acetic Acid	5 ml

Note: Working solutions were mixed no more than 24 hours prior to use.

APPENDIX D

ACKNOWLEDGEMENTS

The project officer gratefully acknowledges the assistance from everyone who worked on this study.

a. Dow Chemical USA supplied the compound and lent the entire vapor generating apparatus.

b. Robert MacKenzie established methods for analysis of chamber concentrations. Douglas Nelson prepared fetuses for Staples' and skeletal examination. Pat Beall prepared the laboratory for necropsy, and cleared and stained fetuses for skeletal examination. Pat Beall, Richard Angerhofer and Daneen Harris examined fetuses for soft tissue and skeletal abnormalities. Daneen Harris examined heads and entered data on the computer. Richard Angerhofer provided technical support throughout the study.

APPENDIX E

ANALYTICAL QUALITY ASSURANCE

The USAEHA Quality Assurance Office certifies the following:

a. These studies were conducted in accordance with:

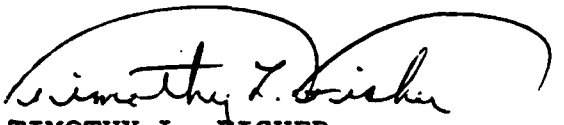
(1) Standing Operating Procedures developed by the Toxicology Division, USAEHA.

(2) Title 21, Code of Federal Regulations (CFR), 1986 rev, Part 58, Good Laboratory Practices for Nonclinical Studies.

(3) Title 40, CFR, 1987 rev, Part 798, Toxic Substances Control Act Test Guidelines.

b. The pilot study was performed from March to April 1988 and the main study was performed from April to November 1988. Facilities were inspected during its operational phase to ensure compliance with paragraph a above.

c. Raw data from the pilot and main studies are housed in Room 3015 of Bldg E 2100 of USAEHA. Tissues from the main study are stored in Bldg E 1958 of USAEHA. The information presented in this report accurately reflects the raw data generated during the course of conducting this study.



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