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Chemical Category	DIMETHYLACETAMIDE		

OFFICE OF TOXIC SUBSTANCES
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DuPont Haskell Laboratory

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November 3, 1997

via Federal Express

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Attention: 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
401 M Street SW
Washington, D.C. 20460-0001

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Dear 8(e) Coordinator:

8EHQ-0494-12956
Dimethylacetamide (DMAC)
CAS # 127-19-5



8EHQ-94-12956

In our April 14, 1997 letter, we informed the Agency of the preliminary results of a recently completed rat developmental toxicity study with the above referenced test material.

Enclosed, please find a copy of the final report.

Sincerely,

A. Michael Kaplan, Ph.D.
Manager-Regulatory Affairs



89980000035

AMK:jat
(302) 366-5260

Enclosure(1): Final Report "Dimethylacetamide (DMAC): Developmental Toxicity Study in Sprague-Dawley Rats" [HL-1997-00203]

TRADE SECRET

Study Title

**Dimethylacetamide (DMAC): Developmental Toxicity
Study in Sprague-Dawley Rats**

Laboratory Project ID

HL-1997-00203

Data Requirements

U.S. EPA Pesticide Assessment Guidelines
Subdivision F, 83-3

OECD Guidelines for Testing of Chemicals
Section 4, No. 414

MAFF Testing Guidelines for Toxicology Studies
NohSan 59, No. 4200

Author

Susan M. Munley, M.A.

Study Completed on

October 24, 1997

Performing Laboratory

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Medical Research Project No. 11131-001

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with EPA FIFRA (40 CFR 160) and EPA TSCA (40 CFR 792) Good Laboratory Practice Standards, OECD Principles of Good Laboratory Practice (C(81)30(Final), Annex 2), and MAFF Japan Good Laboratory Practice Standards (59 NohSan No. 3850).

Submitter: E. I. du Pont de Nemours and Company

Sponsors: BASF AG (Ludwigshafen, Germany)
DuPont Deutschland (Bad Homburg, Germany)
Ertisa S. A. (Madrid, Spain)
Montefibre S. P. A. (Milano, Italy)

Study Director:

Susan M. Munley

Susan M. Munley, M.A.
Research Toxicologist

October 24, 1997

Date

FLAGGING OF STUDIES FOR POTENTIAL ADVERSE EFFECTS

I have applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of the attached study. This study neither meets nor exceeds any of the applicable criteria.

Study Director:

Susan M. Munley

Susan M. Munley, M.A.
Research Toxicologist

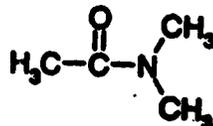
October 4, 1997

Date

GENERAL INFORMATION

Material Tested: Acetamide, N,N-dimethyl-

Structure:



Synonyms:

- DMA
- DMAc
- DMAA
- Acetic acid, dimethylamide
- Dimethylamide acetate
- N,N-dimethylacetamide

CAS Registry Number: 127-19-5

Haskell No.: 22203

Purity: ≥ 99%

Sponsor: BASF AG
DuPont Deutschland
Ertisa S. A.
Montefibre

Study Initiated/Completed: January 2, 1997 (study protocol signed) /
See report cover page (report issue date)

Experiment Initiated/Completed: January 5, 1997 (first dose) /
September 29, 1997 (last fetal evaluation)

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SUMMARY

Dimethylacetamide (DMAC) in water was administered to groups of 25 mated rats over days 7-21 of gestation (days 7-21G) at daily dose levels of 0, 20, 65, 150, or 400 mg/kg. On day 22G, all rats were euthanized and grossly necropsied. Near the end of the study, blood was drawn and evaluated for a battery of clinical chemical parameters. At necropsy, samples of liver and kidney tissue were taken and evaluated histopathologically. The fetuses were removed from the uterus and were weighed, sexed, and examined for external, visceral, head, and skeletal alterations. Significant, adverse maternal and developmental toxicity were produced at 400 mg/kg. Minimal maternal and developmental toxicity were seen at 150 mg/kg.

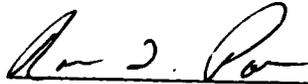
At 400 mg/kg, maternal toxicity was evident as significant, dose-related reductions in mean maternal body weight, weight change, and food consumption. No dose-related, adverse effects on any clinical chemistry parameter were seen at any dose level nor were there any adverse histopathological changes seen in either the liver or the kidney. Developmental toxicity was evident as significantly increased embryoletality (increased resorptions), malformations (synotia, anasarca, micrognathia, naris atresia, malformations of the heart and great heart vessels, distended lateral brain ventricles, fused ribs, absent vertebrae, hemivertebrae), and variations. Mean fetal weight was also significantly decreased.

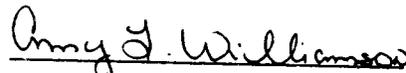
At 150 mg/kg, maternal toxicity was evident as a significant, dose-related reduction in maternal weight gain at the onset of the dosing period. Developmental toxicity was evident as a significant decrease in mean fetal weight. In addition, at 150 mg/kg, there was one fetus with malformations which included naris atresia, heart and vessel malformations, cleft palate, macroglossia, micrognathia, and synotia. Despite the fact that there was only one affected fetus, this may represent the bottom end of the dose response curve for malformations given the similarity of specific malformations relative to those seen at 400 mg/kg.

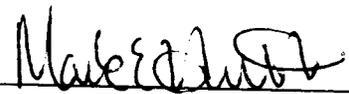
There was no evidence of either maternal or developmental toxicity at 65 or 20 mg/kg.

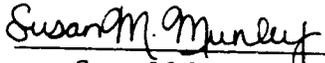
Under the conditions of this study, maternal and developmental toxicity were seen at 150 and 400 mg/kg. The maternal and developmental no-observed-effect level (NOEL) was 65 mg/kg. Thus, the results of this study indicate that DMAC produced fetal toxicity at the same doses that produced maternal toxicity.

SIGNATURE PAGE

Report Prepared By:  10/23/97
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Toxicology Associate

 10/23/97
Amy L. Williamson
Technical Publisher

Reviewed and Approved by:  Oct 24, 1997
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Authored, Reviewed, and
Approved for Issue:  October 24, 1997
Susan M. Munley, M.A.
Study Director
Research Toxicologist

QUALITY ASSURANCE DOCUMENTATION

(H-22203)

Dates of Inspection:

Conduct - 1/10/97

Records, Report(s) - 4/15-17,21-22/97;
5/5-9,13-16/97; 6/2-3/97;
6/27,29/97; 7/1-3/97; 10/23/97

Date(s) Findings Reported to:

Study Director - 4/16,21/97; 5/5,12,16/97; 6/3/97; 7/3/97;
10/23/97

Management - 4/21/97; 5/5,21/97; 6/2/97; 7/3,7/97; 10/23/97

Reported by:

Paul Jeffrey Chapman

P. Jeff Chapman, B.S.
Quality Assurance Auditor

10/23/97
Date

STUDY PERSONNEL

The following individuals participated in the conduct of the in-life portions of the study, maternal postmortem and fetal examinations, and/or in the preparation or review of the final report:

Study Director: Susan M. Munley, M.A.

Management: Mark E. Hurtt, Ph.D.

Primary Technician: Ronald L. Poore

Laboratory Technicians: Sandra E. Doughty, A.A.S.
Mary Ann Jacobs, B.A.
Cindy H. Krans, B.A.
Richard P. Mathena, Jr.
Bernice Dewberry Street, B.S.
Deborah L. Tyler

Technical Assistant: Joseph F. Aschiero

Technical Publisher: Amy L. Williamson

The following individual was responsible for the analysis of dosing solutions:

Chemistry Associate: Janet C. Maslanka, B.S.

The health status of the animals on study was assessed by the attending laboratory veterinarian, Charles E. Cover, V.M.D.

INTRODUCTION

The purpose of this study was to evaluate the developmental toxicity of dimethylacetamide (DMAC), administered by gavage to pregnant rats from around the time of implantation to the end of gestation.

This study conforms to applicable Good Laboratory Practice Standards.⁽¹⁻³⁾ This study also conforms to applicable Test Guidelines⁽⁴⁻⁷⁾ except that the study protocol is not included in this final report; this represents a deviation from the EPA guideline. This deviation has no adverse impact on the study.

MATERIALS AND METHODS

A. Animals

On December 10, 1996, 160 nulliparous female CrI:CD^o(SD)BR rats were received from Charles River Laboratories, Inc. They were 64 days old on the day after arrival, with body weights ranging from 173.8 to 228.5 grams. Males to be used for breeding were from a group which were 76 days old when delivered on July 30, 1996. On the day after arrival, their body weights ranged from 304.1 to 370.2 grams. Each rat was identified by a unique number recorded on its cage card, as well as by a tail tattoo with the last three digits of that number.

The rat was selected for this study because it is a preferred species for developmental toxicity testing as recommended by regulatory agencies. The CrI:CD^o(SD)BR strain was chosen because extensive background information is available from the literature, the supplier, and previous studies with other compounds at Haskell Laboratory. This strain is also considered suitable relative to hardiness and incidence of spontaneous disease.

B. Animal Husbandry

1. Caging: All rats were housed individually in suspended, wire-mesh, stainless steel cages. Nesting material was not provided because the dams were euthanized prior to parturition.
2. Food: Purina^o Certified Rodent Chow^o #5002 was available *ad libitum*.
3. Water: Water from United Water Delaware was available *ad libitum*.

4. **Environmental Conditions:** The animal room was maintained on a 12-hour light/dark cycle (fluorescent light) and targeted at a temperature of $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and a relative humidity of $50 \pm 10\%$. Occasional excursions outside the target ranges were minor and did not affect the study.
5. **Animal Health Monitoring:** Haskell Laboratory has an animal health monitoring program. The following procedures are performed periodically:
 - Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.
 - Food samples are analyzed for the presence of bacteria and fungi.
 - Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Haskell Laboratory uses certified animal food. The food is guaranteed by the manufacturer to meet specified nutritional requirements and to be free of a list of specified contaminants.

The animal health monitoring program is administered by the laboratory animal veterinarian. Data are maintained separately from study records and will not be included in this final report.
6. **Quarantine:** All animals were quarantined for at least 6 days, and then released for the study by the laboratory animal veterinarian.

C. **Experimental Design**

The experimental design is shown below:

<u>Group</u>	<u>Dose^a</u> <u>(mg/kg/day)</u>	<u>Concentration</u> <u>(mg/mL)</u>	<u>No. Mated</u> <u>Females^b</u>
I	0 ^c	0.0	25
II	20	2.0	25
III	65	6.5	25
IV	150	15.0	25
V	400	40.0	25

^a DMAC, administered once daily, by gavage, on Days 7-21 of gestation, at a dosage volume of 10 mL/kg

^b Copulation confirmed

^c Vehicle only (commercially-supplied, HPLC-grade water)

D. Selection of Dose Levels

Dose levels for the current study were based on a pilot developmental toxicity study conducted with DMAC. Dimethylacetamide (DMAC) in water was administered to groups of 8 mated rats over days 7-21 of gestation (days 7-21G) at daily dose levels of 0, 62.5, 125, 250, or 500 mg/kg. On day 22G, all rats were euthanized and grossly necropsied. The fetuses were removed from the uterus and were weighed, sexed, and examined for external alterations. A summary of results by dose group is provided below:

500 mg/kg

- significant reduction in maternal body weight (days 13, 15, 17, 19, 21, 22G)
- significant reduction in adjusted final body weight (adjusted for products of conception) and for weight changes calculated using the adjusted final weight
- significant reduction in maternal weight change (days 11-13, 15-17, 17-19, 19-21, 21-22, 7-22, 1-22G)
- significant reduction in maternal food consumption (days 9-11, 11-13, 15-17, 7-22G)
- significantly increased liver and kidney weights
- all animals observed with red-colored vaginal discharge
- effects on clinical chemistry data (cholesterol, triglycerides, glucose, and minerals); no effect on enzymatic markers of liver and kidney function
- no remarkable postmortem observations
- significant evidence of embryoletality (total litter resorption in 5 of 8 animals); significantly increased resorptions in 3 remaining litters; there were only 5 viable fetuses at this level
- viable fetuses were very small; 4 of 5 fetuses had anasarca and domed heads

250 mg/kg

- significant reduction in maternal weight change (days 11-13G)
- significantly increased liver and kidney weights
- effects on clinical chemistry data (cholesterol, triglycerides); no effect on enzymatic markers of liver and kidney function
- significantly decreased mean fetal weight
- 3 fetuses with malformations (including imperforate anus, cleft palate, filamentous tail, domed head)

125 mg/kg

- significantly increased liver and kidney weights
- effects on clinical chemistry data (cholesterol, triglycerides); no effect on enzymatic markers of liver and kidney function
- 3 fetuses with cleft palate

62.5 mg/kg

- significantly increased liver weights

Consideration was also given to results of an earlier study with DMAC.⁽⁸⁾ Pregnant Sprague-Dawley rats were gavaged over days 6-19 of gestation (days 6-19G, day 0G was the day copulation was confirmed) with solutions of DMAC in deionized water at daily dose levels of 0, 65, 160, and 400 mg/kg/day. Cesarean sections were performed on day 20G. Maternal toxicity was seen at 400 mg/kg; maternal body weight gain was significantly reduced. Developmental toxicity was seen at 160 mg/kg and above; mean fetal weight was significantly reduced at 160 mg/kg and above. Additional developmental toxicity was seen at 400 mg/kg. At this level, there were significantly increased fetal resorptions, and among live fetuses, malformations and variations were significantly increased. The results of this study, however, may have been confounded by the fact that there was evidence of a viral infection across all dose groups. Animals were noted with red and swollen conjunctivae and a characteristic swelling of the neck. Therefore, one purpose of this study is to clarify the findings seen in this earlier study.

Based on these results and the extensive database of information available regarding the effects of DMAC in the rat,⁽⁹⁾ dose levels of 0, 20, 65, 150, and 400 mg/kg were selected for the current study.

E. Preparation, Administration, & Analyses of Dosing Solutions

Solutions of the test material in the vehicle were prepared weekly. The method of mixing the test material with the vehicle was documented in the study records. DMAC was administered by gavage because the oral route is the route which is recommended by regulatory agencies for this type of study. The volume administered was based on the most recent body weight.

Samples from three batches of test solutions were collected. Analyses of all samples addressed concentration. The homogeneity and stability of DMAC dosing solutions was demonstrated previously in the pilot developmental toxicity study. Samples were submitted, shortly after preparation, to the Analytical Group of Environmental Sciences (ES) at Haskell Laboratory.

Analysis for DMAC in dosing formulations was conducted according to the following methods. 10 mL samples (2.0, 6.5, 15.0 and 40.0 mg/mL of DMAC dosing plus one 0 mg/mL sample of water) were received January 6, 23, and 27, 1997. The dosing samples at each concentration were analyzed for dose verification. Duplicate 1 mL samples at each concentration (plus one 0 mg/mL sample) were aliquoted and analyzed.

Each aliquot of the dosing samples was diluted to 100 mL with methanol. All samples were further diluted with methanol to give a nominal concentration of approximately 0.01 mg/mL active ingredient. In order to obtain an equivalent amount of the vehicle in each sample, an appropriate aliquot of the 0 mg/mL sample (initial dilution) was added to this final dilution.

Samples submitted for analysis were analyzed the day the formulations were received.

CHROMATOGRAPHIC CONDITIONS

Instrument:	Hewlett-Packard Model 5890 GC with a 7673 autosampler
Column:	Stabilwax-DB, 15 m; 0.32 mm ID; 0.25 um film thickness
Injector:	Split 49:1; 220° C
Detector:	FID 220° C
Oven (Gradient):	
Initial Temp	80°C for 1.5 min.
Level 1	10°C/min.
Final Temp	100°C for 0.1 min.
Total run time	3.60 min.
Carrier Gas:	Helium at 2.6 mL/min.
Split vent:	100 mL/min.
Injection Volume:	1 microliter

A stock solution of the analytical standard (DMAC, 99.9+% pure) was made in methanol. Appropriate aliquots of the stock were further diluted with methanol to make calibration standards which bracketed the target concentration of the diluted dosing formulations. Before this dilution, an amount of the 0 mg/mL sample (initial dilution) equivalent to the final dilution of the samples was added to each calibration solution. Peak heights from the analysis of these standards were used to construct a calibration curve by least squares regression (see Figure 1 for a representative calibration curve). Measured concentrations for dosing formulations were determined by applying the peak heights from replicate injections of each sample to the calibration curve.

F. Experimental Procedures

1. **Mating:** Females were cohabited with males (1:1) until copulation was confirmed by the presence of a copulation plug in the vagina or on the cageboard. Checks for copulation plugs were made each morning; the day copulation was confirmed was designated day 1 of gestation (day 1G). Mating began on December 29, 1996, with day 1G occurring from December 30, 1996 to January 8, 1997. Females with confirmed copulation dates were assigned to 8 breeding lots, A through H, respectively.

2. **Assignment to Groups/Control of Bias:** Before dosing began, females selected for the study that copulated during the first week of mating were ranked by their body weights on day 1G and randomly assigned to control or experimental groups. Females selected from the second week of mating were similarly assigned. The randomization resulted in a distribution in which the mean body weights for all groups were not statistically different ($p=0.9992$). In addition to random assignment to groups, bias was controlled by coding all females prior to scheduled sacrifice. They remained coded during the collection of the postmortem and fetal data.
3. **Observations:** Observations for morbidity and mortality were made daily. Prior to the start of dosing, females were weighed and examined at least twice. During the study, females were weighed on days 1, 7-22G. Food was weighed on days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 22G. Individual clinical signs were scheduled to be recorded each morning on days 1-22G and each afternoon on days 7-21G (the exposure period). These clinical signs were recorded as scheduled with one exception. Clinical signs were inadvertently not recorded for two animals assigned to the 400 mg/kg group on January 20, 1997 (day 13G). This deviation had no adverse effect on the validity or integrity of the study.
4. **Euthanasia and Postmortem Examinations:** Females were euthanized on day 22G by carbon dioxide asphyxiation. A gross necropsy was conducted on each animal; the organs of the thoracic and abdominal cavities were examined. The uterus was removed, weighed, and opened. The types of implants (live and dead fetuses, and resorptions) were counted and their relative positions were recorded. Then, the empty uterus was weighed. The ovaries were removed and the corpora lutea were counted and recorded. The uterus of each apparently nonpregnant rat was opened and stained with ammonium sulfide⁽¹⁰⁾ to detect very early resorptions; data collected from those animals were used only to determine the incidence of pregnancy and the number of females with total resorptions.

Live fetuses were weighed, sexed, and examined for external alterations. The first live fetus and thereafter every other fetus in each litter was decapitated and examined for visceral alterations⁽¹¹⁾ and the sex verified. Retarded renal development was classified using the scheme of Woo and Hoar.⁽¹²⁾ The heads were fixed in Bouin's fluid and examined.⁽¹³⁾ The remaining fetuses were euthanized by an intraperitoneal injection of sodium pentobarbital. All fetuses were fixed in 70% ethanol, eviscerated (if not done earlier during the visceral examination), macerated in 1% aqueous potassium hydroxide solution, stained with alizarin red, and examined for skeletal alterations.
5. **Clinical Pathology:** Near the end of the dosing period, blood was taken from the orbital sinus of each female rat while it was under light carbon dioxide anesthesia. The following clinical chemistry parameters were measured or calculated:

alkaline phosphatase (ALP)	glucose (GLUCO)
alanine aminotransferase (ALT)	urea nitrogen (BUN)
aspartate aminotransferase (AST)	creatinine (CREAT)
sorbitol dehydrogenase (SDH)	phosphate (PHOS)
bilirubin (BILRN)	calcium (CALC)
cholesterol (CHOL)	sodium (Na)
triglycerides (TRIG)	potassium (K)
total protein (TPROT)	chloride (Cl)
albumin (ALBMN)	
globulin (GLOBN)	

Clinical chemical parameters were measured on a Boehringer Mannheim/Hitachi 717 clinical chemistry analyzer using Boehringer Mannheim reagents.

6. **Pathology:** On day 22G, all female rats were sacrificed and necropsied. Rats sacrificed by design at study termination were euthanized by carbon dioxide asphyxiation.

Liver and kidneys were weighed from final sacrifice rats.

Representative samples of liver and each kidney were saved at necropsy and fixed in 10% neutral buffered formalin. Processed tissues were embedded in paraffin, cut at a nominal thickness of 5 micrometers, and stained with hematoxylin and eosin (H&E).

Sections of liver and each kidney from all adult female rats in all exposure groups were examined microscopically. The key to Appendix M, located at the front of Appendix M, describes the lesion grading system used in this study.

7. **Statistical Evaluation:** Sequential trend testing was applied to the developmental toxicity data for each parameter as tabulated below.⁽¹⁴⁾ If a significant dose-response was detected, data from the top dose group was excluded and the test repeated until no significant trend was detected. For litter parameters, the proportion of affected fetuses per litter or the litter mean was the experimental unit for statistical evaluation.⁽¹⁵⁾

<u>Parameter</u>	<u>Trend Test</u>
Maternal weight Maternal weight changes Maternal food consumption	Linear contrast of means ⁽¹⁶⁾
Live fetuses Dead fetuses Resorptions Implantations Corpora lutea Incidence of fetal alterations	Jonckheere's test ⁽¹⁷⁾
Incidence of pregnancy Clinical observations Maternal mortality Females with total resorptions Abortions/Early deliveries	Cochran-Armitage test ⁽¹⁶⁾
Fetal weight (Covariates: litter size, sex ratio) Sex ratio (Covariate: litter size)	Linear contrast of least square means ⁽¹⁸⁾

For developmental toxicity data, where the data were tied and the standard large sample version of Jonckheere's test was not applicable, exact p values were calculated using permutation methodology.⁽¹⁹⁾

For clinical pathology data, a one-way analysis of variance (ANOVA)⁽¹⁶⁾ and Bartlett's test⁽²⁰⁾ were calculated for each sampling time. Dunnett's test⁽²¹⁾ was used to compare means from the control groups and each of the groups exposed to DMAC. When the results of the Bartlett's test were significant ($p < 0.005$), the Kruskal-Wallis test⁽²²⁾ was employed and the Mann-Whitney U test^(23,24) was used to compare means from the control groups and each of the groups exposed to DMAC.

For pathology data, body weights and organ weights (absolute and relative to body weight) were analyzed by a one-way analysis of variance (ANOVA). Pairwise comparisons between test and control groups were made with Dunnett's test. Body weights and organ weights were also analyzed by Bartlett's test for homogeneity of variances. Increased incidences of microscopic observations were evaluated by the Cochran-Armitage trend test.

For all statistical analyses, significance was judged at $p \leq 0.05$, except for Bartlett's test which was judged at $p \leq 0.005$.

The use of the words "significant" or "significantly" in this report indicates a statistically significant difference between the control and the experimental groups.

G. Archiving

Raw data (including slides and paraffin-blocked tissues) and the final report are stored in the archives of Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, or at Iron Mountain, Wilmington, Delaware (formerly DuPont Records Management Center, Wilmington, Delaware). All skeletal and head preparations are stored at Haskell Laboratory and will be retained as long as the material permits proper evaluation.

REPORT A

RESULTS AND DISCUSSION

A. Maternal Findings

1. Mortality (Table 5)

There were no instances of dose-related mortality; all animals survived to scheduled sacrifice.

2. Body Weights and Weight Changes (Table 2, Appendices B and C)

Mean maternal weight change was reduced during the dosing period for animals dosed at 150 and 400 mg/kg. At 400 mg/kg, maternal weight changes were significantly reduced over the following intervals: days 7-9, 9-11, 15-17, 17-19, 19-21G, and when averaged over the dosing period, days 7-22G. Weight changes calculated using the adjusted final body weight (final body weight minus the products of conception) were significantly reduced at 400 mg/kg as well. At 150 mg/kg, maternal weight changes were reduced over days 7-9G, and significantly reduced over days 9-11G. There were no dose-related effects on maternal weight changes at either 65 or 20 mg/kg.

Maternal body weights were significantly reduced for animals dosed at 400 mg/kg on the following days: days 9, 11, 13, 15, 17, 19, 21, and 22G. The adjusted final body weight was significantly reduced as well. There were no effects on body weight at 150 mg/kg or lower.

3. Food Consumption (Table 3, Appendix D)

Significant, dose-related reductions in mean maternal food consumption were observed at 400 mg/kg over the following intervals: days 7-9, 9-11, 11-13, 15-17, 19-21G, and when averaged over the dosing period, days 7-22G. There were no effects on maternal food consumption at 150 mg/kg or lower.

4. Clinical Observations (Table 4, Appendix E)

There were no dose-related increases in any clinical observation at any dose level.

5. Postmortem Findings (Appendix F)

At 400 mg/kg, the placentas from 14 of 25 dams had white or tan outer edges. This observation is most likely related to and consistent with the adverse

maternal and developmental toxicity seen at this dose level. This observation was also reported for one, zero, two, and two animals from the 0, 20, 65, and 150 mg/kg groups, respectively. There were no other remarkable postmortem findings.

B. Reproductive Effects (Table 5, Appendix G)

There were no compound-related effects at any dose level on the following reproductive outcome parameters: dams with either total resorptions or that delivered early, the mean number of implantations, or mean litter sex ratio.

The mean number of live fetuses per litter was significantly reduced at 400 mg/kg; this is a reflection of the decreased embryofetal viability at this dose level. The mean number of live fetuses per litter was not affected at 150 mg/kg or lower.

C. Fetal Findings

1. Mortality (Table 5, Appendix G)

Embryofetal mortality was evident at 400 mg/kg. The incidence of resorptions (early, late, or when combined) was significantly increased. There was no evidence of dose-related embryofetal mortality at 150 mg/kg or lower.

2. Body Weight (Table 5, Appendices G and I)

Mean fetal weight was significantly reduced at 150 and 400 mg/kg and unaffected at 65 and 20 mg/kg.

3. Malformations (Table 6, Appendix I)

The incidence of fetal malformations was significantly increased at 400 mg/kg. The specific malformations that were significantly increased were synotia, anasarca, micrognathia, naris atresia, malformations of the heart and great heart vessels, distended lateral brain ventricles, fused ribs, absent vertebrae, and hemivertebrae.

For one particular malformation, distended lateral brain ventricles, data were collected regarding the relative severity of this subjective finding. Only one fetus from the 400 mg/kg level was found to have severely distended ventricles. The majority of the remaining fetuses had moderately distended ventricles while the remainder were only slightly distended. At 150 mg/kg, there were four affected fetuses from one litter; two of these fetuses had moderately distended ventricles

and the other two had only slightly distended ventricles. The incidence of this finding at the 400 mg/kg is clearly dose related. At 150 mg/kg, the incidence of this finding was not significantly increased. The toxicological significance of this finding at 150 mg/kg is unclear due at least in part to the fact that all affected fetuses were from one litter.

In addition, at 150 mg/kg, there was one fetus which was malformed. The malformations included naris atresia, heart and vessel malformations, cleft palate, macroglossia, micrognathia, and synotia. Despite the fact that there was only one affected fetus, this may represent the bottom end of the dose response curve for malformations given the similarity of specific malformations relative to those seen at 400 mg/kg.

There were no dose-related malformations seen at 65 mg/kg or lower. At 65 mg/kg, there was one fetus with anasarca and heart and vessel malformations. This is not considered to be a dose-related effect. There was one fetus from the concurrent control group with anasarca, and heart and vessel malformations are seen in variable frequency in control animals based on historical control data compiled by the Middle-Atlantic Reproduction and Teratology Association (MARTA).⁽²⁵⁾ In addition, the 65 mg/kg dose level was previously tested under nearly identical experimental conditions by Johannsen et. al.⁽⁸⁾ and no dose-related malformations were seen at this dose or at 160 mg/kg/day.

4. Variations (Table 7, Appendix I)

The incidence of fetal variations was significantly increased at 400 mg/kg. The specific variations that were significantly increased were patent ductus arteriosus and delayed sternebral ossification. There were no significant increases in any variation at 150 mg/kg or lower.

REPORT B

RESULTS AND DISCUSSION

Analysis and Report by:

Janet C. Maslanka

Janet C. Maslanka
Chemistry Associate

10/23/97

Date

Concentrations of DMAC in dosing formulations prepared January 3, 17, and 24, 1997, were measured by gas chromatography (GC). Dosing formulations at concentrations of 2.0, 6.5, 15.0 and 40.0 mg/mL of DMAC were submitted for concentration verification on January 6, 20, and 27, 1997 from the respective preparations. In addition, a 0 mg/mL sample was submitted with each set of dosing formulations. The vehicle for the study was commercially-supplied water (HPLC grade). Results from analysis of all dosing formulations submitted for concentration verification showed that DMAC was at targeted concentrations ($\pm 8\%$ of nominal). DMAC was not detected in the 0 mg/mL samples.

Chromatography

DMAC eluted from the GC column as a resolved peak with a retention time of approximately 2.7 minutes. Representative GC chromatograms are shown in Figures 2(a - c).

Concentration Verification Samples

Analytical results from dosing formulations prepared January 3, 17, and 24, 1997, and submitted on January 6, 20, and 27, 1997 for concentration verification analysis are shown in Table 1 and Appendix A. Duplicate aliquots from the 10 mL submitted samples were analyzed.

Measured concentrations of DMAC ranged from 93.5 to 100.3% of nominal on January 6, 1997; from 93.5 to 104.3% of nominal on January 20, 1997, and from 97.0 to 108.0% of nominal on January 27, 1997. These data indicate that the test substance was at expected concentrations in the dosing formulations for all samples. Test substance was not detected in the 0 mg/mL samples.

REPORT C

RESULTS AND DISCUSSION

Analysis and Report by:



Glenn S. Elliott, D.V.M., Ph.D.
Diplomate, A.C.V.P.
Clinical Pathologist

10-24-97
Date

Summary of Clinical Pathology Findings

Changes in some clinical chemistry parameters occurred in the 400 mg/kg group but they were not considered to be toxicologically important. Significantly decreased ALP and increased glucose, calcium, and phosphate concentrations were likely secondary changes associated with reproductive effects. Therefore, these changes were not considered to be toxicologically important. Mildly increased serum cholesterol concentration was suggestive of altered lipid metabolism. However, the magnitude of the change was small and biologically inconsequential.

Under the conditions of this study and for the clinical pathology parameters measured, the no-observed-effect level (NOEL) was considered to be the high dose level.

Clinical Chemistry (Table 8, Appendix J)

The 400 mg/kg group had changes in ALP, glucose, calcium, and phosphate concentrations that were likely secondary to reproductive effects.

- Group mean ALP was decreased compared to controls (statistically significant). However, the control, 20 and 65 mg/kg group mean ALP were higher than the historical control range for non-pregnant female rats of similar age. Most likely, some of the measured ALP in the control and low dose groups was of placental origin and this resulted in values which were higher than than the historical control range. Therefore, significantly decreased ALP was likely associated with the reduced number of live fetuses and embryoletality which occurred in the 400 mg/kg group.
- Likewise, increased mean calcium and phosphate concentrations the the 400 mg/kg group (statistically significant) were probably secondary to the reproductive effects. During normal pregnancy, mineral metabolism is altered to support skeletal development of the fetuses. These changes result in increased absorption of minerals

from the gut and increased resorption of minerals from the skeleton of the dam. In a normal pregnancy, serum concentrations of calcium and phosphate would not be expected to change. However, death or resorption of fetuses could result in increased serum calcium and phosphate concentrations because of disruption in the balance between gut absorption/bone resorption and fetal uptake.

- Mildly increased serum glucose concentration the 400 mg/kg group was likely secondary to stress associated with maternal and fetal toxicity.

Since the changes in ALP, glucose, calcium, and phosphate in the 400 mg/kg group were likely secondary to reproductive effects, the clinical chemistry changes were not considered to be toxicologically important.

Serum cholesterol concentration was mildly increased in the 400 mg/kg group. This change in cholesterol concentration was suggestive of altered lipid metabolism which may have been test substance-related. However, the mean value (92.3 mg/dl) was only slightly higher than the historical control range (73 - 86 mg/dl) and the magnitude of the change was not considered to be biologically adverse.

Other statistically significant clinical chemical findings were observed, but they were not considered to be toxicologically important for the following reasons:

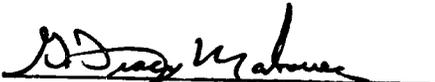
- Significantly decreased ALP in the 150 mg/kg group was not considered to be biologically adverse because ~~decreases~~ in serum enzyme activity are not relevant to organ injury or dysfunction. Conversely, it is ~~increases~~ in serum ALP which may indicate liver injury. It is possible that this change in ALP was associated with reproductive effects which occurred at this dose level. Perhaps decreased mean fetal weight was associated with decreased placental mass and, hence, lower ALP.
- Significant increases in mean glucose and chloride concentration in the 20 and 65 mg/kg groups were not considered to be test substance-related because the mean values did not exhibit a dose-response relationship.

Under the conditions of this study and for the clinical pathology parameters measured, the no-observed-effect level (NOEL) was considered to be the high dose level. Increased mean cholesterol concentration in the 400 mg/kg group was suggestive of altered lipid metabolism; however, the magnitude of the cholesterol change was not considered to be biologically adverse. In the 400 mg/kg group, decreased ALP, and increased glucose, calcium, and phosphate concentrations were likely secondary to test substance-related reproductive effects. In-and-of-themselves these changes in clinical chemistry parameters were not considered to be toxicologically important.

REPORT D

RESULTS AND DISCUSSION

Analysis and Report by:


G. Tracy Makovec, D.V.M.
Diplomate, A.C.V.P.
Staff Pathologist

10/24/97
Date

Organ Weight Data (Table 9, Appendix K)

There were statistically significant increases in mean absolute kidney weight and in mean relative (percent of body weight) liver and kidney weights in the 400 mg/kg dose group. These weight increases were considered to be compound-related but not biologically adverse for the following reasons:

- The mean absolute weight increases were minimal (2% and 8% for liver and kidneys, respectively).
- No microscopic findings were associated with the increased weights.
- Clinical pathology results for liver and kidney function were within normal ranges.

The weight changes in liver and kidneys were considered to be a pharmacologically adaptive response during exposure to a xenobiotic (DMAC).

Gross Observations (Appendix F)

There were no compound-related gross observations noted for the liver or kidneys. Recorded gross observations were considered incidental/spontaneous and are commonly observed in rats of this strain and age.

Microscopic Findings (Tables 10, 11, and 12, Appendix L)

There was a statistically significant increase in the incidence of increased mitotic figures in livers of the 400 mg/kg group. The incidences were 0/25, 0/25, 0/25, 0/25, and 4/25 for the dose groups 0, 20, 65, 150, and 400 mg/kg, respectively. This finding was considered to be compound-related but not biologically adverse for the following reasons:

- Microscopic biologically adverse findings, e.g. hepatocellular swelling, necrosis, or inflammation, were not present.
- Clinical pathology results for the liver were within normal limits.

The presence of increased mitotic figures in hepatocytes in the 400 mg/kg dose group was most likely associated with metabolism of the test compound.

The other microscopic findings noted in this study were considered incidental and/or spontaneous and are routinely seen in rats of this strain and age by experienced toxicologic pathologists.

Under the conditions of this study, the no-observed-effect level (NOEL), based upon organ weights and gross and microscopic pathology, was 400 mg/kg for female rats.

CONCLUSION

Under the conditions of this study, maternal and developmental toxicity were seen at 150 and 400 mg/kg. The maternal and developmental no-observed-effect level (NOEL) was 65 mg/kg. Thus, the results of this study indicate that DMAC produced fetal toxicity at the same doses that produced maternal toxicity.

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SUMMARY TABLES

0 0 3 6

TABLE 1
SUMMARY OF DOSING ANALYSES

<u>Concentration</u> <u>Verification</u> ^a	<u>Dosing Concentration of DMAC (mg/mL)</u>				
	<u>Nominal</u>	<u>2.00</u>	<u>6.5</u>	<u>15.0</u>	<u>40.0</u>
6-Jan-97		1.87 (93.5) ^b	6.39 (98.3)	14.8 (98.7)	39.3 (98.3)
		1.95 (97.5)	6.24 (96.0)	14.9 (99.3)	40.1 (100.3)
20-Jan-97		1.87 (93.5)	6.43 (98.9)	14.9 (99.3)	40.6 (101.5)
		1.88 (94.0)	6.58 (101.2)	14.9 (99.3)	41.7 (104.3)
27-Jan-97		1.97 (98.5)	6.57 (101.1)	15.9 (106.0)	40.1 (100.3)
		1.94 (97.0)	6.71 (103.2)	15.6 (104.0)	43.2 (108.0)

^a Duplicate samples submitted and analyzed.

^b Numbers in parentheses are the respective percent of nominal.

TABLE 2
MEAN MATERNAL BODY WEIGHT
AND ADJUSTED BODY WEIGHT CHANGES (grams)^a

GROUP	DAILY DOSE (mg/kg)	N	DAYS OF GESTATION						
			1-7	7-9	9-11	11-13	13-15	15-17	17-19
I	0	24	30.1	3.6	9.5	8.6	8.3	17.9	24.4
II	20	24	27.9	3.9	8.9	8.5	7.7	15.5	25.1
III	65	24	28.6	3.9	9.5	9.7	7.9	15.9	26.1
IV	150	25	29.1	0.4	6.1*	9.5	8.0	17.1	25.5
V	400	24	32.6	-8.5*	1.8*	6.0	4.7	11.6*	14.5*

GROUP	DAILY DOSE (mg/kg)	N	DAYS OF GESTATION			
			19-21	21-22	7-22	7-22 ^b
I	0	24	29.0	19.0	120.3	32.0
II	20	24	27.4	21.2	118.2	30.9
III	65	24	28.8	19.4	121.1	30.9
IV	150	25	27.5	21.2	115.4	27.6
V	400	24	13.3*	14.6	57.9*	8.2*

^a Data from females that were not pregnant were excluded. Individual data, standard deviations, and standard errors are presented in Appendices B (body weight changes) and C (body weights).

^b Weight changes calculated using the final body weights minus the products of conception.

* Significant trend (linear contrast of means); $p \leq 0.05$.

TABLE 3
MEAN MATERNAL FOOD CONSUMPTION (grams/day)^a

GROUP	DAILY DOSE (mg/kg)	N	DAYS OF GESTATION						
			<u>1-7</u>	<u>7-9</u>	<u>9-11</u>	<u>11-13</u>	<u>13-15</u>	<u>15-17</u>	<u>17-19</u>
I	0	24	23.4	22.1	23.4	23.8	23.4	25.3	26.3
II	20	24	22.7	21.9	22.9	23.0	22.9	24.2	26.1
III	65	24	23.6	22.9	24.1	24.9	24.1	25.9	28.1
IV	150	25	22.7	21.2	21.9	22.6	23.7	25.0	27.2
V	400	24	23.9	19.1*	17.7*	19.3*	21.0	22.3*	24.5

GROUP	DAILY DOSE (mg/kg)	N	DAYS OF GESTATION		
			<u>19-21</u>	<u>21-22</u>	<u>7-22</u>
I	0	24	25.1	21.5	24.0
II	20	24	24.9	23.3	23.7
III	65	24	26.2	23.1	25.0
IV	150	25	25.8	24.0	23.9
V	400	24	22.4*	19.3	20.8*

^a Data from females that were not pregnant were excluded. Individual data, standard deviations, and standard errors are presented in Appendix D.

* Significant trend (linear contrast of means); $p \leq 0.05$.

TABLE 4
CLINICAL OBSERVATIONS^{a,b}

DAY OF GESTATION	OBSERVATION	DAILY DOSE (mg/kg):	GROUP: I II III IV V				
			0	20	65	150	400
1-6	No. Examined		25	25	25	25	25
	No. Affected		1	5	1	1	4
	Alopecia		1	4	1	1	3
	Discharge Vaginal Opening		0	1	0	0	1
7-22	No. Examined		25	25	25	25	25
	No. Affected		8	10	5	4	9
	Alopecia		7	10	3	4	8
	Discharge Nose		0	0	1	0	0
	Discharge Right Eye		1	0	1	0	1

^a Individual clinical observations are presented in Appendix E.

^b No significant trends were detected (Cochran-Armitage test); $p \leq 0.05$.

Note: Statistical Analyses are only conducted on the individual clinical observations. The total number of affected animals is presented for information only.

TABLE 5
REPRODUCTIVE OUTCOME^a

	GROUP:	I	II	III	IV	V
	DAILY DOSE (mg/kg):	0	20	65	150	400
No. Mated		25	25	25	25	25
No. Pregnant		24	24	24	25	24
No. Deaths		0	0	0	0	0
No. With Total Resorptions		0	0	0	0	0
No. Early Deliveries		0	0	0	0	0
No. Litters		24	24	24	25	24
Means Per Litter						
Mean Corpora Lutea ^b		15.0	15.3	15.5	15.7	14.7
Implantations		14.5	14.3	14.8	15.0	13.5
Resorptions:	Total	0.5	0.6	0.6	0.5	3.1*
	Early	0.4	0.6	0.6	0.5	2.8*
	Late	0.0	0.0	0.0	0.0	0.3*
Dead Fetuses		0.0	0.0	0.0	0.0	0.0
Live Fetuses: ^c	Total	14.1	13.6	14.1	14.5	10.4*
	Males	7.3	6.8	6.8	7.3	5.8
	Females	6.8	6.8	7.3	7.2	4.6
Mean Fetal Weight:	Total	4.88	5.03	4.99	4.69*	3.22*
Sex Ratio ^d		0.51	0.50	0.49	0.51	0.58

^a Individual data, standard deviations, and standard errors are presented in Appendix G.

^b Statistical analyses are not conducted on mean corpora lutea data; these data are presented for information only.

^c Statistical analyses are only conducted on the mean total number of live fetuses per litter. The mean numbers of males and females are presented for information only.

^d Number male fetuses/total number fetuses per litter.

* Significant trend; $p < 0.05$.

Note: The pregnancy rate data, adult mortality data, and the total resorption data were statistically analyzed using the Cochran-Armitage test. All litter mean data (except for fetal weight and sex ratio) were analyzed using Jonckheere's test. Fetal weight and sex ratio were analyzed using a linear contrast of least square means.

TABLE 6
INCIDENCE OF FETAL MALFORMATIONS*

	GROUP: I	II	III	IV	V
DAILY DOSE (mg/kg):	0	20	65	150	400
EXTERNAL					
No. Examined ^b	338[24]	327[24]	339[24]	362[25]	250[24]
No. Affected	1[1]	0[0]	1[1]	1[1]	69[17]
Mean percent affected per litter (S.E.) (S.D.)	0.3 (0.28) (1.36)	0.0	0.3 (0.35) (1.70)	0.3 (0.31) (1.54)	31.3 (6.49) (31.77)
Anus - Absent	... ^c	1(1)
Ear - Synotia	1(1)	15(7)*
Entire Body - Anasarca	1(1)	...	1(1)	...	28(8)*
Head - Micrognathia	1(1)	2(1)*
Limb - Short	1(1)
Paw - Adactyly	1(1)
Palate - Cleft	1(1)	...
Snout - Naris Atresia	1(1)	...
Tail	1(1)	33(10)*
- Absent	1(1)
- Vestigial	1(1)
VISCERAL					
No. Examined	184[24]	172[24]	177[24]	190[25]	206[24]
No. Affected	0[0]	0[0]	1[1]	1[1]	113[24]
Mean percent affected per litter (S.E.) (S.D.)	0.0	0.0	0.6 (0.60) (2.92)	0.5 (0.50) (2.50)	57.3 (5.51) (26.98)
Heart &/or Greater Vessels - Malformation	1(1)	1(1)	113(24)*
HEAD					
No. Examined	183[24]	171[24]	177[24]	190[25]	206[24]
No. Affected	0[0]	0[0]	0[0]	5[2]	82[18]

TABLE 6 (CONT.)

INCIDENCE OF FETAL MALFORMATIONS*

	GROUP: DAILY DOSE (mg/kg):	I 0	II 20	III 65	IV 150	V 400
<u>HEAD (CONT.)</u>						
Mean percent affected per litter (S.E.) (S.D.)		0.0	0.0	0.0	4.5 (4.01) (20.05)	38.1 (6.72) (32.93)
Brain - Distended Lateral Ventricles ^d		4(1)	23(8)*
Severe		1(1)
Moderate		2(1)	15(7)
Slight		2(1)	7(2)
Mandible - Micrognathia		1(1)	...
Nares - Naris Atresia		1(1)	70(18)*
Palate - Cleft		1(1)	...
Tongue - Large		1(1)	...
<u>SKELETAL</u>						
No. Examined		338[24]	327[24]	339[24]	362[25]	250[24]
No. Affected		1[1]	0[0]	0[0]	0[0]	13[7]
Mean percent affected per litter (S.E.) (S.D.)		0.3 (0.28) (1.36)	0.0	0.0	0.0	5.1 (2.18) (10.68)
Rib - Fused		5(2)*
Sternebra - Non-fused Vertebra		1(1)	2(2)
- Absent		2(2)*
- Fused		2(1)
- Hemi		4(3)*
TOTAL NUMBER AFFECTED		2(2)	0(0)	1(1)	5(2)	167(24)
MEAN PERCENT AFFECTED PER LITTER (S.E.) (S.D.)		0.6 (0.38) (1.88)	0.0	0.3 (0.35) (1.70)	2.6 (2.29) (11.47)	69.0 (5.41) (26.50)

**TABLE 6 (CONT.)
INCIDENCE OF FETAL MALFORMATIONS^a**

- ^a Individual fetal alterations are presented in Appendix I.
- ^b Number examined and affected, including the number affected with the listed malformations, are expressed as Fetuses [Litters] or Fetuses (Litters).
- ^c For ease of reading, zeros have been replaced with ellipses for the listed malformations.
- ^d Statistical analyses were performed on the combined data. The data broken down by severity are presented for information only.
- ^e Significant trend (Jonckheere's test); $p \leq 0.05$.

Note: Statistical analyses are only conducted on the individual endpoints. The overall total and totals by exam are presented for information only.

TABLE 7

INCIDENCE OF FETAL VARIATIONS^{a,b}

	GROUP: I	II	III	IV	V
DAILY DOSE (mg/kg):	0	20	65	150	400
<u>DEVELOPMENTAL VARIATIONS</u>					
<u>EXTERNAL</u>					
No. Examined ^c	338[24]	327[24]	339[24]	362[25]	250[24]
No. Affected	0[0]	0[0]	0[0]	0[0]	0[0]
Mean percent affected per litter	0.0	0.0	0.0	0.0	0.0
<u>VISCERAL</u>					
No. Examined	184[24]	172[24]	177[24]	190[25]	206[24]
No. Affected	0[0]	0[0]	0[0]	0[0]	0[0]
Mean percent affected per litter ^d	0.0	0.0	0.0	0.0	0.0
<u>HEAD</u>					
No. Examined	183[24]	171[24]	177[24]	190[25]	206[24]
No. Affected	0[0]	0[0]	0[0]	0[0]	0[0]
Mean percent affected per litter	0.0	0.0	0.0	0.0	0.0
<u>SKELETAL</u>					
No. Examined	338[24]	327[24]	339[24]	362[25]	250[24]
No. Affected	5[5]	10[5]	4[4]	9[6]	5[5]
Mean percent affected per litter (S.E.)	1.7 (0.78)	3.3 (1.69)	1.1 (0.51)	2.3 (1.05)	3.5 (1.44)
(S.D.)	(3.82)	(8.28)	(2.51)	(5.23)	(6.46)

TABLE 7 (CONT.)

INCIDENCE OF FETAL VARIATIONS^{a,b}

	GROUP: I	II	III	IV	V
DAILY DOSE (mg/kg):	0	20	65	150	400
<u>DEVELOPMENTAL VARIATIONS</u>					
<u>SKELETAL (CONT.)</u>					
Rib - Rudimentary Cervical	6(5)	10(5)	3(3)	9(6)	4(4)
Sternebra - Misaligned	... ^d	...	1(1)	...	1(1)
TOTAL WITH DEVELOPMENTAL VARIATIONS					
MEAN PERCENT AFFECTED PER LITTER (S.E.)	6(5)	10(5)	4(4)	9(6)	5(5)
(S.D.)	1.7	3.3	1.1	2.3	3.5
	(0.78)	(1.69)	(0.51)	(1.05)	(1.44)
	(3.82)	(8.28)	(2.51)	(5.23)	(6.46)
<u>VARIATIONS DUE TO RETARDED DEVELOPMENT</u>					
<u>EXTERNAL</u>					
No. Examined	338[24]	327[24]	339[24]	362[25]	250[24]
No. Affected	0[0]	0[0]	0[0]	0[0]	0[0]
Mean percent affected per litter	0.0	0.0	0.0	0.0	0.0
<u>VISCERAL</u>					
No. Examined	184[24]	172[24]	177[24]	190[25]	206[24]
No. Affected	57[19]	64[19]	37[18]	20[11]	8[6]
Mean percent affected per litter (S.E.)	31.6	37.0	20.5	11.2	14.0
(S.D.)	(4.96)	(5.80)	(3.55)	(3.15)	(5.43)
	(24.32)	(28.41)	(17.38)	(15.44)	(22.39)

TABLE 7 (CONT.)

INCIDENCE OF FETAL VARIATIONS^{ab}

GROUP:	I	II	III	IV	V
DAILY DOSE (mg/kg):	0	20	65	150	400
<u>VARIATIONS DUE TO RETARDED DEVELOPMENT</u>					
<u>VISCERAL (CONT.)</u>					
Heart &/or Greater Vessels					
Patent Ductus Arteriosus	...	1(1)	8(6)*
Kidney, Papilla ^c	57(19)	64(19)	37(18)	20(11)	...
- Small Papilla - Size 1	4(2)	6(4)	1(1)
- Small Papilla - Size 2	22(12)	28(16)	16(9)	4(3)	...
- Papilla - Size 3	31(17)	30(18)	20(13)	16(11)	...
<u>HEAD</u>					
No. Examined	183[24]	171[24]	177[24]	190[25]	206[24]
No. Affected	0[0]	0[0]	0[0]	0[0]	0[0]
Mean percent affected per litter	0.0	0.0	0.0	0.0	0.0
<u>SKELETAL</u>					
No. Examined	338[24]	327[24]	339[24]	362[25]	250[24]
No. Affected	141[23]	119[22]	84[22]	141[25]	78[20]
Mean percent affected per litter (S.E.)	41.7 (5.84)	36.5 (4.70)	25.7 (3.94)	40.1 (5.37)	95.4 (2.53)
(S.D.)	(28.62)	(23.05)	(19.29)	(26.86)	(11.30)

TABLE 7 (CONT.)

INCIDENCE OF FETAL VARIATIONS^{a,b}

	GROUP: I	II	III	IV	V
DAILY DOSE (mg/kg):	0	20	65	150	400
VARIATIONS DUE TO RETARDED DEVELOPMENT					
<u>SKELETAL (CONT.)</u>					
Rib - Wavy	1 (1)	...	3 (2)	2 (1)	1 (1)
Skull - Retarded Ossification	17 (8)	10 (5)	11 (4)	10 (5)	34 (10)
Sternebra - Retarded Ossification	11 (7)	4 (3)	5 (4)	28 (11)	64 (17)*
Vertebra - Retarded Ossification	126 (23)	107 (21)	70 (22)	119 (24)	54 (19)
TOTAL WITH VARIATIONS DUE TO RETARDED DEVELOPMENT	172 (23)	163 (22)	116 (24)	151 (25)	80 (20)
MEAN PERCENT AFFECTED PER LITTER (S.E.)	50.7	49.9	34.6	42.9	97.2
(S.D.)	(5.20)	(5.18)	(3.84)	(5.18)	(1.95)
(S.D.)	(25.45)	(25.36)	(18.82)	(25.92)	(8.74)
TOTAL NUMBER FETUSES WITH VARIATIONS	176 (24)	167 (23)	119 (24)	156 (25)	81 (20)
MEAN PERCENT FETUSES WITH VARIATIONS (S.E.)	51.8	51.4	35.5	44.1	97.8
(S.D.)	(5.09)	(5.03)	(3.68)	(5.03)	(1.73)
(S.D.)	(24.94)	(24.65)	(18.04)	(25.16)	(7.73)

11-11-4-8

TABLE 7 (CONT.)

INCIDENCE OF FETAL VARIATIONS^{a,b}

- ^a Individual fetal alterations are presented in Appendix I.
- ^b Numbers examined and affected, including the numbers affected with the listed variations, are expressed as Fetuses [Litters] or Fetuses (Litters). Malformed fetuses are included in the counts of fetuses examined, but excluded from the number affected.
- ^d In calculating the percent affected per litter, malformed fetuses are omitted from the numbers examined and the number affected.
- ^d For ease of reading, zeros have been replaced with ellipses for the listed variations.
- ^e Statistical analyses are conducted on the combined incidences of all small renal papillae; the details for each size are presented for information only.
- ^{*} Significant trend (Jonckheere's test); $p \leq 0.05$.

Note: Statistical analyses are only conducted on the individual endpoints. The overall total and totals by exam type are presented for information only.

TABLE 8

SUMMARY OF CLINICAL CHEMICAL FINDINGS FOR FEMALE RATS

TESTS	CONCENTRATION (mg/kg)	
ALP U/L	0	154.(28.)*
	20	143.(36.)
	65	185.(45.)
	150	115.(23.)*
	400	99.(26.)*
ALT U/L	0	61.(9.)
	20	66.(6.)
	65	72.(18.)
	150	70.(7.)
	400	62.(9.)
AST U/L	0	95.(27.)
	20	92.(17.)
	65	109.(28.)
	150	94.(18.)
	400	83.(17.)
SDH U/L	0	20.0(3.7)
	20	17.7(2.5)
	65	21.0(7.0)
	150	20.9(6.2)
	400	16.3(4.1)
BILRN mg/dl	0	0.30(0.05)
	20	0.25(0.10)
	65	0.27(0.11)
	150	0.32(0.10)
	400	0.30(0.15)
CHOL mg/dl	0	67.(12.)
	20	66.(16.)
	65	72.(9.)
	150	75.(18.)
	400	92.(17.)*

TABLE 8 (CONT.)

SUMMARY OF CLINICAL CHEMICAL FINDINGS FOR FEMALE RATS

TESTS	CONCENTRATION	
	(mg/kg)	
TRIG mg/dl	0	372.(150.)
	20	414.(120.)
	65	439.(152.)
	150	519.(167.)
	400	525.(233.)
	0	6.9(0.5)
TPROT g/dl	20	6.8(0.4)
	65	6.9(0.5)
	150	6.8(0.4)
	400	6.9(0.6)
	0	5.1(0.4)
	ALBMN g/dl	20
65		5.2(0.4)
150		5.0(0.3)
400		5.2(0.5)
0		1.8(0.4)
GLOBN g/dl		20
	65	1.8(0.2)
	150	1.7(0.2)
	400	1.8(0.2)
	0	92.(5.)
	GLUCO mg/dl	20
65		105.(17.) ⁺
150		100.(11.)
400		109.(5.) ⁺
0		20.(2.)
BUN mg/dl		20
	65	20.(2.)
	150	21.(3.)
	400	18.(2.)

TABLE 8 (CONT.)

SUMMARY OF CLINICAL CHEMICAL FINDINGS FOR FEMALE RATS

TESTS	CONCENTRATION (mg/kg)	
CREAT mg/dl	0	0.4(0.0)
	20	0.4(0.0)
	65	0.4(0.0)
	150	0.4(0.0)
	400	0.4(0.1)
PHOS mg/dl	0	7.4(0.3)
	20	7.6(0.7)
	65	7.8(0.5)
	150	7.7(0.3)
	400	8.4(0.7)*
CALC mg/dl	0	11.3(0.3)
	20	11.2(0.2)
	65	11.4(0.3)
	150	11.3(0.3)
	400	11.6(0.4)*
Na mmol/L	0	144.(1.)
	20	145.(2.)
	65	145.(3.)
	150	143.(1.)
	400	144.(2.)
K mmol/L	0	6.3(0.3)
	20	6.2(0.4)
	65	6.1(0.5)
	150	6.3(0.4)
	400	6.2(0.4)

TABLE 8 (CONT.)

SUMMARY OF CLINICAL CHEMICAL FINDINGS FOR FEMALE RATS

<u>TESTS</u>	<u>CONCENTRATION</u> <u>(mg/kg)</u>	
	0	100.(2.)
Cl	20	103.(2.)*
mmol/L	65	104.(2.)*
	150	102.(2.)
	400	99.(2.)

-
- * Group means and standard deviations (SD)
 - * Significantly different from control at 5% level by Dunnett criteria
 - * Significantly different from control at 5% level by Mann-Whitney U criteria

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DEVELOPMENTAL TOXICITY STUDY WITH DMAC
TABLE 9
MEAN FINAL BODY AND ORGAN WEIGHTS FROM FEMALE RATS

MEAN FINAL BODY WEIGHTS (grams)		FINAL BODY	NUMBER OF
GROUP	CONCENTRATION (mg/kg)	WEIGHT (grams)	ANIMALS
I	0	319.58 (23.6)	24
II	20	315.60 (13.4)	24
III	65	318.22 (20.6)	24
IV	150	314.79 (17.9)	25
V	400	298.11 (17.9) [#]	24

MEAN ABSOLUTE ORGAN WEIGHTS (grams)		LIVER	KIDNEYS
GROUP	CONCENTRATION (mg/kg)	WEIGHT (grams)	WEIGHT (% of body weight)
I	0	15.14 (1.68)	2.09 (0.25)
II	20	14.49 (1.61)	2.03 (0.20)
III	65	15.20 (1.49)	2.13 (0.15)
IV	150	15.76 (1.63)	2.12 (0.20)
V	400	15.50 (1.76)	2.26 (0.25) [#]

MEAN RELATIVE ORGAN WEIGHTS (% of body weight)		LIVER	KIDNEYS
GROUP	CONCENTRATION (mg/kg)	WEIGHT (%)	WEIGHT (%)
I	0	4.74 (0.46)	0.65 (0.08)
II	20	4.59 (0.43)	0.64 (0.06)
III	65	4.78 (0.42)	0.67 (0.04)
IV	150	5.00 (0.36)	0.67 (0.06)
V	400	5.19 (0.42) [#]	0.76 (0.07) [#]

STANDARD DEVIATION IN PARENTHESES
- SIGNIFICANTLY DIFFERENT (P < 0.05) FROM CONTROL GROUP BY DUNNETT'S TEST

0 0 5 4

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DEVELOPMENTAL TOXICITY STUDY WITH DMAC
TABLE 10
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN FEMALE RATS

TISSUE/LESION	I	II	III	IV	V
GROUP DESIGNATION:	1	20	65	150	400
DOSE (mg/kg):	0	20	65	150	400
NUMBER IN GROUP:	25	25	25	25	25
LIVER					
FATTY CHANGE, MEDIAN CLEFT	25	25	25	25	25
INFLAMMATION, SUBACUTE/CHRONIC	5	3	3	-	1
ISCHEMIC/ATROPHIC LOBE	-	1	-	-	3
MITOTIC FIGURES, INCREASED	-	-	-	-	-
NECROSIS, FOCAL	3	3	1	3	4*
1	-	-	-	-	1
KIDNEYS					
LYTOMEGALY/KARYOMEGALY, TUBULAR (SPONTANEOUS)	25	25	25	25	25
HYDRONEPHROSIS, BILATERAL	1	-	-	-	1
HYDRONEPHROSIS, UNILATERAL	1	-	-	-	-
HYPERPLASIA, TRANSITIONAL CELL	-	1	-	1	3
INFLAMMATION, SUBACUTE/CHRONIC	-	1	-	1	3
NEPHROPATHY, CHRONIC PROGRESSIVE	1	-	-	-	-
TOTAL ANIMALS WITH PRIMARY TUMORS					
	0	0	0	0	0
TOTAL ANIMALS WITH BENIGN TUMORS					
	0	0	0	0	0
TOTAL ANIMALS WITH MALIGNANT TUMORS					
	0	0	0	0	0

NOTES:
 0 THE NUMBER OF ORGANS EXAMINED FOR EACH GROUP IS UNDERLINED.
 * # DEMOTES A STATISTICALLY SIGNIFICANT CORRELATION BETWEEN LESION INCIDENCE AND TREATMENT BY COCHRAN-ARMITAGE TREND TEST (p < 0.05).

DEVELOPMENTAL TOXICITY STUDY WITH DMAC

TABLE 11
INCIDENCES AND LESION GRADES OF MICROSCOPIC OBSERVATIONS IN FEMALE RATS

TISSUE/LESION (P, 1, 2, 3, 4)	GROUP DESIGNATION:		II	III	IV
	DOSE (mg/kg): NUMBER IN GROUP:	0			
LIVER	25	25	25	25	25
FATTY CHANGE, MEDIAN CLEFT INFLAMMATION, SUBACUTE/CHRONIC	5 (-, 5, -, -, -)	3 (-, 3, -, -, -)	3 (-, 3, -, -, -)	3 (-, 3, -, -, -)	3 (-, 3, -, -, -)
ISCHEMIC/ATROPHIC LOBE	-	1 (1, -, -, -, -)	-	-	-
MITOTIC FIGURES, INCREASED NECROSIS, FOCAL	3 (-, 3, -, -, -)	3 (-, 3, -, -, -)	1 (-, 1, -, -, -)	3 (-, 2, 1, -, -)	-
KIDNEYS	25	25	25	25	25
CYTOMEGLY/KARYOMEGLY, TUBULAR (SPONTANEOUS)	1 (-, 1, -, -, -)	-	-	-	-
HYDRONEPHROSIS, BILATERAL	1 (-, 1, -, -, -)	-	-	-	-
HYPERPLASIA, TRANSITIONAL CELL	-	1 (-, 1, -, -, -)	-	-	-
INFLAMMATION, SUBACUTE/CHRONIC	-	-	-	-	-
NEPHROPATHY, CHRONIC PROGRESSIVE	1 (-, 1, -, -, -)	-	-	-	1 (-, 1, -, -, -)

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DEVELOPMENTAL TOXICITY STUDY WITH DMAC

TABLE 11 (Continued)
INCIDENCES AND LESION GRADES OF MICROSCOPIC OBSERVATIONS IN FEMALE RATS

TISSUE/LESION (P, 1, 2, 3, 4)	GROUP DESIGNATION:	
	DOSE (mg/kg):	NUMBER IN GROUP:
	V	400
		25

LIVER		25
FATTY CHANGE, MEDIUM LEFT	1	(-1, 1, -1, -1)
INFLAMMATION, SUBACUTE/CHRONIC	3	(-1, 3, -1, -1)
ISCHEMIC/ATROPHIC LESION	4	(-1, 4, -1, -1)
MITOTIC FIGURES, INCREASED	1	(-1, 1, -1, -1)
NECROSIS, FOCAL		

KIDNEYS		25
CYTOMEGALY/KARYOMEGALY, TUBULAR (SPONTANEOUS)	1	(-1, -1, 1, -1)
HYDRONEPHROSIS, BILATERAL		
HYDRONEPHROSIS, UNILATERAL		
HYPERPLASIA, TRANSITIONAL CELL	3	(-1, 1, 2, -1)
INFLAMMATION, SUBACUTE/CHRONIC	3	(-1, 3, -1, -1)
NEPHROPATHY, CHRONIC PROGRESSIVE		

NOTES:
 o THE NUMBER OF ORGANS EXAMINED FOR EACH GROUP IS UNDERLINED.
 o LESION GRADES: P = PRESENT; 1 = MINIMAL; 2 = MILD; 3 = MODERATE; 4 = SEVERE.
 o LESION GRADES CORRESPOND BY POSITION WITH THE NUMBERS IN PARENTHESES WHICH INDICATE HOW OFTEN EACH GRADE WAS OBSERVED. FOR EXAMPLE: (-1, 2, -1) MEANS NO LESIONS WERE GRADED "PRESENT" (NON-GRADED LESIONS).
 o 1 LESION WAS GRADED "MINIMAL", 2 LESIONS WERE GRADED "MILD", NO LESIONS WERE GRADED "MODERATE" AND NO LESIONS WERE GRADED "SEVERE".

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DEVELOPMENTAL TOXICITY STUDY WITH DMAC

TABLE 12
MICROSCOPIC OBSERVATIONS IN FEMALE RATS LISTING INDIVIDUAL ANIMALS AFFECTED

TISSUE/LESION	GROUP DESIGNATION: DOSE (mg/kg):				IV
	I	II	III	IV	
	0	20	65	150	
	25	25	25	25	
LIVER					
FATTY CHANGE, MEDIAN CLEFT	25	25	25	25	
INFLAMMATION, SUBACUTE/CHRONIC	586691, 586696 586698, 586725 586749	586680, 586681 586728	586703, 586712 586759		
ISCHEMIC/ATROPHIC LOBE		586772			
MITOTIC FIGURES, INCREASED					
NECROSIS, FOCAL					
KIDNEYS					
CYTOMEGALY/KARYOMEGALY, TUBULAR (SPONTANEOUS)	586673, 586696 586724	586759, 586772 586814	586746		586674, 586778 586781
HYDRONEPHROSIS, BILATERAL	25	25	25	25	
HYDRONEPHROSIS, UNILATERAL	586685				
HYPERPLASIA, TRANSITIONAL CELL	586698				
INFLAMMATION, SUBACUTE/CHRONIC		586728			586678
NEPHROPATHY, CHRONIC PROGRESSIVE	586725	586728			586678
TOTAL ANIMALS WITH PRIMARY TUMORS	0	0	0	0	0
TOTAL ANIMALS WITH BENIGN TUMORS	0	0	0	0	0
TOTAL ANIMALS WITH MALIGNANT TUMORS	0	0	0	0	0

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DEVELOPMENTAL TOXICITY STUDY WITH DMAC

TABLE 12 (Continued)
MICROSCOPIC OBSERVATIONS IN FEMALE RATS LISTING INDIVIDUAL ANIMALS AFFECTED

TISSUE/LESION	GROUP DESIGNATION: DOSE (mg/kg): NUMBER IN GROUP:	V 400 25
LIVER		25
FATTY CHANGE, MEDIAN CLEFT	586683	
INFLAMMATION, SUBACUTE/CHRONIC	586693, 586699 586730	
ISCHEMIC/ATROPHIC LOBE		
MITOTIC FIGURES, INCREASED	586727, 586804 586823, 586832	
NECROSIS, FOCAL	586730	
KIDNEYS		25
CYTOMEGALY/KARYOMEGALY, TUBULAR (SPONTANEOUS)	586693	
HYDRONEPHROSIS, BILATERAL		
HYDRONEPHROSIS, UNILATERAL		
HYPERPLASIA, TRANSITIONAL CELL	586677, 586699 586806	
INFLAMMATION, SUBACUTE/CHRONIC	586677, 586699 586806	
NEPHROPATHY, CHRONIC PROGRESSIVE		
TOTAL ANIMALS WITH PRIMARY TUMORS		0
TOTAL ANIMALS WITH BENIGN TUMORS		0
TOTAL ANIMALS WITH MALIGNANT TUMORS		0

NOTES:
0 THE NUMBER OF ORGANS EXAMINED FOR EACH GROUP IS UNDERLINED.

FIGURES

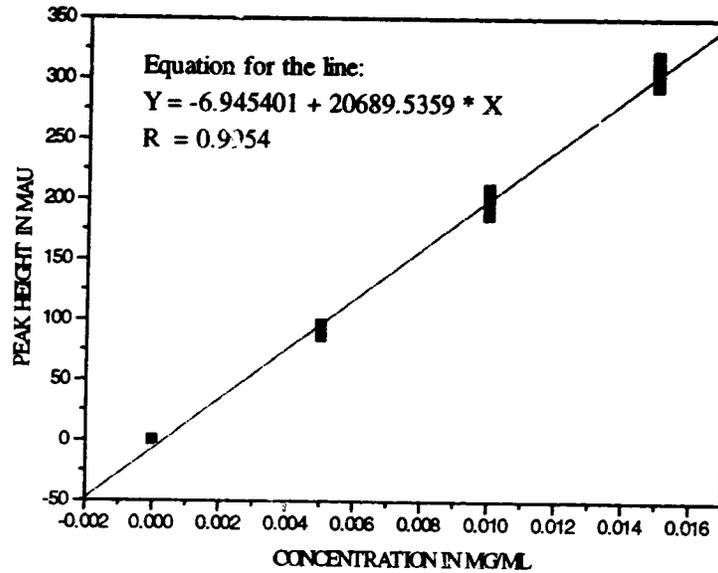


Figure 1: Calibration curve showing linear fit (line) to replicate peak height measurements (squares) for calibration solutions of DMAC in methanol over a concentration range of 0.000 to 0.015 mg/mL.

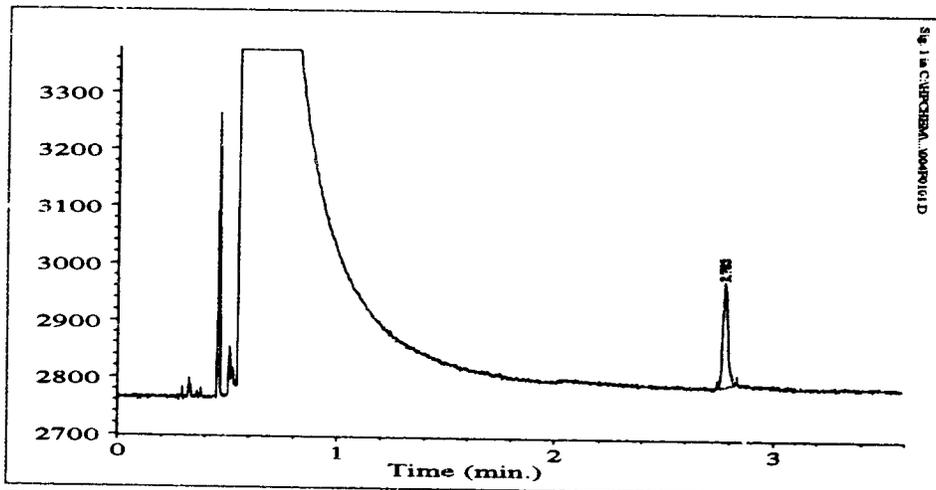


Figure 2a: Representative GC chromatogram of 0.010 mg/mL DMAC standard in methanol (after the addition of 0 mg/mL dosing solution equivalent to the final sample dilution). Retention time is 2.783 minutes.

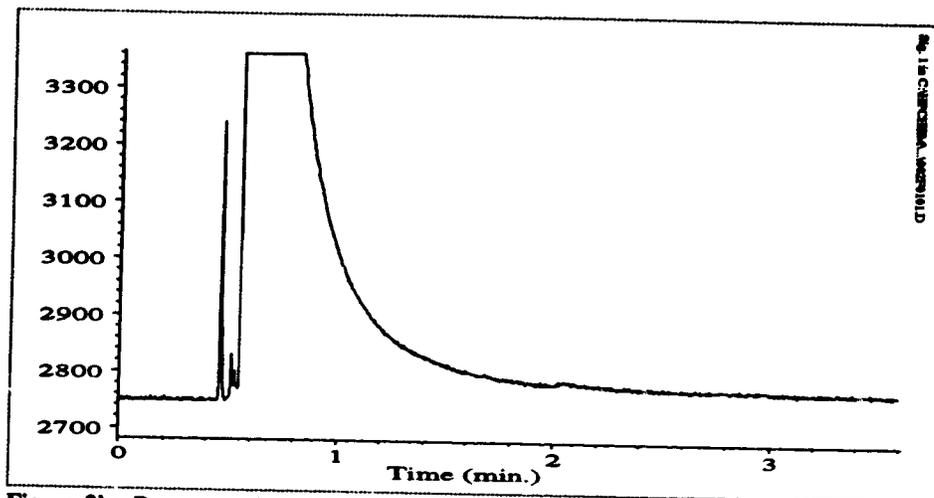


Figure 2b: Representative HPLC chromatogram of 0 mg/mL control dosing solution.

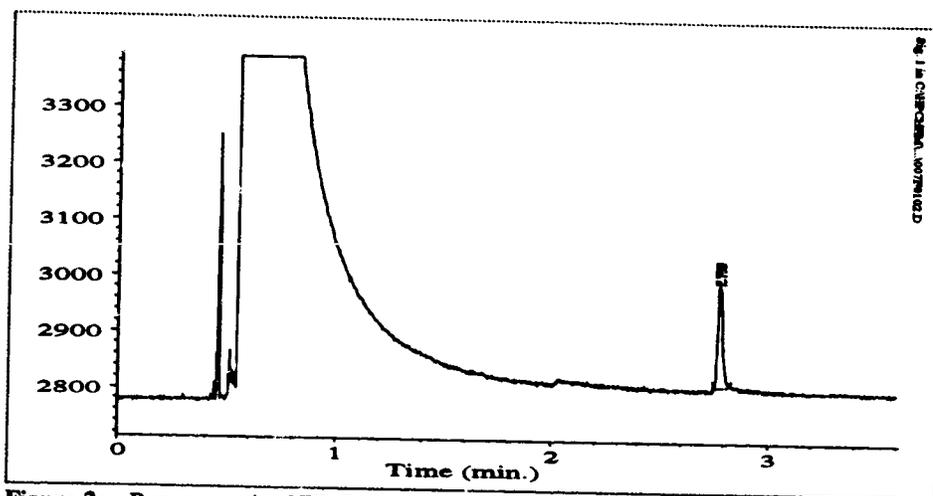


Figure 2c: Representative HPLC chromatogram of 2.0 mg/mL DMAC dosing solution diluted to a nominal concentration of 0.010 ng/mL. Retention time is 2.783 minutes.