28-DAY ORAL TOXICITY OF MDMA IN DOGS

EMD-SC-001

ADDENDUM REPORT
28-DAY ORAL TOXICITY OF METHYLENEDIOXYMETHAMPHETAMINE HYDROCHLORIDE (MDMA) IN DOGS AND RATS
PROTOCOL NO. EMD-SC-001 & EMD-SC-002

SPONSOR:
Earth Metabolic Design Laboratories
2105 Robinson Avenue
Sarasota, Florida 33582

TESTING FACILITY:
Division of Laboratory Animal Medicine
University of Arkansas for Medical Sciences
4301 West Markham
Little Rock, Arkansas 72205

STUDY DIRECTOR:
Charles H. Frith, D.V.M., Ph.D.
Toxicology Pathology Associates
1102 Briar Creek Road
Little Rock, Arkansas 72211

START DATE:
September 17, 1985
Start Dosing

COMPLETION DATE:
October 16, 1985
Completion of In-Life Phase

STUDY DIRECTOR: Charles H. Frith 4-17-86
Charles H. Frith, D.V.M., Ph.D. Date

APPROVED BY:
Sponsor Date

ADDENDUM TO MDMA REPORTS
PROTOCOLS EMD-SC-001 AND EMD-SC-002

This addendum is provided as a result of the reevaluation of the microscopic changes in the brains of both the dogs and rats administered methylenedioxymethamphetamine (MDMA). The lesions seen in the brains of these animals were of an equivocal nature, and their significance could not be determined by either the Study Director (Charles H. Frith, D.V.M., Ph.D.) or the neuropathologist who initially evaluated the brain slides (Louis W. Chang, Ph.D.). After the reports were submitted to the Sponsor (Earth Metabolic Design Laboratories) and talking to the Sponsor on the telephone, the joint decision was made to seek a reevaluation of the brain slides.

Representative lesions were mailed to the following three neuropathologists:

Kevin T. Morgan, B.V.Sc., Ph.D.
Diplomate of the American College of Veterinary Pathologists
Chemical Industry Institute of Toxicology
P.O. Box 12137
Research Triangle Park, North Carolina 27709

James W. Swenberg, D.V.M., Ph.D.
Diplomate of the American College of Veterinary Pathologists
Chemical Industry Institute of Toxicology
P.O. Box 12137
Research Triangle Park, North Carolina 27709

Albee Messing, V.M.D., Ph.D.
Assistant Professor Pathology
University of Wisconsin-Madison
Department of Pathobiological Sciences
Madison, Wisconsin 53706

All three of the above pathologists examined the same slides and reported their findings to the Study Director in writing. Their letters are attached as Appendix 1 to this addendum.

They concluded that all of the lesions seen were consistent with artifacts and were probably not related to the administration of MDMA. Dr. Louis W. Chang examined the letters and concurred with the three neuropathologists. He has also written a letter, and it is also included in Appendix 1.

In summary, it is the conclusion of the Study Director that the toxicity of MDMA when administered daily to both dogs and rats for a 28-day period is much less toxic than indicated in the original final report.

APPENDIX 1

LETTERS FROM NEUROPATHOLOGISTS

Chemical Industry Institute of Toxicology

CIIT

P O Box 12137
Research Triangle Park,
North Carolina 27709
(919) 541-2070

President, Robert A. Neal, Ph.D.
Vice President, Director of Research, James E. Gibson, Ph.D
Vice President, Administration and Secretary, Donald A. Hart, Ed.D

March 25, 1986

Dr. Charles H. Frith
Toxicology Pathology Associates
1102 Briar Creek Road
Little Rock
Arkansas 72211

RE: Interpretation of Brain Sections

Dear Charlie,

Both Dr. Swenberg and I examined slides from a toxicology study on "Ecstasy", referred to in your letter of March 14. Slides were provided from rats (8656-3 and 8661-3b, high dose males) and dogs (XOE5-18,19 and ZOF5-17A, C; high dose males). No control sections were provided. Our joint opinion, based upon independent interpretation of the slides, is as follows:

No changes were detected which could not be ruled out as artifacts of block or section preparation. Longitudinal sections of the rat brain interfere with detection of bilaterally symmetric changes. Bilateral symmetry is often of value for the discrimation of treatment-related changes from artifacts. In the abscence of further material, no other conclusions could be reached concerning possible treatment effects.

Sincerely

K.T. Morgan
B.V.Sc., Ph.D.
Diplomate A.C.V.P.

J.A. Swenberg
D.V.M., Ph.D.
Diplomate A.C.V.P.

University of Wisconsin-Madison
School of Veterinary Medicine
Dept. of Pathobiological Sciences
608-263-9191

2015 Linden Drive West
Madison, WI
53706

March 21, 1986

Dr. Charles Frith
Toxicology Pathology Associates
1102 Briar Creek Road
Little Rock, Arkansas 72211

Dear Dr. Frith:

I have examined the slides which you sent earlier this week and offer the following observations:

rat 8656-3: the LFB stain shows marked vacuolization of the myelin within a fiber tract adjacent to the trigeminal nucleus.

rat 8661-3B: the LFB stain shows similar focal areas of marked vacuolization of myelin within the middle cerebellar peduncle. The area circled on the slide I assume refers to some small tracts adjacent to sensory neurons of the mesencephalic nucleus, but I consider the vacuolization in these tracts to be less convincing.

dog X0E5-18,19: I consider the variation in cytoplasmic staining of the neurons in the cerebellar roof nuclei to be within the range of normal. As noted by Dr. Chang, these neurons do not have eccentric nuclei and the gallocyanin stain shows persistence of Nissl substance. dog Z0F5-17C: The floccular changes noted in the white matter tracts at the dorso-lateral border of the corpus callosum and caudate nucleus is a normal feature of the myelin in this region and I do not believe it represents degeneration of either myelin or axons.

dog Z0F5-17A: There appears to be a focal area of malacia with adjacent glial reaction, but the tissue block has not been completely sectioned and the area of malacia suffers from artifactual fragmentation which makes interpretation difficult. With true leukomalacia one would expect to see a prominent glial reaction and residual lipid-laden macrophages. Even so, we periodically see small focal areas of leukomalacia, perhaps secondary to lacunar infarcts, in otherwise normal dogs.

To summarize, the two lesions that I can recognize in these slides are the apparent vacuolization in some white matter tracts in the rats, and the focal leukomalacia in the dog. In my experience, however, white matter vacuolization, particularly when it is seen in peripheral locations, may be an artifact associated with stretching of the tissue upon removal from the skull, and so one must be extremely cautious in its interpretation. The slide showing apparent malacia in the dog was not ideally sectioned, and even if real the malacia may be an aging change in these animals. The ages,

strains or breeds, and sources (commercial, pound, etc.) of the rats and dogs were not stated. Altogether, I do not consider these lesions to be convincing evidence of a toxic or degenerative effect on the central nervous system. More animals would be needed and careful attention paid to possible sources of artifactual disruption of the CNS tissue.

If I can be of further assistance please let me know.

Sincerely,

Albee Messing

Albee Messing, VMD PhD
Assistant Professor of Pathology

iiversity of
kansas
· Medical
iences

to:

Louis W. Chang
essor of Pathology
artment of Pathology
ege of Medicine
1 West Markham
e Rock, Arkansas
05

1) 661-6400

April 17, 1986

Dr. Charles H. Frith
Toxicology Pathology Associates
1102 Briar Creek Rd.
Little Rock, AR 72211

Dear Charlie,

I have reviewed the general comments from Drs. K.T. Morgan and J.A. Swenberg of CITT and Dr. Albee Messing of Wisconsin who had examined a few tissue slides from your MDMA experiment.

I concur with their opinions that the changes observed are not very prominent and one cannot be conclusive that they are degenerative changes induced by this drug or rule out the possibility of artifacts. As pointed out by Dr. Messing, the focal malacia with some adjacent glial reaction in one dog was present - but again, one cannot conclude that this was a direct result of the drug given.

As I pointed out to you earlier, the neuronal "chromatolysis" observed was not a typical one. Those neurons retained their Nissl substance (by Nissl stain) and did not have eccentric nuclei. The void of hematoxylin staining in these neurons, as I suggested to you in private, may be a result of tissue chemistry changes (e.g. acidosis) rather than actual "lesions" development. However, one cannot be conclusive on this interpretation unless tissue chemical analysis can be performed.

I fully concur with the other reviewers that the present study can only be considered a very preliminary observation. Many more animals and further experimentation must be performed before any conclusive interpretation can be made.

Sincerely,

Louis W. Chang, Ph.D.
Professor & Director
Experimental Pathology

REPORT

28-DAY ORAL TOXICITY OF METHYLENEDIOXYMETHAMPHETAMINE HYDROCLORIDE (MDMA) IN DOGS

PROTOCOL NO. EMD-SC-001

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SPONSOR:
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1102 Briar Creek Road
Little Rock, Arkansas 72211

START DATE:
September 17, 1985
Start Dosing

COMPLETION DATE:
October 16, 1985
Completion of In-Life Phase

STUDY DIRECTOR:
Charles H Frith 2-7-86
Charles H. Frith, D.V.M., Ph.D. Date

APPROVED BY:
Richard Doblin 2-14-86
Sponsor Date

QUALITY ASSURANCE OFFICER:
Gina Caldwell 2-7-86
Gina Caldwell, B.A. Date

FORM QAU/005

QUALITY ASSURANCE AUTHENTICATION

PROJECT NO. EMD-SC-001

The conduct of Project EMD-SC-001 has been subjected to periodic inspections and this report has been audited. The dates of inspection are given below.

Date of QA Inspection	Date of Report to Study Director	Date of Report to Management
9-17-85	9-17-85	10-23-85
9-10-85	9-10-85	10-23-85
10-1-85	10-1-85	10-23-85
10-1-85	10-1-85	10-23-85
10-8-85	10-8-85	10-23-85
10-15-85	10-15-85	10-23-85
10-15-85	10-15-85	10-23-85
10-15-85	10-15-85	10-23-85
10-16-85	10-16-85	10-23-85
10-16-85	10-16-85	10-23-85
1-23-86 to 2-7-86	2-7-86	2-11-86

This report accurately describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Signed: Gina Caldwell Date 2-7-86

Gina L. Caldwell, B.A.
Quality Assurance Officer

SUMMARY:

Major clinical signs associated with the administration of the test article included circling, depression, dilated pupils, hyperactivity, rapid breathing and salivation. Gross observations at necropsy possibly related to administration of the test article included reduced testicular size (one high and one medium dose) and prostatic enlargement in two high dose animals. The medium and the high dose groups in both sexes gained significantly less weight than the control and low dose groups. Food consumption decreased the first week for the high and medium dose groups, but a significant reversal was toward more normal consumption in the following weeks. Hematologic, clinical chemistry and urinalyses values did not appear to be affected by the administration of the test article. Microscopically, testicular atrophy was present in one medium dose and two high dose males. Prostatic hyperplasia was present in two high dose males. [Lesions in the central nervous system attributed to administration of the test article included focal cellular infiltrates in the cerebrum, floccular change in the white matter of the cerebrum, chromatolysis of the neurons of the brain stem and focal malacia in the cerebrum.] See Baldwin for analysis

PURPOSE:

The purpose of this study was to evaluate the toxicological potential of methylenedioxymethamphetamine hydrochloride (MDMA) administered to dogs for a 28-day period.

TEST ARTICLE:

The test article was methylenedioxymethamphetamine hydrochloride (MDMA). The test article was supplied by a collaborator of the study, Dr. David Nichols, Department of Medicinal Chemistry, Purdue University. Two hundred and fifty grams of the MDMA was received from Dr. Nichols on September 12, 1985. It was assigned Sample Number 1. The test article was stored in a safe in the laboratory of Dr. Danny Lattin, Professor of Medicinal Chemistry, College of Pharmacy, University of Arkansas for Medical Sciences.

TEST ARTICLE CHARACTERIZATION:

Data concerning uniformity, composition and additional chemical and physical characteristics were supplied by Dr. Nichols. The vacuum distilled free base, as isolated from the synthesis, had a purity of 99.05%. The recrystallized hydrochloride salt had a purity of 99.75%. The hydrochloride salt had an uncorrrected melting point of 158-159 ^{o}C. Dr. Nichols stated to me over the telephone that as long as the test article remained dry that it would be stable for an indefinite period. Additional information concerning the test article may be found in the in the report submitted by Dr. David Nichols which is located in the Appendices.

CONTROL ARTICLE:

Gelatin Capsules.

TEST SYSTEM:

Twelve (12) male and 12 female beagle dogs were obtained from Ridglan Farms, Mt. Horeb, Wisconsin. The animals weighed between 5.1 and 8.5 kg. the day after receipt. All animals were born during the month of March, 1985. The dog was chosen for this study because it is the commonly accepted species used in studies to evaluate the potential toxicity and pharmacological action of drugs.

QUARANTINE:

The animals were quarantined from August 28, 1985, until September 15, 1985. Each animal was subjected to a thorough physical examination including an ophthalmological examination, a CBC and a heartworm check. The eyes of all of the dogs were examined by Dr. Cynthia Jacobs, Assistant Director of the Division of Laboratory Animal Medicine, University of Arkansas for Mecical Sciences. All ophthalmoscopic examinations were normal, all CBC's were normal, and all heart worm checks were negative. All dogs were determined to be of adequate health at the end of the quarantine period by Dr. Charles H. Frith, the Study Director, and Dr. Harold E. Farris, Director of the Division of Laboratory Animal Medicine.

TEST PROCEDURES:

1. Animal Receipt, Identification and Care

Animals were received and quarantined as described in the Quarantine Section. The existing ear tattoos were used as unique identification numbers (UIN'S) throughout the study for data collection. Animals were housed individually in 4 x 8 foot runs in a room maintained at 72+-8 ^{o}F with a 30-70% relative humidity. The actual temperature and humidity ranges during the study were 70-82 ^{o}F and 33-70%, respectively. A protocol deviation occurred on September 22 and 23, 1985, when the room temperature reached 82 ^{o}F. As no animal appeared adversely affected and since the temperature for the dog recommended in the NIH Guide Supplement of the U.S. Department of Health and Human Services (Vol. 14, No. 8, June 25, 1985) is 64-84 ^{o}F, it was judged that this 2 ^{o}F temperature maximum deviation resulted in no significant impact on the study. The room was maintained on a 12-hour light-dark cycle (0600 hours light onset central standard time). Water was provided via an automatic watering system. Food (Purina Lab Canine Diet 5006) was provided ad libitum except for the 24-hour period prior to sampling of blood for clinical chemistry. There were no dietary contaminants reasonably expected to interfere with the outcome of the study; and therefore, no dietary analyses were performed. Food consumption was measured once a week for a 24-hour period. Allocation to treatment groups was completely random and was based on body weights obtained during the quarantine period (weight stratified randomization).

2. Experimental Design

MDMA was administered orally in gelatin capsules to three male and three female beagle dogs at doses of 0, 3, 9, and 15 mg/kg. The experimental design is outlined in the following table.

EXPERIMENTAL DESIGN

	Sample Size		Treatment
Group	Males	Females	
1	3	3	0 mg/kg
2	3	3	3 mg/kg
3	3	3	9 mg/kg
4	3	3	15 mg/kg
	12 +	12 = 24	

Initially, the high dose was to be 18 mg/kg based on some preliminary data from range finding studies. One high dose female (UIN VEE5) died on the first day of dosing approximately 2 and 1/2 hours after dosing, and the protocol was amended and the high dose was subsequently reduced to 15 mg/kg for the duration of the study.

3. Test Article Administration

The route of exposure was orally via gelatin capsules because oral ingestion is the anticipated route of human exposure. Doses were adjusted weekly to account for changes in body weights that occurred between weighings. Data concerning the degree of absorbtion of the test article were not required to meet the objectives of the study.

4. Observations

All animals were observed for clinical observations approximately 1-2 hours after dosing, in addition to a morning and an afternoon morbidity and mortality check. The following clinical observations were made: nature, onset, severity and duration of all visible toxic or pharmacologic effects. Body weights were determined on a weekly basis and on the day the animals were sacrificed.

5. Clinical Pathology

Each animal was sampled for clinical pathology determinations prior to first dosing and at the conclusion of the study. Blood was collected by venous puncture. Urine was originally to be collected by catheterization. Based on the opinion of the laboratory animal veterinarian, the protocol was amended and the urine was collected by cystocentesis. Hematology measurements included total red cell count, total white cell count, differential leukocyte count, hemoglobin, hematocrit and platelet count. Clinical chemistry measurements included calcium, total protein, potassium, sodium, chloride, SGOT, SGPT, BUN, alkaline phosphatase, cholesterol, creatinine phosphokinase, bilirubin, albumin, globulin, glucose and LDH. Urinalysis measurements included appearence, 24-hour volume, specific gravity, osmolality, pH, microscopic evaluation, glucose, ketones, bilirubin, urobilibubin, protein and blood.

6. Necropsy Examinations

All animals that died or survived to terminal sacrifice were subjected to complete necropsies. Dogs were euthanized with T-61 Euthansia Solution. Gross necropsies included the examination of

external surface, all orifices, cranial cavity, external surface of the brain and spinal cord; the thoracic, abdominal, and pelvic cavities and viscera; and cervical tissues and organs. The following tissues were collected and fixed in 10% neutral buffered formalin for microscopic examination:

Adrenal Glands
Aorta (thoracic)
Bone and Bone Marrow
Brain (cerebrum, cerebellum and pons)
Cecum
Colon
Esophagus
Eye
Gonads
Heart
Kidneys
Liver
Lung
Lymph Node (mesenteric or cervical)
Mammary Gland (females)
Muscle (skeletal)
Optic Nerve
Pancreas
Parathyroid
Pituitary Gland
Prostate
Rectum
Salivary Gland (Submaxillary)
Sciatic Nerve
Small Intestine (duodenum, jejunum and ileum)
Spinal Cord (mid-thoracic, cervical and lumbar)
Spleen
Stomach
Thymus
Thyroid Gland
Urinary Bladder
Uterus

Necropsy observations were originally collected manually on PAT-001 Forms and subsequently entered into Beckman's TOXSYS automated data collection system for generation of individual reports. The following organs were weighed in all animals that survived to terminal sacrifice: liver, kidneys, brain, gonads and adrenal glands. A Gross Summary Table, and individual computer generated gross pathology reports and organ weight reports are included in the Appendices.

7. Histology Examinations

Histology examinations were performed on all animals that died or survived to terminal sacrifice. All organs and tissues collected under Section 6-Necropsy Examinations were examined microscopically

after routinely processing and staining with hematoxylin and eosin. Dr. L. W. Chang examined all sections of the brain, spinal cord and sciatic nerves and recorded his findings manually on PAT-002 Forms. Dr. Frith examined all remaining tissues and entered his findings on Beckman's automated data collection system. Dr. Frith subsequently entered Dr. Chang's findings on the automated data collection system and generated microscopic reports for each animal. The Summary Table and individual animal microscopic reports are included in the appendices.

8. Data Evaluation

Evaluation of results included the relationship, if any, between exposures to the test article and the appearence, incidence, and severity of all abnormalities including clinical observations, food consumption, body weight changes, gross and microscopic and clinical pathology findings. Parametric and/or nonparametric stastical analysis were performed where appropriate.

RESULTS:

1. Clinical Observations

A number of clinical signs of pharmacological and/or toxicological effects of the test article were observed. The most significant of these are summarized in the table in Appendix 1. One female dog (UIN VEE5) died approximately 2 and 1/2 hours after the first dose showing convulsions, vocalization, aggression, and paralysis of the front limbs. The major clinical observations in the treated dogs included circling, hyperactivity, rapid breathing, salivation, and dilated pupils. The dogs began to show clinical signs approximately 45

minutes after dosing and continued to show them for approximately 6-8 hours. The males appeared to show more clinical effects than the females.

2. Body Weights

The control group in both sexes showed the greatest weight gain and the high dose groups in both sexes showed the least weight gain over time. Specific values including tables and graphs are included in the Stastical Report in the Appendices.

3. Food Consumption

Food consumption for the high and medium dose groups was decreased significantly from the control and medium dose groups for the first week, but reverted toward normal and was not significantly different by the fourth week. Graphs and tables are included in the Stastical Report in the Appendices.

4. Organ Weights

The adrenal gland weights and the adrenal gland to body weight ratios of the low dose males were increased significantly compared to the other treatment groups. This is believed to be a random effect and not related to administration of the test article. An apparent treatment-related affect was a sex by dose interaction for the brain weights. Specific values and graphs are included in the Stastical Report in the Appendices.

5. Clinical Pathology

Clinical pathology data including urinalyses, hematology and clinical chemistry showed no consistent changes from the initial and the final period which could be related to administration of the test article. A protocol deviation occurred in that Freeway Medical

Laboratory failed to determine creatinine phosphokinase (CPK) values in the blood samples drawn at the conclusion of the study.

6. Gross Pathology

A small number of incidental gross lesions unrelated to treatment were seen at necropsy, some of which such as the congested lungs may have been related to the administration of the euthansia solution. In the female dog that died, red or dark zones (hemorrhages) were seen in the heart and the thymus and may have been related to an agonal death. The testes of one dog in the 9 mg/kg group and one dog in the 15 mg/kg group were noticeably smaller than normal at necropsy. Two of the dogs in the 15 mg/kg group were noticeably thin and/or emaciated. Two male dogs in the 15 mg/kg group showed gross prostatic enlargement.

7. Histopathology

Microscopically, diffuse mild atrophy was seen in the two male dogs with the grossly small testes. In addition, focal mild atrophy was seen in the testes of one additional dog from the 15 mg/kg group. Hyperplasia of the prostate was evident in the two dogs with grossly enlarged prostates. These lesions are probably related to administration of the test article.

A periventricular cellular infiltrate was evident in the cerebrum of both control and treated dogs and is probably an incidental finding. Focal cellular infiltrates in the cerebrum were present in 2 females in the 3 mg/kg group, 1 female in the 9 mg/kg group and 1 male and 2 females in the 15 mg/kg group suggesting a possible relationship to test article administration. Floccular change in the white matter of the cerebrum, suggesting an early destruction of myelin, was present

in 2 males and 1 female in the 9 mg/kg group and in 1 male in the 15 mg/kg group. Focal malacia was present in the cerebrum in two of the males in the 15 mg/kg group. Chromatolysis of the neurons was present in the brain stem in two females in the 3 mg/kg group, one male and two females in the 9 mg/kg and in three males and three females in the 15 mg/kg group, also suggesting a treatment effect.

Special stains of brain tissue were not originally required in the protocol. After finding treatment related lesions in the brain and talking with the Sponsor, the Protocol was amended to include the Luxol Fast Blue (LFB) for the demonstration of loss of myelin and the Gallocyanine stain for demonstrastion of nissl substance in the neurons in the control and the high dose animals. The LFB stain revealed the breakdown of myelin around the areas of malacia. The floccular changes were also LFB positive suggesting breakdown of the myelin sheaths in these areas. The nissl stain revealed a persistance of the nissl substance indicating that the neurons were still viable. The chromatolysis may be indicative of a metabolic, physiologic or an early degenerative change. Dr. Chang's report, as well as individual animal reports and summary tables, are included in the Appendices.

See deliberations for analysis

RECORDS:

Upon completion of this project, the disposition of study files and study-related material will be determined by the sponsor.

STAFF:

Charles H. Frith, D.V.M., Ph.D.	Study Director and Pathologist
Phillip T. Goad, Ph.D.	Consultant
Louis W. Chang, Ph.D.	Neuropathologist
Harold E. Farris, D.V.M., M.S.	Laboratory Animal Veterinarian
Cynthia Jacobs, D.V.M.	Laboratory Animal Veterinarian
Carolyn Yarbrough, Ph.D.	Clinical Pathology
David R. Nichols, Ph.D.	Collaborator and Supplier of MDMA
Robert Walls, Ph.D.	Statistics
Jack Hamm, Ph.D.	Statistics
Gina Caldwell, B.A.	Quality Assurance
Dan Crowder, B.S.	Technical Assistance
Robert Graves, B.S.	Manager, Animal Facilities

NOTICE:

The conduct of this study was subjected to perodic audits and inspections by the Quality Assurance Officer. Procedures were conducted in accordance with the study protocol and standard operating procedures (SOP's) were followed.

APPENDICES

APPENDIX 1

TABLE 1-CLINICAL SIGNS SUMMARY
PROJECT EMD-SC-001

Number Showing Each Sign

	Males				Females			
Group (mg/kg)	0	3	9	15	0	3	9	15
CLINICAL SIGN								
Aggression	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3*
Circling	0/3	0/3	1/3	3/3	0/3	0/3	1/3	1/3
Convulsions	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3*
Depression	0/3	3/3	1/3	1/3	0/3	3/3	1/3	0/3
Dilated Pupils	0/3	3/3	3/3	3/3	0/3	3/3	3/3	3/3
Hyperactivity	0/3	2/3	3/3	3/3	0/3	1/3	3/3	3/3
Paralysis	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3*
Rapid Breathing	0/3	0/3	3/3	3/3	0/3	0/3	3/3	2/3
Salivation	0/3	0/3	1/3	3/3	0/3	0/3	1/3	0/3
Vocalization	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3*

* Seen in the dog that died on the first day of dosing.

APPENDIX 2

NEUROPATHOLOGY REPORT

28-Day Oral Toxicity of MDMA in Dogs
EMD-SC-001

All brain, cord, and peripheral nerve (sciatic) specimens were fixed in 10% neutral buffered formalin, dehydrated with graded ethanol, embedded in paraffin and sectioned at a thickness of 8-10 microns. All tissue sections were stained with hematoxylin and eosin (H&E) utilizing an automated tissue stainer. In addition to H&E staining, Luxol Fast Blue (LFB) for myelin and the Gallocyamine stain for Nissl substance were performed on the control and high dose groups.

Two male dogs in the high dose group (15 mg/kg) showed isolated areas of encephalomalacia (decomposition of brain substance) in the cerebral cortex. This lesion was particularly large in one dog (Z0F5) showing breakdown of the brain tissue with extensive cellular infiltration. Thinning or breakdown of myelin was demonstrable with the LFB stain around the areas of malacia. Floccular changes of isolated areas of the white substance in the cerebral cortex were observed. This floccular material was LFB positive suggesting myelin origin and breakdown of the myelin sheaths in the areas where the floccular changes were visible. Another prominent finding was a chromatolysis-like change in some of the neurons in the brain stem, particularly those in the cerebellar peduncle area. These neurons appeared very pale (did not stain with H&E) and had a "washed-out" appearence. Unlike chromatolysis in classical Wallerian degeneration, these neurons did not show eccentric nuclei. The Gallocyamine stain revealed a persistance of Nissl substance in these neurons. Such

neuronal alterations may reflect a metabolic, physiologic or an early degenerative change as a result of exposure to MDMA. The precise mechanism resulting in the occurrence of such phenomenon can not be explained on morphological findings. This phenomenon was more prominent in the female than in the male animals, and the incidence appeared to correlate with the dose level of MDMA.

Isolated foci of cellular infiltration were also observed in various tissue sections. The significance of such infiltration is unknown. Since paraventricular cellular infiltration was observed in a number of control animals, it may be considered as a normal finding in dogs or a tissue reaction unrelated to the drug treatment. Cellular infiltration unrelated to ventricles was only present in treated animals and may reflect a treatment-related effect.

No remarkable pathologic findings were seen in the cerebellar cortex, spinal cord or sciatic nerve of any of the animals.

The present study provided an interesting suggestion of neural response to treatment of dogs with MDMA. However, the small number of animals used in the study (3 per sex per group) makes it difficult to derive the full impact of MDMA on the nervous system.

Louis W. Chang, Ph.D., Neuropathologist — Date 2/3/94

APPENDIX 3

GROSS SUMMARY TABLE

Study: 1
Compound: MDMA
Start Date: 17 Sep85

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Non-Neoplastic Morphology Incidence Summary

Format	First Dose	Director
None	17 Sep85	Charles Frith

	0 mg/kg		3 mg/kg		9 mg/kg		15 mg/kg	
	M	F	M	F	M	F	M	F
Animals on Study:	3	3	3	3	3	3	3	3
Animals Logged:	3	3	3	3	3	3	3	3
ESOPHAGUS	3	3	3	3	3	3	3	3
Not Remarkable	2	3	3	3	3	3	3	3
Missing	0	0	0	0	0	0	0	0
Autolysis	0	0	0	0	0	0	0	0
Serosal ecchymotic hemorrhage	1	0	0	0	0	0	0	0
TESTES	3	0	3	0	3	0	3	0
Not Remarkable	3	0	3	0	2	0	2	0
Missing	0	0	0	0	0	0	0	0
Autolysis	0	0	0	0	0	0	0	0
Reduced	0	0	0	0	1	0	1	0
HEART	3	3	3	3	3	3	3	3
Not Remarkable	3	2	3	3	3	3	3	2
Missing	0	0	0	0	0	0	0	0
Autolysis	0	0	0	0	0	0	0	0
Brown pericardial fibrinous material	0	1	0	0	0	0	0	0
Red zones	0	0	0	0	0	0	0	1
LUNG	3	3	3	3	3	3	3	3
Not Remarkable	2	3	2	3	3	2	2	2
Missing	0	0	0	0	0	0	0	0
Autolysis	0	0	0	0	0	0	0	0
Red mottling	1	0	0	0	0	0	1	0
Dark	0	0	1	0	0	0	0	0
Congested	0	0	1	0	0	0	0	1
Did not collapse	0	0	0	0	0	1	0	0
PROSTATE	3	0	3	0	3	0	3	0
Not Remarkable	3	0	3	0	3	0	1	0
Missing	0	0	0	0	0	0	0	0
Autolysis	0	0	0	0	0	0	0	0
Enlarged	0	0	0	0	0	0	2	0
SPLEEN	3	3	3	3	3	3	3	3
Not Remarkable	3	3	3	2	3	3	3	3
Missing	0	0	0	0	0	0	0	0
Autolysis	0	0	0	0	0	0	0	0
Congested	0	0	0	1	0	0	0	0
THYMUS	3	3	3	3	3	3	3	3
Not Remarkable	3	3	3	3	3	3	2	2
Missing	0	0	0	0	0	0	0	0

Removal Reasons Included:
Dead, Terminal Sac.
Removal Dates: 17 Sep85 - 16 Oct85 Data Collection Dates: 13 Nov85 - 25 Nov85

Format	First Dose	Director
None	17 Sep85	Charles Frith

	0 mg/kg		3 mg/kg		9 mg/kg		15 mg/kg	
	M	F	M	F	M	F	M	F
Animals on Study:	3	3	3	3	3	3	3	3
Animals Logged:	3	3	3	3	3	3	3	3
Autolysis	0	0	0	0	0	0	0	0
Reduced	0	0	0	0	0	0	1	0
Dark red	0	0	0	0	0	0	0	1
TONSIL	1	0	0	0	0	0	0	0
Not Remarkable	0	0	0	0	0	0	0	0
Missing	0	0	0	0	0	0	0	0
Autolysis	0	0	0	0	0	0	0	0
Enlarged	1	0	0	0	0	0	0	0
TRACHEA	0	0	0	0	0	1	0	0
Not Remarkable	0	0	0	0	0	0	0	0
Missing	0	0	0	0	0	0	0	0
Autolysis	0	0	0	0	0	0	0	0
Froth	0	0	0	0	0	1	0	0
Oozes fluid from cut surface	0	0	0	0	0	1	0	0

Removal Reasons Included:
Dead, Terminal Sac.
Removal Dates: 17 Sep85 - 16 Oct85 Data Collection Dates: 13 Nov85 - 25 Nov85

APPENDIX 4

MICROSCOPIC SUMMARY TABLE

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Non-Neoplastic Morphology Incidence Summary

Format	First Dose	Director
None	17 Sep85	Charles Frith

	0 mg/kg		3 mg/kg		9 mg/kg		15 mg/kg	
	M	F	M	F	M	F	M	F
Animals on Study:	3	3	3	3	3	3	3	3
Animals Logged:	3	3	3	3	3	3	3	3
Brain/cerebellum	3	3	3	3	3	3	3	3
Not Remarkable	3	3	3	3	3	3	3	2
Missing.	0	0	0	0	0	0	0	0
Autolysis.	0	0	0	0	0	0	0	0
Autolysis	0	0	0	0	0	0	0	1
Brain/cerebrum	3	3	3	3	3	3	3	3
Not Remarkable	3	1	3	1	1	1	1	0
Missing.	0	0	0	0	0	0	0	0
Autolysis.	0	0	0	0	0	0	0	0
Cellular infiltrate	0	0	0	2	0	1	1	2
Floccular change, white matter	0	0	0	0	2	1	1	0
Periventricular cellular infiltrate	0	2	0	2	2	2	0	0
Malacia	0	0	0	0	0	0	2	0
Autolysis	0	0	0	0	0	0	0	1
Brain/stem	3	3	3	3	3	3	3	3
Not Remarkable	3	3	3	1	2	1	0	0
Missing.	0	0	0	0	0	0	0	0
Autolysis.	0	0	0	0	0	0	0	0
Chromatolysis, neurons	0	0	0	2	1	2	3	3
Autolysis	0	0	0	0	0	0	0	1
Heart	3	3	3	3	3	3	3	3
Not Remarkable	3	3	2	3	3	3	3	2
Missing.	0	0	1	0	0	0	0	0
Autolysis.	0	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	1
Lung	3	3	3	3	3	3	3	3
Not Remarkable	3	2	2	2	2	3	2	0
Missing.	0	0	0	0	0	0	0	0
Autolysis.	0	0	0	0	0	0	0	0
Congestion	0	1	1	1	1	0	1	3
Inflammation	0	0	1	0	0	0	0	0
Lymph Node	3	3	3	3	3	3	3	3
Not Remarkable	2	2	3	3	3	3	3	2
Missing.	1	1	0	0	0	0	0	0
Autolysis.	0	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	1
Parathyroid Gland	3	3	3	3	3	3	3	3

Removal Reasons Included:
Terminal Sac, Dead.
Removal Dates: -

Data Collection Dates: 24 Nov85 - 25 Dec85

Study: 1
Compound: MDMA
Start Date: 17 Sep85

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Non-Neoplastic Morphology Incidence Summary

Date: 7 Feb86
Time: 14:54
DMS008 V3.0

Format	First Dose	Director
None	17 Sep85	Charles Frith

	0 mg/kg		3 mg/kg		9 mg/kg		15 mg/kg	
	M	F	M	F	M	F	M	F
Animals on Study:	3	3	3	3	3	3	3	3
Animals Logged:	3	3	3	3	3	3	3	3
Not Remarkable	3	3	1	3	2	2	1	2
Missing	0	0	2	0	1	1	1	1
Autolysis	0	0	0	0	0	0	0	0
Cyst, NOS	0	0	0	0	0	0	1	0
Pituitary Gland	3	3	3	3	3	3	3	3
Not Remarkable	3	1	2	3	1	2	1	2
Missing	0	0	1	0	0	0	1	1
Autolysis	0	0	0	0	0	0	0	0
Cyst, NOS	0	2	0	0	2	1	1	0
Prostate	3	0	3	0	3	0	3	0
Not Remarkable	3	0	2	0	2	0	2	0
Missing	0	0	1	0	0	0	0	0
Autolysis	0	0	0	0	0	0	0	0
Hyperplasia	0	0	0	0	1	0	1	0
Testes	3	0	3	0	3	0	3	0
Not Remarkable	3	0	3	0	2	0	1	0
Missing	0	0	0	0	0	0	0	0
Autolysis	0	0	0	0	0	0	0	0
Atrophy	0	0	0	0	1	0	2	0
Thymus	3	3	3	3	3	3	3	3
Not Remarkable	3	2	0	3	3	3	1	2
Missing	0	1	2	0	0	0	1	0
Autolysis	0	0	0	0	0	0	0	0
Atrophy	0	0	1	0	0	0	1	0
Hemorrhage	0	0	0	0	0	0	0	1
Uterus	0	3	0	3	0	3	0	3
Not Remarkable	0	3	0	3	0	3	0	2
Missing	0	0	0	0	0	0	0	0
Autolysis	0	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	1

Removal Reasons Included:
Terminal Sac, Dead.
Removal Dates: -

Data Collection Dates: 24 Nov85 - 25 Dec85

APPENDIX 5

INDIVIDUAL ANIMAL GROSS NECROPSY REPORTS

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1
Started: 17 Sep85
First Dose: 17 Sep85
Compound: MDMA

Individual Animal Gross Pathology

Date: 22 Oct88
Time: 14:43
GPS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		15 Oct85		Canine/B	M	0 mg/kg		XCF5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:02	FRITA7PATH	FRITA7PATH	JACOBS	Terminal Sac	

Status: Completed.

The following observations were noted:

LUNG
  Red mottling [Diffuse]
TONSIL
  Enlarged [Mild, Bilateral]

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, TESTES, EPIDIDYMIS, HEART, KIDNEY, LIVER, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, PROSTATE, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER.

The following protocol tissues were not marked:
None.

Study: 1
Started: 17 Sep85
First Dose: 17 Sep85
Compound: MDMA

Individual Animal Gross Pathology

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		15 Oct85		Canine/B	M	0 mg/kg		XZF5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:03	FRITH/PATH	FRITH/PATH	JACOBS	Terminal Sac	

Status: Completed.

The following observations were noted:

ESOPHAGUS
Serosal ecchymotic hemorrhage

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, EYE, TESTES, EPIDIDYMIS, HEART, KIDNEY, LIVER, LUNG, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, PROSTATE, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER.

The following protocol tissues were not marked:
None.

Study: 1
Started: 17 Sep85
First Dose:17 Sep85
Compound: MDMA

Individual Animal Gross Pathology

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		15 Oct85		Canine/B	M	0 mg/kg		0VP5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:04	FRITA/PATA	FRITA/PATA	FARRIS	Terminal Sac	

Status: Completed.

The following observations were noted:

No observations noted.

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, TESTES, EPIDIDYMIS, HEART, KIDNEY, LIVER, LUNG, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, PROSTATE, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER.

The following protocol tissues were not marked:
None.

Study: 1
Started: 17 Sep85
First Dose: 17 Sep85
Compound: MDMA

Individual Animal Gross Pathology

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		16 Oct85		Canine/B	F	0 mg/kg		YOE5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:06	FRITH/PATH	FRITH/PATH	JACOBS	Terminal Sac	

Status: Completed.

The following observations were noted:

HEART
Brown pericardial fibrinous material

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, OVARY, KIDNEY, LIVER, LUNG, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER, UTERUS.

The following protocol tissues were not marked:
None.

Comments:
Animal was euthanized by intracardiac puncture.

Study: 1
Started: 17 Sep85
First Dose: 17 Sep85
Compound: MDMA

Individual Animal Gross Pathology

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		16 Oct85		Canine/B	F	0 mg/kg		ZSE5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:07	FRITH/PATH	FRITH/PATH	FARRIS	Terminal Sac	

Status: Completed.

The following observations were noted:

No observations noted.

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, OVARY, HEART, KIDNEY, LIVER, LUNG, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER, UTERUS.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1 | Page: 6
Started: 17 Sep85 | Individual Animal Gross Pathology | Date: 22 Oct88
First Dose:17 Sep85 | Time: 14:44
Compound: MDMA | GPS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		16 Oct85		Canine/B	F	0 mg/kg		0TE5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:07	FRITH/PATH	FRITH/PATH	FRITH/PATH	Terminal Sac	

Status: Completed.

The following observations were noted:

No observations noted.

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, OVARY, HEART, KIDNEY, LIVER, LUNG, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER, UTERUS.

The following protocol tissues were not marked:
None.

Study: 1
Started: 17 Sep85
First Dose:17 Sep85
Compound: MDMA

Individual Animal Gross Pathology

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		15 Oct85		Canine/B	M	3 mg/kg		WEF5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:08	FRITH/PATH	FRITH/PATH	FRITH/PATH	Terminal Sac	

Status: Completed.

The following observations were noted:

No observations noted.

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, TESTES, EPIDIDYMIS, HEART, KIDNEY, LIVER, LUNG, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, PROSTATE, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1
Started: 17 Sep85
First Dose:17 Sep85
Compound: MDMA

Individual Animal Gross Pathology

Date: 22 Oct88
Time: 14:45
GPS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		15 Oct85		Canine/B	M	3 mg/kg		YCF5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:09	FRITH/PATH	FRITH/PATH	FRITH/PATH	Terminal Sac	

Status: Completed.

The following observations were noted:

No observations noted.

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, TESTES, EPIDIDYMIS, HEART, KIDNEY, LIVER, LUNG, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, PROSTATE, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER.

The following protocol tissues were not marked:
None.

Study: 1
Started: 17 Sep85
First Dose: 17 Sep85
Compound: MDMA

Individual Animal Gross Pathology

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		15 Oct85		Canine/B	M	3 mg/kg		VWF5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:10	FRITA/PATH	FRITA/PATH	FARRIS	Terminal Sac	

Status: Completed.

The following observations were noted:

LUNG
Dark
Congested

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, TESTES, EPIDIDYMIS, HEART, KIDNEY, LIVER, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, PROSTATE, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER.

The following protocol tissues were not marked:
None.

Study: 1
Started: 17 Sep85
First Dose: 17 Sep85
Compound: MDMA

Individual Animal Gross Pathology

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		16 Oct85		Canine/B	F	3 mg/kg		YGE5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:10	FRITA7/PATH	FRITA7/PATH	JACOBS	Terminal Sac	

Status: Completed.

The following observations were noted:

No observations noted.

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, OVARY, HEART, KIDNEY, LIVER, LUNG, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER, UTERUS.

The following protocol tissues were not marked:
None.

Study: 1
Started: 17 Sep85
First Dose: 17 Sep85
Compound: MDMA

Individual Animal Gross Pathology

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		16 Oct85		Canine/B	F	3 mg/kg		XRE5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:11	FRITH/PATH	FRITH/PATH	FARRIS	Terminal Sac	

Status: Completed.

The following observations were noted:

SPLEEN
Congested

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, OVARY, HEART, KIDNEY, LIVER, LUNG, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER, UTERUS.

The following protocol tissues were not marked:
None.

Study: 1 | Page: 12
Started: 17 Sep85 | Individual Animal Gross Pathology | Date: 22 Oct88
First Dose: 17 Sep85 | Time: 14:46
Compound: MDMA | GPS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		16 Oct85		Canine/B	F	3 mg/kg		YCE5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:12	FRITH/PATH	FRITH/PATH	FRITH/PATH	Terminal Sac	

Status: Completed.

The following observations were noted:

No observations noted.

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, OVARY, HEART, KIDNEY, LIVER, LUNG, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER, UTERUS.

The following protocol tissues were not marked:
None.

Study: 1
Started: 17 Sep85
First Dose:17 Sep85
Compound: MDMA

Individual Animal Gross Pathology

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		15 Oct85		Canine/B	M	9 mg/kg		YFF5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:13	FRITA/PATH	FRITA/PATH	JACOBS	Terminal Sac	

Status: Completed.

The following observations were noted:

No observations noted.

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, TESTES, EPIDIDYMIS, HEART, KIDNEY, LIVER, LUNG, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, PROSTATE, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER.

The following protocol tissues were not marked:
None.

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		15 Oct85		Canine/B	M	9 mg/kg		YAF5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:14	FRITH/PATH	FRITH/PATH	FRITH/PATH	Terminal Sac	

Status: Completed.

The following observations were noted:

TESTES
Reduced [Bilateral]

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, EPIDIDYMIS, HEART, KIDNEY, LIVER, LUNG, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, PROSTATE, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER.

The following protocol tissues were not marked:
None.

Study: 1
Started: 17 Sep85
First Dose: 17 Sep85
Compound: MDMA

Individual Animal Gross Pathology

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		15 Oct85		Canine/B	M	9 mg/kg		ORE5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:15	FRITA/PATA	FRITA/PATA	FARRIS	Terminal Sac	

Status: Completed.

The following observations were noted:

No observations noted.

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, TESTES, EPIDIDYMIS, HEART, KIDNEY, LIVER, LUNG, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, PROSTATE, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1
Started: 17 Sep85 Individual Animal Gross Pathology
First Dose:17 Sep85
Compound: MDMA

Date: 25 Nov85
Time: 10:14
GPS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		16 Oct85		Canine/B	F	9 mg/kg		YBE5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
25 Nov85 10:14	FRITH/PATH	FRITH/PATH	JACOBS	Terminal Sac	

Status: Completed.

The following observations were noted:

No observations noted.

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, OVARY, HEART, KIDNEY, LIVER, LUNG, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER, UTERUS.

The following protocol tissues were not marked:
None.

Study: 1
Started: 17 Sep85 Individual Animal Gross Pathology
First Dose:17 Sep85
Compound: MDMA

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		16 Oct85		Canine/B	F	9 mg/kg		Y1C5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:18	FRITH/PATH	FRITH/PATH	JACOBS	Terminal Sac	

Status: Completed.

The following observations were noted:

LUNG
Did not collapse
TRACHEA
Froth [Mild]
Oozes fluid from cut surface

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, OVARY, HEART, KIDNEY, LIVER, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER, UTERUS.

The following protocol tissues were not marked:
None.

Study: 1
Started: 17 Sep85
First Dose: 17 Sep85
Compound: MDMA

Individual Animal Gross Pathology

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		16 Oct85		Canine/B	F	9 mg/kg		YPE5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:18	FRITA/PATH	FRITA/PATH	FRITA/PATH	Terminal Sac	

Status: Completed.

The following observations were noted:

No observations noted.

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, OVARY, HEART, KIDNEY, LIVER, LUNG, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER, UTERUS.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1
Started: 17 Sep85
First Dose:17 Sep85
Compound: MDMA

Individual Animal Gross Pathology

Date: 22 Oct88
Time: 14:48
GPS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		15 Oct85		Canine/B	M	15 mg/kg		ZOF5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:22	FRITH/PATH	FRITH/PATH	FARRIS	Terminal Sac	

Status: Completed.

The following observations were noted:

TESTES
Reduced [Bilateral]
THYMUS
Reduced

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, EPIDIDYMIS, HEART, KIDNEY, LIVER, LUNG, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, PROSTATE, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYROID GLAND, URINARY BLADDER.

The following protocol tissues were not marked:
None.

Comments:
Animal was emaciated.

Study: 1
Started: 17 Sep85
First Dose: 17 Sep85
Compound: MDMA

Individual Animal Gross Pathology

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		15 Oct85		Canine/B	M	15 mg/kg		WXF5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
25 Nov85 10:13	FRITH/PATH	FRITH/PATH	JACOBS	Terminal Sac	

Status: Completed.

The following observations were noted:

LUNG
  Red mottling
PROSTATE
  Enlarged

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, TESTES, EPIDIDYMIS, HEART, KIDNEY, LIVER, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1 | Page: 21
Started: 17 Sep85 | Individual Animal Gross Pathology | Date: 22 Oct88
First Dose: 17 Sep85 | Time: 14:48
Compound: MDMA | GPS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		15 Oct85		Canine/B	M	15 mg/kg		XJF5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:23	FRITH/PATH	FRITH/PATH	FRITH/PATH	Terminal Sac	

Status: Completed.

The following observations were noted:

PROSTATE
Enlarged

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, TESTES, EPIDIDYMIS, HEART, KIDNEY, LIVER, LUNG, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER.

The following protocol tissues were not marked:
None.

Study: 1
Started: 17 Sep85
First Dose: 17 Sep85
Compound: MDMA

Individual Animal Gross Pathology

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		17 Sep85		Canine/B	F	15 mg/kg		VEE5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:28	FRITA/PATH	FRITA/PATH	FRITA/PATH	Dead	

Status: Completed.

The following observations were noted:

HEART
  Red zones [Diffuse]
THYMUS
  Dark red [Diffuse]

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, OVARY, KIDNEY, LIVER, LUNG, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYROID GLAND, URINARY BLADDER, UTERUS.

The following protocol tissues were not marked:
None.

Comments:
Organs were not weighed because animal was dead.

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		16 Oct85		Canine7B	F	15 mg/kg		X0E5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:25	FRITH/PATH	FRITH/PATH	FRITH/PATH	Terminal Sac	

Status: Completed.

The following observations were noted:

LUNG
Congested

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECOM, COLON, ESOPHAGUS, EYE, OVARY, HEART, KIDNEY, LIVER, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER, UTERUS.

The following protocol tissues were not marked:
None.

Study: 1
Started: 17 Sep85
First Dose: 17 Sep85
Compound: MDMA

Individual Animal Gross Pathology

Completed	Birth	Death	Age/Days	Species	Sex	Group	Photograph	Animal ID
13 Nov85		16 Oct85		Canine/B	F	15 mg/kg		XBE5

Data Collection	Pathologist	Operator	Prosector	Removal Reason	Cassettes
13 Nov85 12:27	FRITA/PATH	FRITA/PATH	FARRIS	Terminal Sac	

Status: Completed.

The following observations were noted:

No observations noted.

The following tissues were marked as NR:
ADRENAL GLAND, AORTA, BONE, BRAIN, CECUM, COLON, ESOPHAGUS, EYE, OVARY, HEART, KIDNEY, LIVER, LUNG, LYMPH NODE, MAMMARY GLAND, SKELETAL MUSCLE, OPTIC NERVE, PANCREAS, PARATHYROID GLAND, PITUITARY GLAND, RECTUM, SALIVARY GLAND, SUBMAXILLARY, SCIATIC NERVE, INTESTINE, SMALL, SPINAL CORD, SPLEEN, STOMACH, THYMUS, THYROID GLAND, URINARY BLADDER, UTERUS.

The following protocol tissues were not marked:
None.

Comments:
Serous atrophy of body fat was present.

APPENDIX 6

INDIVIDUAL ANIMAL MICROSCOPIC PATHOLOGY REPORTS

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1
Started: 17 Sep85
First Dose:17 Sep85
Compound: MDMA

Individual Animal Histopathology

Date: 24 Nov85
Time: 14:05
DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	M	0 mg/kg		XCF5

Data Collection	Pathologist	Operator	Removal Reason	Slides
24 Nov85 14:05	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

No observations noted.

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Brain/cerebrum, Brain/stem, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Kidney, Liver, Lung, Lymph Node, Pancreas, Exocrine, Pancreas, Islet, Parathyroid Gland, Pituitary Gland, Prostate, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Testes, Epididymis, Thymus, Thyroid Gland, Urinary Bladder.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1 | Page: 1
Started: 17 Sep85 | Individual Animal Histopathology | Date: 24 Nov85
First Dose:17 Sep85 | Time: 14:06
Compound: MDMA | DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	M	0 mg/kg		XZF5

Data Collection	Pathologist	Operator	Removal Reason	Slides
24 Nov85 14:06	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Esophagus (Missing)

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Brain/cerebrum, Brain/stem, Sciatic Nerve, Spinal Cord, Cecum, Colon, Eye, Optic Nerve, Heart, Kidney, Liver, Lung, Lymph Node, Pancreas, Exocrine, Pancreas, Islet, Parathyroid Gland, Pituitary Gland, Prostate, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Testes, Epididymis, Thymus, Thyroid Gland, Urinary Bladder.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1 — Page: 1
Started: 17 Sep85 — Individual Animal Histopathology — Date: 24 Nov85
First Dose:17 Sep85 — Time: 14:06
Compound: MDMA — DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	M	0 mg/kg		XZF5

Data Collection	Pathologist	Operator	Removal Reason	Slides
24 Nov85 14:06	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Esophagus (Missing)

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Brain/cerebrum, Brain/stem, Sciatic Nerve, Spinal Cord, Cecum, Colon, Eye, Optic Nerve, Heart, Kidney, Liver, Lung, Lymph Node, Pancreas, Exocrine, Pancreas, Islet, Parathyroid Gland, Pituitary Gland, Prostate, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Testes, Epididymis, Thymus, Thyroid Gland, Urinary Bladder.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1
Started: 17 Sep85
First Dose: 17 Sep85
Compound: MDMA

Individual Animal Histopathology

Date: 24 Nov85
Time: 14:07
DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	M	0 mg/kg		UVF5

Data Collection	Pathologist	Operator	Removal Reason	Slides
24 Nov85 14:07	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Lymph Node (Missing)

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Brain/cerebrum, Brain/stem, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Kidney, Liver, Lung, Pancreas, Exocrine, Pancreas, Islet, Parathyroid Gland, Pituitary Gland, Prostate, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Testes, Epididymis, Thymus, Thyroid Gland, Urinary Bladder.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1 | Page: 1
Started: 17 Sep85 | Individual Animal Histopathology | Date: 11 Dec85
First Dose:17 Sep85 | Time: 13:35
Compound: MDMA | DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	F	0 mg/kg		YUE5

Data Collection	Pathologist	Operator	Removal Reason	Slides
11 Dec85 13:35	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Brain/cerebrum
  Periventricular cellular infiltrate
Lung
  Congestion [Mild, Diffuse]
Pituitary Gland
  Cyst, NOS [Mild, Focal]

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Brain/stem, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Kidney, Liver, Lymph Node, Mammary Gland, Ovary, Pancreas, Exocrine, Pancreas, Islet, Parathyroid Gland, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Thymus, Thyroid Gland, Urinary Bladder, Uterus.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1
Started: 17 Sep85
First Dose:17 Sep85
Compound: MDMA

Individual Animal Histopathology

Date: 24 Nov85
Time: 14:10
DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	F	0 mg/kg		ZSE5

Data Collection	Pathologist	Operator	Removal Reason	Slides
24 Nov85 14:10	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Brain/cerebrum	
Periventricular cellular infiltrate	
Lymph Node	(Missing)
Stomach	(Missing)
Thymus	(Missing)

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Brain/stem, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Kidney, Liver, Lung, Mammary Gland, Ovary, Pancreas, Exocrine, Pancreas, Islet, Parathyroid Gland, Pituitary Gland, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Thyroid Gland, Urinary Bladder, Uterus.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1
Started: 17 Sep85
First Dose:17 Sep85
Compound: MDMA

Individual Animal Histopathology

Date: 24 Nov85
Time: 14:11
DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	F	0 mg/kg		UTE5

Data Collection	Pathologist	Operator	Removal Reason	Slides
24 Nov85 14:11	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Pituitary Gland	
Cyst, NOS	[Mild, Multifocal]
Urinary Bladder	(Missing)

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Brain/cerebrum, Brain/stem, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Kidney, Liver, Lung, Lymph Node, Mammary Gland, Ovary, Pancreas, Exocrine, Pancreas, Islet, Parathyroid Gland, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Thymus, Thyroid Gland, Uterus.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1
Started: 17 Sep85
First Dose: 17 Sep85
Compound: MDMA

Individual Animal Histopathology

Date: 24 Nov85
Time: 14:12
DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	M	3 mg/kg		WEF5

Data Collection	Pathologist	Operator	Removal Reason	Slides
24 Nov85 14:12	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Pituitary Gland	(Missing)
Prostate	(Missing)
Thymus	
Atrophy	[Minimal, Diffuse]

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Brain/cerebrum, Brain/stem, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Kidney, Liver, Lung, Lymph Node, Mammary Gland, Pancreas, Exocrine , Pancreas, Islet, Parathyroid Gland, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Testes, Epididymis, Thyroid Gland, Urinary Bladder.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1
Started: 17 Sep85
First Dose: 17 Sep85
Compound: MDMA

Individual Animal Histopathology

Date: 24 Nov85
Time: 14:13
DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	M	3 mg/kg		YLF5

Data Collection	Pathologist	Operator	Removal Reason	Slides
24 Nov85 14:13	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Heart	(Missing)
Parathyroid Gland	(Missing)
Thymus	(Missing)

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Brain/cerebrum, Brain/stem, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Kidney, Liver, Lung, Lymph Node, Pancreas, Exocrine, Pancreas, Islet, Pituitary Gland, Prostate, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Testes, Epididymis, Thyroid Gland, Urinary Bladder.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1
Started: 17 Sep85
First Dose: 17 Sep85
Compound: MDMA

Individual Animal Histopathology

Date: 24 Nov85
Time: 14:14
DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	M	3 mg/kg		VWF5

Data Collection	Pathologist	Operator	Removal Reason	Slides
24 Nov85 14:14	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Adrenal Gland	(Missing)
Lung	
Congestion	[Mild, Multifocal]
Inflammation	[Mild, Focal, Chronic]
Parathyroid Gland	(Missing)
Thymus	(Missing)

The following tissues were marked as NR:
Aorta, Bone, Bone Marrow, Brain/cerebellum, Brain/cerebrum, Brain/stem, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Kidney, Liver, Lymph Node, Pancreas, Exocrine, Pancreas, Islet, Pituitary Gland, Prostate, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Testes, Epididymis, Thyroid Gland, Urinary Bladder.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	F	3 mg/kg		YGE5

Data Collection	Pathologist	Operator	Removal Reason	Slides
25 Dec85 12:21	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Brain/cerebrum
  Cellular infiltrate [Focal]
  Periventricular cellular infiltrate
Brain/stem
  Chromatolysis, neurons
Mammary Gland (Missing)

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Kidney, Liver, Lung, Lymph Node, Ovary, Pancreas, Exocrine, Pancreas, Islet, Parathyroid Gland, Pituitary Gland, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Thymus, Thyroid Gland, Urinary Bladder, Uterus.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1
Started: 17 Sep85
First Dose:17 Sep85
Compound: MDMA

Individual Animal Histopathology

Date: 24 Nov85
Time: 14:16
DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	F	3 mg/kg		XRE5

Data Collection	Pathologist	Operator	Removal Reason	Slides
24 Nov85 14:16	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

No observations noted.

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Brain/cerebrum, Brain/stem, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Kidney, Liver, Lung, Lymph Node, Mammary Gland, Ovary, Pancreas, Exocrine, Pancreas, Islet, Parathyroid Gland, Pituitary Gland, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Thymus, Thyroid Gland, Urinary Bladder, Uterus.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1 Page: 1
Started: 17 Sep85 Individual Animal Histopathology Date: 25 Dec85
First Dose:17 Sep85 Time: 12:23
Compound: MDMA DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	F	3 mg/kg		YCE5

Data Collection	Pathologist	Operator	Removal Reason	Slides
25 Dec85 12:23	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Brain/cerebrum
  Cellular infiltrate [Focal]
  Periventricular cellular infiltrate
Brain/stem
  Chromatolysis, neurons
Lung
  Congestion [Mild, Diffuse]

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Kidney, Liver, Lymph Node, Mammary Gland, Ovary, Pancreas, Exocrine, Pancreas, Islet, Parathyroid Gland, Pituitary Gland, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Thymus, Thyroid Gland, Urinary Bladder, Uterus.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1
Started: 17 Sep85
First Dose: 17 Sep85
Compound: MDMA

Individual Animal Histopathology

Date: 24 Nov85
Time: 14:17
DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	M	9 mg/kg		YFF5

Data Collection	Pathologist	Operator	Removal Reason	Slides
24 Nov85 14:17	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Brain/cerebrum
Floccular change, white matter
Periventricular cellular infiltrate

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Brain/stem, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Kidney, Liver, Lung, Lymph Node, Pancreas, Exocrine, Pancreas, Islet, Parathyroid Gland, Pituitary Gland, Prostate, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Testes, Epididymis, Thymus, Thyroid Gland, Urinary Bladder.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1 Page: 1
Started: 17 Sep85 Individual Animal Histopathology Date: 24 Nov85
First Dose:17 Sep85 Time: 14:18
Compound: MDMA DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	M	9 mg/kg		YAF5

Data Collection	Pathologist	Operator	Removal Reason	Slides
24 Nov85 14:18	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Brain/cerebrum
  Floccular change, white matter
  Periventricular cellular infiltrate
Parathyroid Gland (Missing)
Pituitary Gland
  Cyst, NOS [Minimal, Focal]
Testes
  Atrophy [Mild, Diffuse]

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Brain/stem, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Kidney, Liver, Lung, Lymph Node, Pancreas, Exocrine, Pancreas, Islet, Prostate, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Epididymis, Thymus, Thyroid Gland, Urinary Bladder.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1
Started: 17 Sep85
First Dose: 17 Sep85
Compound: MDMA

Individual Animal Histopathology

Date: 25 Dec85
Time: 12:24
DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	M	9 mg/kg		URES

Data Collection	Pathologist	Operator	Removal Reason	Slides
25 Dec85 12:24	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Brain/stem
  Chromatolysis, neurons
Lung
  Congestion [Mild, Diffuse]
Pituitary Gland
  Cyst, NOS [Mild, Focal]
Prostate
  Hyperplasia [Mild, Diffuse]

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Brain/cerebrum, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Kidney, Liver, Lymph Node, Pancreas, Exocrine, Pancreas, Islet, Parathyroid Gland, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Testes, Epididymis, Thymus, Thyroid Gland, Urinary Bladder.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	F	9 mg/kg		YB65

Data Collection	Pathologist	Operator	Removal Reason	Slides
25 Dec85 12:25	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Brain/cerebrum
  Cellular infiltrate [Multifocal]
  Periventricular cellular infiltrate
Brain/stem
  Chromatolysis, neurons

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Kidney, Liver, Lung, Lymph Node, Mammary Gland, Ovary, Pancreas, Exocrine, Pancreas, Islet, Parathyroid Gland, Pituitary Gland, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Thymus, Thyroid Gland, Urinary Bladder, Uterus.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1
Started: 17 Sep85
First Dose:17 Sep85
Compound: MDMA

Individual Animal Histopathology

Date: 25 Dec85
Time: 12:26
DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	F	9 mg/kg		YIE5

Data Collection	Pathologist	Operator	Removal Reason	Slides
25 Dec85 12:26	Frith/Chang	Frith/Path		

Status: Completed.

The following observations were noted:

Brain/cerebrum
  Floccular change, white matter
  Periventricular cellular infiltrate
Brain/stem
  Chromatolysis, neurons
Parathyroid Gland (Missing)
Pituitary Gland
  Cyst, NOS [Minimal, Focal]

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Kidney, Liver, Lung, Lymph Node, Mammary Gland, Ovary, Pancreas, Exocrine, Pancreas, Islet, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Thymus, Thyroid Gland, Urinary Bladder, Uterus.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1 | Page: 1
Started: 17 Sep85 | Individual Animal Histopathology | Date: 24 Nov85
First Dose:17 Sep85 | Time: 14:22
Compound: MDMA | DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	F	9 mg/kg		YPE5

Data Collection	Pathologist	Operator	Removal Reason	Slides
24 Nov85 14:22	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

No observations noted.

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Brain/cerebrum, Brain/stem, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Kidney, Liver, Lung, Lymph Node, Mammary Gland, Ovary, Pancreas, Exocrine, Pancreas, Islet, Parathyroid Gland, Pituitary Gland, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Thymus, Thyroid Gland, Urinary Bladder, Uterus.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1
Started: 17 Sep85
First Dose:17 Sep85
Compound: MDMA

Individual Animal Histopathology

Date: 25 Dec85
Time: 12:27
DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	M	15 mg/kg		WXF5

Data Collection	Pathologist	Operator	Removal Reason	Slides
25 Dec85 12:27	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Brain/cerebrum	
Cellular infiltrate	[Focal]
Malacia	[Focal]
Brain/stem	
Chromatolysis, neurons	
Lung	
Congestion	[Mild, Diffuse]
Parathyroid Gland	
Cyst, NOS	[Mild, Focal]
Pituitary Gland	(Missing)
Prostate	
Hyperplasia	[Mild, Diffuse]
Testes	
Atrophy	[Mild, Focal]

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Kidney, Liver, Lymph Node, Pancreas, Exocrine, Pancreas, Islet, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Epididymis, Thymus, Thyroid Gland, Urinary Bladder.

The following protocol tissues were not marked:
None.

Study: 1
Started: 17 Sep85 Individual Animal Histopathology
First Dose:17 Sep85
Compound: MDMA

Date: 25 Dec85
Time: 12:28
DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	M	15 mg/kg		20F5

Data Collection	Pathologist	Operator	Removal Reason	Slides
25 Dec85 12:28	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Brain/cerebrum	
Floccular change, white matter	
Malacia	[Focal]
Brain/stem	
Chromatolysis, neurons	
Kidney	(Missing)
Liver	(Missing)
Submaxillary Salivary Gland	
	(Missing)
Testes	
Atrophy	[Moderate, Diffuse]
Thymus	
Atrophy	[Mild, Diffuse]

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Lung, Lymph Node, Pancreas, Exocrine, Pancreas, Islet, Parathyroid Gland, Pituitary Gland, Prostate, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Skeletal Muscle, Spleen, Stomach, Epididymis, Thyroid Gland, Urinary Bladder.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1
Started: 17 Sep85
First Dose:17 Sep85
Compound: MDMA

Individual Animal Histopathology

Date: 25 Dec85
Time: 12:30
DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	M	15 mg/kg		XJF5

Data Collection	Pathologist	Operator	Removal Reason	Slides
25 Dec85 12:30	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Brain/stem	
Chromatolysis, neurons	
Parathyroid Gland	(Missing)
Pituitary Gland	
Cyst, NOS	[Minimal, Focal]
Thymus	(Missing)

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Brain/cerebrum, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Kidney, Liver, Lung, Lymph Node, Pancreas, Exocrine, Pancreas, Islet, Prostate, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum , Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Testes, Epididymis, Thyroid Gland, Urinary Bladder.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1 — Page: 1
Started: 17 Sep85 — Individual Animal Histopathology — Date: 25 Dec85
First Dose:17 Sep85 — Time: 12:31
Compound: MDMA — DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	F	15 mg/kg		VEE5

Data Collection	Pathologist	Operator	Removal Reason	Slides
25 Dec85 12:31	Frith/Chang	Frith/Path	Dead	

Status: Completed.

The following observations were noted:

Brain/cerebellum
  Autolysis
Brain/cerebrum
  Autolysis
Brain/stem
  Chromatolysis, neurons
  Autolysis
Heart
  Hemorrhage [Moderate, Multifocal]
Lung
  Congestion [Moderate, Diffuse]
Lymph Node
  Hemorrhage [Mild, Multifocal]
Pituitary Gland (Missing)
Thymus
  Hemorrhage [Marked, Multifocal]
Uterus
  Hemorrhage [Moderate, Multifocal]

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Kidney, Liver, Mammary Gland, Ovary, Pancreas, Exocrine, Pancreas, Islet, Parathyroid Gland, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Thyroid Gland, Urinary Bladder
.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1
Started: 17 Sep85
First Dose:17 Sep85
Compound: MDMA

Individual Animal Histopathology

Date: 25 Dec85
Time: 12:32
DM5001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	F	15 mg/kg		XBE5

Data Collection	Pathologist	Operator	Removal Reason	Slides
25 Dec85 12:32	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Brain/cerebrum	
Cellular infiltrate	[Multifocal]
Brain/stem	
Chromatolysis, neurons	
Optic Nerve	(Missing)
Lung	
Congestion	[Mild, Diffuse]
Parathyroid Gland	(Missing)

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Heart, Kidney, Liver, Lymph Node, Mammary Gland, Ovary, Pancreas, Exocrine, Pancreas, Islet, Pituitary Gland, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Thymus, Thyroid Gland, Urinary Bladder, Uterus.

The following protocol tissues were not marked:
None.

28-DAY ORAL TOXICITY OF MDMA IN DOGS

Study: 1 | Page: 1
Started: 17 Sep85 | Individual Animal Histopathology | Date: 25 Dec85
First Dose:17 Sep85 | Time: 12:33
Compound: MDMA | DMS001 V3.0

Completed	Birth	Death	Age/Days	Species	Sex	Group	Accession	Animal ID
24 Nov85				Canine/B	F	15 mg/kg		X0E5

Data Collection	Pathologist	Operator	Removal Reason	Slides
25 Dec85 12:33	Frith/Chang	Frith/Path	Terminal Sac	

Status: Completed.

The following observations were noted:

Brain/cerebrum
  Cellular infiltrate [Focal]
Brain/stem
  Chromatolysis, neurons
Lung
  Congestion [Mild, Diffuse]

The following tissues were marked as NR:
Adrenal Gland, Aorta, Bone, Bone Marrow, Brain/cerebellum, Sciatic Nerve, Spinal Cord, Cecum, Colon, Esophagus, Eye, Optic Nerve, Heart, Kidney, Liver, Lymph Node, Mammary Gland, Ovary, Pancreas, Exocrine, Pancreas, Islet, Parathyroid Gland, Pituitary Gland, Rectum, Small Intestine/duodenum, Small Intestine/jejunum, Small Intestine/ileum, Submaxillary Salivary Gland, Skeletal Muscle, Spleen, Stomach, Thymus, Thyroid Gland, Urinary Bladder, Uterus.

The following protocol tissues were not marked:
None.

APPENDIX 7

REPORT FROM DR. NICHOLS

(4)

PURDUE UNIVERSITY SCHOOL OF PHARMACY AND PHARMACAL SCIENCES

November 11, 1985

Dr. Charles H. Frith
Toxicology Pathology Associates
1102 Briar Creek Road
Little Rock, Arkansas 72211

Dear Dr. Frith:

Enclosed is the report on the characterization and analysis of the MDMA hydrochloride that you received for your toxicology work (Lot # 5810-09). I trust that this information will be adequate for your report.

Please let me know if you require any additional information.

Sincerely,

Dave

David E. Nichols, Ph.D.
Professor of Medicinal Chemistry

DEN/va

Enclosure

DEPARTMENT OF MEDICINAL CHEMISTRY
AND PHARMACOGNOSY
Robert E. Heine Pharmacy Building
West Lafayette, Indiana 47907

SYNTHESIS OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE HYDROCHLORIDE (Lot No. 5810-09)

(Prepared in the Laboratory of David E. Nichols, Ph.D., School of Pharmacy, Purdue University, West Lafayette, IN 47907)

[The following procedure was carried out in octuplicate]
Aluminum foil, 108 g (4.0 moles) was cut into 1 inch squares and placed into a 4 L Erlenmeyer flask. The foil was amalgamated by covering with 2 L of distilled water followed by addition of 4.51 g of $HgCl_2$ (0.0166 moles). The flask was swirled occasionally over 30 minutes, during which time the water became a cloudy grey color and the surface of the foil darkened slightly and lost its luster. The water was decanted from the foil and the foil was rinsed twice with 2 L portions of distilled water. Then the following reagents were added quickly, in sequence, with swirling.

1. 270 g methylamine hydrochloride in 200 mL water (4.0 moles of $MeNH_2$)
2. 500 mL of 2-propanol
3. a solution of 163.5 g (4.09 moles) of NaOH in 385 mL of water
4. 239 g (200 mL, 1.341 moles) of 3,4-methylenedioxyphenyl-2-propanone
5. 1.0 L of 2-propanol

The flask was intermittently swirled and the internal temperature was maintained between 50-60°C by occasional cooling in an ice bath. (The reaction becomes quite exothermic and an ice bath must be ready.) The reaction was essentially complete within 90 min., as analyzed by disappearance of the piperonyl acetone using tlc. The mixture was then decanted through a buchner funnel, celite was added to the thick residue in the flask, followed by filtration and rinses wih 2 x 200 mL of hot 2-propanol. The filtrate was reduced in volume by rotary vacuum evaporation. The residues from all eight reactions were then combined and purified by vacuum distillation (b.p. 88°C at 0.4 mm Hg) to afford 1420 g of the free base as a nearly colorless oil (68.5% yield.) The base was dissolved in isopropanol (ca 4L) and acidified with concentrated HCl. Upon cooling, MDMA hydrochloride crystallized. The solution was diluted with an equal volume of ethyl acetate, the crystals were broken up and filtered. The crude mass of crystals was triturated in a porcelain mortar and pestle with ethyl acetate, these washed crystals were filtered and briefly air dried. The filtrates and washes were combined and concentrated, the residue was dissolved in fresh hot isopropanol, the solution was cooled, and a second crop of material was obtained which was isolated in an identical fashion. The crystals from the first and second crops were combined and were then dried for 48 hours at room temperature under high vacuum. The crystals were then passed through a 30 mesh stainless steel sieve and the final homogeneous crystalline white powder was weighed and transferred into clean amber screw capped bottles, to yield 1437 g (85.1% recovery of base) of white powder. (Labeled as Lot #5810-09)

The filtrates were combined and concentrated, the residue was dissolved in fresh hot isopropanol, the solution was cooled and a third crop of slightly pink crystals was obtained. This material was collected, redissolved in fresh hot isopropanol and recrystallized a second time to yield 87.8 g (5.2% recovery of base) of white crystals. These were ground in a mortar and pestle and dried at room temperature for 48 hours. This material was labeled 5810-09-1B, and was not analyzed. The filtrates and washes were combined, concentrated, and

basified with NaOH. The liberated free base was extracted into methylene chloride, the organic solution was dried (Na_2SO_4), filtered and concentrated. The dark brown oil was then vacuum distilled to yield 76.7g of base. This was then dissolved in 200 ml 2-PrOH, acidified with conc HCl, diluted with 200 ml ethyl acetate, cooled and filtered to yield 44.0g of white crystalline powder (2.6% recovery of base). This material was labelled 5810-09-2A and was not analyzed. The mother liquor was then evaporated in vacuo and the residue spontaneously crystallized to yield 45.1 g of tan powder. This was labeled as "crude" and stored as such. (2.7% recovery of base).

Total recovery of base as hydrochloride salt = 95.6%

ANALYSIS AND CHARACTERIZATION OF MDMA HYDROCHLORIDE (Lot #5810-09 only).

Gas chromatographic analysis

Gas chromatographic analysis of the product was performed on a Varian Aerograph Series 1400 equipped with a flame ionization detector. A 6 ft x 2 mm glass column, packed with 10% Sp-2330 on 100/120 chromosorb w-awx was used.

Peak areas and retention times were determined with a Hewlett-Packard 3390-A integrator. The carrier gas was nitrogen and the column temperature was programmed for a 4°C rise per minute, starting at an initial temperature of 130°C unless specified otherwise. The injector temperature was 240°C and the detector was 270°C. Solutions of the base in chloroform were prepared for injection into the chromatograph. Analysis of the hydrochloride salt was carried out as follows; 100 mg of the hydrochloride salt was dissolved in 1.0 ml of distilled water and basified with 1 mL of 5N NaOH. The liberated base was extracted into 1 mL of reagent grade chloroform. This organic solution was then passed over a small column (Pasteur pipet) of anhydrous sodium sulfate (about 2 g) briefly to dry it.

Attached are five gas chromatographic traces, identified as A-E. The conditions and interpretation of each are described below.

A. Analysis of the vacuum distilled free base. The major component had a retention time of 10.84 minutes and accounted for 99.05% of the total eluted sample. Minor components were located at retention times of 8.57 min. and 14.86 min. and comprised 0.546 and 0.402% of the sample, respectively. Two trace components at 13.08 and 29.99 minutes were below the detection limit of the integrator. Total run time was 40 minutes.

B. Analysis of the base liberated by neutralization of the hydrochloride salt.

Using the same column conditions as described in "A"., the only component eluting had a retention time of 10.46 minutes. All other impurities were below the detection limit of the detector. The apparent purity was 100.00%. Total run time was 20 minutes.

C. Analysis of the base liberated by neutralization of the hydrochloride salt.

This analysis was carried out on the same material as in "B". above, but a smaller sample was injected to insure that the major peak was a single component. The initial column temperature was 135°, and the total run time was 12 minutes.

D. Analysis of the base liberated by neutralization of the hydrochloride salt.

The column temperature and run time were increased to determine that no late-eluting impurities were contained in the sample. The initial column temperature was 140°C and run time was 35 minutes. The MDMA base had a retention time of 7.47 minutes. There was some indication of a very minor contaminant eluting late in the trace. This led to analysis "E".

E. Analysis of the base liberated by neutralization of the hydrochloride salt.

Higher sample loading on the column was performed to emphasize and sharpen any late-eluting peaks. The initial column temperature was 150°C. Under these column conditions, the MDMA base eluted in 6.46 minutes and comprised 99.75% of the integrated sample. The minor component detected at retention time 18.54 min accounted for 0.24% of the sample.

Infra Red Analysis

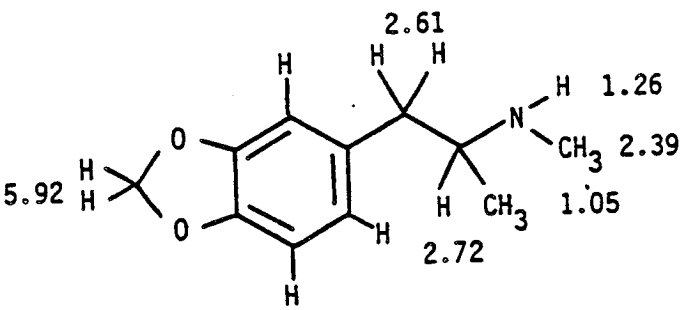
The free base was further characterized by Infra Red analysis as the neat material between NaCl plates. The spectrum, recorded using a Beckman Model IR-33 is attached.

Mass Spectral Analysis

The chemical ionization mass spectrum showed the M+1 molecular ion at m/z 194. The electron impact mass spectrum showed the base peak at 194, also M+1 due to bimolecular collision H^+ transfer.

NMR Analysis

The NMR (80 MHz) of the free base in $CDCl_3$ was consistent with spectra of earlier samples. The chemical shifts, in ppm relative to an internal standard of tetramethylsilane, are shown on the following structure.



ArH 6.76 - 6.61

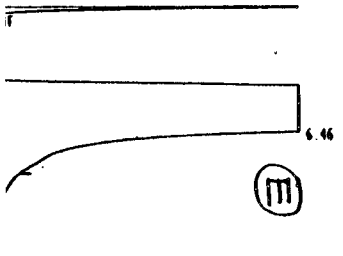
Elemental Composition

The hydrochloride salt had an uncorrected mp of 158-159°C. Elemental composition was determined by the Purdue University Microanalysis laboratory (Analysis number 16772)

Calculated for $C_{11}H_{16}ClNO_2$			Analysis of 5810-09		
%C	%H	%N	%C	%H	%N
57.52	7.02	6.10	57.39	7.19	6.03

CONCLUSIONS

Under these conditions, and with the equipment specified, assuming comparable detector responses to the MDMA base and any impurities, the vacuum distilled free base, as isolated from the synthesis, had a purity of 99.05%. The recrystallized hydrochloride salt had a purity of 99.75%. All other analyses gave results consistent with the expected structure.



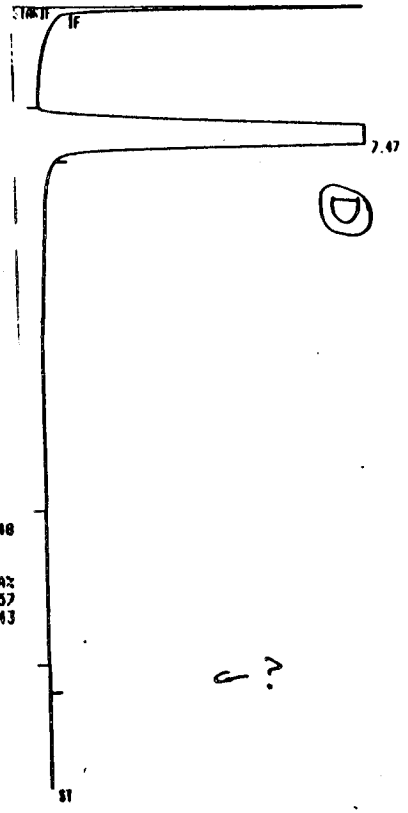
(m)

54

0 80 SEP/30/85 13:01:48

%

T	AREA	TYPE	AR/HT	AREA%
46	4.1938E+07	PB	2.063	99.757
54	102200	BP	0.921	0.243



(o)

RUN 0 87 SEP/30/85 16:05:23

AREA%

RT	AREA	TYPE	AR/HT	AREA%
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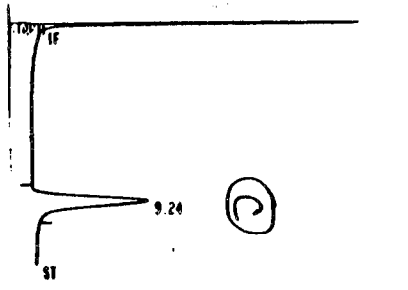

(c)

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AREA%

RT	AREA	TYPE	AR/HT	AREA%
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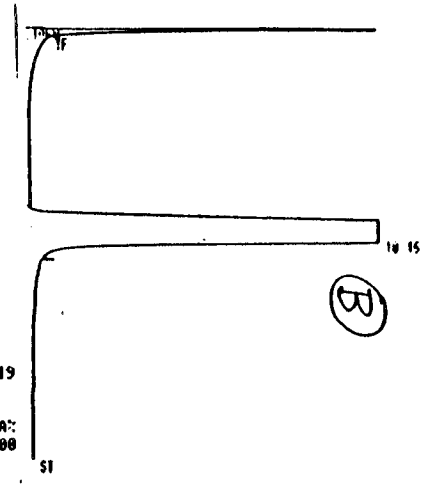

(b)

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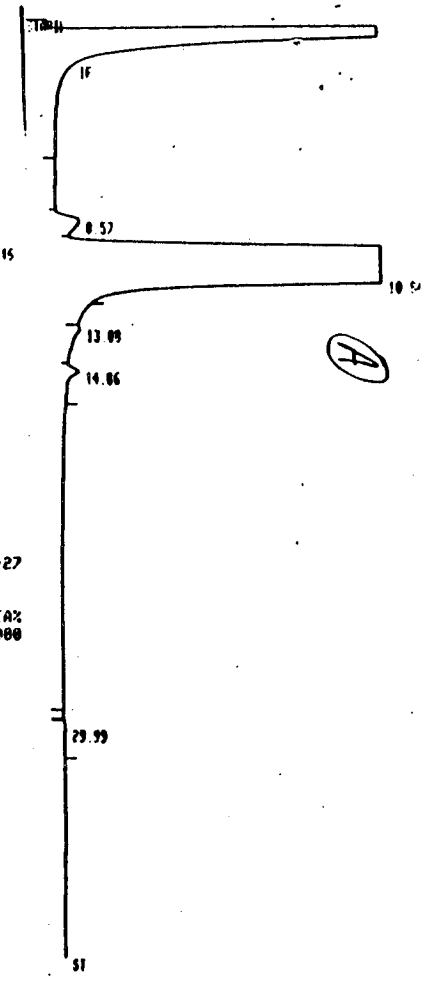
AREA%

RT	AREA	TYPE	AR/HT	AREA%
10.46	1.9082E+07	PB	0.921	100.000



(a)

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AREA%

RT	AREA	TYPE	AR/HT	AREA
8.57	163260	PP	0.421	0 54
10.84	2.9606E+07	PB	1.462	99 85
14.86	120090	PB	0.426	0 40

SPECTRUM NO. _____

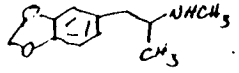
DATE 9/6/85

SAMPLE 5810-09

MDMA Base
Between NaCl Plates

SOURCE Synthesis

STRUCTURE



PATH _____ mm

SOLVENT _____

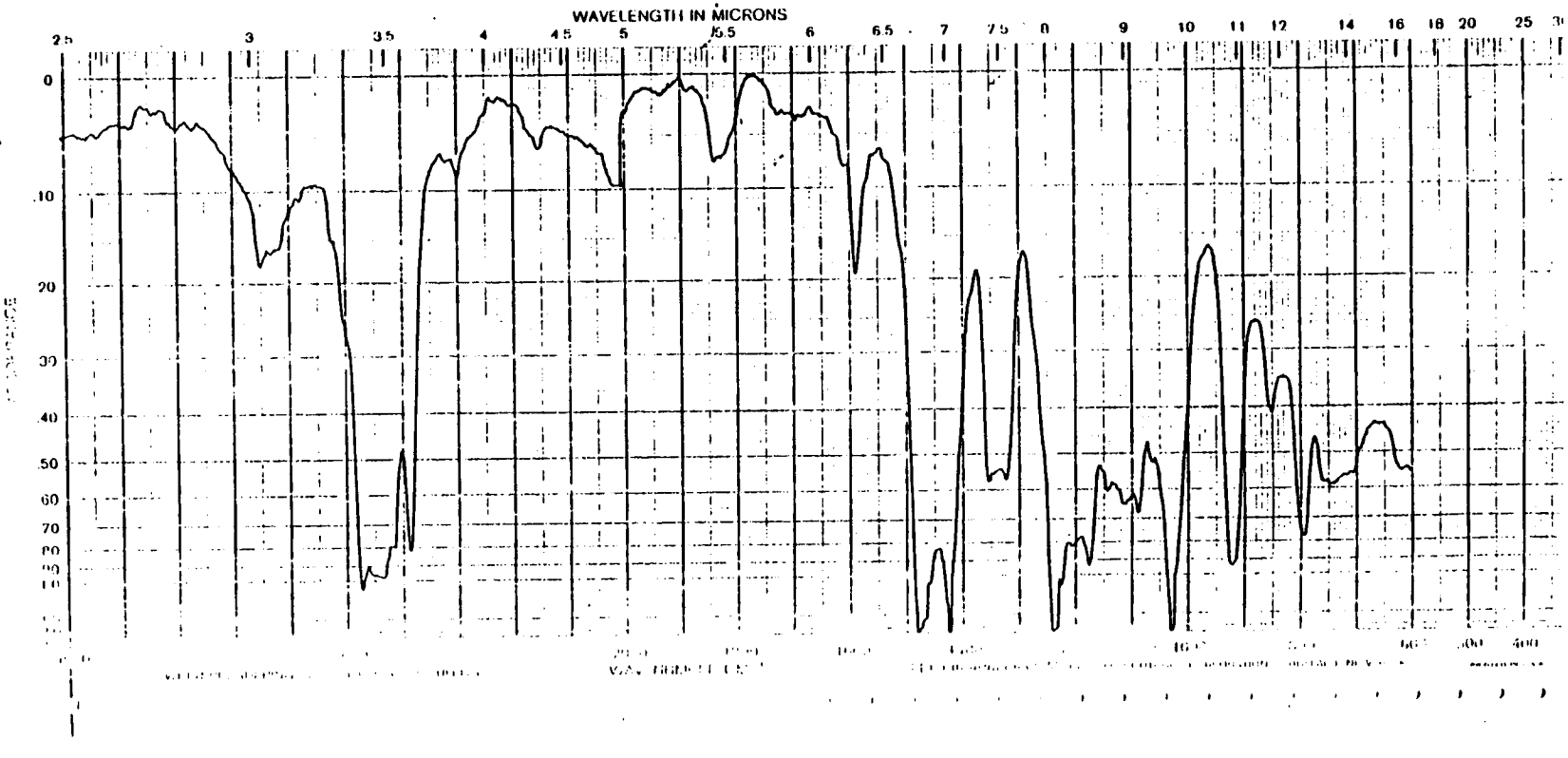
CONCENTRATION Neat

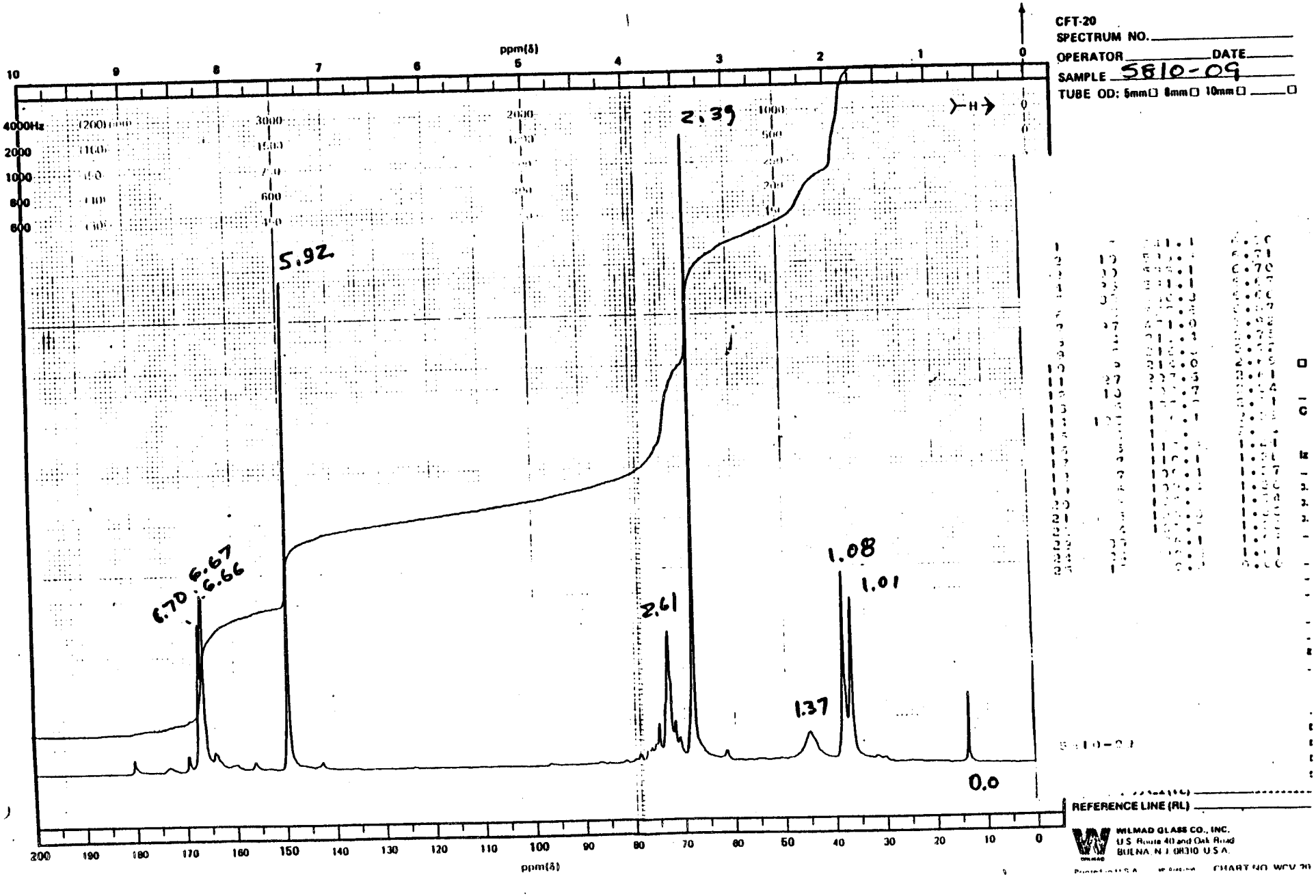
PHA _____

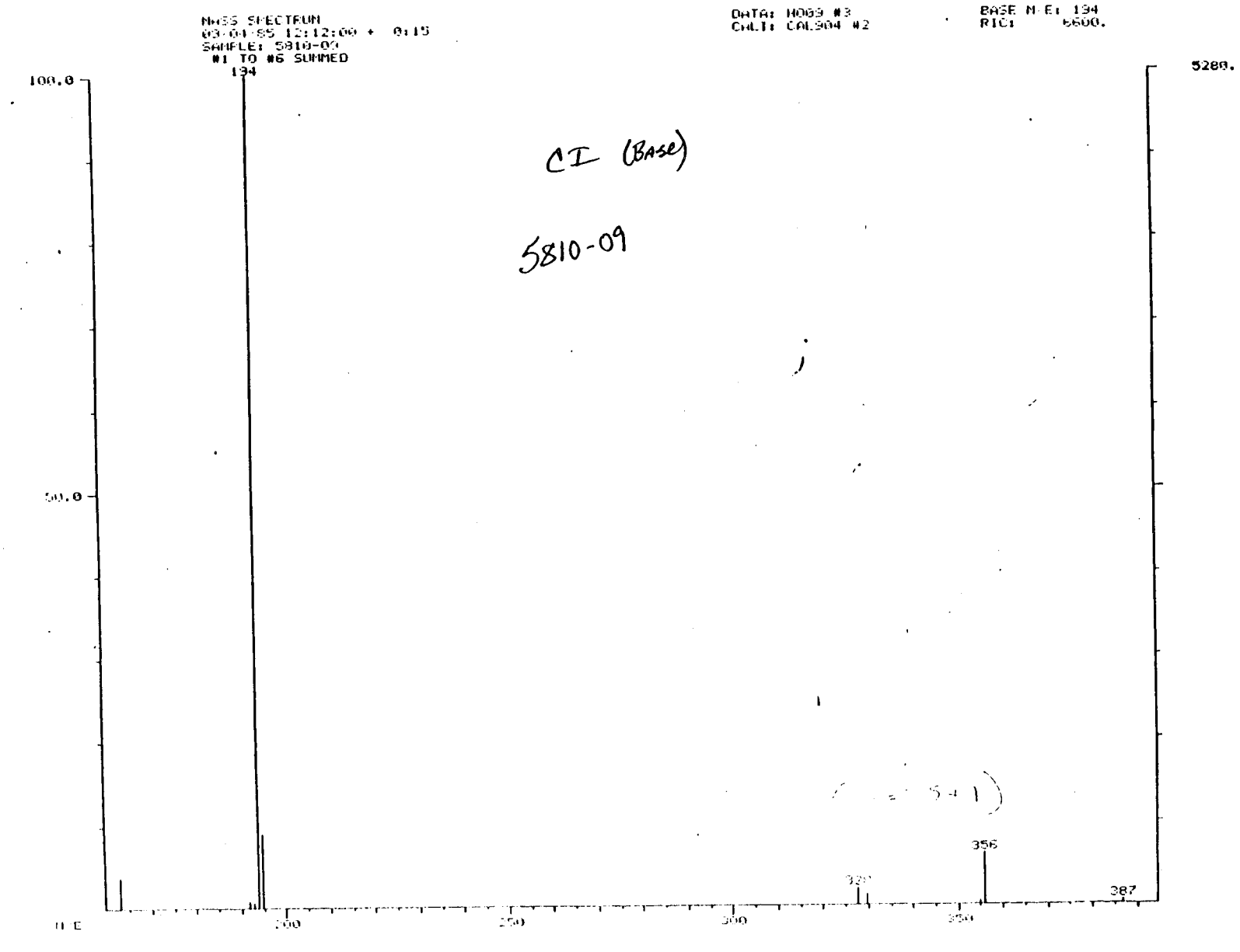
COMMENTS _____

Beckman IR-33

ANALYST ATH




CFT-20
SPECTRUM NO.
OPERATOR
DATE
SAMPLE 5810-09
TUBE OD: 5mm 8mm 10mm
ppm(δ)
4000Hz
2000
1000
800
600
5.92
2.39
6.70
6.67
6.66
2.61
1.08
1.01
1.37
0.0
REFERENCE LINE (RL)
WILMAD GLASS CO., INC.
U.S. Route 40 and Oak Road
BUENA, N.J. 08310 U.S.A.



CI (Base)

5810-09

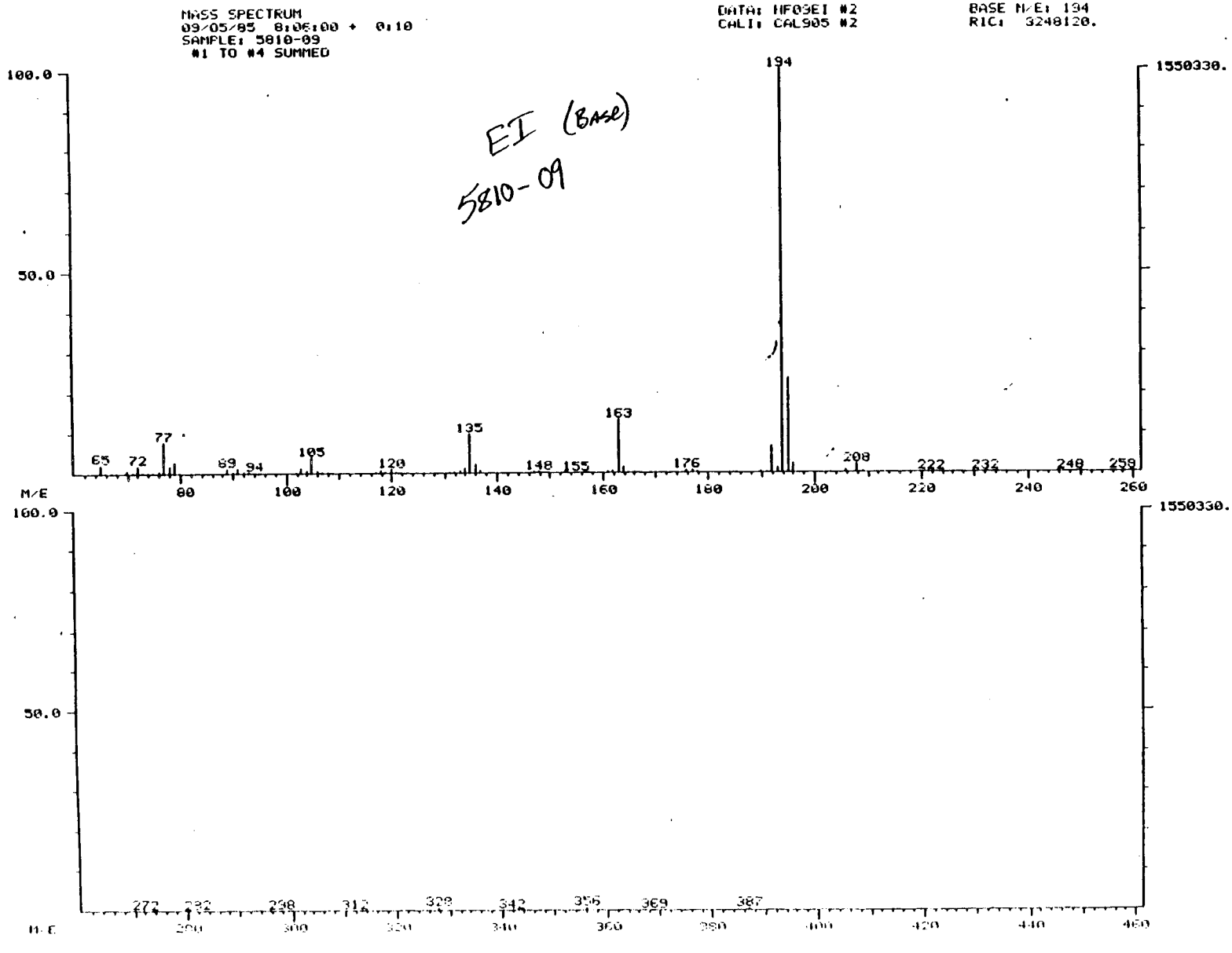
MASS LIST
09/04/85 12:12:00 + 0:15
SAMPLE: 5810-09
#1 TO #6 SUMMED

DATA: H009 # 3
CALI: CAL904 # 2

BASE M/E: 194
RIC: 6600.

163	0.00	0.00	0.	MINIMA	MIN INTEN: 0.
387			# 0	MAXIMA	
MASS	% RA	% RIC	INTEN.		
163.00	3.73	2.98	197.		
192.00	0.89	0.71	47.		
193.00	0.70	0.56	37.		
194.00	100.00	80.00	5280.		
195.00	9.00	7.20	475.		
328.00	2.05	1.64	108.		
330.00	1.17	0.94	62.		
355.00	0.42	0.33	22.		
356.00	6.36	5.09	336.		
387.00	0.53	0.42	28.		

/


MASS SPECTRUM
09/05/85 8:06:00 + 0:10
SAMPLE: 5810-09
#1 TO #4 SUMMED
DATA: HF0SEI #2
CALI: CAL905 #2
BASE M/E: 194
RIC: 3248120.
EI (BASE)
5810-09
100.0
50.0
1550330.
194
163
135
105
77
65
72
89
94
120
148
155
176
208
222
232
240
259
M/E
80
100
120
140
160
180
200
220
240
260
100.0
50.0
1550330.
272
282
298
312
328
342
356
369
387
M/E
300
320
340
360
380
400
420
440
460

MASS LIST
09/05/85 8:06:00 + 0:10
SAMPLE: 5810-09
#1 TO #4 SUMMED

DATA: HF09EI # 2
CALI: CAL905 # 2

BASE M/E 194
RIC: 3248120

65 0.00 0.00 0. MINIMA MIN INTEN: 786.
389 @ 0 MAXIMA

MASS	% RA	% RIC	INTEN.	MASS	% RA	% RIC	INTEN.
65.00?	2.10	1.00	32480.	138.00	0.05	0.02	798.
66.00?	0.41	0.20	6352.	145.00	0.05	0.03	829.
67.00?	0.36	0.17	5520.	146.00	0.15	0.07	2260.
68.00?	0.38	0.18	5832.	147.00	0.26	0.12	4000.
70.00	0.70	0.33	10848.	148.00	0.38	0.18	5848.
71.00	0.35	0.17	5464.	149.00	0.21	0.10	3328.
72.00	1.83	0.87	28352.	160.00	0.13	0.06	2088.
73.00	0.20	0.09	3068.	161.00	0.46	0.22	7144.
74.00	0.23	0.11	3540.	162.00	0.68	0.33	10576.
75.00	0.33	0.16	5184.	163.00	13.04	6.23	202240.
76.00	0.80	0.38	12400.	164.00	1.74	0.83	26976.
77.00	7.72	3.68	119680.	165.00	0.41	0.20	6368.
78.00	1.77	0.85	27456.	166.00	0.46	0.22	7088.
79.00	2.79	1.33	43328.	167.00	0.05	0.02	789.
80.00	0.48	0.23	7448.	175.00	0.14	0.07	2200.
81.00	0.15	0.07	2344.	176.00	0.65	0.31	10016.
82.00	0.12	0.06	1918.	177.00	0.51	0.24	7864.
86.00	0.08	0.04	1182.	178.00	0.40	0.19	6144.
87.00	0.10	0.05	1546.	179.00	0.06	0.03	969.
89.00	1.31	0.63	20384.	180.00	0.06	0.03	939.
90.00	0.26	0.12	4020.	181.00	0.07	0.03	1014.
91.00	1.23	0.59	19136.	190.00	0.27	0.13	4256.
92.00	0.33	0.16	5048.	191.00	0.42	0.20	6560.
93.00	0.22	0.11	3480.	192.00	6.29	3.00	97536.
94.00	0.33	0.16	5120.	193.00	1.39	0.66	21536.
95.00	0.08	0.04	1220.	194.00	100.00	47.73	1550330.
96.00	0.06	0.03	901.	195.00	23.48	11.21	364032.
102.00	0.27	0.13	4240.	196.00	2.09	1.00	32352.
103.00	1.24	0.59	19168.	197.00	0.15	0.07	2324.
104.00	0.71	0.34	11024.	204.00	0.07	0.04	1150.
105.00	3.79	1.81	58816.	206.00	0.72	0.34	11088.
106.00	0.73	0.35	11264.	207.00	0.10	0.05	1544.
107.00	0.39	0.18	6000.	208.00	2.07	0.99	32032.
108.00	0.20	0.09	3072.	209.00	0.30	0.14	4664.
109.00	0.05	0.03	820.	210.00	0.10	0.05	1590.
115.00	0.06	0.03	1000.	222.00	0.13	0.06	1954.
117.00	0.27	0.13	4152.	224.00	0.07	0.04	1158.
118.00	0.58	0.28	9024.	232.00	0.10	0.05	1492.
119.00	0.19	0.09	2980.	234.00	0.08	0.04	1186.
120.00	0.83	0.39	12800.	258.00	0.05	0.02	787.
121.00	0.31	0.15	4848.	272.00	0.06	0.03	919.
122.00	0.07	0.03	1023.	325.00	0.06	0.03	994.
130.00	0.07	0.03	1110.	326.00	0.06	0.03	948.
131.00	0.43	0.21	6720.	328.00	0.80	0.38	12416.
132.00	0.33	0.16	5112.	329.00	0.16	0.08	2504.
133.00	0.61	0.29	9440.	330.00	0.06	0.03	967.
134.00	1.21	0.58	18720.	354.00	0.05	0.03	816.
135.00	9.76	4.66	151296.	356.00	0.57	0.27	8800.
136.00	2.32	1.11	35968.	357.00	0.12	0.06	1848.
137.00	0.49	0.23	7528.	387.00	0.19	0.09	2892.

CHARGE TO: (stamp with charge card in space below)

[illegible] / [illegible]
[illegible] E. Nichols
[illegible]
CH-333-1145

16772

ANAL. NO. _____

REQUEST FOR MICROANALYSIS

Notebook Reference No. 37[illegible]

Identification 5810-[illegible]

Circle Analyses desired.

Single? X Duplicate? _____

	(C)	(H)	(N)	F	Cl	Br	I
Approximate %	[illegible]	[illegible]	[illegible]				
Results %	57.39	7.19	6.03				

	S	Li	Na	K	Cs	Fe	P
Approximate %							
Results %							

	B	Si	Hg	Mol. Wt.	Solvent	Others
Approximate %				[illegible]	2 [illegible]	
Results %						

Remarks _____

Date received _____ Date reported 9/11/85

Analyst H D Lee Notebook Reference No. _____

Analyst M R Daley Notebook Reference No. VI-41

Analyst _____ Notebook Reference No. _____

DATA TO AID OBTAINING THE MOST SATISFACTORY RESULTS:

Element present C, H, N, Cl

Type compound Amine HCl salt

M.P. 159 [illegible] °C B.P. _____ Hygroscopic _____

NOTE: MICROANALYST – STAMP IBM CARD WITH SAME CHARGE CARD AND ANAL. NO. STAMPED AS ABOVE. PAPER CLIP IBM CARD TO BLUE SHEET AND TURN IN TO CHEM STORES OFFICE.

Recorded on Charges for Microanalysis Sheet

Date _____

IBM Card Completed

Date _____

Original (Canary) for Analyst
1st Carbon (Lt. Blue) for Storeroom
2nd Carbon (Lt. Green) for Research Director
3rd Carbon (Pink) for Student

APPENDIX 8

STASTICAL REPORT

28-Day Oral Toxicity of MDMA in Dogs

Protocol No. EMD-SC-001

Statistical Analysis

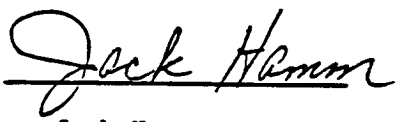
by

Hamm & Walls Associates

Statistical Consultants

Robert C. Walls, Ph.D.

and



Jack Hamm, Ph.D.

for

Earth Metabolic Design Laboratories

and

Charles H. Frith, D.V.M., Ph.D.

Study Director

January, 1986

Hamm & Walls Associates, 301 N. Shackleford Rd., Little Rock, AR 72211

(501) 227-5919

Index

Tables

Figures Page

Appendix 1

Body Weight, Food Consumption; Organ Weights

Item

Appendix 1 (cont'd) Page

Appendix 2

Blood and Urine Laboratory Measures

Item

I. Introduction

This study was designed to evaluate the toxicological potential of a methylenedioxymethamphetamine (MDMA) test article when administered orally to dogs over a four-week period. Twenty-four dogs were randomized to four groups: "control", "low", "medium", and "high" doses. Six dogs, 3 males and 3 females, were assigned to each group. One female died of an overdose at the beginning of the study and was not replaced. As a result of this event, the high dose was reduced and the study was completed with twenty-three animals.

Fifty-one variables including body weight changes, food consumption data, organ weights, blood chemistries and urinalysis measurements were analyzed.

II. Summary of Results

There were no convincing evidences of dose related effects among the several laboratory (blood and urine) tests. A seeming dose effect with the brain to body weight ratio may simply reflect a deleterious effect on growth (weight gain) that selectively spares the brain over this short period of time. A pronounced effect is seen in that dogs on medium and high doses gain significantly less weight than do those on the control and low doses. Food consumption decreases the first week for the high and medium dose groups but shows a significant reversal toward more normal consumption in the following weeks.

III. Study Design and Analysis

The basic study design is a completely randomized design in a 2x4 factorial arrangement (2 sexes by 4 treatment groups) with repeated measures in time. Food consumption was recorded 4 times (weekly totals) as was body weight. Laboratory determinations (blood and urinalysis) were recorded once at baseline and again at the end of the study period (CPK was the one

exception, being measured only at the initial time). Organ weights were determined at time of sacrifice.

Derived variables consisted of the ratio of food consumption to body weight, the ratios of organ weights to body weights and globulin as the difference between total protein and albumin.

The general linear models procedure (GLM Proc) of the Statistical Analysis System (SAS) was utilized with the Duncan's multiple comparisons option to test differences among the dose group means on each of the several variables. With the one missing dog in the female high dose group, the sums of squares in the analysis of variance were not additive. We utilized the Type III sums of squares output and the .05 significance level in assessing statistical significance.

Two basic analyses were performed on the variables that had repeated measures over time: In one analysis the initial measurement on each animal was considered to be the "baseline" value and was subtracted from each subsequent measurement to provide a "change-from-baseline" data set. In the other analysis the original data set consisting of all observation periods was analyzed without transformation.

1. Food Consumption and Body Weight Analyses. The raw data, means, standard errors and other analyses on these data are listed in Appendix 1. The SASLOG, defining all operations, is listed first. The raw data are listed next. The cryptic variable DN stands for "dose number" and was used only as a sorting device.

Other variable names, such as BW for "body weight" should be decipherable. If the letter M is attached, as in MBW, this stands for "mean body weight". If SE is appended, as in BWSE, this denotes the standard error of a body

weight mean. Means and standard errors have been calculated for various combinations of dose, sex and time.

The overall analysis of variance on the 92 data values (23 animals by 4 weeks) is a repeated measures analysis having two basic error terms. The "remainder" error term is automatically applied to all terms in the table but is not appropriate in the case of "dose", "sex" and "dose by sex". The appropriate tests for these effects are listed separately at the end of each analysis of variance. The analyses of variance are followed by Duncan's tests on the dose group means (averaged over all weeks). These results are summarized in Table I.

Separate analyses of variance are then run on the data at each week. Each of these analyses is a straightforward 2x4 factorial with dose, sex and dose by sex effects to be tested. Means are compared by Duncan's test. These results are summarized in Table II.

The analyses described above are then repeated on the "change-from-baseline" data (weeks 2, 3 and 4). In these analyses the variable names each begin with the letter D (such as DBW) to indicate "difference from baseline". Results are summarized in Tables III and IV.

2. Organ Weight Analyses. These data and analyses are listed in the latter portions of Appendix 1. The analyses of variance are of the form of a 2x4 factorial. The ratios of organ weights to body weight are also analyzed. Since testis and ovaries pertain to only one sex the analyses in those cases reduce to one-way (dose groups) analyses of variance. These results are summarized in Table V.

3. Laboratory Parameter Analyses. These data, means, analyses of variance and Duncan's tests are all listed in Appendix 2. The raw data are listed first, followed by means and their standard errors. Overall repeated measures analyses of variance follow. Duncan's test is applied only with those variables for which some significance was indicated in the analysis of variance.

Direct bilirubin was constant throughout the data set; therefore, an analysis is not presented for this variable.

The analyses consisting of simple 2x4 factorials are then done at each of the two measurement times. Again, only selected variables are tested by Duncan's procedure. These variables, PH, TRIGLY, POTM, MCV, GLBN and LYMPHS are clustered on pages 158-187 of Appendix 2.

The "change-from-baseline" analyses follow, beginning on page 188 of Appendix 2. Since there is only one period past baseline for these data, the analysis is a simple 2x4 factorial for each variable.

IV. Discussion of Results

Reference will be made to Tables I through V and Figures 1 through 10.

1. Body Weight over Time. The raw data analysis summarized in Table 1 indicates a dose effect that is time related (significant Time x Dose effect) but when averaged over time the dose groups are not significantly different nor are they different at each time (Table II). However, subtracting the baseline weight adjusts for initial weight differences and clears the picture somewhat. The overall dose means are significantly different (Table III) as are the dose means for DBW at each week 2 through 4 (Table IV). There are significant sex differences but no dose by sex interactions in DBW. Reference to Figures 1 and 2 and Tables III and IV show that the dose groups are ordered

with the control group showing the greatest weight gain and the high dose group the least (a significant difference at each time and over all times).

2. Food Consumption over Time. According to Table II (see also Appendix 1, page 9), food consumption was down the first week for the high and medium dose groups; by week 4 the differences were not significant. Changes from week 1 are shown in Table IV and Figures 3 and 4. These results indicate a significant increase over week 1 for the medium and high dose groups relative to the control and low dose groups.

3. Ratio - Food to Body Weight. The pattern of results on this measure mimics the food consumption pattern almost identically and the same effects are significant.

4. Organ Weights. The results summarized in Table V call attention to the adrenal gland and the brain. For the adrenal gland, Figures 7 and 8 show that the high value for males in the low dose group is the cause of the significant sex and dose by sex interaction effects. However, this is not easily explained as a dose effect and we would rather attribute it to other, perhaps random, causes.

The sex by dose interaction for the brain weight is quite evident (but puzzling) in Figure 9. However, when we consider the brain to body weight ratio in Figure 10 the picture clears somewhat. From this it appears that there may be a dose related effect but we wonder if it may not be due to a decreased body weight in the higher dose groups in which the brain weight is affected less than the remainder of the body.

5. Laboratory Measures. The only variables showing any likelihood of dose related effects were Ph, MCV, CALCM, POTM, GLBN, LYMPHS and TRIGLY. There were 6 tests involving dose that were significant at the .05 level and 4

others that were "close". A total of 217 tests involving dose effects (including interactions) were run in this section of the analysis; eleven would be expected to be "significant" by chance alone if all were independent. Therefore, these few could be spurious results. A closer investigation of the seven variables listed above showed no consistent changes from the initial to the final period that could be related to dose effects. We conclude that there are no real dose effects in these data.

Table I

Summary of Results of Repeated Measures Analyses of Variance:

Body Weight and Food Consumption Data

Plus Duncan's Test on the Dose Means Averaged Over Weeks

(Table entries are the p-values for the F-test of the corresponding source of variation in the ANOVA table. NS stands for "not significant".)

"Sources" in the ANOVA Table	Variable BW	FC	Ratio
Dose	NS	.0015	.0132
Sex	.0008	NS	NS
Dose * Sex	NS	NS	NS
Time	.0001	.0001	.0001
Time * Dose	.0001	.0001	.0001
Time * Sex	.0036	.0532	.0165
Time * Dose * Sex	NS	NS	NS
Duncan's results* on the ranked dose means:	HMLC	HMCL	HMCL

*Means joined by the same underline are not significantly (.05) different by the Duncan's Multiple Range Test.

Table II

Summary of Results of Analyses at each Week:

Body Weight and Food Consumption Data

(Letters indicate the group means listed in order of magnitude. Means that are not significantly different at the .05 level by Duncan's Multiple Range Test are underlined. The p-value from the analysis of variance test on the "dose" effect is given. NS stands for "not significant".)

	Observation Period			
Variable Code	Week 1	Week 2	Week 3	Week 4
BW	NS	NS	NS	NS
FC	.0040 HMCL	.0001 HCML	.0645 MHCL	NS
RATIO	.0024 HMCL	.0164 HCML	NS	NS

Table III

Summary of Results of Repeated Measures Analyses of Variance: Body Weight and Food Consumption "Change-from-Baseline" Data Plus Duncan's Test on the Dose Means Averaged Over Weeks

(Table entries are the p-values for the F-tests of the corresponding "sources" in the analysis of variance table. NS stands for "not significant".)

"Sources" in the ANOVA Table	Variable DBW	DFC	DRATIO
Dose	.0012	.0155	.0075
Sex	.0141	.0607	.0331
Dose * Sex	NS	NS	NS
Time	.0001	NS	.0069
Time * Dose	.0002	.0005	.0003
Time * Sex	NS	NS	NS
Time * Dose * Sex	NS	NS	NS
Duncan's results* on the ranked dose means:	HMLC	LCHM	LCHM

*Means joined by the same underline are not significantly (.05) different by Duncan's Multiple Range Test.

Table IV

Summary of Results of Change-from-baseline Analyses at each Week:

Body Weight and Food Consumption Data

(Letters indicate the group means listed in order of magnitude. Means that are not significantly different at the .05 level by Duncan's Multiple Range Test are underlined. The p-value from the analysis of variance test on the "dose" effect is listed. NS stands for "not significant".)

Variable Code	Week 1 Baseline	Week 2	Week 3	Week 4
DBW	------	.0018	.0013	.0015
		HMCL	HMLC	HMLC
DFC	------	.0376	.0573	.0026
		LCHM	LCMH	LCMH
DRATIO	------	.0252	.0248	.0012
		LCHM	LCMH	LCMH

Table V

Summary of Results of Analyses of Variance
and Duncan's Tests on the Organ Weight Data
(Table entries are p-value for the F-tests.
NS stands for "not significant".)

	Source of Variation			Duncan's Test* on
Variable Code	Dose	Sex	Dose * Sex	Dose Means
BW (at sacrifice)	NS	.0031	NS	-----
LIVER	NS	.0008	NS	-----
KIDNEY	NS	.0521	NS	-----
ADRENAL	NS	.0047	.0478	HCML
BRAIN	NS	NS	.0358	-----
LIVBWR	NS	NS	NS	-----
KIDBWR	NS	NS	NS	-----
ADRBWR	NS	NS	.0283	CHML
BRABWR	.0515	.0003	NS	CLMH
TESTIS	NS	-----	-----	-----
TESBWR	NS	-----	-----	-----
OVARY	NS	-----	-----	-----
OVABWR	NS	-----	-----	-----

*Means joined by the same underline are not significantly (.05) different.

FIGURE 1: MEAN BODY WEIGHT CHANGE FROM BASELINE
FEMALE DOGS

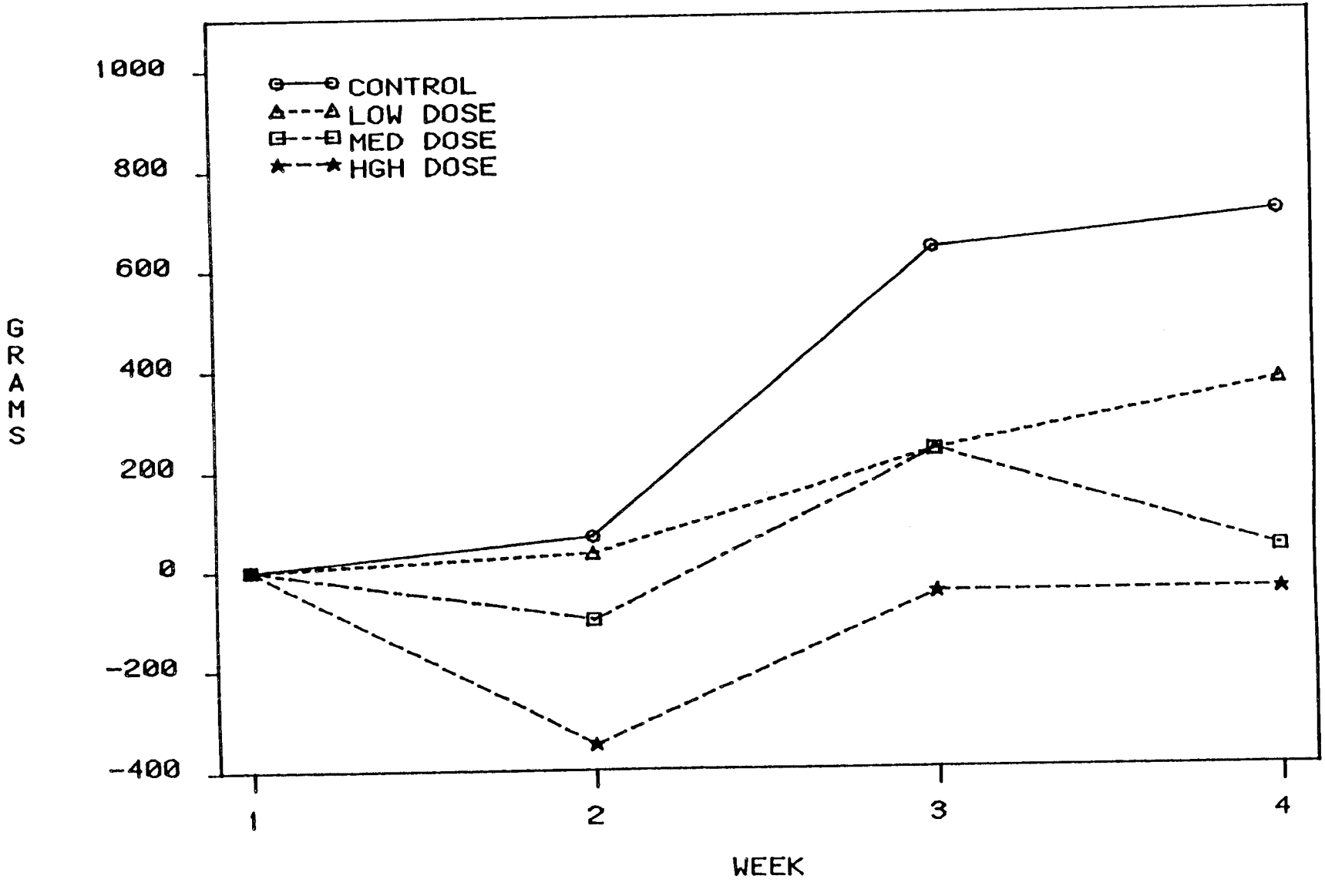


FIGURE 2: MEAN BODY WEIGHT CHANGE FROM BASELINE
MALE DOGS

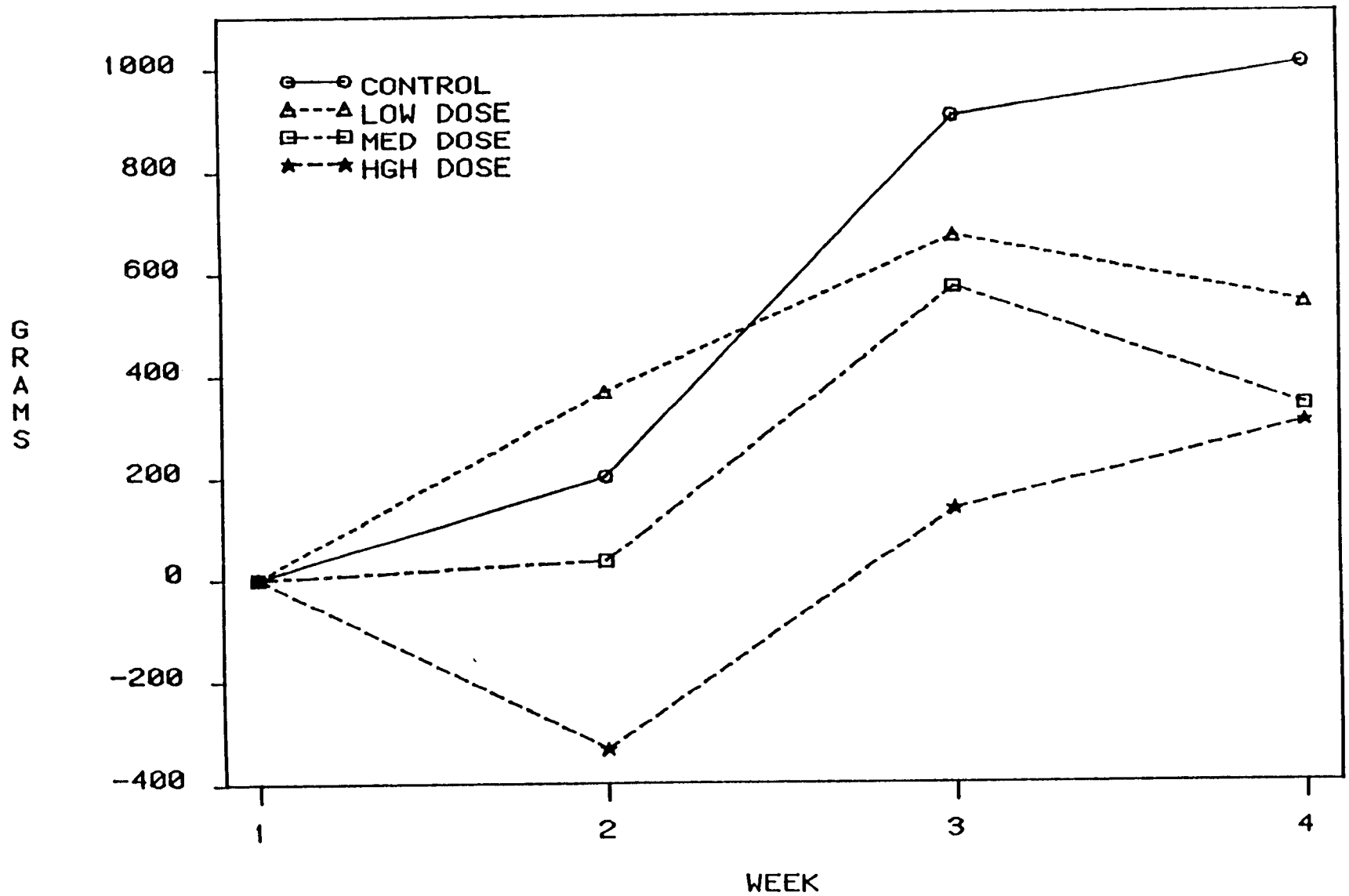


FIGURE 3: MEAN FOOD CONSUMPTION CHANGE FROM WEEK 1
FEMALE DOGS

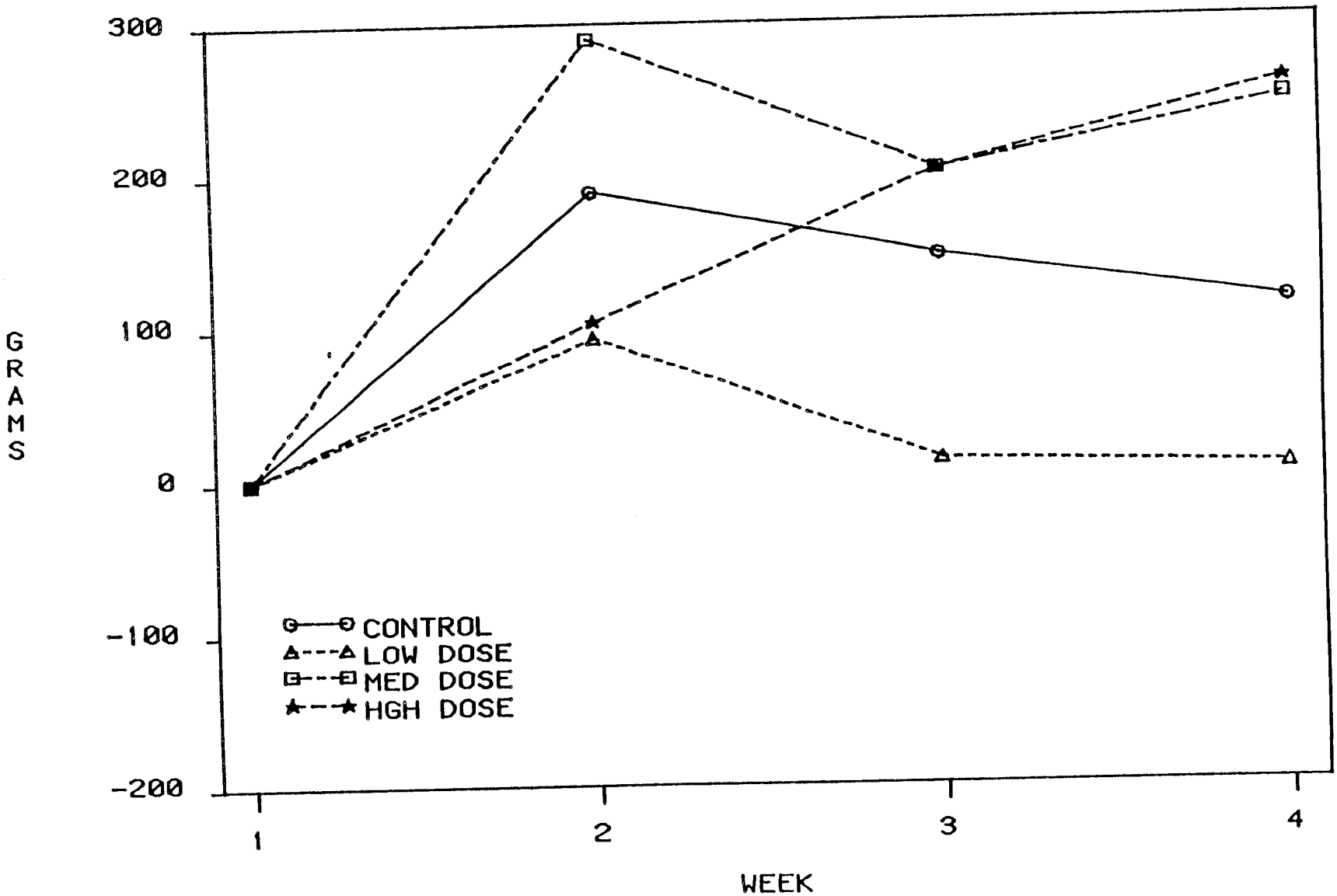


FIGURE 4: MEAN FOOD CONSUMPTION CHANGE FROM WEEK 1
MALE DOGS

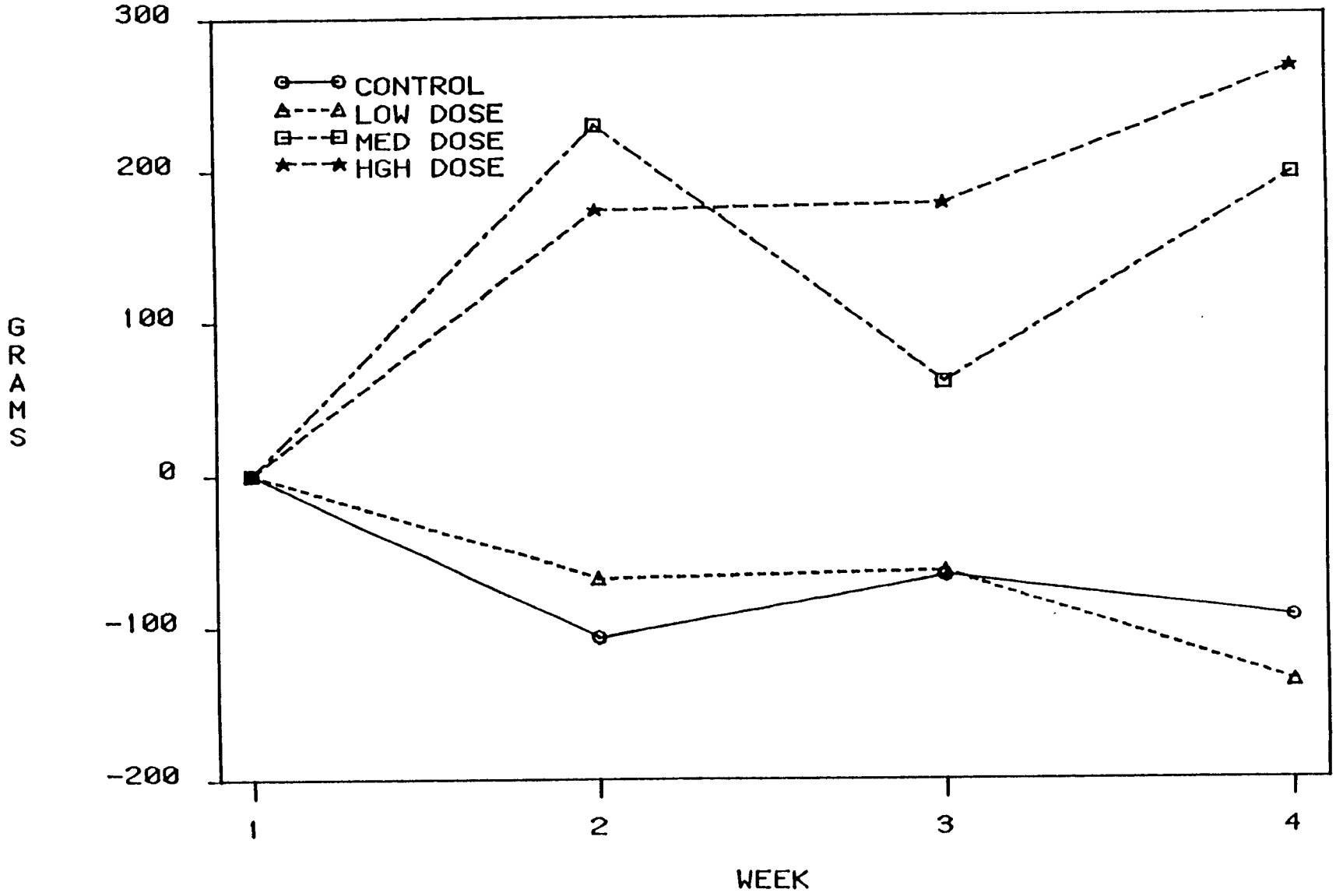


FIGURE 5: MEAN CHANGE - FOOD TO BODY WT RATIO
FEMALE DOGS

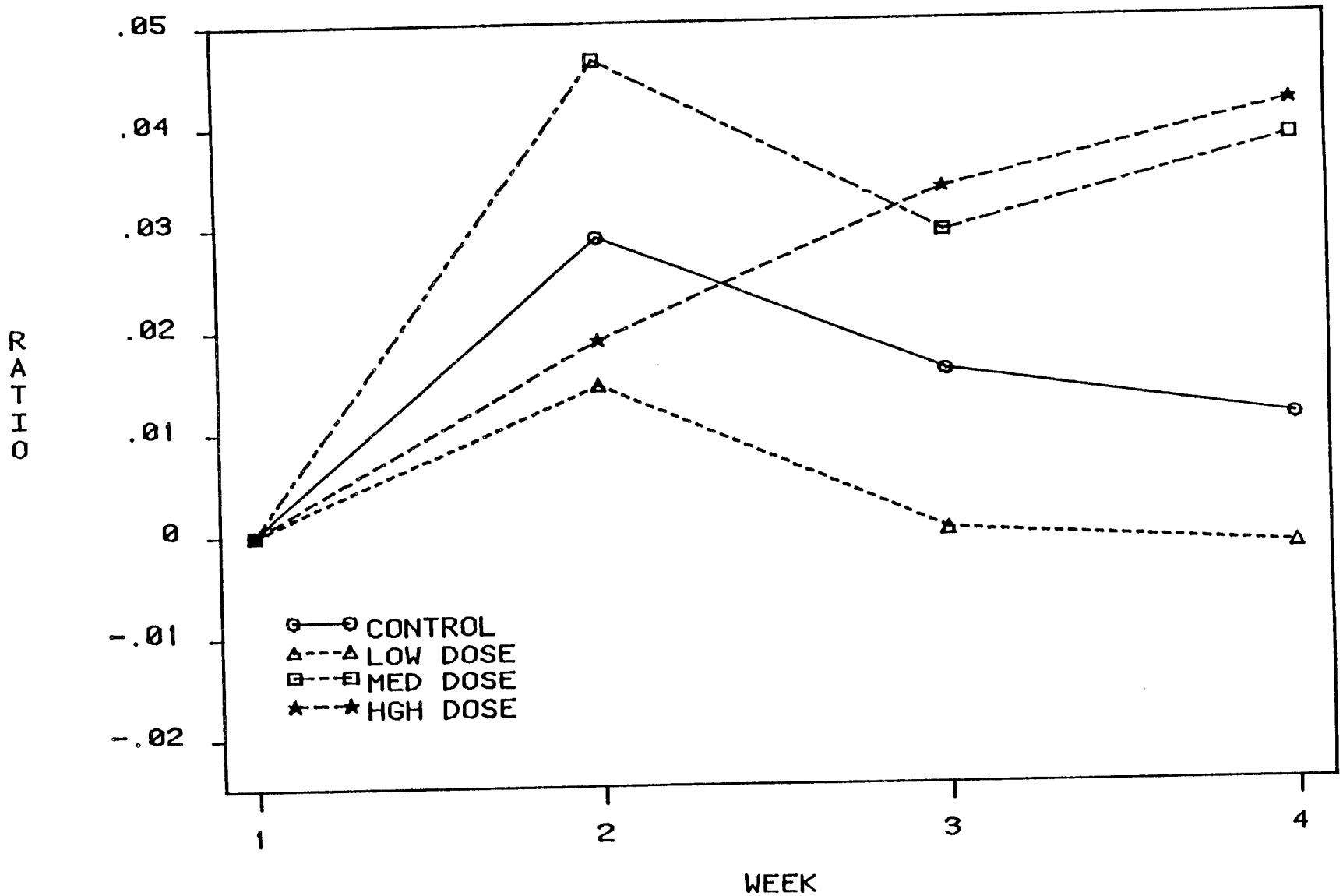


FIGURE 6: MEAN CHANGE - FOOD TO BODY WT RATIO
MALE DOGS

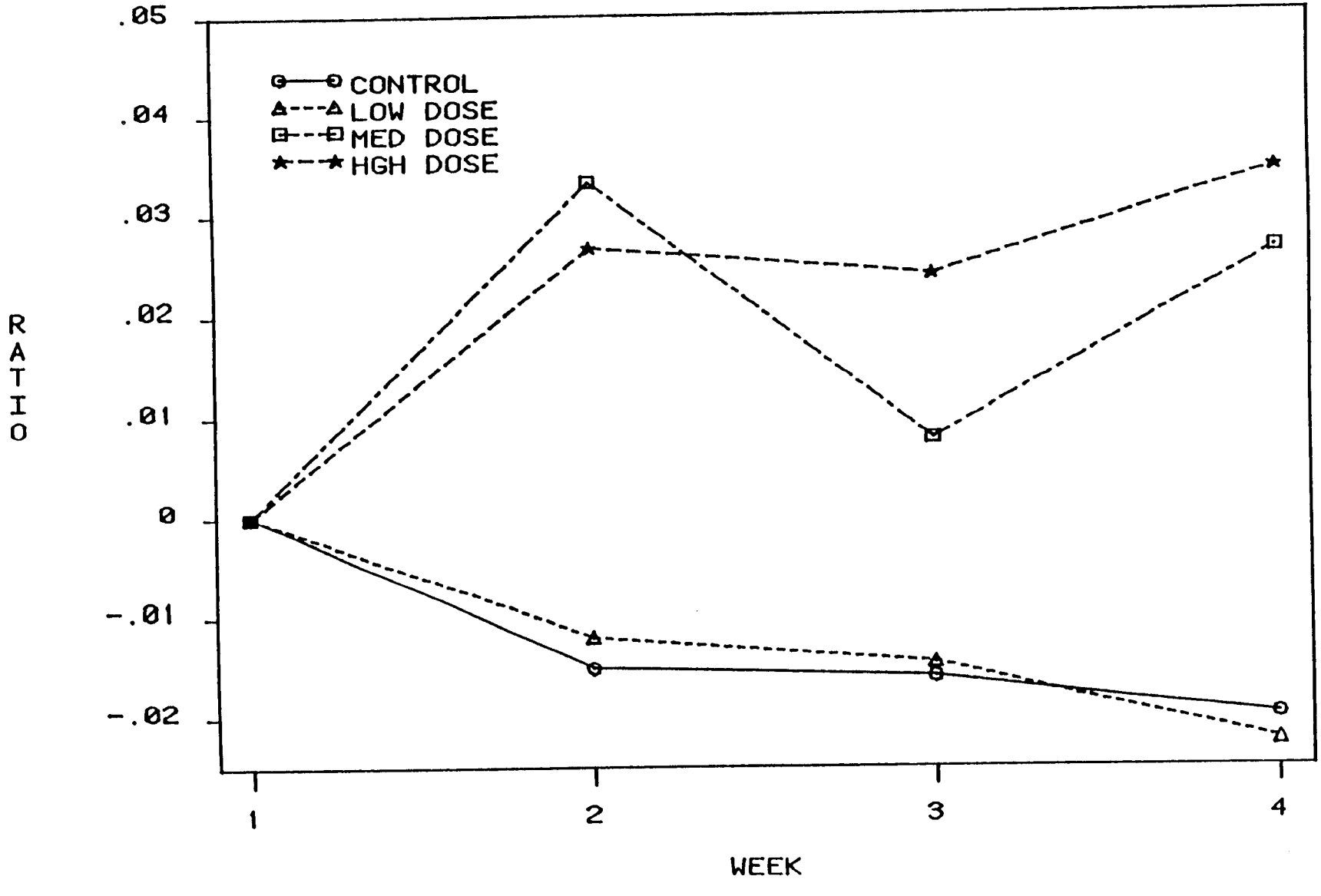


FIGURE 7: ADRENAL GLAND MEAN WEIGHT VERSUS DOSE
BY SEX

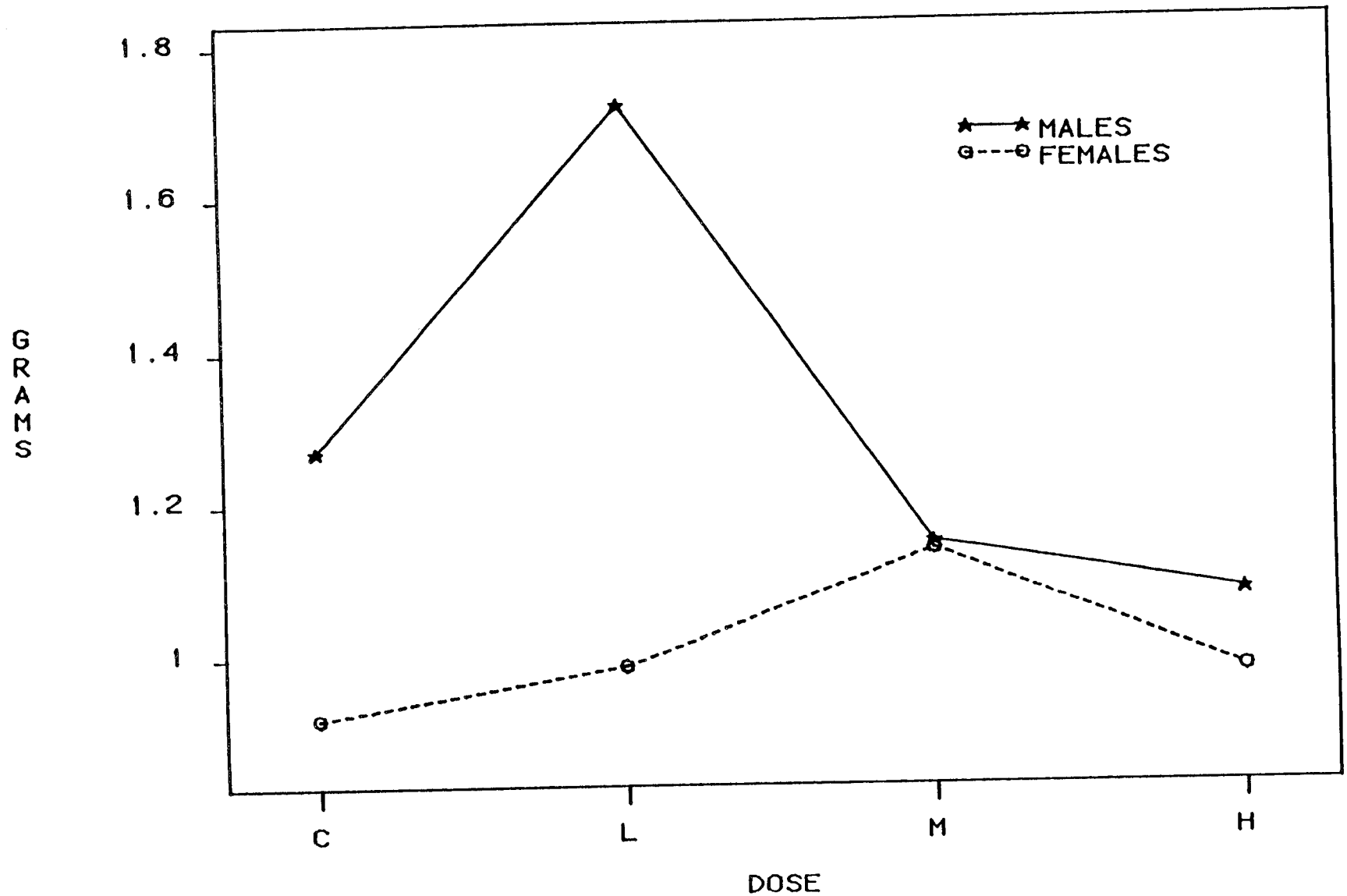


FIGURE 8: ADRENAL TO BODY WT RATIO - MEAN VERSUS DOSE
BY SEX

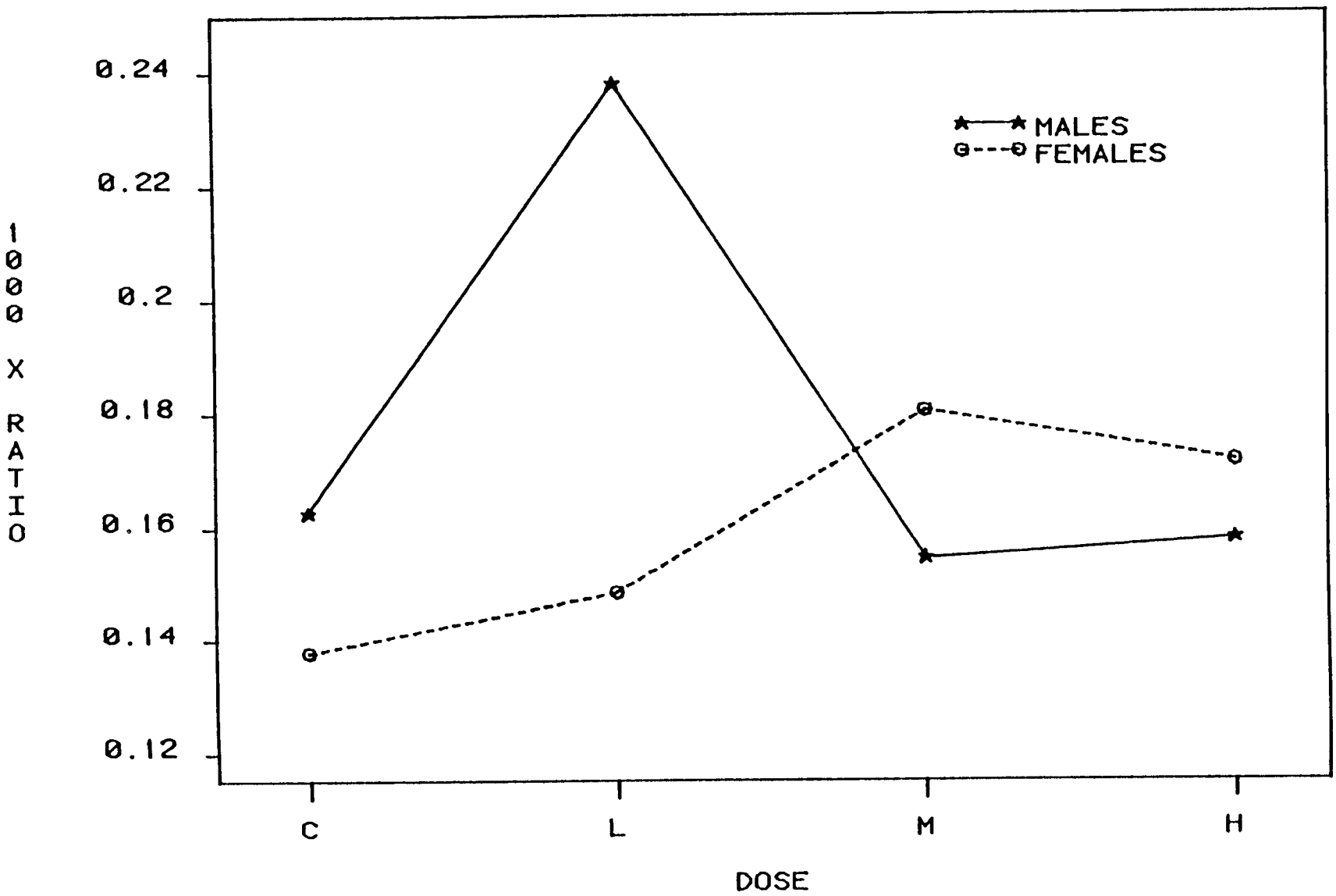


FIGURE 9: MEAN BRAIN WEIGHT VERSUS DOSE
BY SEX

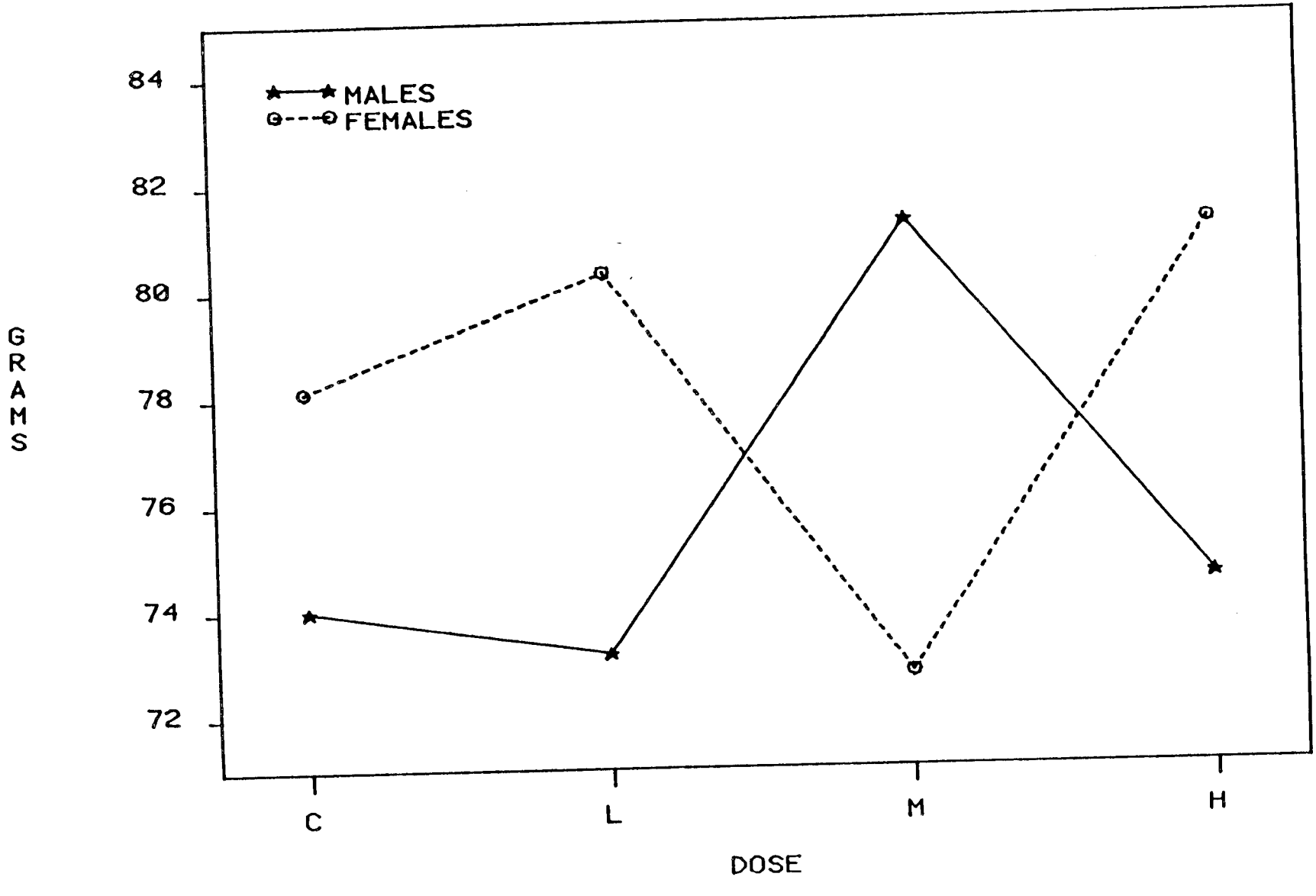


FIGURE 10: BRAIN TO BODY WT RATIO - MEAN VERSUS DOSE
BY SEX