PRELIMINARY REPORT ON THE ACUTE AND SUBACUTE ORAL TOXICITY OF MDMA IN RATS
PROJECT EMD-AT-001

SUMMARY:

This report presents preliminary, unaudited findings of a study designed to obtain information on the effects of a single (acute) and repeated (subacute) oral administration of MDMA to rats. Data from this project will be used to select doses and potential toxicologic endpoints for future studies with this agent.

A total of 20 male and 20 female rats were administered daily doses of MDMA. Beginning with a dose of 25 mg/kg/day, the amount given was increased by 25 mg/kg/day thereafter. An additional group of 10 male and 10 female rats served as the control group, and were dosed with water only. Dosing continued for 13 consecutive days, by which time the dose level had reached 300 mg/kg. (Note: The dose used in humans for therapeutic purposes is approximately 2 mg/kg). This dosing regimen was based on data obtained in a preliminary study in female rats showing mortality at doses of 300 mg/kg and above. Animals went an additional three days without dosing and were then examined for changes in hematology and blood chemistry, and for microscopic damage in the brain, lungs, kidneys, liver, spleen and reproductive organs. After completing the repeated-dosing study, an additional group of 10 male and 10 female rats that had never been exposed to MDMA were given the maximum dose used in the previous study (300 mg/kg), and the survivors were sacrificed three days later. These animals were also examined for hematology and blood chemistry changes and for damage in several major organs, including the brain.

Adverse clinical signs such as excitability, uncontrolled urination, piloerection (raised hair) and bulging eyes were seen at all dose levels above 25 mg/kg. At higher dose levels tremor, muscle spasms, impaired movement, convulsions and death were observed. Deaths with single dosing occurred at 300 mg/kg and above. With repeated dosing, deaths were observed between 150 and 300 mg/kg. Using the preliminary data, the single-dose oral LD50 was estimated to be approximately 325 mg/kg, which is six times higher than previously reported for i.p. injection. Blood chemistry analyses suggested changes consistent with kidney and liver damage in animals dosed repeatedly at high levels. Confirmation of this finding awaits completion of the microscopic examinations of these organs.

The brains from all animals in the repeated-dosing study were examined with a standard histology stain (H&E), with five of the male and five of the female brains from the control and treated groups also examined following staining with three specialized nervous-system stains: cresyl violet, luxol fast blue, and Bodian's silver stain. No evidence of histologic brain damage was noted in any of the control or treated animals in this study.

These preliminary findings showed that MDMA administered orally at a maximum dose approximating 150 times the accepted human therapeutic level resulted in marked adverse clinical reactions, including death, and blood chemistry changes suggestive of liver and kidney damage. No histologic evidence of brain damage was observed with the methods utilized in this study. A final report on this study, including results from all endpoints examined will be available in early August, 1985.

Phillip T. Goad 7-9-85

Phillip T. Goad, Ph.D.
Toxicologist
Intox Laboratories, Inc.

Date

PURPOSE:

The purpose of this study was to evaluate and assess the potential acute and subacute toxicity of methylenedioxymethamphetamine (MDMA) administered orally to rats by single and repeated dosing, respectively; and to provide information to assist in selection of doses for future repeated-dose studies.

TEST ARTICLE:

The test article was methylenedioxymethamphetamine (MDMA). The test article was supplied by Dr. David Nichols, Department of Medicinal Chemistry, Purdue University. Upon receipt the test article was assigned Intox sample number 618. Two lots were received. The first lot of 15 gm was received on May 24, 1985 (lot 5810-07). The second lot of 100 gms was received on June 12, 1985 (lot 5810-08).

TEST ARTICLE CHARACTERIZATION:

Data concerning uniformity, composition and additional chemical/physical characteristics of the test article were supplied by Dr. Nichols. Chemical confirmation was obtained using GC/MS and NMR.

CONTROL ARTICLE:

Distilled water.

TEST SYSTEM:

Male and female Sprague-Dawley rats were obtained from Charles River Laboratories, Inc., Portage, MI. The animals (8-10 weeks of age) weighed between 195-225 grams at receipt. The rat was chosen because data from this study will be used to facilitate selection of dose levels on future repeated dose oral toxicity studies utilizing the rat as the test system. A total of 80 rats (40 male and 40 female) were used.

QUARANTINE:

To insure that sufficient animals of adequate health and weight range were available at the start of dosing, more animals (5 males, 5 females) were ordered than were actually required for the project. The animals were quarantined for five days (SOP/ANH/011). Each animal was assigned a quarantine number at the time of receipt and housed individually in a cage identified with the assigned number. Each animal was weighed on the day following receipt and on the third day of the quarantine period, and examined daily for signs of disease (diarrhea, ectoparasites, rough hair coat, nasal or ocular discharge, injury, etc.). Only those animals with an adequate health status and a body weight on the 3rd day of quarantine within + 20% of the overall group mean were allocated to the study.

TEST PROCEDURES:

1. Animal Receipt, Identification and Care

   Animals were received and quarantined according to procedures described in SOP/ANH/011. Animals used on study were assigned a unique identification number (UIN) according to procedures outlined in SOP/ANH/034, and were ear tagged with a metal tag bearing that number. Animals were housed one per

cage in suspended stainless steel cages in an animal room maintained at an average temperature and humidity of 72 + 5 F and 30-70%, respectively. The room was maintained on a 12-hour light cycle (0600 hours light onset central standard time). Water via an automatic watering system and feed (Purina 5010 Laboratory Rodent Chow) was supplied ad libitum. There were no dietary contaminants reasonably expected to interfere with the outcome of this study. Therefore, no dietary analysis was performed.

2. Experimental Design

a. Preliminary Range-Finding Study

MDMA was administered orally to ten female Sprague-Dawley rats (2/dose) at doses of 100, 200, 300, 400 or 500 mg/kg body weight. All animals were observed frequently on the day of dosing and at least daily thereafter for signs of toxicological and/or pharmacological effects. Animals that died after dosing were subjected to a complete gross necropsy. Survivors and two additional untreated control rats were anesthetized one week later and perfused through the heart with physiologic saline and 10% neutral buffered formalin. Gross necropsies were performed and the brains were removed and processed for histopathologic evaluation following staining with hematoxylin and eosin (a routine stain) and luxol fast blue (a special stain for myelin). The slides were evaluated for histopathologic lesions by the Intox pathologist, Dr. Charles Frith.

b. Phase I

A group of twenty male and twenty female rats received doses of the test article on a daily basis at approximately the same time each day. Dose levels began at 25 mg/kg/day, and were increased daily by 25

mg/kg, until a dose level of 300 mg/kg was reached. (NOTE: On the 5th day of dosing, an error in dose preparation resulted in the administration of 15 mg/kg of MDMA, instead of the anticipated dose of 150 mg/kg. The following day, the 150 mg/kg dose was administered and the increasing-dose regimen continued.) The initial dose level administered was selected based upon data obtained from the preliminary range finding study. The purpose of phase I of the study was to assess the effects of repeated dosing with the test article and, to provide information to assist in selection of doses for future repeated-dose studies. The study protocol specified that dosing would continue until a dose was reached that killed half or more than half of the test animals dosed at that level. As the study proceeded it was apparent that, if the original design was followed, very few animals would be available for Clinical Pathology and Histopathology evaluations. One death was noted at 150 mg/kg, with a total of 10 deaths occurring between 150 and 300 mg/kg. From these data, it was obvious that 300 mg/kg far exceeded the dose that would be used for future repeated-dose studies. Therefore, no useful information would be obtained by continuing the dosing and in fact, continued dosing would have reduced the sample sizes available for clinical pathology and histopathology evaluations. Therefore, the decision was made by the Study Director to halt dosing at 300 mg/kg. A further factor in the decision was the prevention of unnecessary stress to the test animals by continued dosing. Dosing occurred for 13 consecutive days. An additional group of ten male and ten female rats received vehicle (distilled water) only each day that the treated animals were dosed. Allocation of animals to the treated and control groups was made in a weight-stratified manner using the TOXSYS allocation routine. Animals dying during Phase I were sub-

jected to a complete gross necropsy (see section 6). Survivors were submitted for clinical chemistry (section 5) and histopathologic evaluation (see Section 7). Once the 300 mg/kg dose level was reached, Phase I was terminated and Phase II was initiated.

c. Phase II

A group of previously untreated rats, ten males and ten females received a single dose of MDMA at a level of 300 mg/kg. Animals were selected for this phase using a table of random numbers. After dosing the animals were held for observation for three days. Animals dying during Phase II were subjected to a complete gross necropsy. Survivors were submitted for clinical chemistry and histopathologic evaluation.

3. Test Article Administration

The route of exposure was via gastric intubation. This route was chosen since the test article is administered orally to humans. Test article administration was performed according to procedures described in Intox Standard Operating Procedure SOP/GTX/001. The test article was diluted with distilled water so that the final volume dosage for all animals was 10 ml/kg. Dose volumes were adjusted weekly in the Phase I portion of the study to account for changes in body weight that occurred between weighings. Data concerning the degree of absorption of the test article were not required to meet the objectives of the study.

4. Observations

All animals were observed at least once daily. On dosing days, observations were performed approximately 1-2 hours after dosing. The following observations were made: nature, onset, severity, and duration of all gross or visible toxic or pharmacologic effects. In addition to the detailed

clinical observation, each animal was observed twice daily for signs of morbidity/mortality. Times of death were recorded for all animals. Animals were observed for three days after reaching the 300 mg/kg dose in Phase I, and for three days after the initial dosing in Phase II. On the third post-dosing day all survivors were sacrificed. Body weights were determined on the day of dosing, weekly thereafter, and at death/or sacrifice. Changes in weights were calculated and recorded when survival exceeded one day. The procedures followed for weighing and observation of animals are described in Intox Standard Operating Procedures SOP/ANH/015 and SOP/ANH/017.

5. Clinical Pathology

All surviving animals from Phase I and II were sampled for clinical pathology at sacrifice. In a deviation from the original protocol, animals were not fasted overnight prior to sampling. Blood was collected from the orbital sinus.

Hematology measurements included:

Total red blood cell count
Total white blood cell count
Differential Leucocyte count
Hemoglobin
Hematocrit
Reticulocyte count

Clinical chemistry measurements included:

Potassium & Sodium
SGOT
SGPT
BUN
Serum Alkaline Phosphatase
Total Cholesterol
Creatinine Phosphokinase
Glucose
LDH
Albumin
Total Protein
Globulin

6. Necropsy Examinations

All animals dying during the course of the study were subjected to complete necropsies according to SOP/PAT/002. Survivors were perfused through the heart prior to necropsy according to procedures described in SOP/PAT/008. Gross necropsies included examination of the external surface; all orifices; the cranial cavity; the external surfaces of the brain and spinal cord; the thoracic, abdominal and pelvic cavities and their viscera; and the cervical tissues and organs. Tissues identified under Histology Examinations (Section 7, below) were collected at the time of necropsy and fixed in 10% neutral buffered formalin.

7. **Histology Examinations**

All animals that survive to terminal sacrifice had the following tissues examined for histopathological lesions according to INTOX Standard Operating Procedures SOP/PAT/002-005.

Brain (cerebrum, cerebellum and pons)
Peripheral (sciatic) nerve
Gonads
Kidneys
Liver
Lungs
Spleen

Histopathology evaluations were performed by the Intox Pathologist on all the above tissues from the specified animals. In addition, any gross lesions or alterations were submitted for histologic examination. The above tissues from all surviving animals were examined after staining with hematoxylin and eosin (H&E), or luxol fast blue (peripheral nerve). In addition, the brains from five animals per sex per group (total of 20) were examined after staining with cresyl violet, luxol fast blue and Bodian's silver stains.

8. Data Evaluation

Evaluation of results included the relationship, if any, between exposure

to the test article and the appearance, incidence and severity of all abnormalities including: behavioral and clinical abnormalities, gross and histopathologic lesions, clinical pathology, body weight changes, effects on mortality and any other toxicological effects. Parametric and/or non-parametric statistical analyses were performed where appropriate (body weights, mortality, clinical pathology data).

RESULTS:

1. Preliminary Range-Finding Study

All animals showed toxicological and/or pharmacological effects, including piloerection, hyperexcitability, exophthalmos and polyuria. Animals at the higher doses (300 mg/kg and above) showed signs of tremor, muscle spasms, ataxia and/or convulsions. These signs appeared approximately 15-20 minutes following dosing. Mortality at each dose level was as follows: 0/2 at 100 mg/kg, 0/2 at 200 mg/kg, 2/2 at 300 mg/kg, 1/2 at 400 mg/kg and 2/2 at 500 mg/kg. Death occurred between approximately 4 to 24 hours following dosing.

No MDMA-related lesions were noted at necropsy in any of the animals. No histopathologic lesions were observed in the brains of the five surviving animals.

2. Phase I - Repeated Dose Study

a. Clinical Observations - A numbers of clinical signs of pharmacological and/or toxicological effects of the test article were observed. The most significant of these findings are summarized in Table 1. Several of the pharmacological manifestations of the test article, including

hyperactivity, excitability, polyuria, piloerection and exophthalmos, were observed by the first and second day of dosing.

Additional clinical observations included oral/nasal discharge, aggressive behavior, ataxia, stereotypic behavior and convulsion. In approximately 20% of the females and 50% of the males changes in lacrimation resulted in dry, dull corneas that in some males resulted in corneal opacity and/or rupture, possibly due to secondary eye infections. Deaths occurred at doses ranging from 150 to 300 mg/kg. A mortality summary for both Phase I and II is presented in Table 2.

b. Body Weights - Group mean body weights are summarized in Table 3. As can be seen from these data, the treated animals had significantly reduced body weights at seven and 14 days following initiation of dosing.

c. Clinical Pathology - Clinical chemistry and hematology data are summarized in Tables 4 and 5, respectively. Blood urea nitrogen (BUN) was significantly ($p<.05$) increased in females, suggesting potential renal damage in this sex (Table 4). In males, both SGOT and SGPT were significantly ($p<.05$) elevated, suggesting potential hepatic toxicity in this sex.

The hematology data (Table 5) revealed a significant increase in total white blood cell count, ($p<.01$), hemoglobin concentration ($p<.001$), and hematocrit ($p<.01$) in treated males as compared to control. The increased white blood cell count could be related to the possible eye infections referred to above. However, the toxicological significance

of the changes in hematocrit and hemoglobin concentration are unknown. In females, there was a statistically significant ($p<.05$) reduction in mean corpuscular hemoglobin (MCH), and a statistically significant ($p<.05$) increase in mean corpuscular hemoglobin concentration (MCHC). The toxicological significance of these findings is unknown.

d. Gross Pathology - Two gross observations were observed at the time of necropsy that were potentially related to the administration of the test article. These observations included reddened lungs and enlarged urinary bladder. The lung observations were seen in two of the four males and four of the six females that died during the study. Similar observations were not seen in animals that survived to terminal sacrifice. An enlarged urinary bladder was observed in four males and seven females from the treated group, and one female (mild enlargement) from the control group. This finding was observed both in dead and surviving animals, and is likely related to the clinical observation of polyuria noted previously.

e. Histopathology - At the writing of this preliminary report, the H&E brain sections from all 50 animals that survived to terminal sacrifice have been examined. In addition, the luxol fast blue, cresyl violet and Bodian's silver brain sections from the five animals per sex per group selected for special staining have been examined. No microscopic findings were seen in the brains of either control or treated animals.

3. Phase II - Single Dose Study

Toxicological and/or pharmacological effects, similar to those seen in both the preliminary dosing and repeated dosing studies were observed after administering a single 300 mg/kg dose of MDMA. The clinical signs were

more severe in the naive animals administered this dose level as compared to those observed in the multiple dose animals administered the same level. In conjunction, 40% (4/10) of the males and 40% (4/10) of the females died following dosing. This is in contrast to 0/16 deaths in males and 3/17 (18%) deaths in females administered 300 mg/kg in the repeated dose study. The difference in mortality between the two phases was statistically significant for males ($p=0.014$), but not for females ($p=0.204$). These data indicate a possible tolerance development to the acute toxicity (mortality) following repeated administration of the test article. The mortality data from this phase of the study were combined with those obtained from the preliminary range-finding study, and the single dose oral LD50 was estimated at 322 mg/kg.

At the time of necropsy, no gross observations were noted in any of the animals that died following test article administration. However, one of six males and three of six females that survived the treatment had enlarged urinary bladders at necropsy. No other gross observations were noted.

At the time of preparation of this report, no further data are available from this phase of the study.

RECORDS:

The records were maintained according to Intox Standard Operating Procedure SOP/REC/002.

NOTICE:

This study was conducted in accordance with the FDA Good Laboratory Practices for Non-Clinical Laboratory Studies (21 CFR 58) and the criteria of this protocol.

This study was designed in order to control bias and ensure integrity of the data obtained. The methods to achieve these objectives include: 1) Preparations of and adherence to an approved study protocol, 2) Adherence to approved Standard Operating Procedures, 3) Utilization of automated data collection systems, 4) Random allocation of animals to study groups (Phase I and II), and 5) Auditing of protocols, study conduct and reporting by the INTOX Quality Assurance Officer. Note: This preliminary report has <u>not</u> been audited by the Quality Assurance Officer.

TABLES

TABLE 1
PROJECT EMD-AT-001
CLINICAL SIGNS SUMMARY - PHASE I

NUMBER SHOWING EACH SIGN

CLINICAL SIGN	MALE		FEMALE	
	CONTROL	TREATED	CONTROL	TREATED
Aggression	0	6	0	9
Ataxia	0	12	0	9
Convulsions	0	7	0	8
Hyperexcitability	0	20	0	20
Exophthalmos	0	18	0	20
Lacrimation	0	5	0	16
Muscle Spasms	0	18	0	3
Oral/Nasal Discharge	0	19	0	20
Piloerection	0	20	0	20
Polyuria	0	19	0	18
Rough Coat	0	20	0	19
Tremor	0	3	0	6

TABLE 2
PROJECT EMD-AT-001
MORTALITY SUMMARY - PHASE I & II

Dose (mg/kg)	(DEATHS/NUMBER DOSED) Male	Female
150	0	1/20
175	2/20	0
200	1/18	0
225	0	0
250	0	0
275	1/17	2/19
300 (repeated dose)	0/16	3/17
300 (single dose)	4/10	4/10

TABLE 3
PROJECT EMD-AT-001
BODY WEIGHT DATA - PHASE I
(MEAN ± S.D.)

BODY WEIGHT (gm)

DAY OF STUDY[1]	MALE		FEMALE	
	CONTROL	TREATED	CONTROL	TREATED
Day 0 (N)	235 ± 6 (10)	236 ± 7 (20)	219 ± 8 (10)	214 ± 6 (20)
Day 7 (N)	294 ± 11 (10)	248 ± 8[2] (20)	245 ± 12 (10)	214 ± 9[2] (19)
Day 14 (N)	332 ± 17 (10)	254 ± 17[2] (16)	258 ± 11 (10)	228 ± 10[2] (14)

[1] - Day 0 = first dosing day

[2] - Significantly different from control ($p<.0001$)

TABLE 4
PROJECT EMD-AT-001
CLINICAL CHEMISTRY DATA - PHASE I
(MEAN ± S.D.)

CLINICAL CHEMISTRY PARAMETER	MALE		FEMALE	
	CONTROL N=10	TREATED N=16	CONTROL N=10	TREATED N=13
Albumin (g/dl)	4.1 ± 0.3	3.7 ± 1.2	4.6 ± 0.6	3.9 ± 1.1
Alkaline phosphatase (IU/L)	225 ± 47	205 ± 55	121 ± 54	130 ± 32
BUN (mg/dl)	26 ± 3	31 ± 14	21 ± ?	31 ± 11[1]
Cholesterol (mg/dl)	78 ± 17	78 ± 17	88 ± 16	87 ± 16
Creatinine (mg/dl)	0.7 ± 0.1	1.0 ± 0.7	0.6 ± 0.1	0.8 ± 0.3
Glucose (mg/dl)	230 ± 57	214 ± 70	232 ± 181	209 ± 60
LDH (IU/L)	483 ± 329	524 ± 351	560 ± 203	468 ± 242
SGOT (IU/L)	76 ± 19	97 ± 27[1]	85 ± 21	90 ± 26
SGPT (IU/L)	35 ± 5	42 ± 8[1]	36 ± 11	36 ± 7

[1] - $P < .05$

TABLE 5
PROJECT EMD-AT-001
HEMATOLOGY DATA - PHASE I
(MEAN ± S.D.)

HEMATOLOGY PARAMETER	MALE		FEMALE	
	CONTROL N=10	TREATED N=16	CONTROL N=10	TREATED N=13
WBC ($X10^3/mm^3$)	6.8 ± 1.5	9.7 ± 2.2[2]	7.8 ± 1.3	8.1 ± 3.1
RBC ($X10^6/mm^3$)	6.3 ± 0.4	6.7 ± 0.8	5.9 ± 0.4	6.0 ± 0.5
HGB (G/DL)	14.0 ± 0.5	15.6 ± 1.1[3]	14.1 ± 0.5	13.7 ± 0.8
HCT (ml%)	33 ± 1	37 ± 4[2]	32 ± 2	33 ± 3
MCV (μ^3)	53 ± 2	55 ± 2	55 ± 1	54 ± 1
MCH ($\mu\mu g$)	22 ± 1	23 ± 2	24 ± 2	23 ± 1[2]
MCHC (%)	42 ± 1	43 ± 4	44 ± 3	42 ± 2[1]

[1] - $p<.05$

[2] - $p<.01$

[3] - $p<.001$

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August 1, 1985

Mr. Rick Doblin
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Dear Rick:

Enclosed, find two copies each of the protocols for the 28-day rat and dog MDMA studies. Please review them, sign both copies and return one of each to me. I will serve as the Study Director, Phil will serve as a Consultant and Ms. Gina Caldwell will serve as the Quality Assurance Officer.

Ms. Caldwell will contact you concerning her participation. She will review the protocols, do periodic inspections on the ongoing studies, review raw data and review the final report. She will report directly to you and bill you directly for her services. I estimate the cost for her services to be approximately $1,800.

The total cost of the 28-day dog and rat studies will be $60,000. This also includes some additional range finding (30 rats and 6 dogs) which we will complete prior to starting the 28-day studies.

Since we are ordering the rats and dogs relatively soon, the initial down payment of $20,000 will be required upon acceptance of the protocols. The check can be made payable to Toxicology Pathology Associates. The second installment of $20,000 will be due upon final sacrifice of the animals, and the final installment of $20,000 will be due upon receipt of the final reports.

As I mentioned on the phone, a repeat of the University of Chicago study would be much better as a small separate research project which would not have to be done under Good Laboratory Practices. Phil and I are looking at the possibility of doing it jointly between the Medical Center and NCTR. The project should cost less than $5,000. We can put a better cost estimate on it after Phil and I have talked to respective individuals at the Medical Center and NCTR.

The cost for a 90-day subchronic rat study would be approximately $60,000-$65,000.

I have sent copies of the protocols to Dr. Contrera at FDA. I look forward to the start of the studies and to working with you.

Sincerely,

Charlie

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

CHF:plb

Enclosures

Hallucinogenic Amphetamine Selectively Destroys Brain Serotonin Nerve Terminals: Neurochemical and Anatomical Evidence

Abstract. (±)-3-4-Methylenedioxyamphetamine (MDA), an amphetamine analogue with hallucinogenic activity, produced selective long-lasting reductions in the level of rat brain serotonin (5HT), the number of 5HT uptake sites and the concentration of 5-hydroxyindoleacetic acid (5HIAA). Correlative morphological studies suggests that these long-lasting neurochemical deficits were due to 5HT nerve terminal degeneration. These results show that MDA possesses brain 5HT neurotoxic activity and raise the question of whether exposure to MDA and related hallucinogenic amphetamines can produce brain 5HT neurotoxicity in man.

Keywords: Neurotoxicity - Serotonin - Amphetamine - Hallucinogenic Drugs

endogenous neurotoxins, if they exist, could play a role in the etiology of neurodegenerative disorders involving monoamine-containing neurons in the central nervous system of humans.

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12. Male albino Sprague-Dawley rats weighing approximately 250 grams were obtained from the Holtzman Co. (Madison, WI) and housed singly in suspended wire-mesh cages with free access to food (Purina Rat Chow) and water in a colony room maintained at 23±1 C. (±)-MDA hydrochloride was obtained from the National Institute on Drug Abuse. Its purity was confirmed by means of mass spectroscopic analysis. MDA was administered subcutaneously after being dissolved in sterile 0.9% saline at various desired concentrations. Dose (expressed as the free base) was adjusted by injecting each of these MDA solutions on a 1 ml/kg basis. Control rats were injected with an equal volume of saline. Regional brain DA, 5HT and NE levels were determined by high performance liquid chromatography coupled with electrochemical detection. DA and 5HT were measured according to the method of R. Keller, A. Oke, I. Mefford, R. Adams, Life Sciences 19, 995 (1976), as modified in this laboratory (J. Lucot, J. Horwitz, L.S. Seiden, J. Pharmacol. Exp. Ther. 217, 738 (1981). NE was analyzed using the method of R.S. Fenn, S. Siggia, D.J. Curran, Analyt. Chem. 50 (8), 1067 (1978).

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Table 1

Regional Brain Monoamines Two Weeks After Various Doses of MDA[1]

	Striatum		Hippocampus		Rest of Brain		
	DA[2]	5HT	NE	5HT	DA	NE	5HT
Treatment							
Saline	11.6±0.3	0.43±-0.05	0.34±0.02	0.41±0.02	0.19±0.01	0.46±.01	0.32±0.0
MDA							
1.25 mg/kg	10.6±0.4	0.42±0.02	NM[3]	0.39±0.03	0.17±0.01	NM	0.29±0.0
2.5 mg/kg	11.7±0.4	0.37±0.04	NM	0.40±0.02	0.18±0.01	NM	0.34±0.0
5 mg/kg	12.4±0.7	0.36±0.04	NM	0.28±0.04*	0.18±0.01	NM	0.23±0.0
10 mg/kg	11.5±0.6	0.18±0.05*	0.31±0.06	0.16±0.05*	0.18±0.02	0.46±0.04	0.19±0.0
20 mg/kg	10.6±0.4	0.14±0.02*	0.38±0.01	0.10±0.01*	0.17±0.02	0.47±0.01	0.16±0.0
40 mg/kg	10.8±0.5	0.11±0.01*	0.40±0.02	0.10±0.01	0.18±0.02	0.43±0.02	0.15±0.0

[1]Each MDA dose was administered approximately every 12 hours for 4 consecutive days.

[2]Values represent the mean ± S.E.M. expressed in ug/g tissue (N=4).

[3]Not measured since these MDA doses produced little or no effect on 5HT.

*$p < 0.05$, two-tailed student's t-test.

Table 2

Hippocampal 5HT Content Two Weeks After Various 10 mg/kg Regimens of MDA

Regimen Duration	Hipoocampal 5HT	% Decrease
Control	0.41 ± 0.02	-
0.5 day	0.28 ± 0.04*	32
1 day	0.17 ± 0.01*	59
2 days	0.12 ± 0.01*	74
4 days	0.10 ± 0.01*	76

* Significantly different from saline control ($p < 0.05$).

Fig. 1 Legend. Coronal silver-stained sections through the striatum of (A) control rat and (B) a rat administered 10 mg/kg of MDA subcutaneously twice, 12 hours apart. Fink-Heimer method with cresyl-violet counter-stain. 18 hour survival.