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Toxicity of Methylenedioxymethamphetamine (MDMA) in the Dog and the Rat

CHARLES H. FRITH,*† LOUIS W. CHANG,† DANNY L. LATTIN,‡ ROBERT C. WALLS,§ JACK HAMM,‖ AND RICHARD DOBLIN**

*Toxicology Pathology Associates, 1102 Briar Creek Road, Little Rock, Arkanasas, 72211; †Department of Pathology, ‡Department of Medicinal Chemistry, College of Pharmacy, and §Department of Biometry, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205; ‖School of System Science, Arkansas Tech University, Russellville, Arkansas 72801; and **Earth Metabolic Design Laboratories, 2105 Robinson Avenue, Sarasota, Florida 33582

Toxicity of Methylenedioxymethamphetamine (MDMA) in the Dog and the Rat. FRITH, C. H., CHANG, L. W., LATTIN, D. L., WALLS, R. C., HAMM, J., AND DOBLIN, R. (1987). *Fundam. Appl. Toxicol.* **9**, 110–119. Methylenedioxymethamphetamine (MDMA) was administered to dogs and rats orally once a day for a 28-day period to evaluate the morphological and neuropathological effects. Major clinical signs associated with the administration of MDMA in the dog included circling, depression, dilated pupils, hyperactivity, rapid breathing, and salivation. Major clinical signs in the rat included hyperactivity, excitability, piloerection, exophthalmos, and salivation. Gross observations at necropsy in the dog 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. No gross lesions were seen in the rats at necropsy. The medium- and the high-dose groups in both sexes in both the rats and the dogs 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 toward more normal consumption was noted in the following weeks in both the rats and the dogs. Hematologic, clinical chemistry, and urinalysis values did not appear to be affected by the administration of the test article in the dog. In the rat clinical pathology variables showing a trend to decrease with dose included urinary pH, blood urea nitrogen, glucose, creatinine (females), lactate dehydrogenase (LDH) (females), and chloride. Clinical pathology variables showing a trend to increase with dose included total white blood cell count and phosphorus. Microscopically, testicular atrophy was present in one medium-dose and two high-dose male dogs. Prostatic hyperplasia was present in two high-dose male dogs. No test article-related lesions were seen in the brains of either species.

Methylenedioxymethamphetamine (MDMA) is a ring-substituted derivative of phenethylamine obtained from nutmeg or sassafras whose use as a psychotherapeutic adjunct was quietly explored over a 10-year period by some health care professionals. Due to its increasingly widespread use as a recreational drug, and because of its structural similarity to methylenedioxyamphetamine (MDA) which has been reported to selectively destroy brain serotonin nerve terminals in the rat (Ricaurte *et al.*, 1985), the Drug Enforcement Administration invoked their emergency scheduling powers granted by Congress under the Controlled Substances Act and on July 1, 1985, placed MDMA in Schedule I, pending hearings to determine its permanent scheduling. This study was undertaken to evaluate the morphological and neuropathological effects in beagle dogs and Sprague–Dawley rats administered the compound daily for 28 days.

MATERIALS AND METHODS

Test Article

The test article was methylenedioxymethamphetamine hydrochloride. The MDMA was a white, crystal-

line powder. The test article was supplied by a collaborator of the study, Dr. David Nichols, Department of Medicinal Chemistry, Purdue University. 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 uncorrected melting point of 158–159°C.

Dog Study

Twelve male and twelve female beagle dogs were obtained from a licensed vendor. The dogs weighed between 5.1 and 8.5 kg on the day of receipt. They were quarantined for a 2-week period during which time they were subjected to a complete physical examination including an ophthalmological examination, a complete blood count (CBC), and a heartworm examination. The animals were housed individually in 4 × 8-ft runs in a room maintained at 72 ± 8°F with a 30–70% relative humidity. The room was maintained on a 12-hr light/dark cycle. Water was provided *ad libitum* via an automatic watering system and food (Lab Chow 5006, Ralston Purina) was also provided *ad libitum* except for the 24-hr period prior to sampling of blood for clinical chemistry.

Experimental design. Animals were randomly allocated to one of four treatment groups based upon a weight-stratified randomization. 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 dogs were weighed at the beginning of each week, and the dose of MDMA was based upon that weight for 1 week. If the weight changed the following week, the dose was subsequently adjusted. MDMA was administered in gelatin capsules each morning for a 28-day period.

Clinical observations. All animals were observed for clinical observations approximately 1–2 hr after dosing, in addition to a morning and an afternoon morbidity and mortality check. Body weights were obtained on a weekly basis, and food consumption was measured once a week for a 24-hr period.

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 and urine was collected by cystocentesis. Hematology measurements included total red cell count (RBC), total white cell count (WBC), differential leukocyte count, hemoglobin (HGB), hematocrit (HCT), mean RBC volume (MCV), and platelet count. Clinical chemistry measurements included calcium, total protein, potassium, sodium, chloride, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN), alkaline phosphatase, cholesterol, creatinine phosphokinase, bilirubin, albumin, globulin, glucose and lactate dehydrogenase (LDH). Urinalysis measurements included appearance, 24-hr volume, specific gravity, osmolality, pH, microscopic evaluation, glucose, ketones, bilirubin, urobilinogen, protein, and blood.

Necropsy examinations. All animals that died or survived to terminal sacrifice were subjected to complete necropsies. Dogs were euthanatized with T-61 euthansia solution (American Hoechst, Somerville, NJ). Necropsies included the examination of external surface, all orifices, cranial cavity, external surface of the brain; 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, optic nerve, aorta (thoracic), pancreas, bone and bone marrow, parathyroid, brain (cerebrum, cerebellum, and pons), pituitary gland, prostate, cecum, rectum, colon, salivary gland (submaxillary), esophagus, sciatic nerve, eye, small intestine (duodenum, jejunum, and ileum), gonads, heart, spinal cord (cervical, thoracic, and lumbar), kidneys, liver, spleen, lung, stomach, lymph node (mesenteric and cervical), thymus, thyroid gland, mammary gland (females), urinary bladder, muscle (skeletal), and uterus. The following organs were weighed in all animals that survived to terminal sacrifice: liver, kidneys, brain, gonads, and adrenal glands.

Histology examinations. Histology examinations were performed on all animals that died or survived to terminal sacrifice. All organs and tissues collected under Necropsy Examinations were examined microscopically after routinely processing and staining with hematoxylin and eosin.

Rat Study

Fifty-three (53) male and 52 female Sprague–Dawley rats were obtained from The Charles River Company in Portage, Michigan. The animals weighed between 206 and 344 g on the fifth day of quarantine. The animals were quarantined for 1 week prior to the initiation of the study. Each animal was 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 fifth day of quarantine within ±20% of the overall group mean were allocated to the study. The mean weight of the males was 297 g ± 20%, and the mean weight of the females was 220 g ± 20%. At the end of the quarantine period, each animal was randomly assigned a unique identification number (UIN) and tagged with a metal eartag bearing its UIN. Animals were housed individually in suspended stainless-steel cages with undercage pans containing chipped-hardwood bedding. The room was maintained at 72 ± 5°F with a 30–70% relative humidity. The room was maintained on 12-hr light/dark cycle. Water was provided via an automatic watering system. Food (Formulab 5008, Ralston Purina) was provided *ad libi-*

tum except for the 24-hr period prior to sampling of blood for clinical chemistry. Food consumption was measured weekly.

Experimental design. Allocation to treatment groups was completely random and was based on body weights obtained during the quarantine period (weight-stratified randomization). MDMA was administered orally by gavage to 40 male and 40 female Sprague–Dawley rats at doses of 0, 10, 50, and 100 mg/kg. Doses were adjusted weekly to account for changes in body weights that occurred between weighings. The animals were dosed 7 days per week for 28 days.

Clinical observations. All animals were observed approximately 1–2 hr 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 that animals were sacrificed.

Clinical pathology. Each animal was sampled for clinical chemistry and hematologic determinations at the conclusion of the study. Blood was collected by cardiac puncture following anesthetization and prior to sacrifice. Urine was collected on five males and five females from each dose group. Male rats were placed in metabolism cages on Day 20 of the study and female rats were placed in metabolism cages on Day 25 of the study. A 24-hr sample was collected from the rats while they were in the metabolism cages and was measured for volume and osmolality. Fresh urine samples were collected by holding a plastic collection vessel under each rat during forced ether inhalation. Hematology measurements included total red cell count, total white cell count, differential leukocyte count, hemoglobin, hematocrit, mean RBC volume, 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 appearance, 24-hr volume, specific gravity, osmolality, pH, microscopic evaluation, glucose, ketones, bilirubin, urobilinogen, protein, and blood.

Necropsy examinations. All animals that died or survived to terminal sacrifice were subjected to complete necropsies. Rats were anesthetized with a mixture of Acepromazine and Ketaset (1:10) at a dosage of approximately 0.10 ml per 100 g. The abdominal and thoracic cavities were opened, and the animals were perfused through the left ventricle with saline followed by 10% neutral buffered Formalin. The right atrium was incised to allow the blood, and saline and Formalin to flow through the vascular system. Necropsies included the examination of the external surface, all orifices, cranial cavity, external surface of the brain; the thoracic, abdominal, and pelvic cavities and viscera; and cervical tissues and organs. Tissues were collected and fixed in 10% neutral buffered Formalin for microscopic examination. The same tissues collected from the dogs were collected from the rats. In addition, the Harderian gland and the nonglandular stomach were collected.

The following organs were weighed in all animals that survived to terminal sacrifice: liver, kidneys, brain, and gonads.

Histology examinations. Complete histology examinations were performed on all animals that died or survived to terminal sacrifice in the control and the high-dose groups. All organs and tissues collected under Necropsy Examinations were examined microscopically (control and high-dose groups) after routinely processing and staining with hematoxylin and eosin. Special stains (Bodian's silver stain, gallocyanine stain, and luxol fast blue) were performed on the brains of the animals from the control and high-dose groups.

Data Evaluation

Evaluation of results included the relationship, if any, between exposures to the test article and the appearance, incidence, and severity of all abnormalities including clinical observations, food consumption, body weight changes, gross, microscopic, and clinical pathology findings. Response variables were analyzed by repeated-measures analysis of variance (Winer, 1971). Means at each time were contrasted by Duncan's multiple comparisons procedure (Winer, 1971).

Analytical Chemistry

A stability study on MDMA HCl solution in deionized distilled water and a quantitative analysis of the aqueous dosing solutions of the MDMA HCl were performed using uv spectrophotometry. The uv absorbance of the solution did not increase or decrease significantly during a period of 168 hr (1 week), and it was concluded that the aqueous solutions of MDMA HCl refrigerated between 0 and 10°C were stable for up to 1 week. Consequently, the MDMA solutions were prepared only once each week for the 4-week duration of the study. Animals were dosed for the full week based upon the body weight at the beginning of the week.

RESULTS

Dog Study

Clinical observations. A number of clinical signs of pharmacological and/or toxicological effects of the test article were observed (Table 1). One female dog died approximately $2\frac{1}{2}$ hr

TABLE 1

CLINICAL SIGNS SUMMARY FOR DOGS

	Males				Females			
Group (mg/kg): Clinical sign	0	3	9	15	0	3	9	15
Aggression	0/3[a]	0/3	0/3	0/3	0/3	0/3	0/3	1/3[b]
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[b]
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[b]
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[b]

[a] Number showing sign/total group.
[b] Seen in the dog that died on the first day of dosing.

after the first dose with 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 min after dosing and continued to show them for approximately 6–8 hr. The males appeared to show more clinical effects than the females.

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 (Table 2).

Food consumption. Food consumption for the high- and medium-dose groups was decreased significantly from the control and low-dose groups for the first week, but reverted toward normal and was not significantly different by the fourth week (Table 3).

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

TABLE 2

BODY WEIGHT (kg) IN DOGS

Dose (mg/kg)	Sex	Week 1	Week 2	Week 3	Week 4
0	M	7.3	7.5	8.2	8.3
3	M	7.3	7.7	8.0	7.8
9	M	7.4	7.5	8.0	7.8
15	M	7.4	7.0	7.5	7.7
0	F	6.3	6.4	7.0	7.0
3	F	6.6	6.7	6.9	7.0
9	F	6.4	6.3	6.6	6.4
15	F	6.3	5.9	6.2	6.2

TABLE 3

24-HOUR FOOD CONSUMPTION (g) IN DOGS

Dose (mg/kg)	Sex	Week 1	Week 2	Week 3	Week 4
0	M	523	416	457	429
3	M	568	501	504	432
9	M	239	468	298	434
15	M	195	368	372	460
0	F	296	485	442	410
3	F	399	492	411	405
9	F	181	470	383	428
15	F	165	269	367	423

TABLE 4

HEMATOLOGIC FINDINGS IN DOGS

Dose (mg/kg)	Sex	Total WBC	Total RBC	HGB	HCT	MCV	Platelets
0	M	15.5	6.6	15.2	45.3	68.8	462.2
3	M	10.9	6.3	15.0	44.9	71.2	407.5
9	M	11.3	6.1	13.9	41.9	68.8	452.8
15	M	9.4	6.3	14.3	42.5	66.8	426.0
0	F	10.7	6.8	15.5	46.4	67.7	457.4
3	F	11.7	6.5	15.2	45.2	70.0	458.8
9	F	11.1	6.9	15.3	46.0	67.0	401.0
15	F	9.7	6.5	15.2	45.3	69.3	398.8

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.

Clinical pathology. Clinical pathology data including hematology (Table 4), clinical chemistry (Table 5), and urinalyses showed no consistent changes which could be related to administration of the test article.

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.

Histopathology. Microscopically, diffuse mild trophy 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.

Rat Study

Clinical observations. A number of clinical signs of pharmacological and/or toxicological effects of the test article were observed (Table 6). The most significant of these clinical observations in the treated rats included hyperactivity, excitability, piloerection, exophthalmos, and salivation. The rats began to show clinical signs approximately 45 min after dosing and continued to show some of them prior to dosing on the following day.

Food consumption. The male rats generally consumed more food than the female rats (Table 7). The food consumption of all of the animals receiving the test article was depressed compared to the controls during the first week of the study. The food consumption of the low-dose animals was depressed less than the medium- and high-dose groups. By the second week of the study, food consumption of the dosed animals returned to normal; and in the case of the high-dose females, the females actually ate more food than the controls. By the third and fourth weeks, the food consumption of the various treatment groups were not significantly different.

Body weights. Both sexes receiving the test article failed to gain weight at the same rate as the controls (Table 8). The middle- and high-dose groups lost weight during the second

TABLE 5

CLINICAL CHEMISTRY DETERMINATIONS IN DOGS

Dose (mg/kg)	Sex	BUN	Total protein	Bilirubin	Glucose	Creatinine	SGPT	SGOT	Alkaline phosphatase	LDH	Calcium	Phos-phorus	Choles-terol	Albumin	Sodium	Chloride	Potas-sium
0	M	15.8	5.1	0.20	76.5	0.76	32.6	38.3	100.3	99.7	11.2	6.3	244.3	2.9	148.3	111.5	5.4
3	M	13.0	5.4	0.22	82.5	0.80	33.2	38.5	97.5	132.5	11.4	6.3	233.0	3.1	147.8	110.0	5.5
9	M	11.3	5.1	0.15	80.7	0.68	40.5	38.8	94.2	95.2	11.9	5.9	235.3	2.8	145.8	110.8	5.1
15	M	11.7	5.1	0.15	73.5	0.75	39.5	39.8	82.5	138.2	10.9	5.9	188.7	2.8	146.2	110.5	4.8
0	F	13.2	5.3	0.15	83.3	0.88	35.8	42.3	91.2	136.3	11.0	5.8	175.7	3.1	147.5	112.7	4.7
3	F	12.7	5.1	0.15	81.8	0.75	30.2	38.0	108.3	116.3	11.3	5.8	182.1	3.2	148.0	110.7	4.8
9	F	15.7	5.3	0.15	80.8	0.83	29.3	44.5	112.3	158.2	11.4	6.5	228.3	3.1	148.2	110.5	5.3
15	F	11.25	5.4	0.15	73.3	0.75	39.0	36.8	97.3	64.8	11.2	5.7	244.3	3.1	147.5	109.5	4.9

week of the study and did little more than regain the lost weight by the end of the study. Both sexes receiving the test article had a decreased body weight compared to the controls at the time of sacrifice. The effect for males appeared to be greater than for females.

Clinical pathology. Clinical pathology variables showing a trend to *decrease* with increasing dose levels of the test article included urinary pH, lymphocytes in the white blood cell differential, blood urea nitrogen, glucose, creatinine (females), LDH (females), chloride, and possibly albumin (Tables 9 and 10). Clinical pathology variables showing a trend to *increase* with increasing dose levels of the test article included total white blood cell count, creatinine (males, possibly), LDH (males, possibly), phosphorus, and possibly uric acid (Tables 9 and 10). Sodium increased significantly at the low and medium doses but not at the high dose.

Gross pathology. A small number of incidental gross lesions unrelated to treatment were seen at necropsy. No treatment-related lesions were seen.

Organ weights. Organ weight and organ weight to body weight variables that differed significantly between the control and treated animals included decreased liver weight for the males, decreased kidney weight for the males, increased kidney weight to body weight ratio for both sexes, increased brain to body weight ratio for both sexes, and increased testis to body weight ratio in the males.

Histopathology. A variety of histopathology lesions were seen which were not attributed to the administration of the test article. These lesions were present in both control and treated animals or occurred in isolated animals. These included calculi; hyperplasia and inflammation in the urinary bladder of one high-dose female animal; ultimobranchial cysts in the thyroid; atrophy of the thymus; inflammation of the epididymis; atrophy of the testes; inflammation of the coagulating gland and the prostate; cyst in the ovary; congestion, hyperplasia, hemosiderin

TABLE 6

CLINICAL SIGNS SUMMARY FOR RATS

Group (mg/kg): Clinical Sign	Males				Females			
	0	10	50	100	0	10	50	100
Hyperactivity	0/10	0/10	4/10	10/10	0/10	0/10	10/10	10/10
Excitability	0/10	8/10	10/10	10/10	0/10	6/10	10/10	10/10
Piloerection	0/10	10/10	10/10	10/10	0/10	10/10	10/10	10/10
Anogenital area urine stained	0/10	0/10	0/10	6/10	0/10	0/10	0/10	5/10
Ocular discharge	0/10	0/10	2/10	4/10	0/10	0/10	2/10	6/10
Exophthalmos	0/10	10/10	10/10	10/10	0/10	10/10	10/10	10/10
Salivation	0/10	2/10	10/10	10/10	0/10	2/10	10/10	10/10
Nasal discharge	0/10	1/10	3/10	6/10	0/10	0/10	4/10	7/10
Vocalization	0/10	0/10	1/10	1/10	0/10	0/10	0/10	0/10
Rough hair coat	0/10	0/10	0/10	0/10	0/10	0/10	1/10	1/10
Aggression	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10
Polyuria	0/10	0/10	0/10	2/10	0/10	0/10	0/10	0/10

pigment, and plasmacytosis of lymph nodes; lymphocytic infiltrates in the lungs and livers; and mineralization, hydronephrosis, calculi, infarct, inflammation, and chronic progressive nephropathy of the kidneys. These lesions are considered to represent the normal spectrum of spontaneous lesions in rats. No treatment-related microscopic lesions were evident.

DISCUSSION

This study was undertaken to evaluate the clinical, morphological, neuropathological, and clinical pathological effects in both rats and dogs administered MDMA daily for a 28-day period. Ricaurte *et al.* (1985) reported that methylenedioxyamphetamine selectively destroyed serotonin nerve terminals in the rat and produced long-lasting reductions in the level of rat brain serotonin, the number of serotonin uptake sites, and the concentration of 5-hydroxyindoleacetic acid. MDMA is structurally very similar to MDA and it was believed that MDMA might produce neuropathological effects. Due to the increasingly widespread use of MDMA as a recreational drug and its attendant media coverage, and out of concern for the potential damage to the public health, the Drug Enforcement Administration elected to invoke its emergency scheduling powers and on July 1, 1985, placed MDMA in Schedule I of the Controlled Substances Act (Lawn, 1985).

TABLE 7

WEEKLY FOOD CONSUMPTION (g) IN RATS

Dose mg/kg)	Sex	Week 1	Week 2	Week 3	Week 4
0	M	214	229	217	221
10	M	200	213	213	219
50	M	146	190	184	217
100	M	149	212	206	212
0	F	173	169	166	165
10	F	142	152	144	155
50	F	114	173	159	164
100	F	107	195	168	160

Schedule I drugs are those that have been determined to have a high potential for abuse and no recognized medical uses (Seymour, 1986). These include heroin, other nonclinical opiate and opioid pain killers, nonclinical stimulants, and a number of psychotomimetic hallucinogens, including LSD, mescaline, and MDA (Seymour, 1986). Psychotherapists argue that MDMA does have some

TABLE 8

BODY WEIGHT (g) IN RATS

Dose (mg/kg)	Sex	Week 1	Week 2	Week 3	Week 4
0	M	312	361	400	410
10	M	307	338	370	383
50	M	310	300	322	325
100	M	308	299	322	331
0	F	227	240	256	262
10	F	222	226	240	258
50	F	223	216	223	229
100	F	219	211	224	228

recognized medical uses (Greer, 1985). Prior to its classification in Schedule I, MDMA was used as an adjunct to psychotherapy for improvement in interpersonal relationships, couples therapy, and traumatic experiences and in terminal cancer (Greer, 1985). Greer (1985) also stated that MDMA did not lend itself to overuse, since its most desirable effects appeared to diminish with frequency of use.

Yeh *et al.* (1986) injected male Sprague–Dawley rats subcutaneously with saline, MDA, or MDMA twice a day for 4 consecutive days. The animals were sacrificed 2 weeks following the last injection. MDA caused dramatic decreases in serotonin and 5-hydroxyindoleacetic acid in frontoparietal cortex, hippocampus, and hypothalamus. MDMA produced similar but less dramatic changes. In parallel with these changes, significant decreases in the radioactive labeled serotonin uptake sites were demonstrated.

Champney *et al.* (1986) injected male Sprague–Dawley rats with either saline or various dose levels of MDMA intraperitoneally. The animals were killed 4 hr later. The authors reported that both 5-hydroxyindoleacetic acid and serotonin were reduced after a single injection of MDMA and that these depressions may be region specific.

Slikker *et al.* (1986) administered MDMA orally by gavage to adult Sprague–Dawley rats at either 40 or 80 mg/kg once every 12 hr for 4 days. These rats were sacrificed 2 weeks after the first dose with an additional group of rats (80 mg/kg) sacrificed 4 weeks after the first dose. MDMA resulted in a decrease in both serotonin and 5-hydroxyindoleacetic acid in both the caudate nucleus and the hippocampus. The levels of both neurotransmitters remained reduced 4 weeks after the first dose, suggesting that the reductions may be lasting.

O'Hearn *et al.* (1986) administered MDA and MDMA to rats subcutaneously at a dose level of 20 mg/kg every 12 hr for 4 days. The rats were sacrificed 2 weeks later. They reported that MDMA and more markedly MDA produced profound reductions in the

TABLE 9

HEMATOLOGIC FINDINGS IN RATS

Dose (mg/kg)	Sex	Total WBC	Total RBC	HGB	HCT	MCV	Platelets
0	M	6.12	6.96	13.8	40.0	57.2	1067.8
10	M	5.22	7.17	14.5	41.4	57.4	1025.4
50	M	5.29	7.13	13.9	40.2	56.0	1061.7
100	M	7.08	7.20	14.0	41.3	57.2	1253.3
0	F	2.55	6.69	13.2	40.5	60.4	1154.7
10	F	2.19	6.89	13.6	41.3	59.7	1104.4
50	F	3.56	6.45	13.0	38.9	60.1	1138.8
100	F	3.26	6.54	13.2	39.5	60.2	1129.9

TABLE 10

CLINICAL CHEMISTRY DETERMINATIONS IN RATS

Dose (mg/kg)	Sex	BUN	Total protein	Bilirubin	Glucose	Creatinine	SGPT	SGOT	Alkaline phosphatase	LDH	Calcium	Phosphorus	Cholesterol	Albumin	Sodium	Chloride	Potassium
0	M	19.4	5.6	0.19	133.5	0.66	52.8	185.6	210.1	1493	10.3	9.2	72.0	2.88	146.1	102.0	5.6
10	M	17.9	5.5	0.17	136.4	0.69	80.4	206.6	256.9	1633	10.7	10.5	70.1	3.10	147.4	101.4	5.7
50	M	16.0	5.7	0.18	125.1	0.74	66.3	210.1	240.6	1937	10.8	12.9	71.4	2.92	148.6	98.8	6.0
100	M	16.2	5.5	0.18	109.5	0.73	71.4	216.1	277.9	1885	10.8	12.6	65.9	2.86	147.3	99.6	6.4
0	F	20.0	5.8	0.15	108.1	0.73	51.4	185.3	160.9	1895	11.1	8.5	68.5	3.43	147.5	100.5	5.2
10	F	18.6	5.7	0.22	126.6	0.78	52.5	189.6	182.5	1748	11.2	9.7	68.8	3.24	149.8	101.8	5.4
50	F	18.8	5.5	0.21	125.9	0.65	57.5	180.7	206.9	1677	11.1	10.0	72.2	3.12	149.8	101.4	5.7
100	F	17.4	5.7	0.22	113.7	0.64	61.4	193.6	187.3	1544	11.3	9.5	66.0	3.13	146.4	98.4	5.4

density of serotonin axon terminals and was most evident in the cerebral cortex, thalamus, olfactory bulb, and striatum but also in the hippocampus, hypothalamus, and septum.

In the present study, neither the rat brains nor the dog brains were examined for levels of neurotransmitters. The authors can only speculate that serotonin and 5-hydroxyindoleacetic acid would have been reduced in the rat based upon the results of other studies. There have been no similar studies in dogs or other species to date.

MDMA has been referred to as a "designer" drug although it was first synthesized in 1914 (Seymour, 1986). MDMA became newsworthy in the 1980s under the name of "ecstasy." MDMA was studied in animals as early as 1953–1954 (Hardman *et al.,* 1973). MDMA was one of eight compounds (including mescaline, DMPEA, MDPEA, MDA, DMA, TMA, and α-ethyl-MDPEA) studied in five animal species (mouse, rat, guinea pig, dog, and monkey). The study reported that MDMA was one of the more toxic compounds studied. The average LD50s given for MDMA were 97, 49, and 98 mg/kg for the mouse, rat, and guinea pig, respectively.

Braun *et al.* (1980) studied the effects of MDMA and a large collection of *N*-substituted homologs on the analgesic potency and enhancement of motor activity in mice. MDMA was reported to be the most potent analgesic but was not particularly effective as a motor stimulant. In the present study, MDMA resulted in hyperactivity in the dogs and hyperactivity and excitability in the rats.

Both the dogs and the rats in the present study ate less food and either failed to gain or lost weight early in the study. This was not unexpected since MDMA has been considered to be an appetite depressant and also because it is an amphetamine.

A number of histopathologic and clinical pathologic changes occurred in the study. The clinical pathologic changes were most evident in the rat. The significance of these changes is not known. The histopathologic changes in the rats were considered to be within the normal

range and were not considered to be treatment related. The testicular atrophy in the dogs may have been related to treatment of MDMA. With only three dogs per group, a conclusive statement cannot be made. It is of significance that neuropathological changes were not evident in either species, since that was the primary reason for performing the study. It may be that more sophisticated methods such as the silver Fink–Heimer stain (Fink and Heimer, 1967), which was used by Ricaurte in demonstrating certain MDA-induced changes in the CNS (Ricaurte *et al.*, 1985), may be necessary for the demonstration of neuropathological changes.

In summary, MDMA *may* have a use as a psychotherapeutic adjunct in the treatment of certain psychological problems in humans. There have been, as of yet, no well controlled, carefully supervised studies in humans. The lack of demonstrated histopathologic lesions in the central nervous system of the dog and the rat observed in the present studies does not preclude the possibility of lesions in the central nervous system of humans. Additional work is needed in both animals and humans if the true value and risks of MDMA are be determined.

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REFERENCES

BRAUN, U., SHULGIN, A. T., AND BRAUN, G. (1980). Prufung auf zentral aktivitat und analgesie von *N*-substituierten analogen des amphetamin-derivates 3,4-methylenedioxypeunylisopropylamine. *Arzneim. Forsch.* **30,** 825–830.

CHAMPNEY, T. H., GOLDEN, P. T., AND MATTHEWS, R. T. (1986). Reduction in hypothalamic serotonin levels after acute MDMA administration. *Soc. Neurosci. Abst.* **12,** 363.

FINK, R. P., AND HEIMER, L. (1967). Two methods for selective silver impregnation of degenerating axons and their synaptic endings in the central nervous system. *Brain Res.* **4,** 369–374.

GREER, G. (1985). Using MDMA in psychotherapy. *Advances* **2,** 57–59.

HARDMAN, H. F., HAAVIK, C. O., AND SEEVERS, M. H. (1973). Relationship of the structure of mescaline and seven analogs to toxicity and behavior in five species of laboratory animals. *Toxicol. Appl. Pharmacol.* **25,** 299–309.

LAWN, J. C. (1985). Schedules of controlled substances: Temporary placement of 3,4-methylenedioxymethamphetamine (MDMA) in Schedule I. *Fed. Regist.* **50,** 106.

O'HEARN, E., BATTAGLIA, G., DESOUZA, E. B., KUBAR, M. J., AND MOLLIVER, M. E. (1968). Systemic MDA and MDMA, psychotropic substituted amphetamines. *Soc. Neurosci. Abst.* **12,** 1233.

RICAURTE, G., BRYAN, G., STRAUSS, L., SEIDEN, L., AND SCHUSTER, C. (1985). Hallucinogenic amphetamine selectively destroys brain serotonin nerve terminals: Neurochemical and anatomical evidence. *Science* **229,** 986–988.

SLIKKER, W., JR., ALI, S. F., SCALLET, A. C., AND FRITH, C. H. (1986). Methylenedioxymethamphetamine (MDMA) produces long lasting alterations in the serotonergic system of the brain. *Soc. Neurosci. Abst.* **12,** 363.

WINER, B. J. (1971). *Statistical Principles in Experimental Design,* 2nd ed. McGraw-Hill, New York.

YEH, S. H., BATTAGLIA, G., O'HEARN, E., MOLLIVER, M. E., KUHAR, M. J., AND DE SOUZA, E. B. (1986). Effects of MDA and MDMA (ecstasy) on brain monoaminergic systems: *In vivo* studies. *Soc. Neurosci. Abst.* **12,** 1234.