

**UNITED STATES AIR FORCE
ARMSTRONG LABORATORY**

**Range-Finding Study for a
Reproductive Screen of Modular
Artillery Charge System
(XM231/XM232) Administered in the
Diet of Sprague-Dawley Rats**

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER



TERRY A. CHILDRRESS, Lt Col, USAF, BSC
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PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted at the Toxic Hazards Research Unit, ManTech Environmental Technology, Inc. This document serves as a final report on the range-finding study for a subsequent, more definitive reproduction study of Modular Artillery Charge System (XM231/XM232) administered in the diet of Sprague-Dawley rats. The research described in this report began in February 1995 and was completed in March 1995 under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. A08). Lt Col Terry A. Childress served as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory. This study was sponsored by the U.S. Army under the direction of LTC Daniel J. Caldwell, USAMRD/WRAIR.

TABLE OF CONTENTS

SECTION	PAGE
Preface.....	2
List of Tables.....	4
Abbreviations.....	5
I INTRODUCTION.....	6
II MATERIALS AND METHODS.....	9
Test Agent and Doses.....	9
Diet Preparation, Homogeneity, Stability, and Analysis.....	9
Test Animals, Group Assignments, Clinical Measurements.....	10
III RESULTS.....	11
Diet Preparation and Stability.....	11
General Toxicity.....	11
IV DISCUSSION.....	12
V REFERENCES.....	13
TABLES.....	16-22
Appendix A.....	23
Appendix B.....	24

LIST OF TABLES

PAGE

TABLE 1	Mean Food Consumption of Male Rats Treated with MACS for Three Weeks	16
TABLE 2	Mean Food Consumption of Female Rats Treated with MACS for Three Weeks	17
TABLE 3	Dosages of Rats Treated with MACS for Three Weeks	18
TABLE 4	Body Weights (g) of Rats Treated with MACS for Three Weeks	19
TABLE 5	Absolute (g) and Relative Organ Weights of Male Rats Treated with MACS for Three Weeks	20
TABLE 6	Absolute (g) and Relative Organ Weights of Female Rats Treated with MACS for Three Weeks	21
TABLE 7	Methemoglobin Values of Rats Following Three Weeks Treatment with MACS	22

ABBREVIATIONS

μ l	Microliter
C	Celcius
g	Gram
GTN	Glyceryl trinitrate
h	Hour
IP	Intraperitoneal
kg	Kilogram
LD ₅₀	Median lethal dose
METHB	Methemoglobin
mg	Milligram
min	Minute
mL	Milliliter
mm	Millimeter
N	Number
NC	Nitrocellulose
nm	Nanometer
NQ	Nitroguanidine
P	Probability
qt	Quart
rpm	Revolutions per minute
SIDS	Screening Information Data Set
SEM	Standard error of the mean
THB	Total hemoglobin
TNB	1,3,5-trinitrobenzene

SECTION I

INTRODUCTION

Modular Artillery Charge System (MACS) is a propellant under development by the US Army for autoloading howitzer artillery, consisting of a single increment of propellant charge contained within a rigid combustible casing. This technology will simplify autoloader requirements, result in a significant reduction in propellant production and logistics requirements, and eliminate propellant waste. The MACS propellant is a granular mixture of nitrocellulose (NC), nitroglycerin (GTN; glyceryl trinitrate), nitroguanidine (NQ), ethylcentralite (N,Ndiethyl, N,Ndiphenylurea), cryolite, and graphite. Since NC, GTN, and NQ make up >98% of MACS propellant, it is commonly referred as "triple-based" propellant mixture.

Nitroguanidine is a primary component (approximately 47%) of the triple-based propellant mixture. Previously conducted acute oral and percutaneous studies on NQ found the compound to be relatively nontoxic. The compound was not irritating to either rabbit skin or eyes using the standard Draize technique (Hiatt et al., 1986; Morgan et al., 1986). Nitroguanidine did not kill rats by oral gavage at a limit test dose of 5 g/kg (Brown et al., 1988), while the LD₅₀s for male and female mice are reported as 5.0 g/kg and 4.3 g/kg, respectively (Hiatt et al., 1988).

Nitroguanidine did not produce treatment-related effects when administered to Sprague-Dawley rats at doses as high as 1000 mg/kg/diet for 14 days. However, after 90 days that dose level produced a decrease in body weight gain and food consumption (Reddy and Korte, 1988). No evidence of developmental toxicity in rats or rabbits was reported at doses of 1000 mg/kg diet.

Nitroglycerin is also a component (approximately 22%) of the triple-based propellant. The toxic, physiologic, and pharmacologic effects of GTN have been studied extensively in animals and man (NIOSH, 1978). Nitroglycerin is readily absorbed by ingestion, inhalation, sublingual contact, and through the skin. When both inhalation and dermal exposure to GTN occur, dermal contact is thought to make the major contribution to the total amount absorbed by the body (NIOSH, 1978). Vasodilation following exposure to GTN can occur within minutes, regardless of route of exposure. This effect has led to sudden death and chronic cardiac disease (Carmichael and Lieben, 1963). Small amounts can cause headaches which can persist for hours or days. Larger

doses can cause hypotension, cyanosis, and methemoglobinemia. These effects are potentiated by alcohol consumption (NIOSH, 1978). Symptoms also include nausea and vomiting, and in some instances, diarrhea. The mouse IP LD₅₀ of GTN is 194 mg/kg (Kylin et al., 1964) and the rat IP LD₅₀ has been reported as 93 mg/kg (Burginson et al., 1962).

The third major component (approximately 28%) of the triple-based propellant is nitrocellulose or cellulose nitrate. Cellulose nitrate is relatively nontoxic but is known for its extreme flammability (Montgomery, 1982). Dry NC may explode when subjected to heat or sudden shock; NC is therefore generally handled wet with either water or alcohol. Toxic combustion products are generated when the compound is ignited, primarily carbon monoxide and oxides of nitrogen (Montgomery, 1982).

Munition workers exposed to nitrate containing explosives have experienced skin irritation, liver damage, and anemia (Hathaway, 1977; Morton et al., 1976; Stewart et al., 1945). These compounds have also been found to cause methemoglobin formation, liver and spleen hypertrophy, and degeneration of the seminiferous tubules resulting in decreased spermatogenesis (Cody et al., 1981; Levine et al., 1983, 1984; Furedi et al., 1984a,b). A recently completed reproductive screen in this laboratory with 1,3,5-trinitrobenzene (TNB), resulted in testicular atrophy and decreased spermatogenesis in the male rats. Female rats displayed clinical signs of altered locomotion during the postpartum period and both sexes had brain lesions at necropsy (Kinkead et al., 1994a,b). Neurotransmitter analyses showed statistically significant increase in norepinephrine, epinephrine, serotonin and dopamine in the TNB-treated female rats (Narayanan et al., 1995). Changes in neurotransmitter levels in specific regions may be one of the mechanisms responsible for TNB-induced neurological disorder. A reproductive screen on another nitrate containing explosive compound, liquid propellant XM46, resulted in hemolytic anemia (Kinkead et al., 1994c). Loss of embryos and/or death of fetuses were observed following treatment with ammonium dinitramide indicating that this compound is a reproductive toxicant (Kinkead et al., 1994d).

Nitrate-containing explosive compounds have produced adverse reproductive effects in both male and female rats. Testicular atrophy and decreased spermatogenesis are common in male rats; lack of live litter production has been noted in female rats. Brain lesions and loss of motor

skills, methemoglobinemia, and hemolytic anemia are common findings in the reproductive screens that were performed within this laboratory with nitrate-containing explosives.

An objective of this range-finding study was to determine the dose levels in the diet that rodents could tolerate and to provide information on clinical signs, methemoglobin formation, and possible target organs. Data from this range-finding study was used to determine dose levels for a 90-day modified Screening Information Data Set (SIDS) protocol that was used to evaluate the developmental and reproductive toxicity of MACS in rats.

SECTION II

MATERIALS AND METHODS

Test Agent

The MACS was provided by the U.S. Army, Picatinny Arsenal, NJ. The pellets were ground to granular form before receipt. Pertinent chemical and physical properties of the test compound are listed below:

MACS

Synonyms:	Smokeless powder Modular Artillery Charge System XM231/XM232
CAS#:	None assigned
Specific gravity:	1.5880
Vapor pressure:	Negligible
Appearance:	Hard cylindric pellet white to reddish orange, coated with graphite

Diet Preparation, Homogeneity, Stability, and Analysis

MACS was administered by the oral route, mixed appropriately in the diet. The test material was mixed in powdered Purina Formulab # 5002 (Ralston Purina, St. Louis MO) certified rodent diet meal. The high concentration diet was prepared by adding 20g MACS to 10 kg rodent diet to produce a diet concentration of 2g MACS/kg diet. Diet concentration of 1.0, 0.5 and 0.25 g MACS/kg were made by serial dilutions adding weighed amounts of feed to equal amounts of prepared diet. Each batch of diet was mixed for 30 minutes.

The MACS analysis was performed using a gas chromatographic method described in Appendix A. Total MACS concentrations were based on nitroguanidine concentrations measured in five gram samples of diet.

To assess the efficiency of the mixing process, analyses were performed on samples taken from the top, middle and bottom of the mixing bowl. Duplicate samples were taken for each measurement. Because the diets could be stored in polyethylene containers for as long as 3 weeks

or in the feed jars for up to 7 days, stability of the diets was measured over those time frames. Duplicate samples were measured for each.

Test Animals, Group Assignments, Clinical Measurements

Fifteen male and 15 female Sprague-Dawley-derived outbred albino rats [CrI:CD®BR] known as Charles River CD-rats, were purchased from Charles River Breeding Laboratories, Raleigh, NC. The rats were 9 weeks of age upon arrival and 11 weeks of age at initiation of the treatment period. All rats were identified by tail tattoo and were subjected to a two-week acclimatization period. Water from a reverse-osmosis system and feed were available *ad libitum* to the animals. Animal rooms were maintained on a 12-h light/dark cycle (fluorescent light) and targeted at a temperature of 23 ± 2 °C with a relative humidity of $55 \pm 15\%$.

The rats were single housed in clear plastic cages with hardwood-chip bedding (Bettachip®, Northeastern Products Corp. Warrensburg, NY). There were five study groups with target diet concentrations of 0.0, 0.25, 0.5, 1.0, and 2.0 g MACS/kg diet. Feed jars were cleaned on a weekly basis at such time all leftover diet was discarded. Rats were assigned to groups of three per sex by means of a computer-generated randomization, stratified by body weight such that the mean body weight of all groups were homogeneous by statistical analysis at study initiation.

Rats were observed twice daily for signs of toxic stress. Body weights were measured weekly, food consumption was measured and doses were calculated as mg MACS/kg body weight/day.

All rats received treated or control diet for 21 days and were necropsied on day 22. Animals were not fasted prior to necropsy. Blood was collected via the vena cava for hemoglobin and methemoglobin determinations. Wet weights were recorded for liver, kidney, testes, brain, and spleen. Animals were examined carefully for gross lesions.

Body weights, organ weights, food consumption, hemoglobin, methemoglobin and MACS dose calculations were treated for statistical significance using a one-factorial analysis of variance with Bonferroni multiple comparisons (Rosner, 1990). Significant differences were inferred when $p < 0.1$.

SECTION III

RESULTS

Diet Preparation and Stability

Diet target concentrations were 2.0, 1.0, 0.5, and 0.25 g MACS/kg diet. Measured diet concentration were 1.98, 0.95, 0.47 and 0.2 g MACS/kg diet. Analyses of samples taken from the top, middle and bottom of the diet preparation bowl for homogeneity determined that the test material was uniformly distributed throughout the diet in the high and moderate level concentrations (means per measurement level within 15%). The concentrations per level of the mid and low level showed a greater variation (Appendix B).

Analysis of the diet taken from feed jars and polyethylene storage containers showed no degradation in MACS concentration over the 7 to 21 day time period. Samples were removed from the middle of the containers and represented time periods diet would be stored in either container.

General Toxicity

No mortality occurred during the three-week study. Food consumption did not differ between the treatment groups of either sex (Tables 1 and 2). Based on the diet consumption rate male rats received 141, 71, 35, and 19 mg MACS/kg/day and female rats received 154, 81, 40, and 20 mg MACS/kg/day (Table 3). Mean body weights of male and female treated rats did not differ significantly from their respective control groups at any of the measurement periods during the study (Table 4).

Absolute and relative organ weights of male rats treated with MACS were compared with controls. There were no statistically significant differences (table 5). Absolute organ weights of the female rats did not differ from controls; however, relative liver weights of the high-dose female group were significantly greater than those of the respective control group (Table 6). Total hemoglobin and methemoglobin concentrations measured at necropsy indicated no differences between treated and control rats (Table 7). At necropsy, all rats utilized in this study were in good general condition. No lesions were noted during the gross pathologic examination and no tissues were removed for further evaluation.

SECTION IV

DISCUSSION

Diet concentrations measured in the total mix were well within the expected values, $< \pm 6\%$ of target concentrations. The homogeneity analysis showed a variation between the levels measured in the mixing bowl. One of the reasons for this is that the test compound is of irregular granular form and has particles of varying sizes. A relatively large granule in a small feed sample (5 grams) may result in an abnormally high concentration value. The measurements of the total mix is more representative of the actual concentration of the diet the rodents are received. However, to ensure complete mixing, the length of time for mixing of diet for the reproductive screening study will be increased from 30 to 90 minutes per batch.

The purpose of this study was to establish dose levels for use in a 90-day modified SIDS reproductive study. The only effect of note in this range-finder was the increase ($p < 0.1$) in the relative liver weights of the high-dose female rats. Because the liver is naturally stressed during pregnancy and increases greatly in relative size, this effect was considered to be of potential toxicologic importance. Based on the increased liver weights, diet concentrations used in the reproductive study are 2.0, 1.0, 0.2, and 0.0 g. The high diet concentration (2.0 g/kg) is equivalent to an approximate concentration level of 1.0 g nitroguanidine/kg diet, a level that resulted in decreased body weight gains in a 90-day feeding study (Reddy and Korte, 1988).

REFERENCES

- Brown, L.D., C.R. Wheeler, and D.W. Korte.** 1988. Oral Toxicity of Nitroguanidine in Male and Female Rats. Letterman Army Institute of Research, LAIR Report No. 264. Presidio of San Francisco, San Francisco, CA.
- Burginson, Lu, Crowley and Kranth.** 1962. Studies on a new coronary vasodilator, 1-chloro-2,3-propanediol dinitrate. *Angiology* 13: 412.
- Carmichael, P. and J. Lieben.** 1963. Sudden Death in Explosive Workers. *Archives of Environmental Health* 7: 50-65.
- Cody, T.E., S. Witherup, L. Hastings, K. Stemmer, and R.T. Christian.** 1981. 1,3-Dinitrobenzene: Toxic effect *in vivo* and *in vitro*. *J. Toxicol. Environ. Health* 7(5):829-847.
- Furedi, E.M., B.S. Levine, D.E. Gordon, V.S. Rac, and P.M. Lish.** 1984a. *Determination of the chronic mammalian toxicologic effects of TNT (Twenty-four month chronic toxicity/carcinogenicity study of trinitrotoluene (TNT) in the Fischer 344 rat)*. Final Report-Phase III, Vol. 1. IIT Research Institute, Project No. L6116, Study No. 11, Chicago, IL. DAMD17-79-C-9120. AD-A168 754.
- Furdei, E.M., B.S. Levine, J.W. Sagartz, V.S. Rac, and P.M. Lish.** 1984b. *Determination of the chronic mammalian toxicologic effects of TNT (Twenty-four month chronic toxicity/carcinogenicity study of trinitrotoluene (TNT) in the B6C3F₁ hybrid mouse)*. Final Report-Phase IV, Vol. 1. IIT Research Institute, Project No. L6116, Study No. 11, Chicago, IL. DAMD17-79-C-9120. AD-A168 754.
- Hathaway, J.A.** 1977. Trinitrotoluene: A review of reported dose-related effects providing documentation for a workplace standard. *J. Occup. Med.* 19(5):341-345.
- Hiatt, G.F.S., S.K. Sano, and D.W. Korte.** 1986. Primary Eye Irritation Potential of Nitroguanidine in Rabbits. Letterman Army Institute of Research, AD-A164675. Presidio of San Francisco, San Francisco, C.A.
- Hiatt, G.F.S., S.K. Sano, C.R. Wheeler, and D.W. Korte.** 1988. Acute Oral Toxicity of Nitroguanidine in Mice. Letterman Army Institute of Research, LAIR Report No. 265. Presidio of San Francisco, San Francisco, CA.
- Kinkead, E.R., R.E. Wolfe, S.A. Salins, C.D. Flemming, D.J. Caldwell, C.R. Miller, and J.R. Latendresse.** 1994a. Range-Finding Study for a Reproductive Assessment of 1,3,5-Trinitrobenzne Administered in the Diet of Sprague-Dawley Rats. AL-OE-TR-1994-0072 /WRAIR/TR-94-0006 Wright-Patterson Air Force Base, OH: Armstrong Laboratory and Walter Reed Army Institute of Research.

Kinkead, E.R., R.E. Wolfe, C.D. Flemming, D.J. Caldwell, C.R. Miller, and G.B. Marit. 1994b. Reproductive Toxicity Screen of 1,3,5-Trinitrobenzene Administered in the Diet of Sprague-Dawley Rats. AL-OE-TR-1994-0144/WRAIR-TR-94-0016. Wright-Patterson Air Force Base, OH: Armstrong Laboratory and Walter Reed Army Institute of Research.

Kinkead, E.R., R.E. Wolfe, S.A. Salins, C.D. Flemming, H.F. Leahy, D.J. Caldwell, C.R. Miller, and G.B. Marit. 1994c. Reproductive Toxicity Screen of Liquid Propellant XM46 Administered in the Drinking Water of Sprague-Dawley Rats. AL-OE-TR-1994-0101/WRAIR/TR-94-0008. Wright-Patterson Air Force Base, OH: Armstrong Laboratory and Walter Reed Army Institute of Research.

Kinkead E.R., R.E. Wolfe, C.D. Flemming, H.F. Leahy, D.J. Caldwell, C.R. Miller, and G.B. Marit. 1994d. Reproductive Toxicity Screen of Ammonium Dinitramide Administered in the Drinking Water of Sprague-Dawley Rats. AL-OE-TR-1994-0162/WRAIR/TR-94-0015. Wright-Patterson Air Force Base, OH: Armstrong Laboratory and Walter Reed Army Institute of Research.

Kylin, B., A. Englund, H. Ehiner-Samuel, and S. Yllner. 1964. A Comparative Study on the Toxicology of Nitroglycerin, Nitroglycol and Propylene Glycol Dinitrate. *International Congress of Occup. Health*. Proc. 15th. Vienna. 3: 191-195.

Levine, B.S., J.H. Rust, J.M. Burns, and P.M. Lish. 1983. *Determination of the chronic mammalian toxicological effects of TNT (Twenty-six week subchronic oral toxicity study of trinitrotoluene (TNT) in the beagle dog)*. Phase II, Final Report, IIT Research Institute, Report No. L6116, Study No. 5, Chicago, IL. DAMD 17-79-C-9120, AD-A157 082.

Levine, B.S., E.M. Furedi, D.E. Gordon, P.M. Lish, and J.J. Barkely. 1984. Subchronic toxicity of trinitrotoluene in Fischer 344 rats. *Toxicology* 32:253-265.

Montgomery, Ruth. 1982. Polymers. In: *Patty's Industrial Hygiene and Toxicology*. 3rd Ed., IIc. pp. 385-4386.

Morgan, E.W., S.K. Sano, and D.W. Korte. 1986. Primary Dermal Irritation Potential of Nitroguanidine in Rabbits. Letterman Army Institute of Research, LAIR Report No. 220. Presidio of San Francisco, San Francisco, CA.

Morton, A.R., M.V. Ranadive, and J.A. Hathaway. 1976. Biological effects of trinitrotoluene from exposure below the threshold limit value. *Am. Ind. Hyg. Assoc. J.* 37(1):56-60.

Narayanan, L., D.J. Caldwell, and C.R. Miller. 1995. Alteration in Neurotransmitters and Their Metabolite Levels in 1,3,5-Trinitrobenzene-Treated Rats. AL-TR-1995- ; WRAIR/TR-95 Wright-Patterson Air Force Base, OH: and Walter Reed Army Institute of Research.

NIOSH. 1978. Criteria for a Recommended Standard. *Occupational Exposure to Nitroglycerin and Ethylene Glycol Dinitrate*.

Reddy, G. and D.W. Korte. 1988. Mammalian Toxicity Studies with Nitroguanidine. *Proceedings of the 13th Annual Environmental Quality R&D Symposium.* pp. 436-449.

Rosner, B. 1990. *Fundamentals of Biostatistics.* PWS-Kent: Boston, MA.

Stewart, A., L.T. Witts, G. Higgins, et al. 1945. Some early effects of exposure to trinitrotoluene. *Br. J. Ind. Med.* 2:74-82.

TABLE 1. MEAN FOOD CONSUMPTION^a (G) OF MALE RATS TREATED WITH MACS FOR THREE WEEKS

Day	Diet Concentrations				
	Control	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
-11	25.17 ± 0.62	25.37 ± 1.66	25.43 ± 1.90	25.30 ± 2.38	26.20 ± 0.87
-8	29.03 ± 0.92	29.20 ± 1.36	30.50 ± 1.85	28.03 ± 1.81	29.67 ± 1.70
-6	31.47 ± 1.64	32.10 ± 2.2	32.50 ± 1.40	31.13 ± 1.90	32.60 ± 1.86
-4	30.43 ± 0.85	34.30 ± 0.80	32.90 ± 2.62	29.63 ± 0.73	32.03 ± 3.43
-1	27.40 ± 0.95	30.80 ± 1.50	30.70 ± 3.05	29.30 ± 1.23	30.80 ± 2.48
2	29.77 ± 0.45	31.40 ± 0.79	30.13 ± 1.99	28.60 ± 1.17	31.80 ± 3.04
4	29.93 ± 0.69	32.83 ± 0.62	29.90 ± 2.05	31.50 ± 2.22	30.87 ± 2.83
6	29.50 ± 1.08	33.47 ± 0.92	30.37 ± 2.15	29.20 ± 1.40	27.10 ± 1.76
8	29.40 ± 0.45	32.33 ± 0.62	31.57 ± 2.23	30.10 ± 0.87	31.13 ± 2.25
10	29.60 ± 0.69	33.43 ± 1.92	28.03 ± 0.89	29.53 ± 1.52	28.30 ± 1.58
12	31.40 ± 1.37	33.87 ± 1.92	31.83 ± 2.23	30.10 ± 0.87	31.13 ± 2.25
14	30.40 ± 0.10	32.80 ± 2.06	32.37 ± 2.11	29.27 ± 2.13	31.10 ± 3.48
16	31.70 ± 1.25	35.03 ± 1.68	28.07 ± 3.02	29.40 ± 1.73	29.33 ± 2.56
18	31.73 ± 0.72	32.90 ± 5.48	32.20 ± 1.14	29.13 ± 1.13	28.50 ± 1.70
20	30.13 ± 1.05	36.50 ± 0.80	29.33 ± 2.97	30.13 ± 0.82	31.50 ± 2.69

^a Mean ± SEM, N=3.

TABLE 2. MEAN FOOD CONSUMPTION^a (G) OF FEMALE RATS TREATED WITH MACS FOR THREE WEEKS

Day	Diet Concentrations				
	Control	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
-11	17.30 ± 1.38	17.63 ± 1.37	17.40 ± 1.40	15.83 ± 2.19	18.83 ± 1.42
-8	18.23 ± 0.42	19.53 ± 2.19	20.27 ± 0.69	19.53 ± 2.27	19.53 ± 2.07
-6	19.10 ± 0.36	20.87 ± 3.12	21.67 ± 2.33	18.47 ± 1.88	20.60 ± 1.11
-4	22.07 ± 2.08	22.23 ± 1.89	21.93 ± 0.58	22.57 ± 3.21	20.13 ± 1.59
-1	18.07 ± 1.02	20.37 ± 1.56	18.93 ± 1.23	19.63 ± 2.15	19.57 ± 0.58
2	18.97 ± 0.37	20.33 ± 0.72	18.70 ± 1.66	18.60 ± 1.93	18.60 ± 1.30
4	19.67 ± 1.09	21.23 ± 3.24	19.23 ± 1.21	20.33 ± 3.07	18.87 ± 0.99
6	26.13 ± 7.07	22.17 ± 0.62	19.97 ± 1.99	20.23 ± 19.77	19.77 ± 0.18
8	19.80 ± 1.45	21.00 ± 2.58	19.73 ± 1.39	21.67 ± 3.40	19.67 ± 0.23
10	18.73 ± 0.32	20.30 ± 1.04	19.63 ± 1.57	19.77 ± 2.58	18.73 ± 1.32
12	19.73 ± 1.38	22.13 ± 3.68	20.10 ± 2.31	21.17 ± 3.54	20.57 ± 0.44
14	20.27 ± 1.77	21.10 ± 1.36	19.10 ± 3.17	20.80 ± 2.60	20.23 ± 0.99
16	19.43 ± 1.33	21.97 ± 3.09	20.57 ± 2.09	21.87 ± 2.85	19.70 ± 0.76
18	18.90 ± 0.53	12.50 ± 6.25	21.47 ± 2.26	20.63 ± 2.18	19.07 ± 0.95
20	18.53 ± 1.87	21.93 ± 2.77	22.43 ± 2.15	22.30 ± 4.15	19.57 ± 0.60

^a Mean ± SEM, N=3.

TABLE 3. DOSAGES^a OF RATS TREATED WITH MACS FOR THREE WEEKS

Day	Diet Concentrations				
	Control	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
Male					
2	0	19.70 ± 0.35	38.13 ± 1.39	73.70 ± 1.46	161.00 ± 11.73
4	0	20.60 ± 0.55	37.83 ± 1.56	81.33 ± 6.04	156.37 ± 10.81
6	0	21.00 ± 0.40	38.40 ± 1.65	75.27 ± 2.58	137.43 ± 5.97
8	0	18.03 ± 0.48	36.43 ± 1.70	71.40 ± 2.66	147.53 ± 5.54
10	0	18.60 ± 0.76	32.43 ± 0.20	69.97 ± 2.90	134.27 ± 3.26
12	0	18.86 ± 1.02	36.70 ± 2.19	77.07 ± 6.94	146.80 ± 3.54
14	0	18.20 ± 0.55	37.33 ± 1.37	69.20 ± 3.17	146.87 ± 11.10
16	0	17.47 ± 0.81	28.93 ± 2.09	62.30 ± 3.15	122.97 ± 5.31
18	0	16.17 ± 2.00	33.40 ± 0.78	61.73 ± 1.28	119.90 ± 3.76
20	0	17.45 ± 1.15	30.23 ± 1.61	63.90 ± 0.70	132.00 ± 5.09
Female					
2	0	20.63 ± 0.88	38.03 ± 2.88	74.57 ± 3.12	151.17 ± 14.35
4	0	21.23 ± 2.01	39.20 ± 2.35	81.03 ± 7.09	152.40 ± 4.73
6	0	22.60 ± 1.77	40.60 ± 3.52	81.53 ± 5.04	160.10 ± 4.65
8	0	20.13 ± 1.42	39.53 ± 2.74	85.57 ± 7.53	159.10 ± 3.67
10	0	19.63 ± 0.64	39.10 ± 1.72	78.37 ± 4.77	151.93 ± 13.32
12	0	21.10 ± 2.41	40.27 ± 4.66	83.43 ± 8.68	166.37 ± 5.00
14	0	20.43 ± 1.49	37.83 ± 5.17	82.50 ± 4.78	163.97 ± 11.01
16	0	19.67 ± 1.47	38.83 ± 4.16	81.67 ± 5.53	147.63 ± 5.76
18	0	10.87 ± 5.46	40.30 ± 3.43	77.33 ± 2.45	143.00 ± 8.36
20	0	19.63 ± 1.19	42.37 ± 4.19	82.33 ± 8.88	146.63 ± 4.88

^aMean ± SEM, expressed as mgMACS/kg body weight/day, N=3.

TABLE 4. BODY WEIGHTS^a (G) OF RATS TREATED WITH MACS FOR THREE WEEKS

Day	Diet Concentrations				
	Control	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
Male					
-1	387.1 ± 15.3	399.1 ± 17.2	394.4 ± 11.5	387.7 ± 8.3	393.6 ± 10.0
6	420.9 ± 21.7	449.5 ± 16.9	432.5 ± 13.9	421.9 ± 11.0	421.1 ± 15.9
14	465.4 ± 26.0	502.7 ± 26.2	482.8 ± 22.0	471.5 ± 9.8	475.5 ± 23.1
Female					
-1	240.7 ± 6.6	247.4 ± 14.0	245.3 ± 4.2	248.3 ± 15.2	247.3 ± 6.3
6	249.7 ± 2.8	259.0 ± 12.9	250.1 ± 10.6	250.1 ± 17.4	247.4 ± 4.8
14	258.3 ± 9.2	276.6 ± 17.5	265.8 ± 12.4	266.2 ± 24.6	267.0 ± 2.8

^aMean ± SEM, N=3.

TABLE 5. ABSOLUTE (G) AND RELATIVE ORGAN WEIGHTS^a OF MALE RATS TREATED WITH MACS FOR THREE WEEKS

Organs	Diet Concentrations				
	Control	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
Body	466.3 ± 30.7	504.2 ± 30.9	476.1 ± 23.1	474.0 ± 12.5	465.8 ± 24.6
Brain	2.07 ± 0.03	2.13 ± 0.05	2.15 ± 0.08	2.11 ± 0.06	2.16 ± 0.06
Ratio ^b	— 0.45 ± 0.03	0.03 ± 0.02	0.06 ± 0.03	0.45 ± 0.02	0.46 ± 0.01
Liver	14.10 ± 1.42	16.78 ± 1.99	15.11 ± 1.73	15.66 ± 1.30	14.16 ± 1.07
Ratio	3.00 ± 0.12	3.31 ± 0.22	3.16 ± 0.22	3.29 ± 0.20	3.03 ± 0.07
Kidneys	3.75 ± 0.11	3.76 ± 0.13	3.59 ± 0.16	3.73 ± 0.07	3.47 ± 0.13
Ratio	0.81 ± 0.07	0.75 ± 0.02	0.75 ± 0.00	0.79 ± 0.02	0.75 ± 0.01
Spleen	0.87 ± 0.10	0.98 ± 0.15	0.92 ± 0.07	0.86 ± 0.06	0.96 ± 0.09
Ratio	0.19 ± 0.02	0.19 ± 0.03	0.19 ± 0.01	0.18 ± 0.01	0.21 ± 0.01
Testes	3.39 ± 0.27	3.59 ± 0.04	3.61 ± 0.08	3.63 ± 0.06	3.61 ± 0.37
Ratio	0.73 ± 0.07	0.72 ± 0.04	0.76 ± 0.04	0.77 ± 0.02	0.77 ± 0.05

^aMean ± SEM, N=3.

^bOrgan weight/body weight x 100.

TABLE 6. ABSOLUTE (G) AND RELATIVE ORGAN WEIGHTS^a OF FEMALE RATS TREATED WITH MACS FOR THREE WEEKS

Organs	Diet Concentrations				
	Control	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
Body	245.1 ± 7.6	262.5 ± 19.1	255.1 ± 9.9	256.7 ± 30.2	250.3 ± 2.0
Brain	1.93 ± 0.05	1.98 ± 0.03	1.96 ± 0.03	1.88 ± 0.04	1.86 ± 0.05
Ratio ^b	0.79 ± 0.01	0.76 ± 0.06	0.77 ± 0.02	0.75 ± 0.08	0.75 ± 0.02
Liver	6.68 ± 0.10	7.59 ± 0.60	7.39 ± 0.27	7.47 ± 0.75	7.82 ± 0.29
Ratio	2.73 ± 0.07	2.89 ± 0.02	2.90 ± 0.13	2.92 ± 0.05	3.13 ± 0.14 ^c
Kidneys	1.89 ± 0.17	1.92 ± 0.12	1.82 ± 0.09	1.91 ± 0.20	1.79 ± 0.08
Ratio	0.77 ± 0.05	0.73 ± 0.02	0.72 ± 0.05	0.75 ± 0.03	0.71 ± 0.04
Spleen	0.57 ± 0.06	0.48 ± 0.04	0.42 ± 0.04	0.57 ± 0.09	0.49 ± 0.02
Ratio	0.23 ± 0.02	0.18 ± 0.00	0.17 ± 0.01	0.22 ± 0.01	0.20 ± 0.01

^aMean ± SEM, N=3.

^bOrgan weight/body weight x 100.

^cSignificant at p<0.10

TABLE 7. METHEMOGLOBIN VALUES* OF RATS FOLLOWING THREE WEEKS TREATMENT WITH MACS

Variable	Diet Concentrations				
	Control	0.25 g/kg	0.5 g/kg	1.0 g/kg	2.0 g/kg
Male					
THB	15.80 ± 0.17	15.27 ± 0.41	15.63 ± 0.20	15.73 ± 0.37	16.00 ± 0.40
METHB	1.23 ± 0.07	1.13 ± 0.03	1.27 ± 0.03	1.27 ± 0.07	1.40 ± 0.12
Female					
THB	16.83 ± 0.24	16.03 ± 0.41	16.70 ± 0.31	16.27 ± 0.20	15.70 ± 0.87
METHB	1.20 ± 0.06	1.13 ± 0.03	1.43 ± 0.12	1.40 ± 0.10	1.37 ± 0.09

*Mean ± SEM, N=3; THB = Total hemoglobin; METHB = Methemoglobin

Appendix A

MACS composition as listed in the Hercules Material Safety Data Sheet dated December 15, 1992 is:

47.7% nitroguanidine

28.0% nitrocellulose

22.5% nitroglycerin

1.5% Ethylcentralite

0.3% Cryolite

0.2% Graphite

An analysis of the MACS material used to prepare the rat diets was 46.4% nitroguanidine. The main component, nitroguanidine, was detectable by spectrometry (UV absorption at 265 nm wavelength). Because two secondary components are not readily detected by absorption, the MACS analysis was based on the nitroguanidine analysis.

Standards were prepared by adding weighed amounts of MACS to 5.0 g rodent diet and 10 mL of methanol in a 20 mL scintillation vial. The standards were mixed for 20 min. on a Haak-Buchler (Haak-Buchler Instruments, Inc., Saddlebrook, NJ) vortex mixer. After centrifugation for 5 min. at 2000 rpm, 1-mL samples were pipetted into 2-mL autosampler vials. Control diet samples and treated diet samples were treated similarly.

Samples were analyzed using a Hewlett-Packard High Performance Liquid Chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a 4.6 mm X 220 mm Spheri 5 RP-8S, 5 μ particle size, reverse phase column (Alltech Associates, Inc., Deerfield, IL). The carrier flow was set at 0.4 mL/min of 50/50 methanol and water with an injection volume of 1.0 μ L. A variable wavelength detector set at 265 nm provided maximum sensitivity for nitroguanidine. All rodent diet analysis concentrations are expressed as mg MACS/g rodent chow.

Bottom 2	1.87	1.85 ± 0.20 ^a
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Moderate Level (Target: 1.0 mg/g diet)

Top	0.99	
Middle	0.88	
Bottom	0.89	0.92 ± 0.06

Mid Level (Target: 0.5 mg/g diet)

Top	0.41	
Middle	0.41	
Bottom	0.85	0.56 ± 0.25

Low Level (Target: 0.25 mg/g diet)

Top 1	0.19	
Top 2	0.27	
Middle 1	0.19	
Middle 2	0.17	
Bottom 1	0.26	
Bottom 2	0.18	0.23 ± 0.05

^aMean ± S.D.

Diet Batch Stability

Stability was determined for various time periods up to 21 days in the polyethylene storage containers and for 7 days in the open glass rodent feeders. All analyses were determined on high concentration (2 mg/g) diet.

Polyethylene Storage Container

0 day sample 1	2.04	
0 day sample 2	2.71	2.38 ± 0.47 ^a
7 days, sample 1	2.27	
7 days, sample 2	1.66	1.96 ± 0.43
14 days, sample 1	1.80	
14 days, sample 2	2.52	2.16 ± 0.51
21 days, sample 1	1.99	
21 days, sample 2	2.16	2.08 ± 0.12

Appendix B

Diet Batch Analysis

Duplicate samples of approximately 20g each, were taken from the top, middle, and bottom of each prepared batch of diet. The samples were mixed thoroughly and analyzed in duplicate for MACS concentration.

High Level (Target: 2.0 mg/g diet)

Analysis: 2.04
2.71 2.38 ± 0.47^a

Moderate Level (Target: 1.0 mg/g diet)

Analysis: 0.95
1.02 $.99 \pm 0.05$

Mid Level (Target: 0.5 mg/g diet)

Analysis: 0.38
0.28 0.33 ± 0.07

Low Level (Target: 0.25 mg/g diet)

Analysis: 0.21
0.11 0.16 ± 0.07

^amean \pm S.D.

Diet Batch Homogeneity

Two samples each from the top, middle and bottom of the mixing bowl of the high and low diets were analyzed. In addition, one sample from the top, middle, and bottom of the moderate and mid levels were analyzed.

High Level (Target: 2.0 mg/g diet)

Top 1	1.95
Top 2	1.48
Middle 1	2.07
Middle 2	1.94
Bottom 1	1.79

Glass Rodent Feeder

0 day sample 1	2.04	
0 day sample 2	2.71	2.38 ± 0.47
7 days, sample 1	2.33	
7 days, sample 2	2.29	2.31 ± 0.03

^aMean \pm S.D.