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OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

February 7, 1996

MEMORANDUM

SUBJECT: Mepiquat Chloride: Review of the Following Studies: 2-Year Rat Feeding, 1-Year Dog Feeding and Mouse Carcinogenicity

Rereg. Case No. 2375 Chemical Code No. 109101
CAS Reg. No. 24307-26-4 Tox. Chem. No. 380 AB
Sponsor for all studies: BASF Corporation, Agricultural Products Group, Research Triangle Park, NC

FROM: Krystyna K. Locke, Toxicologist
Section I, Toxicology Branch I
Health Effects Division (7509C)

Krystyna K. Locke 2/7/96

THRU: Roger L. Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (7509C)

Roger Gardner 2-14-96

Karl P. Baetcke, Branch Chief
Toxicology Branch I
Health Effects Division (7509C)

KB 2/20/96

TO: Mark Wilhite/Patric Dobak, PM 51
Accelerated Reregistration Branch
Special Review and Reregistration Division (7508W)

Toxicology Branch I/HED has completed an evaluation of the following studies:

Guideline No.	MRID No.	DP Barcode	Acceptability
82-1(a)	42412102	D183221	[redacted]
82-1(a)	42412101	D183221	[redacted]
82-1(b)	43264401	D205089	[redacted]
83-1(a)	43264402	D205089	Yes
83-1(b)	41488105	D216220	Yes *
83-1(b)	43264403	D205089	Yes **
83-2(b)	43264404	D205089	Yes

[redacted] This is a 4-week range-finding study with rats which was not

included as an APPENDIX in the review of the 2-year rat feeding study (MRID 43264402).

■■ This is a 3-month range-finding study with mice which was not reviewed separately. A brief review of this study was included as an APPENDIX A in the review of the mouse carcinogenicity study (MRID 43264404).

■■■ This is a 4-week range-finding study with dogs which was not reviewed separately. Findings observed in this study were included, at appropriate points, in the review of the 1-year dog feeding study (MRID 43264403).

* In this 1-year dog feeding study, 3 doses of Mepiqut Chloride were tested, but a definitive NOEL was not determined. Another 1-year feeding study with one high dose of Mepiquat Chloride (**) was, therefore, conducted 3 years later. Considered together, these studies satisfy the guideline requirements for a chronic oral study in dogs (83-1b).

Results obtained in the above studies are summarized below.

MRID 43264402 - In this study, Wistar strain rats, 20/sex/dose, were fed Mepiquat Chloride in the diet for 24 months at the following levels: 0, 290, 2316 and 5790 ppm (active ingredient). These levels were equivalent to 0, 13, 106 and 268 mg/kg/day, respectively, for males and 0, 18, 146 and 371 mg/kg/day, respectively, for females. Treatment-related findings, observed only in the high-dose group, were: **(1)** Decreased body weights and body weight gains for males and females; **(2)** Increases in urinary crystals for males; and **(3)** Pathological changes in the adrenal cortex (foci, vacuolated cell foci, hemosiderin pigment and nodular hyperplasia) in females. Based on these findings, **the systemic NOEL is 2316 ppm (mg/kg/day: 106 for males and 146 for females) and the systemic LOEL is 5790 ppm (mg/kg/day: 268 for males and 371 for females).** This study is classified as **Acceptable (Core-Minimum)** and satisfies the guideline requirements for a chronic feeding study in rats (83-1a).

MRID 41488105 and 43264403 - In these studies, beagle dogs, 6/sex /dose, were fed Mepiquat Chloride in the diet for 12 months at the following levels: 0, 200, 600 or 1800 ppm **(MRID 41488105)**, and 0 or 6000 ppm **(MRID 43264403)**. These levels were equivalent to 0, 6.3, 19.9, 58.4 and 170.0 mg/kg/day, respectively. Treatment-related effects, observed only in the high-dose (6000 ppm) males and females, were: **(1)** Salivation; **(2)** Early mortality (one female was sacrificed moribund on study day 17); **(3)** Kidney vacuolization; and **(4)** Increased hemosiderin storage in spleen (males only). Salivation (an indicator of impaired neurological functions) occurred in all dogs at 2 hours after each feeding and

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lasted for 4 hours. The moribund dog showed signs of impaired neurological functions (weakness and ataxia of the hind legs, lateral position, and extension spasm), abnormal body temperature (no details), stomach lesion, lung focus and cyst in the pituitary gland. Based on these findings, **the systemic NOEL for male and female dogs is 1800 ppm (58.4 mg/kg/day) and the systemic LOEL is 6000 ppm (170 mg/kg/gay).** Considered singly, the study with 3 dose levels (MRID 41488105) is classified as Acceptable and the study with 1 dose level (MRID 43264403) is classified as Supplementary, and neither study satisfies the 83-1b data requirement. **Considered together, the study (MRID 41488105 and MRID 43264403) is Acceptable (Core-Guideline)** and satisfies the guideline requirements for a chronic oral study in dogs (83-1b). The Flagging Criteria Statement [6(a)(2)] was submitted because of the neurotoxic effects observed in dogs fed 6000 ppm (170 mg/kg/day) of Mepiquat Chloride.

MRID 43264404 - In this carcinogenicity study, B6C3F1/CrlBr mice, 50/sex/dose, were fed Mepiquat Chloride in the diet for 24 months at the following levels: 0, 500, 2000 and 7500 ppm (active ingredient). These levels were equivalent to 0, 74, 297 and 1140 mg/kg/day, respectively, for males and 0, 85, 328 and 1348 mg/kg/day, respectively, for females. There were no treatment-related effects of Mepiquat Chloride administration on all of the parameters examined. Based on these findings, **the systemic NOEL is ≥ 7500 ppm (mg/kg/day: 1140 for males and 1348 for females).**

Mepiquat Chloride was not carcinogenic in this study. Because there were no treatment-related findings, Mepiquat Chloride was not administered at the MTD (maximum tolerated dose). However, the high dose (7500 ppm) was sufficient to assess carcinogenicity since the limit dose of 1000 mg/kg/day was exceeded.

This study is classified as **Acceptable (Core-Minimum)**, satisfying the guideline requirements for an oncogenicity (carcinogenicity) study in mice (83-2b).

Special Review criteria (40 CFR 154.7): None

Mepiquat Chloride Chronic Oral Study 83-1(b)

EPA Toxicologist: Krystyna K. Locke K.K. Locke, Date 4/7/96
Review Section I, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Roger L. Gardner, Date 2/14/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic Oral Toxicity (feeding) - Dog; OPPTS 870.4100 [§83-1 (b)]

DP BARCODE: D205089
P.C. CODE: 109101
TEST MATERIAL (PURITY): 56.05% of Mepiquat Chloride in water
SUBMISSION CODE: S469238
TOX. CHEM. NO.: 380 AB
REREG. CASE NO.: 2375

SYNONYMS: Pix; Reg. No. 85 559

CITATION: Mellert, W. (1994) Supplementary Study of the Toxicity of Mepiquat Chloride in Beagle Dogs - Administration Via the Diet Over 12 Months (BASF, Germany); Report No. 33D0001/92001; May 5, 1994. MRID 43264403 Unpublished

SPONSOR: BASF Corporation, Agricultural Chemicals Group, Research Triangle Park, NC

EXECUTIVE SUMMARY:

In a chronic toxicity supplementary study (**MRID 43264403**), mepiquat chloride, 56.05% a.i.(w/w) in water, was administered to 6 beagle dogs/sex/dose in diet at dose levels of 0 and 6000 ppm (170 mg/kg/day) for 12 months. The following effects were observed in the treated group: salivation in all dogs; early mortality (one female was sacrificed moribund on study day 17); kidney vacuolization in 4/6 males (controls: 1/6) and 5/6 females (controls: 2/6); and increased hemosiderin storage in spleen of male dogs. Salivation (an indicator of impaired neurological functions) occurred at 2 hours after each feeding, was slight at first, moderate to severe during the next 4 hours and then gradually disappeared. The moribund dog showed weakness and ataxia of the hind legs, lateral position, extension spasm, abnormal body temperature (no details), stomach lesion, lung focus and cyst in the pituitary gland. The kidney vacuolization was minimal (grade 1) to slight (grade 2) in the treated dogs and minimal in the controls. The hemosiderin storage in the spleen of the male dogs showed a higher intensity in the treated group (grade 1) than in the controls (grade 2).

This study should be considered together with an earlier study (MRID 41488105) in which 3 doses of mepiquat chloride were tested (200, 600 or 1800 ppm, equivalent to 6.3, 19.9 or 58.4 mg/kg/day, respectively), but in which a definitive NOEL was not determined. Based on both studies, **the LOEL for males and females is 6000 ppm (170 mg/kg/day) and the NOEL is 1800 ppm (58.4 mg/kg/day)**, and

the guideline requirement for a chronic feeding study (83-1b) in dogs is satisfied. By itself, the current study is classified as Supplementary and does not satisfy the 83-1b data requirement.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality and Flagging statements were provided. This study was conducted in accordance with the OECD Principles of Good Laboratory Practices (Paris, 1981) and does not meet all of the requirements for 40 CFR 160, Good Laboratory Practices, in the following way: the stability of the test substance was not fully confirmed according to the GLP Guidelines. The Flagging Criteria Statement [6(a)(2)] was submitted because of the neurotoxic effects observed in dogs treated with 6000 ppm (170 mg/kg/day) of mepiquat chloride.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Mepiquat Chloride
   Description: Aqueous solution
   Lot/Batch #: 91-2 PCP 01826
   Purity: about 56.05% a.i. (w/w)
   Stability of compound: Stable throughout the study at room temperature
   CAS #: 24307-26-4

2. Vehicle and/or positive control: N/A

3. Test animals: Species: Dog
   Strain: Beagle
   Age and weight at study initiation: 6-9 months; 11.6 (9.7-13.5) kg, males and 10.3 (8.4-12.4) kg, females.
   Source: BASF Breeding Unit
   Housing: Singly, in kennels with a floor area about 9 m^2
   Diet: Dog maintenance KLIBA laboratory diet 335, obtained from Klingentalmuhle AG, Switzerland.
   Water: Blended water (fully demineralized water adjusted with drinking water to about 2° German hardness) was available ad libitum.
   Environmental conditions: Temperature: Not reported.
   Humidity: Not reported.
   Air changes: Not reported.
   Photoperiod: "Natural day/night rhythm, with additional artificial light as required during working hours".
   Acclimation period: 5 days.

B. STUDY DESIGN:

1. In life dates - start: January 27, 1992
   - end: January 26-28, 1993 (Necropsy days)

2. Animal assignment

Animals were assigned randomly (random number generator) to the test groups as is shown in TABLE 1. The secondary criterion for the randomization was an approximately equal mean body weight in the individual groups.

TABLE 1: STUDY DESIGN

Test Group	Conc. in Diet (ppm)	Dose to animal (mg/kg) *	Main Study 12 months		Interim Sac. __ months	
			male	female	male	female
Control	0	0	6	6	-	-
Low (LDT)	-	-	-	-	-	-
Mid (MDT)	-	-	-	-	-	-
High (HDT)	6000	170	6	6	-	-

* Actual values; mg/kg of body weight/day.

3. Dose selection rationale: Doses selected for this study were based on the results of two feeding studies with male and female beagle dogs: a 12-month study (dated 1989; MRID 41488105) and a 4-week range-finding study (dated 1994; MRID 43264401). In the 12-month study, the only effect observed at the 1800 ppm dose (58.4 mg/kg; HDT) was a very slightly increased iron pigment storage in the spleen and liver of the male dogs. In the 4-week study, salivation after feeding was observed in all dogs (2/sex/group) at the 6000 ppm dose (185 mg/kg; LDT) and salivation and death at the 12000 ppm dose (308 mg/kg; HDT). In general, salivation started at 2 hours after feeding and continued for 2-4 hours. Based on these findings, a dose of 8000 ppm was initially selected for the current 12-month supplementary study. However, following the death of 3 dogs on treatment day 1, the study was started again 5 days later (on January 27, 1992) with 3 new (replacement) dogs and a lower dose of 6000 ppm (170 mg/kg/day).

The 4-week range-finding study (No. 30D0112/89109; MRID 43264401) was conducted during October 8-28, 1991 and the report was completed on May 5, 1994. The test material used in this study was an aqueous solution of about 57.2% (w/w) of mepiquat chloride (Batch No. WW 262/CP 1490).

4. Diet preparation and analysis:

Diets were prepared daily by mixing powdered feed pellets (350 g) with drinking (tap) water (350 mL) immediately before administration to each dog. Appropriate amount of mepiquat chloride was added to the powdered pellets and and this mixture was usually prepared once a week. The pellets and the powdered mixture were stored at room temperature. Homogeneity and stability of mepiquat chloride in the diet, and concentration of mepiquat chloride

in the dosing solution were not determined in this study.

5. Statistics - Means and standard deviations were calculated for the food consumption, body weight, body weight change and the intake of the test substance data. Body weight, body weight change, hematology and clinical chemistry data were also analyzed using the Mann-Whitney U-test.# The urinalysis data were evaluated by the Fisher's exact test.# Body weights at the termination of the study and absolute and relative (organ/body weight ratios) organ weights were analyzed by the Wilcoxon test.##

Siegel, S. (1956): Non-parametric statistics for the behavioral sciences. McGraw-Hill New York.

Hettmansperger, T.P. (1984): Statistical inference based on ranks. John Wiley and Sons New York.

C. METHODS:

1. Observations:

Animals were inspected once daily (several times when signs occurred) for signs of toxicity. A check was made for any moribund or dead animals twice a day on Mondays through Fridays and once a day on weekends and holidays. During the study days 1-90, the dogs were examined for salivation 4 times a day (before feeding and at 2, 4 and 6 hours after feeding) generally each working day. From study day 91 onward, this examination was carried out generally at weekly intervals.

2. Body weight:

Animals were weighed weekly.

3. Food consumption and compound intake:

Food consumption for each animal was determined and mean daily diet consumption was calculated as g food/animal/day. Food efficiency was calculated for each test group every 4 weeks on the basis of body weight changes and the total amount of food consumed during this period. Compound intake (mg/kg of body weight/day) values were calculated weekly as averages from the food consumption and body weight data.

4. Ophthalmoscopic examination:

All dogs were examined before the start of the study and

at the end of the treatment period, using a KOWA-RC 2 fundus camera.

5. Blood was collected in the morning for hematology and clinical analyses, from all animals (fasted), as follows: 3 days before the beginning of treatment and 91, 186 and 361 days after treatment. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*		
X	(Thromboplastin time)		
X	# (Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for chronic studies based on Subdivision F Guidelines
\# Partial

b. Clinical Chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
		X	Total serum protein (TP)*
X	ENZYMES	X	Triglycerides
X	Alkaline phosphatase (ALK)		Serum protein electrophores
	Cholinesterase (ChE)		
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)*		
X	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for chronic studies based on Subdivision F Guidelines

6. Urinalyses

Urine was collected overnight from all animals (fasted) 4 days before treatment and after 92, 184 and 359 days of treatment. The animals received about 500 ml of drinking water overnight. The CHECKED (X) parameters were examined.

X		X	
X	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*	X	Nitrate
X	Protein*	X	Urobilinogen

* Required for chronic studies

7. Sacrifice and Pathology:

All animals in the study were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	X	Heart*	X	Periph.nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*		
X	Duodenum*	X	Spleen*	X	Pituitary*
X	Jejunum*	X	Thymus*	X	Eyes (optic n.)*
X	Ileum*				
X	Cecum*		UROGENITAL		
X	Colon*	XX	Kidneys*+		GLANDULAR
X	Rectum*	X	Urinary bladder*	XX	Adrenal gland*
XX	Liver*+	XX	Testes*+		Lacrimal gland
X	Gall bladder*		Epididymides	X	Mammary gland*
X	Pancreas*	X	Prostate	XX	Parathyroids*++
			Seminal vesicle	XX	Thyroids*++
	RESPIRATORY	XX	Ovaries*+		
X	Trachea*	X	Uterus*		
X	Lung*				OTHER
	Nose			X	Bone*
	Pharynx			X	Skeletal muscle*
	Larynx			X	Skin*
				X	All gross lesions and masses*

*. Required for chronic studies based on Subdivision F Guidelines.
+ Organ weight required in chronic studies.
++ Organ weight required for non-rodent studies.

All of the above tissues were examined.

II. **RESULTS:**

A. Observations

1. Toxicity - Salivation was observed in all treated dogs. In general, slight salivation occurred at 2 hours after each feeding, became moderate to severe during the next 4 hours and then gradually disappeared. Other toxic signs were not observed.

2. Mortality - One female dog in the treated group was sacrificed moribund on study day 17. This dog showed weakness and ataxia of the hind legs, lateral position, extension spasm and subnormal body temperature on study days 7, 16 and 17.

B. Body weight was not affected by treatment. These data are summarized below.

TABLE 2: BODY WEIGHT CHANGES (kg)

Test material (ppm)	0	6000	0	6000
Study Days	Males		Females	
0-28	0.3	0.2	0.7	0.5
28-63	0.4	0.5	0.6	0.4
63-91	0.2	0.3	0.3	0.3
91-154	0.7	0.7	0.6	0.7
154-259	0.1	0.3	0.1	0.7
259-336	- 0.3	- 0.1	- 0.1	- 0.2
336-364	- 0.3	- 0.2	- 0.3	- 0.2

This table is based on TABLES 049-054, pages 112-117, of the submitted report (MRID 43264403). (-) Sign before a number denotes weight loss, whereas the remaining values represent weight gains.

C. Food consumption and compound intake

1. Food consumption was not affected by treatment. Male dogs in the control and the 6000 ppm groups consumed all of their food every day during the study. In the case of the female dogs, decreases in the food consumption were observed in both groups at various times during the study, as follows:

Mepiquat chloride (ppm):	**0**	**6000**
Percent of daily ration consumed	84-98	86-98
Total number of study days involved	106	61
Days when decreases were observed	79-347	0-315

The above decreases in the food consumption were statistically insignificant. The numbers of the female dogs affected in the control and the 6000 ppm groups were 2 and 4, respectively.

The above data are based on TABLES 002-042, pages 65-105, and on TABLES A 049-094, pages 265-310, of the submitted report (MRID 43264403).

2. Compound consumption - The group mean intake of mepiquat chloride was reported for the 7-day periods during the entire study. These data are summarized below.

TABLE 3: GROUP MEAN INTAKE OF MEPIQUAT CHLORIDE (mg/kg b.w./day)

Mepiquat chloride (ppm)	6000	
Study Days	Males	Females
0	188.4	196.2
14	185.6	196.8
49	181.1	193.9
105	169.4	183.1
161	161.7	172.6
217	156.3	164.2
273	159.4	163.9
329	159.4	165.2
357	162.5	169.8
0-357	166.0	173.0

	Males and Females
0-357	170.0

This table is based on TABLES 056-061, pages 119-124, of the submitted report (MRID 43264403).

3. Food efficiency was not affected by mepiquat chloride. The mean food efficiency values, determined at 4-week intervals, varied considerably within one group and between groups. Regarding the food efficiency from day 0 to day 363, reduced values were obtained for both the

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control and the treated dogs. These data are summarized below.

TABLE 4: - FOOD EFFICIENCY (Group Means)

Mepiquat chloride (ppm)	0		6000	
Study Days	Males	Females	Males	Females
0-27	3.1	7.1	2.0	5.1
56-83	2.0	3.1	4.1	3.1
112-139	2.0	3.2	3.1	3.1
168-195	3.3	2.2	3.3	5.5
224-251	0	-1.1	0	1.0
280-307	0	1.0	0	-2.1
336-363	-3.3	-3.4	-2.2	-2.2
0-363	0.9	1.5	1.3	1.8

This table is based on TABLE 055, page 118, of the submitted report (MRID 43264403).

D. Ophthalmoscopic examination revealed no treatment-related effects in the male and female dogs.

E. Blood work

1. Hematology - Mepiquat chloride had no effect on all hematological parameters examined in the male and female dogs.

2. Clinical chemistry - Mepiquat chloride, at all doses tested, had no effect on clinical chemistry.

F. Urinalysis were not affected by mepiquat chloride.

G. Sacrifice and Pathology

1. Organ weight - Mepiquat chloride had no effect on the absolute and relative organ weights in this study. (Relative organ weight = ogran/body weight ratio). In the absence of macroscopic and microscopic findings, the 14% increase ($P \leq 0.05$) in the absolute weight of the adrenals and the 5 % decrease ($P \leq 0.05$) in the relative weight of the kidneys, in the treated male dogs, were considered in the pathology report for this study as incidental.

2. Gross pathology - One control and 3 treated male dogs had lung foci. The following findings were observed in the treated females: lung induration and cyst in the pituitary gland in one dog, stomach lesion and lung foci in another dog (which was sacrificed moribund on treatment day 17), and induration in the third dog. According to the pathologist who examined the tissues, all gross lesions were spontaneous in origin. This does not appear to be true in the case of the moribund dog.

3. Microscopic pathology

a) Non-neoplastic - The only treatment-related findings observed were epithelial vacuolization of renal distal tubules in males and females, and increased homosiderin storage in spleen of male dogs. These and other predominant findings are summarized below.

TABLE 5: INCIDENCE (NUMBER OF DOGS AFFECTED) OF MICROSCOPIC FINDINGS

Mepiquat chloride (ppm):	0	6000	0	6000
Organ	Males		Females	
Kidneys:				
Vacuolization	1	4	2	5
Spleen:				
Hemosiderin storage	6	6	6	6
Liver:				
Hemosiderin storage	6	6	6	6
Pituitary gland:				
Cyst/hematocyst	1	0	2	3

This table is based on data reported on pages 441 and 442 of the submitted report (MRID 43264403).

The epithelial vacuolization noted in the kidneys was minimal (grade 1) to slight (grade 2) in the treated dogs and minimal in the controls. The hemosiderin storage in the spleen of the male dogs showed a higher intensity in the treated group (grade 2) than in the controls (grade 1) and was, therefore, attributed to treatment by the testing facility. The hemosiderin storage in the spleen of the female dogs (both groups) and in the liver of all dogs was mostly of the same intensity (grade 1).

b) Neoplastic findings were not observed.

III. **DISCUSSION**

A. Review of the final report indicates that the design of this supplementary study was not adequate. Since no definite toxic effects were observed at the 1800 ppm dose (58.4 mg/kg/day; HDT) in an earlier 12-month dog feeding study (MRID 41488105), the aim of the current study was to establish definitive NOEL and LOEL. However, only one dose, 6000 (170 mg/kg/day) was tested. There is too big a gap between the 1800 ppm and the 6000 ppm dose levels. Two doses, like 2500 ppm (100 mg/kg/day; 4-week study, dated 1976; not submitted to EPA for review) and 6000 ppm, would have been a better selection. The 2500 ppm dose in the 4-week study was the lowest dose at which toxic signs (decreased body weight gain and food consumption, and anemia) were observed.

B. Study deficiencies: With the exception of poor dose selection, noted above, there are no major deficiencies in this study. Although lactic acid dehydrogenase, gamma glutamyl transferase and glutamate dehydrogenase were not determined, the absence of these data does not affect the classification of this study (Supplementary). This study should be considered together with an earlier study (MRID 41488105).

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DATA EVALUATION REPORT

MEPIQUAT CHLORIDE

Study Type: ONCOGENICITY FEEDING - MOUSE (83-2)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Health Sciences Research Division
Oak Ridge National Laboratory*
Oak Ridge, TN 37831
Task Order No. 95-07 F, G

Primary Reviewer:
C.S. Jamison, Ph.D.

Signature: RHR for C.S. Jamison
Date: 11-20-95

Secondary Reviewers:
A.F. Francis, M.S., D.A.B.T.

Signature: RHR for A.F. Francis
Date: 11-20-95

Robert H. Ross, M.S., Group Leader

Signature: RHR
Date: 11-20-95

Quality Assurance:
Susan Chang, M.S.

Signature: [illegible]
Date: [illegible]

Disclaimer

The final Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Oak Ridge National Laboratory personnel.

*Managed by Lockeed Martin Energy Systems, Inc., for the U.S. Department of Energy under Contract No. DE-AC05-84OR21400

EPA Reviewer: William Greear, M.P.H., D.A.B.T. Date: 12/14/95
Review Section IV, Toxicology Branch I (7509C)
EPA Section Head: Marion Copley, D.V.M., D.A.B.T. Date: 12/14/95 1/4/95
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity Feeding – Mouse (83-2)

TOX. CHEM. NO: 380AB

P.C.CODE: 109101

MRID NO.: 432644-04 (main study); 424121-01 (range-finding study)

TEST MATERIAL: Mepiquat Chloride

SYNONYMS: 1, 1-Dimethylpiperidinium chloride

ROUTE OF ADMINISTRATION: Oral, mixed with diet

TREATMENT DURATION: 24 months

STUDY NUMBER: 80S0112/89107

SPONSOR: BASF Corporation, Agricultural Products Group, P.O. Box 13528, Research Triangle Park, NC 27709-3528

TESTING FACILITY: BASF Aktiengesellschaft, Department of Toxicology, D-67056 Ludwigshafen/Rhine, FRG

TITLE OF REPORT: Carcinogenicity Study with Mepiquat Chloride in B6C3F1 Mice-Administration in the Diet for 24 Months.

AUTHOR: W. Mellert

REPORT ISSUED: May 4, 1994 (Study completion date)

EXECUTIVE SUMMARY: In a oncogenicity study, Mepiquat Chloride was administered in the diet for 24 months to 50 B6C3F1/CrlBr mice/sex/dose and for 12 months to 10 mice/sex/dose at concentrations of 0, 500, 2000, and 7500 ppm (active ingredient). For main group mice, these respective doses are equivalent to 0, 74, 297, and 1140 mg/kg/day for males and 0, 85, 328, and 1348 mg/kg/day for females, averaged over the 24-month feeding study.

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There were no treatment-related effects of Mepiquat Chloride administration on group mean body weights or body weight gains over the 24 month treatment period. There were no treatment-related macroscopic or non-neoplastic microscopic pathological findings for males or females. **The NOEL for Mepiquat Chloride administered for 2 years in food is 7500 ppm for male and female B6C3F1 mice.**

There were no treatment-related neoplastic findings for males or females treated with Mepiquat Chloride. Thus, Mepiquat Chloride does not exhibit carcinogenic potential in a 2-year feeding study involving male and female B6C3F1 mice over this dose range. Based upon the lack of treatment-related findings, Mepiquat Chloride was not administered at the MTD. However, the high dose (7500 ppm) for the study was sufficient to assess carcinogenicity since the limit dose of 1000 mg/kg/day was exceeded.

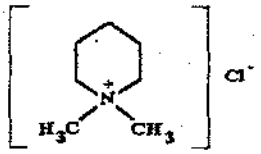
This study is classified as **core-minimum**, satisfying the guideline requirements for an oncogenicity study in mice (§83-2a).

Special Review Criteria (40 CFR 154.7) None

A. MATERIALS

1. Test material: Mepiquat Chloride

   Description: aqueous solution
   Lot/Batch No.: WW 262/CP 1490
   Purity: 57.9% a.i. (w/w in water)
   Stability of compound: stable for 2 years
   CAS No.: 24307-26-4
   Structure:



2. Vehicle and/or positive control

   Test material was mixed with diet. Negative control group was fed diet. No positive control was described.

3. Test animals

   Species: mouse
   Strain: B6C3F1/CrlBr
   Age and weight at study initiation: 49 days, 20.7-27.0 g (males), 17.0-22.0 g (females)

Source: Charles River Breeding Lab., Wilmington, MA
Housing: individually in type MII Makrolon cages with mesh wire tops
Environmental conditions:
Temperature: 20-24°C
Humidity: 30-70%
Air changes: not described
Photoperiod: 12 hour light/dark cycle
Acclimation period: 10 days

B. STUDY DESIGN

1. Animal assignment

Mice (50/sex/dose in the main group, 10/sex/dose in the satellite group) were assigned to the test groups in Table 1 in a randomized fashion.

TABLE 1. STUDY DESIGN FOR MEPIQUAT CHLORIDE ONCOGENICITY IN MALE AND FEMALE B6C3F1 MICE

Dose Group	Conc. in Diet (ppm)	Main Group				Satellite Group			
		Dose (mg/kg/day)[a]		No. of Animals		Dose (mg/kg/day)[a]		No. of Animals	
		Male	Female	Male	Female	Male	Female	Male	Female
1 Control	0	0	0	50	50	0	0	10	10
2 Low	500	74	85	50	50	83	95	10	10
3 Mid	2000	297	328	50	50	314	414	10	10
4 High	7500	1140	1348	50	50	1249	1607	10	10

Data adapted from pp. 24 and 39, MRID No. 432644-04.
[a] Due to spillage, intake amounts are estimates

Dose selection rationale: Doses were selected on the basis of a preliminary 3-month feeding study (MRID No. 424121-01, summarized in the Appendix to this report). There were no treatment-related findings for male or female B6C3F1 mice treated with Mepiquat Chloride at 300, 900, 2700, or 8100 ppm. On the basis of these results, doses of Mepiquat Chloride for the oncogenicity study were chosen as 500, 2000, and 7500 ppm (active ingredient, corresponding to inclusion levels of 864, 3454, and 12,953 ppm of test material containing 57.9% Mepiquat Chloride, respectively). The medium and high doses are 4 and 15 times the low dose, respectively.

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2. Diet preparation and analysis

Concentrated diet was prepared by adding Mepiquat Chloride (57.9% w/w in water) to ground Kliba maintenance diet rat/mouse/hamster, 343 meal (Klingentalmühle AG, Kaiseraugst, Switzerland) and mixing with a BOSCH household mixer. The concentrate was diluted to the appropriate level with diet and mixed (GEBR. LÖDIGE mixer) for 10 minutes.

Results -

a. Homogeneity analysis - Diet was prepared with Mepiquat Chloride at concentrations of 290 and 5790 ppm and 6 samples of each diet preparation was analyzed. The concentrations of Mepiquat Chloride ranged from 85.2%-92.0% (mean: 89.2%, coefficient of variation: 3.12%) of the theoretical inclusion level for the 290 ppm diet, and from 97.1%-101.8% (mean: 99.7%, coefficient of variation: 1.78%) of the theoretical inclusion levels for the 5790 ppm diet.

b. Stability analysis - Mepiquat Chloride stability was assessed after storage of diet (prepared at 500 ppm) at room temperature for 0, 11, and 32 days. Mepiquat Chloride in diet after 0, 11, and 32 days at room temperature was 101.2%, 99.2%, and 95.5% of the theoretical inclusion level.

c. Concentration analysis - Samples of diet containing Mepiquat Chloride at inclusion levels of 0, 500, 2000, and 7500 ppm were prepared on 5 dates from August 7, 1991 to July 26, 1993. The preparations were stored in the freezer and concentrations analyzed within 10 months. Mean concentrations of Mepiquat Chloride for the 500, 2000, and 7500 ppm preparations ranged from 89.5%-98.8%, 95.8-100.2%, and 94.0%-100.1%, respectively, of the theoretical inclusion levels.

3. Diet

Animals were fed ground Kliba maintenance diet rat/mouse/hamster, 343 meal (Klingentalmühle AG, Kaiseraugst, Switzerland). Food and drinking water were available *ad libitum*. Drinking water and food were subjected to chemical and microbiological analyses.

4. Statistics

The mean and standard deviation were calculated for food consumption, body weights, body weight changes, terminal body weights, absolute organ weights, relative organ weights, food efficiency, substance intake, and hematology parameters. Data for body weights, body weight changes, and absolute and relative organ weights were analyzed using a one-way analysis of variance with the F-test, and statistical significance was evaluted at $p \leq 0.05$. If statistical significance was established with the ANOVA and F-test, comparisons between dose groups and controls were performed using Dunnett's test (2-sided).

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5. Signed and dated GLP and quality assurance statements were present.

C. METHODS AND RESULTS

1. Observations

Mice were inspected twice a day on Monday through Friday, and once a day on Saturdays, Sundays, and holidays for mortality and signs of toxicity. Comprehensive clinical examinations were performed at least once a week.

Results – Cumulative mortality (found dead or sacrificed moribund) from days 0-714 for males treated with Mepiquat Chloride at 0, 500, 2000, and 7500 ppm was 8/50 (16%), 2/50 (4%), 2/50 (4%), and 7/50 (14%), respectively. There was no mortality for satellite group males prior to termination. There were no treatment-related clinical signs for main group or satellite group males. Cumulative mortality (found dead or sacrificed moribund) from days 0-714 for females treated with Mepiquat Chloride at 0, 500, 2000, and 7500 ppm was 9/50 (18%), 6/50 (12%), 9/50 (18%), and 8/50 (16%), respectively. There was no mortality for satellite group females prior to termination. There were no treatment-related clinical signs for main group or satellite group females.

2. Body weight

Animals were weighed prior to the beginning of the treatment period in order to assign the animals to test groups, then at day 0, then once each week for the first 14 weeks, then at 4 week intervals for the remainder of the treatment period, with an additional weighing at the interim sacrifice for the satellite group.

Results – For the overall study period, group mean body weights and body weight gains for males or females in the main or satellite groups were not statistically significantly different between treated groups and controls (Table 2). However, on certain days during the first year of treatment primarily at the high dose, there were statistically significant differences in group mean body weights and body weight gains for main group males and satellite group females treated with Mepiquat Chloride as compared to controls (Tables 3 and 4). The body weights in main group males, although statistically significant at certain time points, never dropped below 93% of control body weights (Table 3) during the first year. Body weight gains in main group males during the first year showed a dose response decrease in weight gain only for days 0-63 (Table 4).

3. Food consumption and compound intake

Food consumption (g/mouse/day), food efficiency (body weight gain, (g)/food consumption (g) per unit time X 100)), and substance intake (mg/kg body weight) were determined weekly for each dose group for the first 14 weeks of the study and approximately every 4 weeks for the remainder of the study period.

TABLE 2. GROUP MEAN BODY WEIGHTS AND BODY WEIGHT GAINS FOR MALE AND FEMALE B6C3F1 MICE TREATED WITH MEPIQUAT CHLORIDE FOR 12 or 24 MONTHS

Day of Study	Group	Treatment Group/Exposure Level (ppm)							
		Males				Females			
		0	500	2000	7500	0	500	2000	7500
0	Main	23.4	23.3 (100%)[b]	23.3 (100%)	23.1 (99%)	19.0	19.1 (100%)	19.1 (100%)	18.7 (98%)
	Satellite	23.8	23.6 (99%)	23.6 (99%)	23.5 (99%)	18.8	18.9 (100%)	18.7 (99%)	18.5 (98%)
350	Main	40.8	39.0 (96%)	39.8 (98%)	38.1* (94%)	34.4	35.4 (103%)	36.8 (107%)	35.3 (103%)
364	Satellite	40.1	37.7 (94%)	40.4 (101%)	41.2 (103%)	37.3	34.7 (93%)	35.8 (96%)	32.7 (88%)
714	Main	38.0	36.7 (96%)	36.9 (97%)	35.6 (94%)	34.9	35.0 (100%)	35.7 (102%)	34.9 (100%)
Terminal[a]	Main	34.2	33.2 (97%)	33.3 (97%)	32.8 (96%)	32.1	32.4 (101%)	33.1 (103%)	32.0 (100 %)
	Satellite	35.0	33.1 (95%)	35.7 (102%)	36.3 (104%)	33.7	31.6 (94%)	32.5 (96%)	29.5 (88%)
Weight gain (g)									
0-364	Satellite	16.3	14.1 (87%)	16.8 (103%)	17.7 (109%)	18.4	15.8 (86%)	17.2 (93%)	14.2 (77%)
0-350	Main	17.4	15.7 (90%)	16.5 (95%)	15.0* (86%)	15.4	16.4 (107%)	17.7 (115%)	16.7 (109%)
0-714	Main	14.6	13.4 (92%)	13.6 (93%)	12.5 (86%)	15.8	16.0 (101%)	16.5 (105%)	16.2 (103%)

Data adapted from Tables 15-44 (pp. 60-73, 162-177) and tables of absolute weights, pp. 496, 497, 500, 501 MRID No. 432644-04.

[a]Terminal weights at sacrifice, used for determination of relative organ weights.

[b] Percent of control

*$p<0.05$, **$p<0.01$

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TABLE 3. STATISTICALLY SIGNIFICANT CHANGES IN GROUP MEAN BODY WEIGHTS DURING THE FIRST YEAR OF TREATMENT FOR MAIN GROUP MALE AND SATELLITE GROUP FEMALE B6C3F1 MICE TREATED WITH MEPIQUAT CHLORIDE

Day of Study	Treatment Group/Exposure Level (ppm)							
	Main Group Males				Satellite Group Females			
	0	500	2000	7500	0	500	2000	7500
0	23.4	23.3 (100%)	23.3 (100%)	23.1 (99%)	18.8	18.9 (100%)	18.7 (99%)	18.5 (98%)
49	27.8	28.1 (101%)	27.9 (100%)	27.4 (98%)	23.4	22.5 (96%)	21.4** (92%)	21.6** (92%)
56	28.2	27.5 (97%)	27.7 (98%)	27.2 (96%)	23.5	22.9 (98%)	21.8** (93%)	22.1* (94%)
63	29.1	28.4 (98%)	28.3 (97%)	27.5** (95%)	24.2	24.3 (100%)	23.0 (95%)	22.4** (93%)
70	29.8	29.0 (97%)	29.1 (98%)	28.3** (95%)	24.7	24.4 (99%)	22.9* (93%)	22.6** (92%)
77	30.5	29.9 (98%)	30.0 (98%)	29.3 (96%)	25.2	24.4 (97%)	23.4* (93%)	23.3* (93%)
84	31.1	30.0 (96%)	30.6 (98%)	29.6* (95%)	26.0	24.7 (95%)	24.0 (92%)	23.9 (92%)
91	32.0	30.4* (95%)	30.8 (96%)	30.1** (94%)	26.5	24.5 (92%)	24.6 (93%)	23.9* (90%)
98	32.3	30.8* (96%)	31.4 (97%)	30.3** (94%)	27.7	25.2* (91%)	25.7 (93%)	25.0* (90%)
126	33.8	32.2 (95%)	33.3 (98%)	32.0* (95%)	27.5	26.0 (95%)	26.2 (95%)	25.7 (93%)
154	34.8	33.1 (95%)	34.0 (98%)	32.8* (94%)	30.1	28.2 (94%)	28.6 (95%)	27.1 (90%)
182	36.2	33.8* (93%)	35.5 (98%)	33.7** (93%)	29.7	28.7 (97%)	28.6 (97%)	27.3 (92%)
210	37.1	35.2 (95%)	36.1 (97%)	34.3** (93%)	31.8	30.8 (97%)	30.5 (96%)	28.2 (89%)
350	40.8	39.0 (96%)	39.8 (98%)	38.1* (94%)	37.3	34.8 (94%)	35.7 (96%)	31.6 (85%)

Data adapted from Tables 15-17, 28-30 (pp. 60-62, 73, 162-163), MRID No. 432644-04.
*p≤0.05, **p≤0.01

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TABLE 4. STATISTICALLY SIGNIFICANT CHANGES IN GROUP MEAN BODY WEIGHT GAINS DURING THE FIRST YEAR OF TREATMENT FOR MAIN GROUP MALE AND SATELLITE GROUP FEMALE B6C3F1 MICE TREATED WITH MEPIQUAT CHLORIDE

Range of Days of Study	Treatment Group/Exposure Level (ppm)							
	Main Group Males				Satellite Group Females			
	0	500	2000	7500	0	500	2000	7500
0-49	4.5	4.8 (109%)	4.6 (103%)	4.2 (95%)	4.6	3.6 (79%)	2.8** (61%)	3.1* (69%)
0-56	4.8	4.2 (87%)	4.4 (91%)	4.1 (84%)	4.6	4.0 (86%)	3.2** (69%)	3.6 (78%)
0-63	5.7	5.1 (90%)	5.0 (87%)	4.4** (78%)	5.4	5.4 (100%)	4.4 (81%)	3.9* (73%)
0-70	6.4	5.7 (89%)	5.7 (89%)	5.1** (80%)	5.8	5.5 (94%)	4.2** (73%)	4.2** (72%)
0-77	7.1	6.6 (93%)	6.7 (94%)	6.1 (86%)	6.4	5.5 (86%)	4.7* (74%)	4.9* (77%)
0-84	7.7	6.7 (86%)	7.2 (94%)	6.4* (83%)	7.2	5.8 (82%)	5.4* (75%)	5.4* (76%)
0-91	8.6	7.1** (83%)	7.5 (87%)	7.0** (81%)	7.6	5.6* (73%)	6.0 (78%)	5.4* (71%)
0-98	8.9	7.5* (85%)	8.0 (91%)	7.2** (81%)	8.8	6.3* (72%)	7.0 (80%)	6.5* (74%)
0-126	10.4	8.9 (85%)	9.9 (95%)	8.8* (85%)	8.7	7.1 (82%)	7.5 (87%)	7.2 (83%)
0-182	12.8	10.5* (82%)	12.1 (94%)	10.6* (82%)	10.8	9.8 (90%)	10.0 (92%)	8.9 (82%)
0-210	13.7	11.9 (87%)	12.8 (93%)	11.2** (82%)	13.0	11.9 (92%)	11.0 (91%)	9.8 (75%)
0-350	17.4	15.7 (90%)	16.5 (95%)	15.0* (86%)	18.5	15.9 (86%)	17.0 (92%)	13.1 (71%)

Data adapted from Tables 31-34 and 42-44 (pp. 164-167, 175-177), MRID No. 432644-04.
*p≤0.05, **p≤0.01

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Results –

a. Food consumption – The study author commented that due to spillage of food and the housing of the animals (Macrolon cages with bedding) it was not possible to determine actual food consumption. The author's estimates of food consumption indicated that test animals compared favorably with controls. The lack of significant differences in termination body weights supports this contention.

b. Compound consumption – Compound consumption (Table 1) for mice in the medium and high dose groups was approximately 4 and 15 times the low dose group, respectively.

c. Food efficiency – Since the amount of total food consumed was not accurately determined, overall food efficiency could not be reliably calculated. Using their estimates of daily food consumption, the study author calculated daily food efficiencies. No treatment related effects were observed.

4. Ophthalmoscopic examination

Results – Ophthalmoscopic examinations are not required for oncogenicity studies in mice and were not conducted.

5. Blood was collected from all mice in the satellite and main groups after decapitation at study termination, and differential blood smears from the control and 7500 ppm dose groups were evaluated. For all mice found moribund and sacrificed during the study, blood was collected and differential blood counts were performed. Differential blood smears were analyzed after staining with Wright's stain.

Results – There were no treatment-related effects on white blood cell counts or on cell morphology for males or females receiving the test material.

a. Hematology – There were no treatment-related effects on white blood cell counts or on morhhology of white or red cells for males or females receiving the test material.

b. Clinical chemistry – Clinical chemistry analyses are not required for oncogenicity studies in mice and were not conducted.

6. Urinalysis

Urinalysis is not required for oncogenicity studies in mice.

7. Sacrifice and pathology

Mice were euthanitized by decapitation after carbon dioxide inhalation following a fasting period of 16-20 hours. Necropsy was performed on all animals. Tissues were fixed in 4% formaldehyde and stained with hematoxylin-eosin prior to microscopic examination.

All the tissues checked below were examined in all mice from the control and high dose (7500 ppm) groups and in all mice that died or were sacrificed during the treatment period. The lungs, liver, kidneys, adrenal glands, preputial glands, and gross lesions from all animals in the main study were subjected to microscopic pathological examination. The lungs, liver, kidneys, and gross lesions were microscopically examined for all mice in the 12-month satellite study. Statistical analysis was performed on terminal body weights and absolute and relative organ weights, but was not performed by the study authors on the incidences of macroscopic or microscopic pathological findings. All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	Digestive system
	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
X	Gall Bladder*
X	Pancreas*

	Respiratory
X	Trachea*
X	Lung*
	Nose
	Pharynx
	Larynx

X	Cardiovasc./Hemat.
X	Aorta*
X	Heart*
X	Bone marrow*
X	Lymph nodes*
X	Spleen*
X	Thymus*

	Urogenital
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
X	Epididymides*
X	Prostate
X	Seminal vesicle
X	Ovaries*
X	Uterus*

X	Neurologic
XX	Brain*+
X	Periph. nerve*
X	Spinal cord (3 levels)*
X	Pituitary*
X	eye (optic n.)*

	Glandular
XX	Adrenal gland*
	Lacrimal gland
X	Mammary gland*
X	Parathyroids*
X	Thyroids*

	Other
X	Bone*
X	Skeletal muscle*
X	Skin*
X	All gross lesions and masses*

* Required for oncogenicity studies.
+ Organ weight required in oncogenicity studies.

Results –

a. Organ weight – For main group males, there were statistically significant increases in relative organ weights for the adrenals (500, 2000, and 7500 ppm dose groups, each 118%of control, $p \leq 0.05$, calculated by reviewer), and increases in absolute organ weights for the adrenals (500, 2000, and 7500 ppm dose groups respectively: 109% (not significant), 114%, $p \leq 0.05$, 108% (not significant)), as compared to controls. There were decreases in relative organ weights for the liver (500 ppm: 94%, $p \leq 0.05$) and for the kidneys (2000 ppm: 97%, $p \leq 0.05$) for satellite group females as compared to controls. These changes were not dose-related.

b. Gross pathology – As shown in Table 5, significant differences were seen only in the forestomach (males), liver lymph node (females) and mediastinal lymph node (females), and none exhibited a dose response.

TABLE 5. INCIDENCE OF MACROSCOPIC PATHOLOGICAL FINDINGS FOR MALE AND FEMALE B6C3F1 MICE TREATED WITH MEPIQUAT CHLORIDE IN FOOD FOR 714 DAYS

Pathology	Exposure Level (ppm)							
	Males				Females			
	0	500	2000	7500	0	500	2000	7500
Forestomach - Foci	0/50	5/50*	2/50	4/50*	1/50	1/50	0/50	3/50
Thymus - Enlarged	0/50	0/50	2/50	0/50	0/50	4/50*	2/50	1/50
Liver lymph node - Enlarged	4/50	0/50	0/50	3/50	1/50	7/50*	5/50*	1/50
Mediastinal lymph node -Enlarged	1/50	0/50	1/50	0/50	1/50	7/50*	2/50	3/50

Macroscopic pathological incidences were adapted from the Pathology Report, pp. 505-508 MRID No. 432644-04.
*p≤0.05, statistical significance calculated by the reviewer using Fisher's Exact Test.

c. Microscopic pathology –

1) Non-neoplastic – The only statistically significant non-neoplastic histopathological finding was in the kidney where an increased incidence of cysts ($p \leq 0.05$, calculated by reviewer using Fisher's Exact Test) and tubular hyperplasia ($p \leq 0.05$) was seen in main group male mice at 2000 ppm but not at 500 or 7500 ppm.

2) Neoplastic – There were no neoplastic changes occurring in a dose-related frequency in treated males or females in the main or satellite groups (see APPENDIX B. below). Tumor incidences and total tumor burden for main group males and females are presented in Table 6.

D. DISCUSSION

In an oncogenicity feeding study, Mepiquat Chloride was administered to 50 male and 50 female B6C3F1 mice for 24 months and 10 mice/sex/dose for 12 months (satellite group) at 500, 2000, and 7500 ppm (equivalent to 74, 297, 1140 mg/kg/day for males and 85, 328, 1348 mg/kg/day for females respectively). The doses of Mepiquat Chloride for the current study were selected on the basis of a preliminary 3-month feeding study with doses of Mepiquat Chloride at 300, 900, 2700, or 8100 ppm (MRID No. 424121-01, summarized in the Appendix to this report). There were no treatment-related findings for male or female B6C3F1 mice in the 3-month feeding study.

There were statistically significant decreases in body weights and body weight gains for main group males and satellite group females primarily at the high dose, during the first year of the study. However, the decreases in body weights and body weight gains relative to controls

TABLE 6. TUMOR INCIDENCE FOR MAIN GROUP MALE AND FEMALE B6C3F1 MICE TREATED WITH MEPIQUAT CHLORIDE IN FOOD FOR 714 DAYS

Number of Tumors	Exposure Level (ppm)							
	Males (50/dose group)				Females (50/dose group)			
	0	500	2000	7500	0	500	2000	7500
Number of Animals with:								
Primary neoplasms	29	18	22	23	25	24	21	17
One primary neoplasm	21	17	17	19	21	17	18	15
Two or more primary neoplasms	8	1	5	4	4	7	3	2
Benign neoplasms	15	10	13	13	11	13	10	6
Malignant neoplasms	16	8	11	12	16	15	13	12
Total:								
Primary neoplasms	38	19	29	27	29	32	24	19
Benign neoplasms	22	11	18	13	12	16	11	6
Malignant neoplasms	16	8	11	14	17	16	13	13

Tumor incidences were adapted from the Pathology Report, p. 532, MRID No. 432644-04.

for main group males are not matched by decreases in the satellite males and body weights never fell below 93% of control levels. In addition, group mean body weights and body weight gains during the second year of the study, and at the end of the study were not statistically significantly different from controls. Therefore, the decreased body weight gains for high dose main group males during the first year are not biologically significant. The decreases in group mean body weights and body weight gains for satellite group females as compared to controls are also not biologically significant since the effect was not corroborated in main group females which had a sample size 5 times greater than the satellite group.

There were a few sporadic changes in organ weights, primarily relative weights, but there was no dose response. The only non-neoplastic microscopic finding was an increased incidence of cysts and tubular hyperplasia ($p \leq 0.05$) in main group male mice at 2000 ppm but not at 500 or 7500 ppm.

Based upon the lack of toxicologically significant findings for main group males or females in the high dose group, the NOEL for a 2-year oncogenicity feeding study is 7500 ppm (active ingredient) for male and female B6C3F1 mice.

There were no neoplastic changes occurring at a higher frequency in treated animals than in controls. Therefore, treatment of B6C3F1 mice with Mepiquat Chloride at 7500 ppm in feed for 2 years does not result in an increased incidence of carcinogenesis for males or females. The lack of effect of Mepiquat Chloride administration on group mean body weights and body weight gains suggest that the test material was not administered at or near the MTD. However, the compound intake for high dose males and females was greater than 1000 mg/kg/day, satisfying the guidelines for an oncogenicity study (§83-2).

E. STUDY DEFICIENCIES

The pages in Book 1 of the study report were out of order. Tables A17-A104 (individual values for food consumption and body weights) were inserted between tables 28 (p.73) and 29 (p.162, group mean body weights for satellite group females). The incidence of the macroscopic and microscopic pathological findings were not statistically analyzed by the study authors. These deficiencies are considered minor and do not alter the conclusions of the study.

APPENDIX A

Dose Selection Study in Mice

MRID No.: 424121-01
Study Type: Subchronic Feeding-Mouse (82-1a, range-finding)
Test Material: Mepiquat Chloride
Study No.: 53S0112/89013
Testing Facility: BASF Aktiengesellschaft, Dept. of Toxicology, Limburgerhof, Germany
Study Title: Oral Toxicity of Mepiquat Chloride in B6C3F1 Mice, Administration in the Diet over 3 months (Range-Finding)
Author: K. Schilling
Study Completed: June 12, 1992

Methods:
Test Animals: B6C3F1/CrlBR mice (Charles River WIGA GmbH, D-W8741, Sulzfeld, FRG); 49 days old; 24.7-28.2 g (males), 18.4-21.4 g (females)
Group Size: 10/sex/dose
Test material:
Concentrations: Mepiquat Chloride, in diet at 0, 300, 900, 2700, or 8100 ppm (a.i.), corresponding to respective daily compound intake values of 0, 60, 186, 526, and 1731 mg/kg body weight for males and 0, 83, 265, 705, and 2422 mg/kg body weight for females.

Results:
Clinical signs: There were no treatment-related findings. All mice were generally healthy. Food consumption was similar between controls and treated groups.
Mortality: There were no deaths prior to termination.
Bodyweight Gain: Group mean body weight gains for treated mice were not statistically significantly different from controls.
Clinical Pathology:
Hematology: There were no statistically significant differences between treated groups and controls for any of the hematology parameters.
Biochemistry: There were no statistically significant differences between treated groups and controls for any of the biochemical parameters.
Urinalysis: Urinalysis was not performed.
Organ Weights: Group mean relative organ weights for liver and kidneys for males in the high dose group were statistically significantly elevated (7% and 9%, respectively, $p<0.05$) above controls. Absolute organ weights were not significantly affected by treatment. Organ weights for the brain, heart, lungs, and ovaries were not determined.
Macroscopic and Microscopic Pathology: There were no treatment-related macroscopic or microscopic pathological findings for males or females.

Conclusions:

There were no treatment-related findings for Mepiquat Chloride administered in diet for 3 months. The NOEL for 3-month dietary administration of Mepiquat Chloride to male and female B6C3F1 mice is 8100 ppm (a.i.). Based upon this study, doses of Mepiquat Chloride for the 2-year oncogenicity study (MRID No. 432644-04) were chosen as 500, 2000, and 7500 ppm (a.i.).

Core Classification: Not applicable; dose range-finding study.

APPENDIX B

APPENDIX. SELECTED TUMOR INCIDENCE IN ALL MALE AND FEMALE B6C3F1 MICE TREATED WITH MEPIQUAT CHLORIDE IN FOOD FOR 24-MONTHS[1]

Number of Tumors	Exposure Level (ppm)							
	Males				Females			
	0	290	2316	5790	0	290	2316	5790
Liver: Hepatocellular adenomas	3/50	4/50	4/50	2/50	4/50	4/50	4/50	2/50
Liver: Hepatocellular carcinomas	6/50	5/50	5/50	4/50	1/50	0/50	3/50	3/50
Lung: Bronchial alveolar adenomas	6/50	3/50	7/50	7/50	1/50	2/50	1/50	1/50
Lung: Bronchial alveolar adenocarcinoma	2/50	2/50	1/50	4/50	1/50	1/50	0/50	2/50
Malignant lymphoma	5/50	0/50	1/50	3/50	11/50	7/50	6/50	5/50

[1] Tumor incidences were adapted from the Pathology Report, pages 525-526, MRID No. 432644-04.

APPENDIX. SELECTED TUMOR INCIDENCE IN SURVIVING MALE AND FEMALE B6C3F1 MICE TREATED WITH MEPIQUAT CHLORIDE IN FOOD FOR 24-MONTHS[1]

Number of Tumors	Exposure Level (ppm)							
	Males				Females			
	0	290	2316	5790	0	290	2316	5790
Liver: Hepatocellular adenomas	3/42	4/48	4/48	1/43	4/41	4/42	4/40	2/42
Liver: Hepatocellular carcinomas	5/42	4/48	5/48	2/43	1/41	0/42	3/40	3/42
Lung: Bronchial alveolar adenomas	5/42	3/48	7/48	7/43	1/41	2/42	1/40	1/42
Lung: Bronchial alveolar adenocarcinoma	2/42	2/48	1/48	4/43	1/41	0/42	0/40	1/42
Malignant lymphoma	2/42	0/48	0/48	1/43	8/41	5/7	2/3	1/42

[1] Tumor incidences were adapted from the Pathology Report, pages 527-528, MRID No. 432644-04.

APPENDIX. SELECTED TUMOR INCIDENCE IN NON-SURVIVORS MALE AND FEMALE B6C3F1 MICE TREATED WITH MEPIQUAT CHLORIDE IN FOOD FOR 24-MONTHS[1]

Number of Tumors	Exposure Level (ppm)							
	Males				Females			
	0	290	2316	5790	0	290	2316	5790
Liver: Hepatocellular adenomas	0/8	0/2	0/2	1/7	NR	NR	NR	NR
Liver: Hepatocellular carcinomas	1/8	1/2	0/2	2/7	NR	NR	NR	NR
Lung: Bronchial alveolar adenomas	1/8	0/2	0/2	0/7	0/9	1/8	0/10	1/8
Lung: Bronchial alveolar adenocarcinoma	0/8	1/2	0/2	0/7	0/9	0/8	1/10	0/8
Malignant lymphoma	3/8	0/2	1/2	2/7	3/9	2/8	4/10	4/8

[1] Tumor incidences were adapted from the Pathology Report, pages 529-530, MRID No. 432644-04.

EPA Toxicologist: Krystyna K. Locke KK Locke, Date 2/7/96
Review Section I, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Roger L. Gardner Roger Gardner Date 2/14/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic Oral Toxicity (feeding) - Dog; OPPTS 870.4100 [§83-1 (b)]

DP BARCODE: D216220
P.C. CODE: 109101
TEST MATERIAL (PURITY): ≥ 99.5%
Reg. No. 85 559 (Mepiquat chloride)

SUBMISSION CODE: S488415
TOX. CHEM. NO.: 380 AB
REREG. CASE NO.: 2375

SYNONYMS: None

CITATION: Hellwig [1st name not reported] (1989) Report on the Study of the Toxicity of Reg. No. 85 559 in Beagle Dogs: Administration Via the Diet Over 12 Months (BASF, Germany); Report No. 33D0453/8565; September 25, 1989. **MRID** 41488105 Unpublished

SPONSOR: BASF Corporation, Agricultural Chemicals Group, Research Triangle Park, NC

EXECUTIVE SUMMARY:

In a chronic toxicity study (**MRID 41488105**), Reg. No. 85 559 (mepiquat chloride), 99.5 % a.i., was administered to 6 beagle dogs/sex/dose in diet at dose levels of 0, 200, 600 or 1800 ppm (0, 6.3, 19.9 or 58.4 mg/kg/day, respectively) for 12 months.

The only possibly treatment-related effect, observed at the 1800 ppm dose, was a very slightly increased (Grade 2 on the scale 1-5) storage of the iron pigment in the spleen of 3 male dogs and in the liver of 2 male dogs. All of the remaining male and female dogs in this group, and all of the males and the majority of the females in the remaining groups, including the controls, also had iron pigment in the spleen and liver, but of slightly lesser severity (Grade 1). There were no compound-related effects in mortality, clinical signs, body weight, food consumption, ophthalmoscopic findings, hematology, clinical chemistry, urinalysis, organ weights, gross pathology and, with the exception of the iron pigment noted above, histologic pathology.

Since no definite toxic effects were detected in the current study (MRID 41488105), another one-year dog feeding Supplementary Study (MRID 43264403) with two doses of mepiquat chloride (0 and 6000 ppm) was started on January, 1992 and completed on May 5, 1994. Both studies were, therefore, considered in establishig a NOEL and determining whether the guideline requirement 83-1b was satisfied.

Based on both studies (MRIDs 41488105 and 43264403), **the LOEL for males and females is 6000 ppm (170 mg/kg/day) and the NOEL is 1800 ppm (58.4 mg/kg/day).** Toxic signs observed at the 6000 ppm dose in all dogs were: (1) impaired neurological functions (salivation after feeding and, in one female, lateral position, extension spasm and ataxia of the hind limb); (2) epithelial vacuolization of the renal distal tubules; and (3) increased hemosiderin (iron pigmenrt; Grade 2) in the spleen (males only).

Considered together with another study (MRID 43264403), this chronic toxicity study in the dog (MRID 41488105) is acceptable and satisfies the guideline requirement for a chronic oral study (83-1b) in dogs.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality and Flagging statements were provided. This study was conducted in accordance with the OECD Principles of Good Laboratory Practices (Paris, 1981) and does not meet all of the requirements for 40 CFR 160, Good Laboratory Practices, in the following way: the stability of the test substance was not fully confirmed according to the GLP Guidelines.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Reg. No. 85 559
   Description: White-greyish powder
   Lot/Batch #: n 48
   Purity: ≥ 99.5 % a.i.
   Stability of compound: Stable for 2 years at room temperature
   CAS #: 24307-26-4

2. Vehicle and/or positive control: N/A

3. Test animals: Species: Dog
   Strain: Beagle
   Age and weight at study initiation: 7-9 months; 9.5 (6.7-13.0) kg, males and 8.6 (6.4-10.4) kg, females.
   Source: BASF Breeding Unit
   Housing: Singly, in kennels about 2.7 m^2
   Diet: Dog maintenance KLIBA laboratory diet A, obtained from Klingentalmuhle AG, Switzerland.
   Water: Blended water (fully demineralized water adjusted with drinking water to about 4° German hardness) was available ad libitum.
   Environmental conditions: Temperature: Not reported.
   Humidity: Not reported.
   Air changes: Not reported.
   Photoperiod: "Natural day/night rhythm, with additional artificial light as required during working hours".
   Acclimation period: 13-20 days.

B. STUDY DESIGN:

1. In life dates - start: May 13, 1986; end: May 27, 1987 (last necropsy day).

2. Animal assignment

Animals were assigned randomly (random number generator) to the test groups as shown in TABLE 1. The secondary criterion for the randomization was an approximately equal mean body weight in the individual groups.

TABLE 1: STUDY DESIGN

Test Group	Conc. in Diet (ppm)	Dose to animal (mg/kg) *	Main Study 12 months		Interim Sac. __ months	
			male	female	male	female
Control	0	0	6	6	-	-
Low (LDT)	200	6.3	6	6	-	-
Mid (MDT)	600	19.9	6	6	-	-
High (HDT)	1800	58.4	6	6	-	-

* Actual values; mg/kg of body weight/day

3. Dose selection rationale: Doses selected for this study were based on the results of two feeding studies with male and female beagle dogs: a 4-week study (dated 1976; not submitted to the Agency for review) and a 3-month study (dated 1977; MRID 00135720).

4. Diet preparation and analysis:

Diets were prepared daily by mixing powdered feed pellets (350 g) with drinking (tap) water (350 mL) immediately before administration to each dog. Appropriate amounts of mepiquat chloride were added to the powdered pellets and these mixtures were usually prepared once a week. The pellets and the powdered mixtures were stored at room temperature. Homogeneity and stability of the test substance in the diet were tested at the beginning of the study, one week later and about 5 months later. During the study, samples of treated food (all doses) were analyzed for concentration of mepiquat chloride on day 7, at quarterly intervals thereafter and at the end of the study.

Results - Homogeneity Analysis: The diets were homogeneous with respect to mepiquat chloride as is shown below.

Target concentration (ppm):	200	600	1800
Mean analytical values (ppm):	205	588	1888
Percent of target concentr.:	102	92	105
Range of values (ppm):	194-212	566-613	1753-2020
Percent of target concentr.:	97-106	94-102	97-112

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Stability Analysis: Mepiquat chloride at doses of 200 and 600 ppm (only doses tested for stability) was stable in diets as is shown below.

Concentration at 0 time (ppm):	200	600
After 10 days at room temp. (ppm):	194	549
Percent of original concentration:	97	91
Range of values (ppm):	181-205	531-567
After 32 days at room temp. (ppm):	194	585
Percent of original concentration:	97	97
Range of values (ppm):	193-201	572-602

Concentration Analysis: The analytical data shown below indicate that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

Target dose (ppm):	200	600	1800
Analytical values (ppm):	191	605	1797
Percent of target dose:	96	101	100
Range of values (ppm):	180-199	574-661	1758-1826
Percent of target dose:	90-100	96-110	98-101

The above tables are based on data reported on pages 829-849 of the submitted report (MRID 41488105). Diet samples were stored at -18°C or -20°C before analyses.

5. Statistics - Feed consumption, body weight, body weight change, test substance intake, and hematology, clinical chemistry and urinalysis data were analyzed using the Kruskal-Wallis (1956) and Mann-Whitney (1956) tests. Body weights at the termination of the study and absolute and relative (organ/body weight ratios) organ weights were analyzed by the procedure of Dunnett (1955 and 1964). The statistical procedures used appeared adequate. (Details are in Attachment I).

C. METHODS:

1. Observations:

Animals were inspected once daily (several times when signs occurred) for signs of toxicity. A check was made for any moribund or dead animals twice a day on Mondays through Fridays and once a day on weekends and holidays.

2. Body weight:

Animals were weighed weekly.

3. Food consumption and compound intake:

Food consumption for each animal was determined and mean daily diet consumption was calculated as g food/animal/day. Food efficiency was not calculated. Compound intake (mg/kg/day) values were calculated weekly as averages from the food consumption and body weight data.

4. Ophthalmoscopic examination:

Eyes were examined before the start of the study (all dogs) and at the end of the treatment period (controls and high-dose groups).

5. Blood was collected in the morning for hematology and clinical analysis, from all animals (fasted), as follows: 11 or 8 days before the beginning of treatment and 93 or 94, 184 or 188, and 367 days after treatment. The CHECKED (X) parameters were examined.

a. Hematology.

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*		
X	(Thromboplastin time)		
X	# (Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for chronic studies based on Subdivision F Guidelines
# Partial

b. Clinical Chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
		X	Total serum protein (TP)*
X	ENZYMES	X	Triglycerides
	Alkaline phosphatase (ALK)		Serum protein electrophores
	Cholinesterase (ChE)		
	Creatine phosphokinase		
X	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)*		
	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for chronic studies based on Subdivision F Guidelines

6. Urinalysis

Urine was collected overnight from all animals (fasted) 6 days before treatment and after 92, 183 or 185, and 365 days of treatment. The animals received about 500 ml of drinking water overnight. The CHECKED (X) parameters were examined.

X		X	
	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*	X	Nitrate
X	Protein*	X	Urobilinogen

* Required for chronic studies

7. Sacrifice and Pathology:

All animals in the study were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs,

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in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	X	Heart*	X	Periph.nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*		
X	Duodenum*	X	Spleen*	X	Pituitary*
X	Jejunum*	X	Thymus*	X	Eyes (optic n.)*
X	Ileum*				
X	Cecum*		UROGENITAL		GLANDULAR
X	Colon*	XX	Kidneys*+	XX	Adrenal gland*
X	Rectum*	X	Urinary bladder*		Lacrimal gland
XX	Liver*+	XX	Testes*+	X	Mammary gland*
X	Gall bladder*		Epididymides	XX	Parathyroids*++
X	Pancreas*	X	Prostate	XX	Thyroids*++
			Seminal vesicle		
	RESPIRATORY	XX	Ovaries*+		OTHER
X	Trachea*	X	Uterus*	X	Bone*
X	Lung*			X	Skeletal muscle*
	Nose			X	Skin*
	Pharynx			X	All gross lesions and masses*
	Larynx				

* Required for chronic studies based on Subdivision F Guidelines.
+ Organ weight required in chronic studies.
++ Organ weight required for non-rodent studies.
All of the above tissues were examined.

II. RESULTS:

A. Observations

1. Toxicity - None observed.

2. Mortality - None.

B. Body weight was not affected by treatment. These data are summarized below.

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TABLE 2: BODY WEIGHT CHANGES (kg)

Test material (ppm)	0	200	600	1800
Study Days		Male Dogs		
0-28	0.8	1.0	0.7	0.8
28-63	0.9	0.7	0.8	0.7
63-91	0.6	0.7	0.6	0.5
91-154	0.2	0.3	0.2	0.3
154-259	- 0.6	- 0.3	- 0.4	- 0.3
259-336	0.0	0.2	0.1	0.1
336-364	0.4	0.1	0.4	0.2
0-364	2.3	2.7	2.4	2.3
		Female Dogs		
0-29	0.6	0.8	0.6	0.8
29-63	0.7	0.7	0.6	0.5
63-91	0.5	0.2	0.3	0.4
91-154	- 0.1	0.4	0.2	0.1
154-259	0.0	0.2	- 0.2	0.0
259-336	0.2	0.1	0.2	0.4
336-364	0.3	0.3	0.1	0.2
0-364	2.2	2.7	1.8	2.4

This table is based on TABLES 109-122, pages 169-182, of the submitted report (MRID 41488105). (-) Sign before a number denotes weight loss, whereas the remaining values represent weight gains.

C. Food consumption and compound intake

1. Food consumption - Male dogs in the control, 200 ppm and 600 ppm groups consumed all of their food every day during the study, as did 5/6 male dogs in the 1800 ppm group. One male dog in the 1800 ppm group consumed less food (91-99% of the daily ration) during 65 days of the study. These decreases in food consumption occurred at various times during study days 0-361, but were statistically insignificant. In the case of the female dogs, decreases in the food consumption were observed in all groups at various times during the study, as follows:

Mepiquat chloride (ppm):	**0**	**200**	**600**	**1800**
Percent of daily ration consumed	76-98	78-98	74-98	70-98
Total number of study days involved	189	99	158	134
Days when decreases were observed	4-363	10-360	10-363	26-362

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Decreases in the food consumption, observed for the female dogs, were also statistically insignificant. The numbers of the female dogs affected in the control, low-dose, mid-dose and high-dose groups were 5, 5, 4 and 5, respectively.

The above data are based on findings reported on pages 61-107 and 277-368 (males), and 108-154 and 369-462 (females) of the submitted report (MRID 41488105).

2. Compound consumption - The group mean intake of mepiquat chloride was reported for the 7-day periods during the entire study. These data are summarized below.

TABLE 3: GROUP MEAN INTAKE OF MEPIQUAT CHLORIDE (mg/kg b.w./day)

Mepiquat chloride (ppm)	200	600	1800
Group	1	2	3
Study Days	Male Dogs		
0	7.4	22.4	67.9
14	6.9	21.3	65.1
49	6.4	19.6	60.4
105	5.9	18.3	55.9
161	5.7	18.0	55.2
217	5.8	18.3	56.5
273	5.9	18.7	56.5
329	5.8	18.7	56.1
357	5.8	18.2	52.3
	Female Dogs		
0	8.2	25.5	73.8
14	7.7	23.4	69.8
49	7.2	22.0	61.2
105	6.8	21.4	60.9
161	5.9	18.3	57.6
217	6.6	19.9	52.9
273	5.7	20.7	60.3
329	6.5	20.6	59.5
357	6.1	20.7	58.0
	Male and Female Dogs		
0-357	6.3	19.9	58.4

This table is based on TABLES 123-136, pages 183-196, of the submitted report (MRID 41488105).

3. Food efficiency was not determined.

D. Ophthalmoscopic examination revealed no treatment-related effects in the 1800 ppm male and female group, the only mepiquat chloride-treated group examined at the termination of the study. One female had an opacity (about the size of the pin head) in the right eye before the study was started and this opacity remained unchanged at the end of the study.

E. Blood work

1. Hematology - Mepiquat chloride, at all doses tested, had no effect on hematology. A few statistically significant differences from the control group were observed in the parameters listed below.

TABLE 4: CHANGES (%) IN HEMATOLOGICAL PARAMETERS RELATIVE TO CONTROL VALUES

Mepiquat chloride (ppm):		200	600	1800
Study Day	Parameter Affected		Male Dogs	
93	HGB ↑	5*	-	-
	PTT ↓	-	-	6*
	RETI ↑	-	-	100**
184	HGB ↑	6**	-	-
	RBC ↑	7**	-	-
	HCT ↑	7**	-	6**
	RETI ↑	117**	100**	133**
			Female Dogs	
188	PTT ↓	5*	-	-
367	RETI ↓	-	-	77**

This table is based on TABLES 140-178, pages 200-238, of the submitted report (MRID 41488105). * P<0.05 and ** P<0.02 (Kruskal-Wallis Anova and Mann-Whitney U-tests).

Abbreviations: HGB (Hemoglobin); PTT (Partial Thromboplastin Time); RETI (Reticulocytes); RBC (Red Blood Cells); and HCT (Hematocrit). ↑ = Increase and ↓ = Decrease

According to the submitted report (pages 52-53), the above findings were not treatment-related for one or more of the following reasons: (1) The values lie within the range of biological variations; (2) There was a random

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increase in the control value; (3) A dose-response relationship is absent; and (4) Similar effect is absent in both sexes.

2. Clinical chemistry - Mepiquat chloride, at all doses tested, had no effect on clinical chemistry. A few statistically significant differences from the control group were observed in the parameters listed below.

TABLE 5: CHANGES (%) IN CLINICAL CHEMISTRY PARAMETERS RELATIVE TO CONTROL VALUES

Mepiquat chloride (ppm):			200	600	1800
Study Day	Parameter Affected			Male Dogs	
184	Total protein	↑	-	-	6*
367	Total protein	↑	-	-	4*
				Female Dogs	
94	Chloride	↑	-	-	4*
	Urea	↑	-	-	28**
188	Potassium	↑	-	6*	-

This table is based on TABLES 179-202, pages 239-262, of the submitted report (MRID 41488105). * $P<0.05$ and ** $P<0.02$ (Kruskal-Wallis Anova and Mann-Whitney U-tests). ↑ = Increase and ↓ = Decrease

According to the submitted report (pages 52-53), the above findings were not treatment-related for one or more reasons listed under TABLE 4 in this review.

F. Urinalysis were not affected by mepiquat chloride. According to Tables 203-210 (pages 263-270) and Tables B 161-192 (pages 703-734) of the submitted report (MRID 41488105), findings observed in 1 or 2 dogs/group 6 days before treatment (blood, bilirubin, urobilinogen, fat, crystals, epithelial tesselated cells, erythrocytes, leucocytes and bacteria) were also observed after treatment. During the first test interval (day 92) and thereafter, a few epithelial kidney and/or round cells were also noted in 1 or 2 control and treated dogs/group.

G. Sacrifice and Pathology

1. Organ weight - Mepiquat chloride had no effect on the absolute and relative organ weights in this study.

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(Relative organ weights = ogran/body weight ratios).

2. Gross pathology - Nothing remarkable was observed in all treated groups during necropsy. The predominant findings were lung foci in the males and females, and lung discoloration in the females, but the incidences were dose-unrelated. The incidences of lung foci in the control, low-dose, mid-dose and high-dose male groups were 3/6, 4/6, 3/6 and 5/6, respectively. The corresponding values for the female groups were 3/6, 5/6, 5/6 and 4/6, respectively. The incidence of lung discoloration in the female groups was 2/6, 1/6, 1/6 and 1/6, respectively. Other findings were single incidences in the control, low-dose or mid-dose groups only, as follows: focus and retraction in the spleen (males) and thickening of the jejunal wall, foci in the kidneys, and cysts in the brain and the pituitary gland (females).

3. Microscopic pathology

a) Non-neoplastic - The only treatment-related finding was a very slightly increased iron pigment storage in the spleen and liver of the male dogs, as follows:

TABLE 6: INCIDENCE AND SEVERITY OF IRON PIGMENT STORAGE IN THE LIVER AND SPLEEN

Mepiquat chloride (ppm):	0	200	600	1800
Organ	Number of male dogs affected			
Liver:	6	6	6	6
Grade 1	6	6	6	4
Grade 2	-	-	-	2
Spleen:	6	6	6	6
Grade 1	6	5	6	3
Grade 2	-	1	-	3
	Number of female dogs affected			
Liver:	6	4	5	5
Grade 1	6	4	5	5
Grade 2	-	-	-	-
Spleen:	6	6	6	6
Grade 1	5	5	5	4
Grade 2	1	1	1	2

This table is based on data reported on pages 741 and 749 of the submitted report (MRID 41488105). Grade 1 = Minimal storage Grade 2 = Slight storage

Other predominant but treatment-unrelated findings were observed in the stomach (lympho. hyperplasia), duodenum (glandular dilation), lung (infiltrates, hemorrhage and inflammation), kidneys (calcification), testes (absence of spermiogenesis), thymus (cysts), axillary lymph nodes (deposition of pigment), brain (hemorrhage), pituitary gland (cyst/hematocysts) and sternum (fibrous dysplasia). The incidences of these lesions are in Attachment II of this review.

b) Neoplastic - The only neoplastic (and treatment-unrelated) lesions observed were cystadenoma and granuloma. Cystadenoma occurred in the ovaries of one control female and granuloma, in the liver of one low-dose female. Granulomas were also observed in the lungs of all dogs, as follows: 5/6, 4/6, 4/6 and 2/6 in the control, low-dose, mid-dose and high-dose male groups, respectively; and 5/6, 3/6, 2/6 and 1/6 in the control, low-dose, mid-dose and high-dose female groups, respectively.

III. DISCUSSION

A. Review of the final report indicates that the design and conduct of the study were adequate. Also, this study is well reported and all procedures used were referenced and/or described.

Although the doses used in the current study were based on the results of two range-finding studies, a definite NOEL was not determined in the current study, according to the author. The doses used in the 4-week range-finding study (dated 1976) were 0, 400, 1000, 2500 and 6250 ppm (0, 16, 40, 100 and 250 mg/kg b.w., respectively). The doses used in the 3-month range-finding study (dated 1977) were 0, 100, 300, 1000 and 3000 ppm (0, 4, 12, 40 and 120 mg/kg b.w., respectively). The lowest dose at which toxic signs (decreased body weight gain and food consumption, and anemia) were observed was 2500 ppm.

In the current study, the only treatment-related effect observed at the 1800 ppm dose (HDT) was a very slightly increased storage of the iron pigment in the spleen of 3 male dogs and in the liver of 2 male dogs (TABLE 6 in this review). According to the author of the current study, this increased iron pigment storage was interpreted as a sign of the transient anemia because more pronounced signs of anemia were noted in the range-finding studies at the 2500 ppm dose and above. However, unlike in the range-finding studies, reduced hemoglobin and erythrocyte levels were not observed in the current study. Therefore, in the

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case of male dogs, the 1800 ppm dose seemed to be either a NOEL or a borderline NOEL.

Since no definite toxic effects were detected in the current study (MRID 41488105), another one-year dog feeding Supplementary Study (MRID 43264403) with two doses of mepiquat chloride (0 and 6000 ppm) was started on January 27, 1992 and completed on May 5, 1994. Therefore, both studies must be considered in establishing a NOEL and in determining whether the guideline requirement 83-1b was satisfied.

B. Study deficiencies: There are no major deficiencies in this study. Although food efficiency was not calculated and serum aspartate aminotransferase, gamma glutamyl transferase and glutamate dehydrogenase were not determined, the absence of these data does not affect the classification of this study.

Attachment I

0186-06

Mepiquat Chloride MRID 43264402, 03, 04

Page _____ is not included in this copy.

Pages 55 through 62 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

EPA Reviewer: William Greear, M.P.H., D.A.B.T. William R. Greear Date: 1/4/96
Review Section IV, Toxicology Branch I (7509C)
EPA Section Head: Marion Copley, D.V.M., D.A.B.T. Marion Copley Date: 1/4/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Chronic Feeding – Rat (83-1a)

TOX. CHEM. NO: 380AB

P.C.CODE.: 109101

MRID NO.: 432644-02 (Main Study), 424121-02 (Range-finding study)

TEST MATERIAL: Mepiquat Chloride

SYNONYMS: 1,1-Dimethylpiperidinium chloride

STUDY NUMBER: 71S0112/89091 (Main study), 30S0112/89012 (Range-finding study)

SPONSOR: BASF CORPORATION, Agricultural Products Group, P.O. Box 13528, Research Triangle Park, NC 27709-3528

TESTING FACILITY: BASF Aktiengesellschaft, Department of Toxicology, D-67056 Ludwigshafen/Rhine, FRG

TITLE OF REPORT: Chronic Toxicity Study with Mepiquat Chloride in Wistar Rats-Administration in the Diet for 24 months

AUTHOR: W. Mellert

REPORT ISSUED: May 3, 1994 (Study completion date)

EXECUTIVE SUMMARY: In a chronic feeding study (MRID No. 432644-02), Mepiquat Chloride (58%, Lot No. WW 262/CP 1490) was administered for 24 months in the diet to 20 Wistar rats/sex/dose at concentrations of 0, 290, 2316, and 5790 ppm (active ingredient), equivalent to doses of 0, 13, 106, 268 mg/kg/day for males and 0, 18, 146, and 371 mg/kg/day for females, respectively.

Total food consumption for rats in the high (5790 ppm) and medium (2316 ppm) dose groups was decreased 8% and 1% (not significant), respectively, for males and 6% and 2%, respectively, for females relative to controls. Body weights for rats in the high dose group were decreased 12% for males and 12% for females (not significant), relative to

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controls at day 728 of the treatment period. Body weight gains from day 0 to 728 were decreased relative to controls for males (17%) and females (20%, not significant) in the high dose group. There was an increase in the macroscopic pathological finding of focus for the adrenal cortex (control: 9/20; 5790 ppm: 16/20) and in microscopic pathological findings of vacuolated cell foci (control: 3/20; 5790 ppm: 9/20), hemosiderin pigment, and nodular hyperplasia for the adrenal cortex for females in the high dose group. There was an increased incidence of urinary crystals for males in the high dose group (12/20) as compared to controls (4/20) at day 99 and the increased incidence persisted (not significant) through day 541 of the treatment period. **The NOEL is 2316 ppm (active ingredient). The LOEL is 5790 ppm (active ingredient) based upon decreased body weights and body weight gains for males and females, increases in urinary crystals for males and pathological changes in the adrenal cortex in females.**

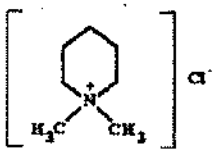
This study is classified as **core-minimum,** satisfying the guideline requirements for a chronic feeding study in rats (§83-1a).

Special Review Criteria (40 CFR 154.7) None

A. MATERIALS

1. Test material: Mepiquat Chloride

   Description: not provided in the study report
   Lot/Batch No.: WW 262/CP 1490
   Purity: 58% a.i. (w/w in water)
   Stability of compound: Stable for 2 years
   CAS No.: unknown
   Structure:



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2. Vehicle and/or positive control

Test material was mixed with diet. Negative control group was fed diet. No positive control was described.

3. Test animals

Species: rat
Strain: Wistar (Chbb:Thom (SPF))
Age and weight at study initiation: 42 days, 180-216 g (Males), 140-165 g (Females)
Source: Dr. Karl Thomae GmbH, Biberach/Riss, FRG
Housing: individually in stainless steel wire cages
Environmental conditions:
Temperature: 20-24°C
Humidity: 30-70%
Air changes: not described
Photoperiod: 12 hour light/dark cycle
Acclimation period: 14 days

B. STUDY DESIGN

1. Animal assignment

Rats (20/sex/dose) were assigned to the test groups in Table 1 in a randomized fashion, giving approximately equal mean body weights among each of the dose groups.

Dose selection rationale: Doses were selected on the basis of two preliminary studies, a 4-week feeding study in rats (Project No. 30S0112/89012, MRID No. 424121-02, see Appendix to this Report) and a 3-month feeding study in rats (Project No. 31S0112/89053 and supplementary Project No. 31S0112/89077). Treatment of rats with Mepiquat Chloride at 8000 ppm (active ingredient) for 4 weeks resulted in decreases in food consumption, body weights, absolute and relative organ weights, and changes in clinical chemistry. Mepiquat Chloride administration at 500 or 2000 ppm (active ingredient) for 4 weeks did not result in treatment-related findings. In the 3-month feeding study, Mepiquat Chloride administration at 145, 579, 2316, or 4632 ppm (active ingredient) did not result in treatment-related toxicological findings. In the supplementary study, Mepiquat Chloride administration at 12000 ppm (active ingredient) for 3 months resulted in decreased food consumption, body weight gain (32% reduction for males, 17% for females, as compared to controls), food efficiency, increased incidence of clinical signs, changes in hematology and clinical chemistry, and neurological findings. The neurological findings were considered to result from reversible effects of the test compound on the acetylcholine-activated receptors. Based on the findings from the preliminary studies, the low, medium, and high doses of Mepiquat Chloride for the

current study were chosen as 290, 2316, and 5790 ppm (active ingredient, corresponding to inclusion levels of 500, 4000, and 10,000 ppm of test material containing 57.9% Mepiquat Chloride, respectively).

TABLE 1. STUDY DESIGN

Dose Group	Conc. in Diet (ppm (a.i.))	Dose (mg/kg/day)		No. of Animals	
		Male	Female	Male	Female
1 Control	0	0	0	20	20
2 Low (LDT)	290	13	18	20	20
3 Mid (MDT)	2316	106	146	20	20
4 High (HDT)	5790	268	371	20	20

Data for dose (mg/kg/day) taken from daily substance intake data in Table 4.2.4, p. 49, MRID No. 432644-02.

2. Diet preparation and analysis

Diet was usually prepared weekly. Concentrated diet was prepared by adding Mepiquat Chloride to ground Kliba maintenance diet rat/mouse/hamster, 343 meal and mixing with a BOSCH mixer. The concentrate was diluted to the appropriate level by adding diet and mixing with a GEBR. LÖDIGE mixer for 10 minutes.

Results –

a. Homogeneity analysis – In the initial test, diet was prepared with Mepiquat Chloride at concentrations of 500, 2000, and 8000 ppm. Six samples of the 500 and 8000 ppm preparations were analyzed and the mean concentrations were 98.9% and 101.7% of theoretical inclusion levels, respectively. In the second test, diet was prepared with Mepiquat Chloride at concentrations of 290 and 5790 ppm and six samples from each preparation analyzed. The mean concentrations of the 290 and 5790 ppm preparations were 89.2% and 99.7% of theoretical inclusion levels, respectively.

b. Stability analysis – The stability of Mepiquat Chloride at 500 ppm in diet preparations was assessed during storage at room temperature for 0, 11, and 32 days. The Mepiquat Chloride concentration in diet after 32 days at room temperature was 95.5% of the theoretical inclusion level.

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c. Concentration analysis – Samples of diet with Mepiquat Chloride at inclusion levels of 290, 2316, and 5790 ppm were prepared on 5 dates from May 8, 1991 to February 2, 1993. The preparations were stored in the freezer and concentrations analyzed within 10 months. Mean concentrations of Mepiquat Chloride for the 290, 2316, and 5790 ppm preparations ranged from 82.8%-103.1%, 92.4%-105.1% (if one determination is disregarded), and 94.6%-102.1%, respectively, of the theoretical inclusion levels.

3. Diet

Animals were fed ground Kliba maintenance diet rat/mouse/hamster, 343 meal (Klingentalmühle AG, Kaiseraugst, Switzerland). Drinking water and food were available *ad libitum*.

4. Statistics

Body weights and body weight changes, clinical chemistry, and hematology results were analyzed using a one-way analysis of variance with the F-test. If $p \leq 0.05$, comparisons between dose groups and controls were performed using Dunnett's test (2-sided). Neurofunctional test data were analyzed using the one-way Kruskal-Wallis non-parametric test. If $p \leq 0.05$, comparisons between each dose group and the control were performed using the Mann-Whitney U-test (2-sided). For urinalysis, a comparison of each dose group with the control was performed with Fisher's exact test.

5. Signed and dated GLP and quality assurance statements were present.

C. METHODS AND RESULTS

1. Observations

Rats were inspected twice a day on Monday through Friday, and once a day on Saturdays, Sundays, and holidays for mortality and signs of toxicity. Comprehensive clinical examinations were performed once a week on the day of weighing. Rats were also inspected for neurological function once prior to beginning treatment, and at the end of the first, second, third, and sixth month of treatment.

Results – Mortality for males during the treatment period (days 0 to 728) was similar between controls and treated animals (controls: 5/20 (25%); 290 ppm: 6/20 (30%); 2316 ppm: 1/20 (5%), 5790 ppm: 6/20 (30%)). Mortality for females during the treatment period (days 0 to 728) was similar between controls and treated animals (controls: 5/20 (25%); 290 ppm: 5/20 (25%); 2316 ppm: 6/20 (30%); 5790 ppm: 5/20 (25%)). There were no clinical signs for males indicative of a response to treatment, except for the observation of unpalpable testes in the

scrotum in 2/20 rats in the 5790 ppm dose group. There were no clinical signs for females indicative of a response to treatment. There were no abnormal neurological signs for any of the males or females.

It is important to note that the mortality data presented by the study author for males and females in the 2316 ppm dose group is 1/20 and 6/20, respectively, in the table for Cumulative Mortality (Tables 71, 72), but is 2/20 for males and 7/20 for females in the table for Summary of Clinical Observations (Tables 57, 58). The reason for this discrepancy is not known. It can be surmised that since the data in the Cumulative Mortality table is for days 0 to 728 and the data in the Summary of Clinical Observations table is for days 0 to 735, there was an additional death in each of the sexes during that 7 day period beyond the end of the treatment period. However, this was not discussed by the study author.

2. Body weight

Animals were weighed prior to the beginning of the administration period in order to assign the animals to test groups, then at day 0, then once each week for the first 14 weeks, and then at 4 week intervals for remainder of the treatment period.

Results – Group mean body weights (Table 2) for males in the high dose group (5790 ppm) were significantly decreased at day 728 (87.5% of control, $p<0.05$). The decrease at the end of the treatment period reflected decreases occurring throughout the treatment period: days 7-182 (all $p<0.01$), from days 210-462 (all $p<0.05$), day 490 ($p<0.01$), days 518-728 (all $p<0.05$). Body weight gains for males in the high dose group were consistently significantly decreased as compared to controls throughout the treatment period, and from day 0 to day 728 were 83% of control ($p<0.05$). Group mean body weights for females in the high dose group were 88.1% and 88.3% of controls at days 546 ($p<0.05$) and 728 (not statistically significant), respectively. The difference in statistical significance reflects the lesser variation in body weights for females in the high dose group at day 546 (standard deviation of 29.6) than at day 728 (standard deviation of 40.2). Group mean body weights for females in the high dose group were similar to controls for days 7-91, were statistically significantly decreased as compared to controls for days 98-266 (all $p<0.05$), 294-546 (all $p<0.01$), and 574 ($p<0.05$), and were not statistically significantly different from controls for days 602-728. Group mean body weight gain from days 0 to 546 was 80% of control ($p<0.01$) and from days 0 to day 728 was 80% of control (not statistically significant) for females in the high dose group. As was the case for group mean body weights, the similar percentages, but different statistical significances reflect the lesser variation at day 546 (standard deviation of 28.0) versus day 728 (standard deviation of 41.4) for females in the high dose group. Group mean body weight gains for females in the high dose group were decreased as compared to controls at day 7 and 14 ($p<0.05$), and days 98 ($p<0.05$), 126 ($p<0.01$), 154 ($p<0.05$), 182 ($p<0.05$), and 210-574 ($p<0.01$). Body weights and

body weight gains for males or females in the low (290 ppm) or medium (2316 ppm) dose groups were similar to controls throughout the treatment period.

TABLE 2. GROUP MEAN BODY WEIGHTS AND BODY WEIGHT CHANGES FOR MALES AND FEMALES TREATED WITH MEPIQUAT CHLORIDE FOR 728 DAYS

Day of Study	Treatment Group/Exposure Level (ppm)							
	Males				Females			
	0	290	2316	5790	0	290	2316	5790
0	194.5	195.1 (100%)	194.6 (100%)	193.3 (99%)	151.4	150.9 (100%)	151.6 (100%)	150.5 (99%)
182	562.1	572.9 (102%)	554.6 (99%)	506.9** (90%)	306.6	311.0 (101%)	300.3 (98%)	287.6* (94%)
378	659.2	662.7 (101%)	642.0 (97%)	595.3* (90%)	343.4	349.6 (102%)	336.7 (98%)	310.4* (90%)
546	719.2	716.2 (100%)	695.0 (97%)	639.2* (89%)	371.1	374.8 (101%)	359.0 (97%)	326.8** (88%)
728	715.9	705.0 (99%)	661.1 (92%)	626.6* (88%)	385.7	369.1 (96%)	381.7 (99%)	340.6 (88%)
Terminal[a]	693.6	670.6 (97%)	632.6 (91%)	598.2* (86%)	360.1	344.3 (96%)	356.4 (99%)	316.7 (88%)
Weight Gain (g)								
Days 0-182	367.6	377.8 (103%)	360.0 (98%)	313.6** (85%)	155.3	160.1 (103%)	148.7 (96%)	137.2* (88%)
Days 0-378	464.7	467.6 (101%)	447.4 (96%)	401.9* (86%)	192.0	198.7 (103%)	185.1 (96%)	159.8** (83%)
Days 0-546	525.1	520.9 (99%)	500.4 (95%)	446.4* (85%)	219.8	224.0 (102%)	207.8 (95%)	176.0** (80%)
Days 0-728	521.2	510.8 (98%)	466.0 (89%)	434.3* (83%)	235.6	216.9 (92%)	230.6 (98%)	188.6 (80%)

Data adapted from Tables 11-30 (pp.75-94), and table of absolute weights, pp. 996-997, MRID No. 432644-02.
[a]Terminal weights at sacrifice, used for determination of relative organ weights.
*p≤0.05, ANOVA+Dunnett's test (two-sided)
**p≤0.01, ANOVA+Dunnett's test (two-sided)

3. Food consumption and compound intake

Food consumption (g/rat/day), food efficiency (body weight gain, (g)/food consumption (g) per unit time X 100)), and substance intake (mg/kg body weight) were determined weekly for each dose group for the first 14 weeks of the study and

approximately every 4 weeks for the remainder of the study period. Total food consumption and overall food efficiency were not presented by the study author and were calculated by the reviewer.

Results –

a. Food consumption – The total food consumption (Table 3) for males in the 290, 2316, and 5790 ppm treatment groups was 101%, 99%, and 92% of controls, respectively. Total food consumption for females in the 290, 2316, and 5790 ppm treatment groups was 100%, 98%, and 94% of controls, respectively.

b. Compound consumption – Compound consumption values for rats in the medium and high dose groups were 8 and 20 times the low dose group, respectively (Table 1).

c. Food efficiency – Food efficiency for males in the 290, 2316, and 5790 dose groups was 104%, 100%, and 88% (not statistically significant) of controls. Food efficiency for females in the 290, 2316, and 5790 dose groups was 92%, 85%, and 85% (not statistically significant) of controls.

TABLE 3. GROUP MEAN FOOD CONSUMPTION AND FOOD EFFICIENCY FOR MALE AND FEMALE WISTAR RATS TREATED WITH MEPIQUAT CHLORIDE IN FOOD FOR 728 DAYS

Parameter	Exposure Level (ppm)							
	Males				Females			
	0	290	2316	5790	0	290	2316	5790
Group Mean Daily Food Consumption (g/rat/day)	26.7	27.0 (101%)	26.5 (99%)	24.6** (92%)	20.4	20.5 (100%)	20.1* (98.5%)	19.2** (94%)
Total Food Consumption (g/rat)	19458	19672 (101%)	19306 (99%)	17922** (92%)	14887	14930 (100%)	14654* (98%)	14005** (94%)
Food Efficiency [((bodyweight gain (g))/(Food consumed (g))]x100	2.5	2.6 (104%)	2.5 (100%)	2.2 (88%)	1.3	1.2 (92%)	1.1 (85%)	1.1 (85%)

Mean daily and total food consumption were calculated by the reviewer from data for days 7-728, obtained from Tables 1-10 (pp. 65-74), and food efficiency was calculated by the reviewer from data for days 14, 42, 70, 98, 126-686, and 728 (4-week intervals) obtained from Tables 31-40 (pp. 95-104), MRID No. 432644-02.
*p<0.05, ANOVA + Dunnett's test (2-sided), statistical analysis performed by the reviewer
**p<0.01, ANOVA + Dunnett's test (2-sided), statistical analysis performed by the reviewer

4. Ophthalmoscopic examination

Four days prior to and 723 days after the beginning of treatment, the eyes of all rats in the control and in the high dose groups were examined using an ophthalmoscope (Heine Focalux). The eyes of rats in the low and medium dose groups were not examined.

Results – There were no findings attributable to treatment with Mepiquat Chloride.

5. Blood was collected for hematology and clinical chemistry analysis from the retroorbital venous plexus in the morning from non-fasted rats. EDTA was used as the anticoagulant. Blood samples were analyzed at days 99, 183, 365, 547, and 725. The hematology parameters WBC, RBC, HGB, HCT, MCV, MCH, MCHC, and platelets were measured with an S Plus Coulter particle counter, reticulocytes with a Hematrak 480 (Geometric Data, Munich), differential WBC was assessed visually, and blood clotting assessed with a ball coagulometer (KC 10 A, Amelung, Lemgo). Clinical chemistry parameters were assessed using an automated analyzer (Hitachi 737, Boehringer, Mannheim).

a. Hematology

X		X	
X	Hematocrit(HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular Hb (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular Hb conc.
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time, APTT)		
	(Clotting time)		
X	(Prothrombin time, PT)		

*Required for chronic studies.

Results – Group mean WBC counts were decreased slightly for males in the 2316 and 5790 ppm dose groups as compared to controls at days 183, 365, 547, and for males in the 5790 ppm dose group at day 725. These decreases were not statistically significant, except at day 183 for males in the 2316 ppm dose group (13% decrease, $p < 0.05$). The decreases were not toxicologically significant.

b. Clinical chemistry

X		X	
Electrolytes		Other	
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
X	Magnesium*	X	Blood urea nitrogen*
X	Phosphorus*	X	Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
Enzymes		X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)	X	Triglycerides
	Cholinesterase (ChE)		Serum protein electrophoresis
	Creatinine pbosphokinase (CK)*	X	Bilirubin*
	Lactic acid dehydrogenase (LDH)*		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
	Ornithine carbamoyltransferase (OCT)		

* Required for chronic studies.

Results - There were statistically significant changes in serum alanine aminotransferase (SGPT) levels for males and females (Table 4). SGPT levels were significantly decreased as compared to controls for males treated with Mepiquat Chloride at 5790 ppm at days 99 (81%, $p<0.01$), 183 (83%, $p<0.01$), and 547 (67%, $p<0.01$), 2316 ppm at days 99 (87%, $p<0.05$) and 547 (71%, $p<0.01$), and at 290 ppm at day 547 (77%, $p<0.05$). Gamma glutamyl transferase (GGT) levels were increased at day 183 for males treated with Mepiquat Chloride at 5790 ppm (9 nkat/L, $p<0.05$) as compared to controls (1 nkat/L). SGPT levels were significantly decreased as compared to controls for females treated with Mepiquat Chloride at 5790 ppm at days 99 (84%, $p<0.01$) and 547 (83%, $p<0.05$), at 2316 ppm at days 99 (84%, $p<0.01$), 365 (85%, $p<0.05$), and 547 (79%, $p<0.01$).

There were other, incidental, statistically significant changes in clinical chemistry parameters for males and females treated with Mepiquat Chloride as compared to controls. For males, there were increases in chloride levels at day 99 for rats treated with Mepiquat Chloride at 290 (101%, $p<0.01$), 2316 (101%, $p<0.05$), or 5790 ppm (101%, $p<0.01$), and at day 547 for males treated with 5790 ppm (101%, $p<0.05$). There was an increase in glucose levels (106%, $p<0.05$) at day 183, in creatinine levels (112%, $p<0.01$) at day 725, and in triglyceride levels at days 183 (148%, $p<0.01$) and 547 (137%, $p<0.05$), and a decrease in total bilirubin levels at day 183 (68% of control, $p<0.05$) for males treated with Mepiquat Chloride at 290 ppm. For females, there was

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a decrease in triglyceride levels at days 99 (70% of control, $p<0.05$) and 725 (44%, $p<0.01$) and an increase in total bilirubin levels at day 547 (160%, $p<0.05$) for rats treated with Mepiquat Chloride at 5790 ppm. Serum aspartate aminotransferase (SGOT) levels at day 547 were statistically significantly decreased as compared to controls for females treated with Mepiquat Chloride at 5790 (83%, $p<0.05$) and 2316 ppm (77%, $p<0.01$). These changes, although statistically significant, were not toxicologically significant.

TABLE 4. SERUM ALANINE AMINOTRANSFERASE (SGPT) LEVELS FOR MALE AND FEMALE RATS TREATED ORALLY WITH MEPIQUAT CHLORIDE IN FEED FOR 728 DAYS

Day of Study	Exposure Level (ppm)							
	Males				Females			
	0	290	2316	5790	0	290	2316	5790
99	0.99[a]	0.95 (96%)[b]	0.86* (87%)	0.80** (81%)	0.94	0.87 (93%)	0.79** (84%)	0.79** (84%)
183	1.05	1.02 (97%)	0.93 (89%)	0.87** (83%)	0.88	0.82 (93%)	0.76 (86%)	0.91 (103%)
365	1.02	0.95 (93%)	0.93 (91%)	0.86 (84%)	0.95	0.91 (96%)	0.81* (85%)	0.87 (92%)
547	1.13	0.87* (77%)	0.80** (71%)	0.76** (67%)	1.03	0.90 (87%)	0.81** (79%)	0.86* (83%)
725	0.72	0.74 (103%)	0.73 (101%)	0.62 (86%)	0.91	0.87 (96%)	0.81 (89%)	0.83 (91%)

Data adapted from Tables 4.3.3.1 and 4.3.3.2 (pp.52-53), MRID No. 432644-02.
[a]SGPT levels are presented in µkat/L.
[b]% of control, calculated by the reviewer.
*$p<0.05$, ANOVA + Dunnett's test (2-sided)
**$p<0.01$, ANOVA + Dunnett's test (2-sided)

6. Urinalysis*

Urinalysis was performed on days 92, 177, 359, 541, and 716 of the treatment period. Urine was collected from the rats during an overnight fast (food and water withheld). The levels of nitrite, *p*H, protein, glucose, ketones, urobilinogen, bilirubin, and blood in the urine samples were determined semi-quantitatively using test strips (Combur-9-test RL, Boehringer Mannheim) and a reflection photometer. The specific gravity was determined using a urine refractometer. Urine sediment was examined microscopically. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	Sediment (microscopic)*	X	Blood*
X	Protein*	X	Nitrite
X	Urobilinogen*	X	*p*H

* Required for chronic studies.

Results – There was a treatment-related finding (Table 5) of increases in crystals in males treated with Mepiquat Chloride at 5790 ppm, as compared to controls. The increased levels of masses of urine crystals in sediment in high dose (5790 ppm) males were found at days 92, 177, 359, and 541, although statistically significant only at day 92 ($p<0.05$). There were other findings for males, but these were not treatment-related as they occurred at single timepoints, or did not represent a trend over the treatment period. At days 92, 177, 359, 541, and 716 of the treatment period, the incidence of erythrocyte levels >1 for controls were 3/20, 6/20, 4/20, 8/20, and 0/20, respectively, and the incidence for males treated with Mepiquat Chloride at 290 ppm was 3/20, 7/20, 10/20 ($p<0.05$), 11/20, and 9/20 ($p<0.01$), respectively. There was an increase in incidence of bacteria levels for males treated with Mepiquat Chloride at 290 ppm at day 92 (5/20, $p<0.05$), as compared to controls (0/20). Instead of nitrate levels, the authors measured nitrite levels.

TABLE 5. INCIDENCE OF CRYSTALS IN URINE FOR MALE AND FEMALE WISTAR RATS TREATED WITH MEPIQUAT CHLORIDE IN FOOD FOR 726 DAYS

Day of Study	Exposure Level (ppm)							
	Males				Females			
	0	290	2316	5790	0	290	2316	5790
92	4/20	6/20	4/20	12/20*	0/20	1/20	3/20	2/20
177	1/20	0/20	1/20	6/20	1/20	1/20	0/20	2/20
359	0/20	0/20	1/20	4/20	1/20	0/20	1/20	5/20
541 (males), 542 (females)	0/20	0/20	0/20	2/20	0/20	0/20	1/20	1/20
716	0/20	0/20	0/20	0/20	0/20	1/20	0/20	0/20

Data adapted from Tables 158-162 (pp. 222-226) and Tables 168-172 (pp. 232-236), MRID No. 432644-02.
*$p<0.05$, Fisher's exact test
**$p<0.01$, Fisher's exact test

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7. Sacrifice and pathology

Rats were euthanitized by decapitation after carbon dioxide inhalation and complete necropsies were performed on all animals. Tissues were stained with hematoxylin-eosin prior to microscopic examination. All the tissues checked below were examined in all rats from the control and high dose (5790 ppm) groups and in all rats that died or were sacrificed during the treatment period. For rats in the low (290 ppm) and medium (2316 ppm) dose groups that survived to termination, the lungs, liver, kidneys, and gross lesions were the only tissues subjected to microscopic pathological examination. Statistical analyses were performed on the organ weights, but were not performed by the study author on the incidences of macroscopic or microscopic pathological findings. All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	Digestive system
	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
	Gall Bladder*
X	Pancreas*
	Respiratory
X	Trachea*
X	Lung*
	Nose
	Pharynx
	Larynx

X	Cardiovasc./Hemat.
X	Aorta*
X	Heart*
X	Bone marrow*
X	Lymph nodes*
X	Spleen
X	Thymus*
	Urogenital
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
X	Epididymides
X	Prostate
X	Seminal vesicle
X	Ovaries*+
X	Uterus*

X	Neurologic
XX	Brain*+
X	Periph. nerve*
X	Spinal cord (3 levels)*
X	Pituitary*
X	Eye (optic n.)*
	Glandular
XX	Adrenal gland*
	Lacrimal gland
X	Mammary gland*
X	Parathyroids*
X	Thyroids*
	Other
X	Bone*
X	Skeletal muscle*
X	Skin*
X	All gross lesions and

* Required for chronic studies.
\+ Organ weight required in chronic studies.

Results -

a. Organ weight - There was a significant decrease in absolute brain weight (4% decrease, $p < 0.05$) for males in the high dose group (5790 ppm) as compared to controls. This is not toxicologically significant as there were no associated microscopic pathological findings.

b. Gross pathology - There were no gross pathological findings for males occurring at a higher frequency in treated animals than in controls. There was an increased incidence of "focus" in the adrenal cortex for treated females, as compared to controls. The respective incidences for controls and females in the 290, 2316, and 5790 ppm dose groups were 9/20, 13/20, 13/20, and 16/20 ($p < 0.05$). The incidences of macroscopic pathological findings were not statistically analyzed by the study author.

c. Microscopic pathology -

1) Non-neoplastic - There was an increased incidence of vacuolated cell foci, hemosiderin pigment, and nodular hyperplasia in the adrenal cortex for treated females, as compared to controls. The respective incidences of these findings for females treated with Mepiquat Chloride at 0, 290, 2316, or 5790 ppm were: vacuolated cell foci, 3/20, 5/17, 5/18, 9/20 ($p < 0.05$); hemosiderin pigment, 3/20, 5/17, 8/18 ($p < 0.05$), 6/20; and nodular hyperplasia, 3/20, 6/17, 4/18, 6/20. The increased incidence of vacuolated cell foci (statistically significant for the high dose group) is toxicologically significant as it correlates with the macroscopic finding of focus for the adrenal cortex (statistically significant for the high dose group). The increased incidences of hemosiderin and nodular hyperplasia were increased in all dose groups (statistically significantly different from control for hemosiderin pigment in the 2316 ppm dose group) and may also correlate with the macroscopic pathological finding for the adrenal cortex. The incidences of microscopic pathological findings were not statistically analyzed by the study author.

2) Neoplastic - There were no neoplastic changes occurring at a higher frequency in treated males as compared to controls. For females, there was a slightly increased frequency of fibroadenomas and adenocarcinomas in the mammary gland. The respective incidences of neoplastic findings in the control, 290, 2316, and 5790 ppm dose groups were: fibroadenomas, 3/20, 2/8, 3/11, and 4/20; and adenocarcinomas, 0/20, 2/8 ($p < 0.05$), 2/11 ($p < 0.05$), and 2/20. However, as the increased incidences were not large, were only statistically significantly different from controls for adenocarcinomas in the low and medium dose groups, and were not accompanied by macroscopic or non-neoplastic pathological findings for mammary glands in females, the increases in incidences of fibroadenomas and adenocarcinomas for females treated with Mepiquat Chloride are not

likely toxicologically significant. The neoplastic findings were not statistically analyzed by the study author.

8. Neurological examination

Examinations were performed at days -3, 30, 58, 86, and 179 to assess qualitatively the general condition of the rats and to observe for: piloerection, skin color, posture, respiration, behavior, activity, tremors, convulsions, ataxia, paresis/paralysis, pupil size, lacrimation, secretion of pigmented tears, salivation, vocalization, body tone, urination, feces, sensitivity of the body surface, righting response, winking reflex, pupillary reflex, vision, audition, olfaction, toe pinch, tail pinch, and any other miscellaneous abnormal responses. The rats were observed quantitatively for grip strength of the forelimbs and the hindlimbs, and a hot-plate test was performed.

Results – All responses were within the normal range and there was no evidence of neurological abnormality or deficit in any of the male or female rats.

D. DISCUSSION

The doses for the current study were chosen based upon 2 previous dose selection studies. The first was a 4-week feeding study with doses of Mepiquat Chloride at 500, 2000, and 8000 ppm (active ingredient). The results are summarized in the Appendix to this DER. In the second study, Mepiquat Chloride was administered in feed at 145, 579, 2316, 4632, and 12000 ppm (active ingredient) for 3 months. The second study report was not seen by the reviewer. In both of these studies, there were changes in food consumption, body weight gain, changes in clinical chemistry and slight changes in organ weights. Based upon the results from the preliminary dose-selection studies, the doses of Mepiquat Chloride for the current study were chosen as 290, 2316, and 5790 ppm (active ingredient).

Oral administration of Mepiquat Chloride at 5790 ppm (active ingredient) for 728 days to male and female Wistar rats resulted in decreased weight gain and decreased food consumption for males and females, decreases in SGPT levels for males and females, an increase in urinary crystal formation for males, and an increased incidence of macroscopic and microscopic pathological findings for the adrenal cortex for females. The finding of crystals in urine sediment was noted in the preliminary 3-month feeding study. However, as there were no macroscopic or microscopic pathological findings to correlate with the finding of crystals in urine sediment, the toxicological significance is somewhat questionable. The statistically significant decreases in SGPT levels for males and females in the high dose group were not toxicologically significant. These decreases were within the historical data range for a 2-year study, were not correlated with macroscopic or microscopic pathological findings, and the decreases were not found in the 3-month study. The LOEL is 5790 ppm.

There were no treatment-related effects seen in the low (290 ppm) and medium (2316 ppm) dose groups. The only statistically significant change noted for the low dose group was the decrease in SGPT levels in males at day 547 (77% of control, $p<0.05$). SGPT levels in the low dose group were similar to controls at all other timepoints (days 99, 183, 365, and 725), therefore this incidental increase is not toxicologically significant. For rats in the 2316 ppm dose group, there were increases in urinary crystals (males, not statistically significant) and decreases in SGPT levels for males at days 99 (87%, $p<0.05$) and 547 (71%, $p<0.01$). As noted above for the high dose group, the SGPT findings are not toxicologically significant. The increases in urinary crystals are of questionable significance as discussed above and the increase for males in the 2316 ppm dose is not statistically significant. Therefore, as there were no toxicologically significant findings for the medium and low dose groups, the NOEL for 728 day feeding of Mepiquat Chloride in male and female Wistar rats is 2316 ppm.

E. STUDY DEFICIENCIES

Lactate dehydrogenase (LDH) and creatinine phosphokinase (CPK) levels were not assessed in the clinical chemistry evaluations. A test for nitrate was not performed in the urinalysis (a test for nitrite was performed). The inclusion of these data would add little to the study report. The study author did not perform statistical evaluations of differential WBC counts, and did not statistically analyze the macroscopic or microscopic pathological data. The lack of these statistical evaluations is a major flaw in the study. There were statistically significant increases in pathological findings for the adrenal cortex that were not identified by the study author. These findings support the identification of a LOEL and should have been discussed by the study author.

APPENDIX

Dose Selection Study in Rats

MRID No.: 424121-02
Study Type: Subchronic Feeding-Rat (4-week, §82-1a)
Test Material: Mepiquat Chloride
Study No.: 30S0112/89012
Testing Facility: BASF Aktiengesellschaft, Department of Toxicology, Limburgerhof, Germany
Study Title: Oral toxicity of Mepiquat Chloride in Wistar rats, administration in the diet over 4 weeks (Range-Finding)
Author: K. Schilling
Report Issued: June 12, 1992 (study completion date)

Methods
Test Animals: Male and female Wistar rats, Chbb=THOM(SPF); 42 days old, 177-191 g (males), 139-159 g (females)
Group Size: 5/sex/dose
Test Concentrations: Daily diet of Mepiquat Chloride at 0, 500, 2000, or 8000 ppm (approximately equivalent to 0, 46, 183, or 661 mg/kg body weight, respectively).

Results
Clinical signs: There were no clinical signs of toxicity for any of the animals.
Mortality: There was no mortality.
Body weight: Group mean body weights for males in the high dose group (8000 ppm) were statistically significantly decreased relative to controls at days 7, 14, 21, and 28 (82%, 81%,82%, and 83%, respectively, $p<0.02$ for each). Group mean body weights for females (8000 ppm) were statistically significantly decreased as compared to controls at day 7 and 14 (91% and 91%, $p<0.02$), and were decreased relative to controls at days 21 and 28 (93% and 95%, respectively, not statistically significant). Group mean body weights in other dose groups were similar to controls at all timepoints.
Food Consumption: Total food consumption was decreased to 75% and 85% of controls, for males and females, respectively, in the high dose group (8000 ppm), primarily due to decreased food consumption during the first week of treatment. Total food consumption was unaffected in other dose groups.
Clinical Pathology:
Hematology: There were no treatment-related effects on hematology parameters.
Biochemistry: Group mean glucose (88%, $p<0.02$), total protein (93%, $p<0.02$), albumin (93%, $p<0.02$), globulin (92%, $p<0.02$), and triglycerides (46%, $p<0.02$) were decreased relative to controls and cholesterol (122%, $p<0.02$) was increased relative to controls for males treated with Mepiquat Chloride at 8000 ppm. Triglyceride levels were decreased relative to controls (73%, $p<0.05$) for males treated at 2000 ppm. There were no effects noted for females.
Urinalysis: There were no treatment-related effects on urinalysis parameters.
Organ Weight Gain:

Group mean absolute organ weights for liver (males: 72%, $p<0.05$; females: 89.5%, $p<0.02$) and for kidney (males: 86%, $p<0.05$; females: 88%, $p<0.05$) for males and females (8000 ppm) were decreased relative to controls. Group mean relative organ weights for liver (88%, $p<0.05$) were decreased relative to controls for males (8000 ppm). Relative organ weights for the adrenals (15% increase, $p<0.05$) and the testes (26% increase, $p<0.02$) were increased relative to controls for males (8000 ppm).

Macroscopic and Microscopic Pathology: There were no treatment-related findings.

[The following findings were summarized for the 12000 ppm group in the 3-month study : severely reduced food intake (males -19%, females - 8%); reduced body weight (males - 32%, females - 17%); impaired food efficiency; reduced general and impaired behavior including tremors, impaired gait, ataxia, posture abnormalities, vocalizations and feces abnormalities; reduced grip strength of forelimbs and hindlimbs; increased reaction time in the hot-plate test; decreases in calcium, creatinine, glucose, total protein, albumin, globulin, and triglycerides; increase in thromboplastin time and chloride; and increased urinary crystals in males.]

Conclusions: There was no significant toxicity for rats in any of the dose groups. The reduced food consumption for males and females was likely a result of decreased palatability of the food/test material mixture, thus resulting in reduced body weight gain. The decreased triglycerides would be consistent with a slight reduction in food consumption. The decreases in organ weights and other clinical chemistry parameters are not toxicologically significant, as there were no macroscopic or microscopic pathological findings, and may simply reflect reduced food consumption. On the basis of the lack of toxicity at 500, 2000, or 8000 ppm, dose levels of Mepiquat Chloride of 0, 145, 579, 2316, and 4632 ppm (and 12000 ppm in a supplementary study) were chosen for a 90-day feeding study. The results of the 90-day feeding study were not seen by the reviewer. On the basis of the 2 subchronic feeding studies (4-week and 90-day), doses of 0, 290, 2316, and 5790 ppm were chosen for use in the 52-week chronic feeding-rat study (MRID No. 432644-02).

Core Classification: Not applicable; dose range-finding study

Division F 8/22/91
Magnesium Chloride MRID No. 423644-02
95-7
Chronic feeding - rat

83-1 Chronic Feeding in the Rodent and Nonrodent

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?:

1. ✓ Technical form of the active ingredient tested.
2. ✓ At least 20 rodents or 4 nonrodents/sex/group (3 test groups and control group).
3. ✓ Dosing duration in rodents minimum 12 month nonfood use, 24 months food use; in nonrodents minimum 12 months[1].
4. ✓ Doses tested include signs of toxicity at high dose but no lethality in nonrodents or a limit dose if nontoxic (1,000 mg/kg).
5. * ✓ Doses tested include a NOEL.
6. * ✓ Analysis for test material stability, homogeneity and concentration in dosing medium
7. ✓ Individual daily observations.
8. ✓ Individual body weights.
9. ✓ Individual or cage food consumption.
10. * ✓ Opthalmoscopic examination (at least pretest and at term) control and high dose.
11. ___ Clinical pathology data for all nonrodents and at least 10 rodents/group consisting of 12, 13 & 14.
13. ✓ Hematology at 6 month intervals consisting of at least:
    - ✓ Erythrocyte count
    - ✓ Hemoglobin
    - ✓ Hematocrit
    - ✓ Leucocyte count
    - * ✓ Differential count
    - ✓ Platelet count (or clotting measure)
14. ✓ Clinical chemistry at 6 month intervals consisting of at least:
    - ✓ Alkaline phosphatase
    - ✓ Aspartate aminotransferase
    - X * Creatinine kinase
    - X Lactic dehydrogenase
    - ✓ Glucose
    - ✓ Bilirubin
    - ✓ Cholesterol
    - * ✓ Creatinine
    - ✓ Total Protein
    - ✓ Albumin
    - ✓ Urea
    - ✓ Inorganic phosphate
    - ✓ Calcium
    - * ✓ Potassium
    - ✓ Sodium
    - * ✓ Chloride
15. ✓ Urinalysis at 6 month intervals consisting of at least:
    - ✓ Blood
    - ✓ Protein
    - ✓ Ketone bodies
    - ✓ Appearance
    - ✓ Glucose
    - ✓ Total bilirubin
    - * ✓ Urobilirubin
    - ✓ Sediment
    - ✓ Specific gravity (osmolality)
    - * ✓ Volume
16. ✓ Individual necropsy of all animals.
17. ✓ Histopathology of the following tissues performed on all nonrodents and rodents, all control and high dose animals, all animals that died or were killed on study, all gross lesions on all animals, target organs on all animals and lungs, liver and kidneys on all other animals.

Criteria marked with a * are supplemental and may not be required for every study.

- eyes
- caecum
- colon
- duodenum
- brain†
- skin
- heart† X
- testes†
- pituitary
- ileum
- trachea
- bone marrow
- liver†
- lung† X
- lymph nodes
- stomach
- mammary gland
- spleen†
- musculature
- epididymis
- adrenals†
- uterus
- kidneys†
- esophagus
- ovaries† X
- oviduct
- pancreas
- rectum
- spinal cord (3x)
- thyroid / parathyroids
- salivary glands
- thymus
- urinary bladder

† organs to be weighed

Six month dog studies may be acceptable. (?)

Deficiencies:

X = lungs, & heart not weighed

ovaries

Ø = Oviduct not examined

LDH

CPK not evaluated

Criteria marked with a * are supplemental and may not be required for every study.

330	0	GOAT-FAT W/O BONE	000.180000	01.000 01.000 AR as per MARC decision 11/4/97
331	U	GOAT-KIDNEY	002.980000	01.000 01.000 AR as per MARC decision 11/4/97
332	U	GOAT-LIVER	002.310000	01.000 01.000 AR as per MARC decision 11/4/97
131	U	GOAT-LEAN (FAT/FREE) W/O BONE	000.480000	01.000 01.000 AR as per MARC decision 11/4/97
134	U	HORSEMEAT	002.980000	01.000 01.000 AR as per MARC decision 11/4/97
315	U	RABBIT	002.980000	01.000 01.000 AR as per MARC decision 11/4/97
116	U	SHEEP-MEAT BYPRODUCTS	002.980000	01.000 01.000 AR as per MARC decision 11/4/97
337	U	SHEEP-OTHER ORGAN MEATS	002.980000	01.000 01.000 AR as per MARC decision 11/4/97
138	U	SHEEP-FAT W/O BONE	000.180000	01.000 01.000 AR as per MARC decision 11/4/97
339	U	SHEEP-KIDNEY	002.980000	01.000 01.000 AR as per MARC decision 11/4/97
340	U	SHEEP-LIVER	002.110000	01.000 01.000 AR as per MARC decision 11/4/97
141	U	SHEEP-LEAN (FAT FREE) W/O BONE	000.480000	01.000 01.000 AR as per MARC decision 11/4/97
342	U	PORK-MEAT BYPRODUCTS	002.980000	01.000 01.000 AR as per MARC decision 11/4/97
143	U	PORK- OTHER ORGAN MEATS	002.980000	01.000 01.000 AR as per MARC decision 11/4/97
144	U	PORK-FAT W/O BONE	000.380000	01.000 01.000 AR as per MARC decision 11/4/97
345	U	PORK-KIDNEY	002.980000	01.000 01.000 AR as per MARC decision 11/4/97
346	U	PORK-LIVER	002.110000	01.000 01.000 AR as per MARC decision 11/4/97
147	U	PORK-LEAN (FAT FREE) W/O BONE	000.480000	01.000 01.000 AR as per MARC decision 11/4/97
355	V	TURKEY-BYPRODUCTS	000.110000	01.000 01.000 AR as per MARC decision 11/4/97
356	V	TURKEY-GIBLETS (LIVER)	000.110000	01.000 01.000 AR as per MARC decision 11/4/97
157	V	TURKEY--FAT W/O BONES	000.017000	01.000 01.000 AR as per MARC decision 11/4/97
158	V	TURKEY-LEAN/FAT FREE W/O BONE	000.021000	01.000 01.000 AR as per MARC decision 11/4/97
360	V	POULTRY-OTHER-LEAN (FAT FREE)	000.023000	01.000 01.000 AR as per MARC decision 11/4/97
361	V	POULTRY-OTHER-GIBLETS(LIVER)	000.110000	01.000 01.000 AR as per MARC decision 11/4/97
362	V	POOLTRY-OTHBR-FAT W/O BONES	000.017000	01.000 01.000 AR as per MARC decision 11/4/97
366	V	CHICKEN-BYPRODUCTS	000.110000	01.000 01.000 AR as per MARC decision 11/4/97
367	V	CHICKEN-GIBLETS(LIVER)	000.110000	01.000 01.000 AR as per MARC decision 11/4/97
368	V	CHICKEN-FAT W/O BONES	000.017000	01.000 01.000 AR as per MARC decision 11/4/97
169	V	CHICKEN-LEAN/FATFREE W/O BONE	000.021000	01.000 01.000 AR as per MARC decision 11/4/97
385	V	CHICKEN-GIBLETS (EXCL. LIVER)	000.110000	01.000 01.000 AR as per MARC decision 11/4/97
188	O	CORN GRAIN/SUGAR-MOLASSES	000.050000	01.500 00.160 TLT 6F04631 exp. 4/30/01
398	X	MILK-BASED WATER	000.042000	01.000 01.000 AR as per MARC decision 11/4/97
399	O	OATS-BRAN	000.100000	01.000 00.260 TLT 6F04611 exp. 4/30/03
408	O	RICE-BRAN	000.100000	01.000 00.260 TLT 6F04631 exp. 4/10/01
409	O	RICE-WILD	000.100000	01.000 00.260 TLT 6F04611 exp. 4/30/01
424	U	VEAL-FAT W/O BONES	000.180000	01.000 01.000 AR as per MARC decision 11/4/97
425	U	VEAL-LEAN (FATFREE) W/O BONES	000.480000	01.000 01.000 AR as per MARC decision 11/4/97
426	U	VEAL-KIDNEY	002.980000	01.000 01.000 AR as per MARC decision 11/4/97
427	U	VEAL-LIVER	002.310000	01.000 01.000 AR as per MARC decision 11/4/97
428	U	VEAL-(THER ORGAN MEATS	002.980000	01.000 01.000 AR as per MARC decision 11/4/97
429	U	VEAL-DRIED	000.480000	01.920 01.000 AR as per MARC decision 11/4/97
430	U	VEAL-MEAT BYPRODUCTS	002.980000	01.000 01.000 AR as per MARC decision 11/4/97
437	O	WHEAT-GERM OIL	001.000000	01.000 00.260 TLT 6F04631 exp. 4/10/03 0.1 + 99OR0001 N 0.9
449	V	TURKEY-OTHER ORGAN MEATS	000.110000	01.000 01.000 AR as per MARC decision 11/4/97
482	A	SOYBEANS-PROTEIN ISOLATE	000.100000	01.000 00.260 TLT 6F04631 exp. 4/30/03