Mepiquat Chloride Chronic Oral Study 83-1(b)

EPA Toxicologist: Krystyna K. Locke K.K. Locke, Date 2/7/96
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 MRID 43264403 Unpublished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

(1)

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. 30DO112/89109; 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		GLANDULAR
X	Colon*	XX	Kidneys*+		
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.

7

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

9

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.

10

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).