UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

February 1, 1996

MEMORANDUM

SUBJECT: Mepiquat Chloride - Review of a Rat Carcinogenicity Study

DP Barcode No. D208423 Rereg. Case No. 2375
Chemical Code No. 109101 Tox. Chem. No. 380 AB
CAS Registry No. 24307-26-4
Sponsor: BASF Corporation, Agricultural Products Group, Research Triangle Park, NC

FROM: Krystyna K. Locke, Toxicologist
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TO: Kathryn Davis/Ruby Whiters, PM 52
Accelerated Reregistration Branch
Special Review and Reregistration Division (7508W)

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

Guideline No.	MRID No.	Acceptability
83-2(a)	43396001	Yes

In this carcinogenicity study, Wistar strain rats, 50/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, 105 and 269 mg/kg/day, respectively, for males and 0, 17, 141 and 370 mg/kg/day, respectively, for females.

Treatment-related findings, observed only in the high-dose male and female rats were: (1) Decreased food consumption, food efficiency and body weight gain; (2) Macroscopic pathological findings in the adrenal cortex (foci) and the ovaries (cysts); and (3) Non-neoplastic pathological findings in the liver (biliary cysts, in females), the kidneys (tubular casts in females and tubular atrophy in males), the prostate (alveolar atrophy), the ovaries (dilated bursa), the uterus (stromal and squamous hyperplasia), the sublingual glands (acinar atrophy), and the mammary glands (secretion). Treatment-related neoplastic findings were not observed. There were no toxicologically significant findings in the mid-dose and the low-dose groups.

Based on the above findings, **the systemic NOEL is 2316 ppm (mg/kg/day: 105 for males and 141 for females) and the systemic LOEL is 5790 ppm (mg/kg/day: 269 for males and 370 for females).**

Mepiquat Chloride was not carcinogenic in this study. Based on the decreased body weight gains, the highest dose tested, 5790 ppm, was a maximum tolerate dose (MTD) for both sexes and was adequate for identifying the carcinogenic potential.

This study is classified as **Acceptable (Core-Minimum)** and satisfies the guideline requirements for an oncogenicity (carcinogenicity) study in rats (83-2a).

Special Review Criteria (40 CFR 154.7) None

011837

DATA EVALUATION REPORT

MEPIQUAT CHLORIDE

Study Type: ONCOGENICITY FEEDING - RAT (83-2a)

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. 95-7 E

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

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Date: 11-1-95

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Susan Chang, M.S.

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Date: 11-1-95

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 Lockheed Martin Energy Systems 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: 1/7/96
Review Section IV, Toxicology Branch I (7509C)
EPA Section Head: Marion Copley, D.V.M., D.A.B.T. Date: 12/14/95
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity Feeding – Rat (83-2a)

TOX. CHEM. NO: 380AB

P.C.CODE.: 109101

MRID NO.: 433960-01

TEST MATERIAL: Mepiquat Chloride

SYNONYMS: 1,1-Dimethylpiperidinium chloride

ROUTE OF ADMINISTRATION: Oral, mixed with diet

TREATMENT DURATION: 24 months

STUDY NUMBER: 7150112/89092

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 Wistar Rats-Administration in the Diet for 24 Months.

AUTHOR: W. Mellert

REPORT ISSUED: September 15, 1994 (Study completion date)

EXECUTIVE SUMMARY: In a oncogenicity study, Mepiquat Chloride was administered for 24 months in the diet to 50 Wistar rats/sex/dose at concentrations of 0, 290, 2316, and 5790 ppm (active ingredient), equivalent to doses of 0, 13, 105, 269 mg/kg/day for males and 0, 17, 141, and 370 mg/kg/day for females, respectively.

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For male and female rats treated with Mepiquat Chloride at 5790 ppm, group mean daily and total food consumption were 88% ($p<0.01$) and 93% ($p<0.01$) of controls, respectively, group mean body weights at day 728 were 82% ($p<0.01$) and 81% ($p<0.01$) of controls, respectively, group mean body weight gains from day 0 to day 728 were 77% ($p<0.01$) and 71% ($p<0.01$) of controls, respectively, and food efficiency was 90% ($p<0.05$) and 78% ($p<0.01$) of controls, respectively. For males and females in the 5790 ppm dose group relative to controls, there were macroscopic pathological findings for the adrenal cortex (focus: 5/50 vs. 16/50, $p<0.01$ for males; 31/50 vs. 39/50, $p<0.05$ for females) and the ovaries (cyst: 14/50 vs. 24/50, $p<0.05$), and non-neoplastic pathological findings for the liver (biliary cysts: 8/50 vs. 16/50, $p<0.05$ for females), the kidneys (tubular casts: 6/50 vs. 13/50, $p<0.05$ for females; tubular atrophy: 5/50 vs. 12/50, $p<0.05$ for males), the prostate (alveolar atrophy: 1/50 vs. 7/50, $p<0.05$), the ovaries (dilated bursa: 1/50 vs. 13/50, $p<0.01$), the uterus (stromal hyperplasia: 2/50 vs. 11/50, $p<0.01$; squamous hyperplasia: 5/50 vs. 14/50, $p<0.05$), the sublingual glands (acinar atrophy: 4/49 vs. 14/50, $p<0.01$), and the mammary glands (secretion: 30/47 vs. 45/50, $p<0.01$). There were no toxicologically significant findings for the medium or low dose groups. There was no excess mortality for treated rats relative to controls. **The LOEL for males and females is 5790 ppm,** based upon decreased body weights, body weight gains, food consumption, food efficiency, and macroscopic and non-neoplastic microscopic pathological findings. **The NOEL for males and females is 2316 ppm.**

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 Wistar rats over this dose range. Based upon the decreased body weights and body weight gains, 5790 ppm is a maximum tolerated dose (MTD) for Mepiquat Chloride for 2-year feeding to male and female Wistar rats, and is adequate for identifying carcinogenic potential.

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

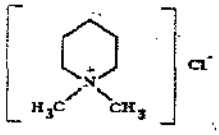
Special Review Criteria (40 CFR 154.7) None

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: rat
Strain: Wistar (Chbb:THOM (SPF))
Age and weight at study initiation: 42 days, 164-193 g (Males), 127-155 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: 8 days

B. STUDY DESIGN

1. Animal assignment

Rats (50/sex/dose) were assigned to the test groups in Table 1 in a randomized fashion, giving approximately equal mean body weights between 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) and a 3-month feeding study in rats (Project No. 31S0112/89053 and supplementary Project No. 31S0112/89077). In the 4-week study treatment of rats with Mepiquat Chloride at 8000 ppm (active ingredient) resulted in decreases in group mean total food consumption (75% of controls for males, 85% of controls for females), group mean body weights (83% of controls for males, $p<0.02$), decreases in glucose, total protein, albumin, globulin, and triglycerides, and increases in cholesterol for males, decreases in absolute organ weights for liver and kidney for males and females, decreases in relative organ weights for liver and increases in relative organ weights for adrenals and testes for

TABLE 1. STUDY DESIGN FOR ONCOGENICITY OF MEPIQUAT CHLORIDE IN MALE AND FEMALE WISTAR RATS

Dose Group	Conc. in Diet (ppm)	Dose (mg/kg/day)[a]		No. of Animals	
		Male	Female	Male	Female
1 Control	0	0	0	50	50
2 Low (LDT)	290	13	17	50	50
3 Mid (MDT)	2316	105	141	50	50
4 High (HDT)	5790	269	370	50	50

Data taken from pp. 22 and 33, MRID No. 433960-01.
[a]Calculated by the study authors as an average of the substance intake data for days 14, 42, 70, 98, 126-728 (approximately 4-week intervals). The calculations were confirmed by the reviewer using the data in Tables 41-50,. pp. 82-91, except that for females in the 5790 ppm dose group, compound intake was 368 mg/kg/day.

males. Administration of Mepiquat Chloride at 500 or 2000 ppm (active ingredient) for 4 weeks did not result in treatment-related findings. In the second study, treatment with Mepiquat Chloride at 145, 579, 2316, or 4632 ppm (active ingredient) for 3 months did not result in treatment-related toxicological findings. Treatment with Mepiquat Chloride at 12,000 ppm (active ingredient) for 3 months resulted in decreased food consumption and body weight gain (32% reduction for males, 17% for females, as compared to controls), reduction in food efficiency, increased incidence of clinical findings including tremors, impaired gait, ataxia, posture abnormalities, abnormal respiration, and vocalization and feces abnormalities, 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). The medium and high doses are 8 and 20 times the low dose, respectively.

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 (Klingentalmühle AG, Kaiseraugst, Switzerland) and mixing with a BOSCH household mixer. The concentrate was diluted to the appropriate level by adding diet and mixing with a GEBR. LÖDIGE mixer for 10 minutes.

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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 and one sample of the 2000 ppm diet were analyzed and the concentrations were 98.9% (mean), 100.8%, and 101.7% (mean) of theoretical inclusion levels, respectively. In the second test, diet was prepared with Mepiquat Chloride at concentrations of 290, 2316, and 5790 ppm. Six samples of the 290 and 5790 ppm diet preparations were analyzed. The concentrations of Mepiquat Chloride ranged from 97.1%-101.8% (mean: 99.7%, coefficient of variation: 1.78%) of theoretical inclusion levels for the 5790 ppm diet, and from 85.2%-92.0% (mean: 89.2%, coefficient of variation: 3.12%) of the theoretical inclusion level for the 290 ppm diet.

b. Stability analysis - The results of stability analyses for Mepiquat Chloride were assessed prior to the 4-week dose range-finding study and were presented in that study report (MRID No. 424121-02). In summary, Mepiquat Chloride stability was assessed during storage of diet (prepared at 500 ppm) 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.

c. Concentration analysis - Samples of diet containing Mepiquat Chloride at inclusion levels of 290, 2316, and 5790 ppm prepared on 5 dates from May 8, 1991 to February 2, 1993, 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%-268.6%, and 94.6%-102.1%, respectively, of the theoretical inclusion levels. Four samples of the 2316 ppm diet contained mean concentrations of Mepiquat Chloride ranging from 92.4%-105.1%. One sample of dietary mixture for the 2316 ppm diet prepared on November 6, 1991 was found (and results confirmed by a second analysis) to contain 242.3%-268.6% of the theoretical concentration. The diet prepared on November 6, 1991 corresponds to day 260 of the study.

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 and body weight changes 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).

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 and holidays for mortality and signs of toxicity. Comprehensive clinical examinations were performed once a week.

Results - Cumulative mortality for males was slightly greater for controls than for treated animals (controls: 23/50 (46%); 290 ppm: 12/50 (24%); 2316 ppm: 14/50 (28%), 5790 ppm: 9/50 (18%)). Cumulative mortality data includes male rats found moribund and sacrificed: 4, 3, 1, and 3 males in the 0, 290, 2316, and 5790 ppm dose groups, respectively. There were no clinical signs for males indicative of a response to treatment. Cumulative mortality for females was slightly greater for controls than for treated animals (controls: 17/50 (34%); 290 ppm: 11/50 (22%); 2316 ppm: 10/50 (20%); 5790 ppm: 13/50 (26%)). Cumulative mortality data includes animals found moribund and sacrificed: 3, 5, 2, and 3 females in the control, 290, 2316, and 5790 ppm dose groups, respectively. There were no clinical signs for females indicative of a response to treatment.

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 - Respective group mean body weights (Table 2) for males in the low (290 ppm), medium (2316 ppm), and high (5790 ppm) dose groups were 97% (not significant), 94% ($p<0.01$), and 85% ($p<0.01$) of control at day 378, and were 92% ($p<0.05$), 88% ($p<0.01$), and 82% ($p<0.01$) of control at day 728. Interestingly, group mean body weights for males in the high dose group were 98% of control ($p<0.05$), prior to administration of the test material. This statistically significant decrease was not discussed by the study authors, but is not biologically relevant. Body weight gains for males in the low (290 ppm), medium (2316 ppm), and high (5790 ppm) dose groups were lower than controls from day 0 to day 378 (97% of control, not significant, 93%, $p<0.01$, 80%, $p<0.01$, respectively) and from day 0 to day 728 (89% of control, $p<0.01$, 85%, $p<0.01$, 77%, $p<0.01$, respectively). Group mean body weights for females in the high dose group were 87% ($p<0.01$ of controls at day 378

TABLE 2. GROUP MEAN BODY WEIGHTS AND BODY WEIGHT CHANGES FOR MALE AND FEMALE WISTAR RATS 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	180.9	180.7 (100%)	179.3 (99%)	177.9* (98%)	142.4	141.8 (100%)	141.1 (99%)	141.2 (99%)
378	700.8	682.8 (97%)	661.0** (94%)	594.7** (85%)	351.1	347.6 (99%)	338.0 (96%)	304.8** (87%)
728	777.7	712.6* (92%)	687.5** (88%)	637.7** (82%)	401.8	411.0 (102%)	393.9 (98%)	325.4** (81%)
Terminal[a]	747.4	681.4** (91%)	657.2** (88%)	611.5** (82%)	380.6	383.3 (101%)	372.1 (98%)	305.6** (80%)
Weight Gain (g)								
Days 0-378	520.1	502.2 (97%)	481.6** (93%)	416.8** (80%)	208.6	205.8 (99%)	196.9 (94%)	163.5** (78%)
Days 0-728	597.3	531.8** (89%)	508.4** (85%)	459.8** (77%)	259.2	269.3 (104%)	253.0 (98%)	184.0** (71%)

Data adapted from Tables 11-30 (pp. 52-71), and table of absolute weights, pp. 419-420, MRID No. 433960-01.
[a]Terminal weights at sacrifice, used for determination of relative organ weights.
*$p \leq 0.05$
**$p \leq 0.01$

and were 81% ($p<0.01$) of controls at day 728. Group mean body weight gain for females in the high dose group from days 0 to 378 was 78% of control ($p<0.01$) and from days 0 to day 728 was 71% of control ($p<0.01$). There were no statistically significant differences in group mean body weights or body weight gains for females treated with Mepiquat Chloride at 2316 ppm or 290 ppm as compared to controls.

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 authors and were calculated by the reviewer.

Results –

a. Food consumption – The group mean daily and total food consumption (Table 3) for males in the 290, 2316, and 5790 ppm treatment groups were 98% ($p<0.01$), 96% ($p<0.01$), and 88% ($p<0.01$) of controls, respectively. Group mean daily and total food consumption for females in the 290, 2316, and 5790 ppm treatment groups were 99%, 99%, and 93% ($p<0.01$), respectively.

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)	27.8	27.2** (98%)	26.6** (96%)	24.6** (88%)	19.9	19.7 (99%)	19.7 (99%)	18.5** (93%)
Total Food Consumption (g/rat)	20,261	19,810** (98%)	19,351** (96%)	17,889** (88%)	14,506	14,323 (99%)	14,355 (99%)	13,473** (93%)
Food Efficiency [((Total bodyweight gain (g))/(Total food consumed (g)))x100	2.9	2.7 (93%)	2.6* (90%)	2.6* (90%)	1.8	1.9 (105%)	1.8 (100%)	1.4** (78%)

Mean daily and total food consumption were calculated by the reviewer from data for days 14, 42, 70, 98, 126-728 (4-week intervals), obtained from Tables 1-10 (pp. 42-51), MRID No. 433960-01.
*$p < 0.05$, calculated by the reviewer
**$p < 0.01$, calculated by the reviewer

b. Compound consumption – Compound consumption (Table 1) for rats in the medium and high dose groups was 8 and 20 times the low dose group, respectively. Compound consumption values for the medium dose group were not corrected by the study authors for the increased inclusion levels noted in the concentration analysis for day 260.

c. Food efficiency – Food efficiency for males in the 290, 2316, and 5790 dose groups was 93%, 90% ($p < 0.05$), and 90% ($p < 0.05$) of controls, respectively. Food efficiency for females in the 290, 2316, and 5790 dose groups was 105%, 100%, and 78% ($p < 0.01$) of controls, respectively.

4. Ophthalmoscopic examination

Results – Ophthalmoscopic examinations were not performed.

5. Blood was collected from rats in the control and the high (5790 ppm) dose group after decapitation at study termination. Blood was also collected for differential blood counts from all rats found moribund and sacrificed during the study. Differential blood smears were analyzed after staining with Wright's stain.

Results –

a. Hematology – There were no treatment-related findings for males or females in the high (5790 ppm) dose group in WBC differential blood counts. Data for WBC

total counts was not presented. There were no grossly observable abnormalities of red blood cells for males or females in the high (5790 ppm) dose group as compared to controls. Differential blood cell data were not collected for males or females in the low (290 ppm) or medium (2316 ppm) dose groups, except for animals found moribund and sacrificed prior to termination. There were no treatment-related abnormalities for males or females sacrificed prior to study termination in the low, medium, or high dose groups, as compared to controls.

b. Clinical chemistry

Clinical chemistry analyses were not performed.

6. Urinalysis

Urinalysis was not performed.

7. Sacrifice and pathology

Rats were euthanitized by decapitation after carbon dioxide inhalation and complete necropsy 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 analysis was performed on the 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*+
	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 chronic studies.
+ Organ weight required in chronic studies.

Results –

a. Organ weight – The mean absolute organ weights (Table 4) for liver were decreased relative to controls for males treated with Mepiquat Chloride at 5790 ppm (17% decrease, $p<0.01$) and at 2316 ppm (9% decrease, $p<0.05$). Mean absolute organ weights for kidneys were decreased as compared to controls for males in the 5790 ppm dose group (15%, $p<0.01$). Absolute organ weights for adrenals were decreased to 48%, 47%, and 44% of controls for males treated with Mepiquat Chloride at 290, 2316, and 5790 ppm, respectively (not statistically significant due to large standard deviation for controls). Relative organ weights for brain were increased (19% increase, $p<0.05$) for males in the high dose group as compared to controls. For females (5790 ppm), absolute organ weights for liver were decreased 17% relative to controls ($p<0.01$), relative organ weights for brain were increased 20% ($p<0.01$) and kidneys were increased 19% ($p<0.01$) relative to controls. As there were no correlations between the changes in absolute and relative organ weights, the changes in organ weights are not toxicologically significant.

TABLE 4. MEAN ABSOLUTE (RELATIVE) ORGAN WEIGHTS FOR MALE AND FEMALE WISTAR RATS TREATED WITH MEPIQUAT CHLORIDE IN FOOD FOR 728 DAYS

Organ	Exposure Level (ppm)							
	Males				Females			
	0	290	2316	5790	0	290	2316	5790
Brain	2.3 (0.31)	2.3 (0.36)	2.3 (0.35)	2.2 (0.37*)	2.1 (0.56)	2.0 (0.54)	2.0 (0.56)	2.0 (0.67**)
Liver	21.3 (2.88)	20.0 (2.95)	19.4* (2.96)	17.6** (2.89)	12.1 (3.21)	11.8 (3.10)	11.3 (3.06)	10.1** (3.39)
Kidneys	4.6 (0.62)	4.3 (0.65)	4.3 (0.66)	3.9** (0.64)	2.8 (0.75)	2.8 (0.74)	2.8 (0.76)	2.7 (0.89**)
Adrenals	199.3 (0.029)	94.9 (0.014)	93.8 (0.015)	88.5 (0.015)	205.7 (0.054)	141.6 (0.037)	205.9 (0.057)	233.8 (0.085)

Data were adapted from the Pathology Report, pp. 419-422, MRID No. 433960-01.

b. Gross pathology – There were gross pathological findings for males (focus in the adrenal cortex, enlarged testes, focus in the testes, cysts in the liver, and enlarged prostate) and for females (focus in the adrenal cortex, cysts in the liver, cysts in the ovaries) that occurred at a higher frequency in treated groups, as compared to controls (Table 5). The increases in incidences of these findings were not statistically analyzed by the study authors.

c. Microscopic pathology –

1) Non-neoplastic – There were non-neoplastic microscopic pathological findings for ileum, liver, kidneys, prostate, ovaries, uterus, iliac lymph node, sublingual glands, and mammary gland that occurred at a higher incidence in treated animals than in controls (Table 6). There was a strong dose-relationship for the findings for kidneys (tubular casts) and ovaries (dilated bursa) for females. The findings for liver (biliary cysts), ovaries (dilated bursa), and prostate (alveolar atrophy) may be related to macroscopic pathological findings for these tissues. Thus, in contrast to the study authors contention, the non-neoplastic microscopic pathological findings for males and females in the high dose group are toxicologically significant. The incidences of the pathological findings were not statistically analyzed by the study authors.

2) Neoplastic – There were no neoplastic changes occurring in a frequency related to dose in treated males or females in the study (see APPENDIX below). The tumor incidences and total tumor burden for each dose group are presented in Table 5.

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TABLE 5. INCIDENCE OF MACROSCOPIC PATHOLOGICAL FINDINGS FOR MALE AND FEMALE WISTAR RATS TREATED WITH MEPIQUAT CHLORIDE IN FOOD FOR 728 DAYS

Pathology	Exposure Level (ppm)							
	Males				Females			
	0	290	2316	5790	0	290	2316	5790
Adrenal Cortex - Focus	5/50 (10%)	10/50 (20%)	7/50 (14%)	16/50** (32%)	31/50 (62%)	36/50 (72%)	39/50* (78%)	39/50* (78%)
Testes - Enlarged	12/50 (24%)	19/50 (28%)	16/50 (32%)	16/50 (32%)	--	--	--	--
- Focus	15/50 (30%)	15/50 (30%)	16/50 (32%)	18/50 (36%)	--	--	--	--
Liver - Cyst	1/50 (2%)	3/50 (6%)	1/50 (2%)	1/50 (2%)	7/50 (14%)	12/50 (24%)	15/50* (30%)	12/50 (24%)
Ovaries - Cyst	--	--	--	--	14/50 (28%)	20/50 (40%)	16/50 (32%)	24/50* (48%)
Prostate -Enlarged	0/50 (0%)	4/50* (8%)	2/50 (4%)	3/50* (6%)	--	--	--	--

Macroscopic pathological incidences were adapted from the Pathology Report, pp. 423-429, MRID No. 433960-01.
*$p<0.05$, calculated by the reviewer
**$p<0.01$, calculated by the reviewer

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TABLE 6. INCIDENCE OF NON-NEOPLASTIC MICROSCOPIC PATHOLOGICAL FINDINGS IN MALE AND FEMALE WISTAR RATS TREATED WITH MEPIQUAT CHLORIDE IN FOOD FOR 728 DAYS

Pathology	Exposure Level (ppm)							
	Males				Females			
	0	290	2316	5790	0	290	2316	5790
Ileum -Lymphoid Hyperplasia	0/49 (0%)	0/10 (0%)	0/10 (0%)	5/46** (11%)	1/46 (2%)	0/10 (0%)	0/10 (0%)	0/47 (0%)
Liver -Biliary cysts	4/50 (8%)	5/50 (10%)	6/50 (12%)	4/50 (8%)	8/50 (16%)	15/50* (30%)	14/50 (28%)	16/50* (32%)
Kidneys -Tubular casts	3/50 (6%)	2/50 (4%)	3/50 (6%)	5/50 (10%)	6/50 (12%)	6/50 (12%)	11/50 (22%)	13/50* (26%)
-Tubular atrophy	5/50 (10%)	3/50 (6%)	3/50 (6%)	12/50* (24%)	9/50 (18%)	9/50 (18%)	13/50 (26%)	16/50 (32%)
Prostate -Alveolar atrophy	1/50 (2%)	2/20 (10%)	4/18** (22%)	7/50* (14%)	--	--	--	--
Ovaries -Dilated bursa	--	--	--	--	1/50 (2%)	2/31 (6%)	2/27 (7%)	13/50** (26%)
Uterus -Stromal hyperplasia	--	--	--	--	2/50 (4%)	3/19* (16%)	1/24 (4%)	11/50** (22%)
-Stromal fibrosis	--	--	--	--	35/50 (70%)	13/19 (68%)	10/24* (42%)	42/50* (82%)
-Squamous hyperplasia	--	--	--	--	5/50 (10%)	5/19* (26%)	4/24 (17%)	14/50* (28%)
-Endometritis	--	--	--	--	1/50 (2%)	3/19* (16%)	2/24 (8%)	5/50* (10%)
Iliac lymph node -Erythrophagocytosis	7/26 (27%)	10/34 (29%)	5/24 (21%)	12/27 (44%)	1/8 (13%)	1/6 (17%)	2/12 (17%)	5/8* (63%)
Sublingual glands -Acinar atrophy	4/50 (8%)	3/12* (25%)	3/14 (21%)	6/50 (12%)	4/50 (8%)	2/11 (18%)	0/9 (0%)	14/49** (29%)
Mammary gland -Secretion	--	--	--	--	30/47 (64%)	9/16 (56%)	7/14 (50%)	45/50** (90%)
-Galactocele(s)	--	--	--	--	14/47 (30%)	6/16 (38%)	6/14 (43%)	21/50 (42%)

Microscopic pathological incidences were adapted from the Pathology Report, pp. 498-510, MRID No. 433960-01.
*$p < 0.05$, calculated by the reviewer
**$p < 0.01$, calculated by the reviewer

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TABLE 6. TUMOR INCIDENCE FOR MALE AND FEMALE WISTAR RATS TREATED WITH MEPIQUAT CHLORIDE IN FOOD FOR 728 DAYS

Number of Tumors	Exposure Level (ppm)							
	Males				Females			
	0	290	2316	5790	0	290	2316	5790
Number of Animals with:								
Primary neoplasms	46	42	35	43	50	44	42	47
One primary neoplasm	11	11	15	17	19	21	16	16
Two or more primary neoplasms	35	31	20	26	31	23	26	31
Benign neoplasms	39	36	31	38	46	41	39	44
Malignant neoplasms	28	18	14	11	19	10	15	21
Total:								
Primary neoplasms	114	95	68	90	111	83	85	102
Benign neoplasms	83	73	52	79	89	71	70	78
Malignant neoplasms	31	22	16	11	22	12	15	24

Tumor incidences were adapted from the Pathology Report, p. 473, MRID No. 433960-01.

D. DISCUSSION

The doses of Mepiquat Chloride for the current study were chosen based upon a 4-week feeding study with Mepiquat Chloride at 500, 2000, and 8000 ppm (MRID No. 424121-02) and upon a 3-month feeding study with Mepiquat Chloride at 145, 579, 2316, 4632, and 12,000 ppm. 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, Mepiquat Chloride was administered at 290, 2316, and 5790 ppm in a 2-year oncogenicity feeding study.

The LOEL for oral administration of Mepiquat Chloride for 728 days is 5790 ppm for males and females, based upon decreased body weight gain, food consumption, food efficiency, and macroscopic and non-neoplastic microscopic pathological findings. For males treated with Mepiquat Chloride at 5790 ppm, group mean body weights at day 728 were 82% of control ($p < 0.01$), group mean body weight gains from day 0 to 728 were 77% of control ($p < 0.01$), group mean daily and total food consumption were 88% of control ($p < 0.01$), and food

efficiency was 90% of control ($p<0.05$). There was a statistically significantly increased incidence of macroscopic pathological findings for the adrenal cortex and prostate and of non-neoplastic microscopic pathological findings for the ileum, kidneys, and prostate for males treated with Mepiquat Chloride at 5790 ppm. For females treated with Mepiquat Chloride at 5790 ppm, group mean body weights at day 728 were 81% of control ($p<0.01$), group mean body weight gains from days 0 to 728 were 71% of control ($p<0.01$), group mean daily and total food consumption were 93% of control ($p<0.01$), and food efficiency was 78% of control ($p<0.01$). There was a statistically significantly increased incidence of macroscopic pathological findings for the adrenal cortex, liver, and ovaries, and non-neoplastic microscopic pathological findings for liver, kidneys, ovaries, uterus, iliac lymph node, sublingual glands, and mammary gland for females treated with Mepiquat Chloride at 5790 ppm. For high dose females compared to controls, the largest increased incidences of microscopic pathological findings were in the ovaries (dilated bursa, 1/50 vs. 13/50, $p<0.01$), the uterus (stromal hyperplasia, 2/50 vs. 11/50, $p<0.01$; squamous hyperplasia, 5/50 vs. 14/50, $p<0.05$), the sublingual glands (acinar atrophy, 4/49 vs. 14/50, $p<0.01$), and the mammary glands (secretion, 30/47 vs. 45/50, $p<0.01$).

The NOEL is 2316 ppm for 728 day administration of Mepiquat Chloride to males and females, even though there were statistically significant changes in certain parameters. For males treated with Mepiquat Chloride at 2316 ppm, there were statistically significant decreases, as compared to controls, in group mean body weights at the end of the treatment period (day 728: 88%, $p<0.01$), group mean body weight gains over the entire treatment period (85%, $p<0.01$), daily and total food consumption (96%, $p<0.01$), food efficiency (96%, $p<0.05$), and increases in incidences of macroscopic and non-neoplastic pathological findings. None of these findings are toxicologically relevant. There were several deaths of low body weight males in the control group resulting in an increase in group mean body weights for controls during year 2. In addition, the increased group mean body weight gain seen for controls in this study during year 2 was not noted in a concurrent 2-year chronic study. The group mean body weights for controls at the end of the 2-year chronic study were approximately 700-725 g, similar to the low dose (290 ppm) group in that study and the low dose (290 ppm) group and the medium dose (2316 ppm) group in the current oncogenicity study. Thus, the statistically significantly decreases as compared to controls in body weights and body weight gains for males in the 2316 ppm dose group did not result from toxicological effects. The small decreases in food consumption and food efficiency are not toxicologically important. The minor pathological findings are of questionable toxicological relevance due to the lack of clear dose-related patterns or correspondence between macroscopic and microscopic pathological findings. For females treated with Mepiquat Chloride at 2316 ppm, food consumption and food efficiency were not significantly different from controls. Although there was an increased incidence of macroscopic and non-neoplastic microscopic pathological findings for females, the findings are rarely correlated or dose-related and are not toxicologically signifieant.

There were no toxicologically significant findings for males or females in the low dose (290 ppm) group.

There were no neoplastic changes occurring at a higher frequency in treated animals than in controls. Therefore, treatment of Wistar rats with Mepiquat Chloride at 5790 ppm in feed for 2 years does not result in an increased incidence of neoplasms for males or females. The high dose (5790 ppm) in the current study is a MTD.

E. STUDY DEFICIENCIES

Differential white blood cell evaluations were only performed at termination, and were not performed at 12 and 18 months, as required by guidelines for an oncogenicity study (§83-5). The heart, lungs, and ovaries were not weighed.

APPENDIX. SELECTED TUMOR INCIDENCE IN ALL MALE AND FEMALE WISTAR RATS TREATED WITH MEPIQUAT CHLORIDE IN FOOD FOR 728 DAYS[1]

Number of Tumors	Exposure Level (ppm)							
	Males				Females			
	0	290	2316	5790	0	290	2316	5790
Testes: interstitial cell tumor	22/50	24/39	17/39	20/50	-	-	-	-
Pituitary: adenoma[2]	13/50	12/21	6/20	7/50	44/50	36/42	34/41	38/50
Adrenal medulla: pheochromocytoma	8/49	7/23	4/22	4/50	5/49	5/42	3/46	4/49
Mammary gland: fibroadenoma	-	-	-	-	6/47	3/16	3/14	5/50
Mammary gland: adenocarcinoma	-	-	-	-	4/47	1/16	3/14	6/50

[1] Tumor incidences were adapted from the Pathology Report, pages 458-462, MRID No. 433960-01.
[2] No pituitary adenocarcinomas were noted in the report.

APPENDIX. SELECTED TUMOR INCIDENCE IN SURVIVING MALE AND FEMALE WISTAR RATS TREATED WITH MEPIQUAT CHLORIDE IN FOOD FOR 728 DAYS[1]

Number of Tumors	Exposure Level (ppm)							
	Males				Females			
	0	290	2316	5790	0	290	2316	5790
Testes: interstitial cell tumor	15/27	20/27	13/25	19/41	-	-	-	-
Pituitary: adenoma[2]	8/27	9/9	4/6	7/41	31/33	29/31	28/31	31/37
Adrenal medulla: pheochromocytoma	5/27	5/12	2/8	3/41	4/32	4/31	3/36	3/36
Mammary gland: fibroadenoma	-	-	-	-	3/32	3/6	2/5	3/37
Mammary gland: adenocarcinoma	-	-	-	-	4/32	1/6	1/5	5/37

[1] Tumor incidences were adapted from the Pathology Report, pages 463-467, MRID No. 433960-01.
[2] No pituitary adenocarcinomas were noted in the report.

APPENDIX. SELECTED TUMOR INCIDENCE IN NON-SURVIVING MALE AND FEMALE WISTAR RATS TREATED WITH MEPIQUAT CHLORIDE IN FOOD FOR 728 DAYS[1]

Number of Tumors	Exposure Level (ppm)							
	Males				Females			
	0	290	2316	5790	0	290	2316	5790
Testes: interstitial cell tumor	7/23	4/12	4/14	1/9	-	-	-	-
Pituitary: adenoma[2]	5/23	3/12	2/14	0/9	13/17	7/11	6/10	7/13
Adrenal medulla: pheochromocytoma	3/22	2/11	2/14	1/9	1/17	1/11	0/10	1/13
Mammary gland: fibroadenoma	-	-	-	-	3/15	0/10	1/9	2/13
Mammary gland: adenocarcinoma	-	-	-	-	0/15	0/10	2/9	1/13

[1] Tumor incidences were adapted from the Pathology Report, pages 468-472, MRID No. 433960-01.
[2] No pituitary adenocarcinomas were noted in the report.

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Chemical:	**Mepiquat chloride**
PC Code:	**109101**
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