Krystyna K. Locke 9/23/94

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

for **Secondary Review by:** Roger Gardner, Section Head Pamela M. Hurley 10/3/94
Section I, Toxicology Branch I
Health Effects Division (7509C)

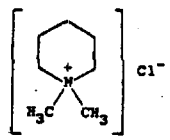
DATA EVALUATION RECORD

STUDY TYPE: 82-1 Subchronic Feeding in the Rodent

EPA IDENTIFICATION NUMBERS:

MRID No. 42337103	DP Barcode No. D182069
EPA ID No. 109101	Submission No. S424471
Case No. 819426	Rereg. Case No. 2375
P.C. Code No. 109101	Tox. Chem. No. 380 AB

TEST MATERIAL: Mepiquat chloride (Pix); 1,1-Dimethylpiperidinium chloride; yellowish liquid; purity (active ingredient or Mepiquat chloride content): 57.9%; batch no. WW 262/CP 1490; test substance no. 89/112; CAS Reg. No. 24307-26-4; stable at room temperature; plant growth regulator. Structure:



REPORT NUMBER: 31SO112/89053 and 92/10433

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

TESTING FACILITY: BASF Aktiengesellschaft, Department of Toxicology, Limburgerhof, Germany.

TITLE OF REPORT: Study on the Oral Toxicity of Mepiquat Chloride in Wistar Rats - Administration in the Diet Over 3 Months.

AUTHOR: K. Schilling, DVM

STUDY COMPLETION DATE: May 15, 1992

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EXECUTIVE SUMMARY

In this subchronic feeding toxicity study, Wistar strain rats, 10/sex/group, received Mepiquat chloride (purity or active ingredient content: 57.9%) in the diet for 3 months. Based on the results of a 4-week range-finding study, the dose levels of Mepiquat chloride selected for this study were 0, 250, 1000, 4000 or 8000 ppm. However, due to an error in the preparation of the diets (no adjustment for purity of Mepiquat chloride was made), which was discovered after the life part of the study was completed, the rats received less Mepiquat chloride throughout the study than was intended. **The actual dose levels of Mepiquat chloride fed were 145, 579, 2316 or 4632 ppm. According to the submitted report, these dose levels were equivalent to about 11, 44, 176 or 346 mg of Mepiquat chloride/kg of body weight, respectively.**

Mepiquat chloride, at all levels tested, had no effect on any of the parameters examined in this study and there were no unscheduled deaths. Based on these findings, **the systemic toxicity NOEL is > 4632 ppm (approx. 346 mg/kg/day; HDT).**

Because the NOEL has not been determined, this study is classified as Core-Supplementary and, by itself, does not satisfy the requirement, § 82-1, for a subchronic feeding study in the rat. However, **considered together with another study (Project No. 31SO 112/89077; MRID 42337102)** in which rats were fed diets containing 0 and 12000 ppm (about 889 mg/kg body weight) of Mepiquat chloride for 3 months and in which toxic effects were observed in the treated group, **the current study (No. 31SO112/89053; MRID 42337103) is classified as Core-Minimum and satisfies the requirement, § 82-1, for a subchronic feeding study in the rat.**

EXPERIMENTAL PROCEDURES

The rats were received in the testing facility on January 22, 1990 and dosing was carried out from January 31, 1990 to May 4, 1990 (last day of necropsy).

Wistar strain rats, 10/sex/group, received Mepiquat chloride in the diet for 3 months. The doses used were 0, 145, 579, 2316 or 4632 ppm, and were based on the results of the 4-week range-finding study (MRID No. 42412102) in which Mepiquat chloride at dose levels of 500, 2000 or 8000 ppm was tested. According to this submission, the dose levels used for the four treated groups in the 90-day rat feeding study were equivalent to about 11, 44, 176 or 346 mg of Mepiquat chloride/kg of body weight, respectively. The dose levels used in the range-finding study were equivalent to about 46, 183 or 661 mg of Mepiqut chloride/kg of body weight, respectively. The diets were prepared weekly and were not pelleted. **The rats were:**

(1) Obtained from Karl Thomae, Biberach/Riss, Germany.

(2) Acclimated for 9 days before assignment to groups on the basis of weight. When the dosing was started, the rats were 42 days old. The mean weight of males was 181 (166-196) g and of females 145 (131-156) g.

(3) Housed singly in stainless steel wire cages (floor area about 800 cm^2), at temperatures of 20-24°C, relative humidity of 30-70%, and 12 hours light/12 hours dark cycles.

(4) Identified by numbers tattooed on the inside and outside of the left ear.

(5) Allowed free access to food (ground Kliba rat/mouse/hamster maintenance diet, supplied by Klingentalmuhle Ag., Switzerland) and tap water.

(6) Sacrificed, at the end of the study, after a fasting period of 16-20 hours.

The following parameters were examined for all rats on the study unless indicated otherwise:

(1) **Clinical Observations:** Twice daily during the week and once daily on the week-ends and holidays, for signs of toxicity. Once a week, each animal was removed from the cage and examined thoroughly for abnormalities and clinical signs of toxicity, including tissue masses.

(2) **Body Weight:** Before the initiation of treatment, weekly thereafter and at the terminal sacrifice.

(3) **Food Consumption:** Weekly, throughout the study.

(4) **Food Efficiency:** Was calculated for each rat at the same time at which simultaneously body weight and food consumption were determined. The formula used to calculate food efficiency was included in the submission.

(5) **Ophthalmoscopy:** One day before the start of the study and on day 85, the eyes of the rats in the control and the 4632 ppm groups were examined with a slit lamp.

(6) **Hematology and Clinical Chemistry:** On study day 86, in the morning, blood was taken from the retroorbital venus plexus of all rats, which were non-fasted and not anesthetized. The blood samplings and the subsequent analyses of the blood and serum samples were carried out in a randomized sequence. The following determinations were performed:

Hematology: Leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemolglobin concentration, platelets, differential blood count, reticulocytes and thromboplastin time.

Clinical Chemistry: alanine and aspartate aminotransferases, alkaline phosphatase and serum gamma glutamyl transferase.

Sodium, potassium, chloride, inorganic phosphate, urea, calcium, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol and magnesium.

(7) **Urinalyses:** On study day 79, the rats were transferred to metabolism cages and urine was collected overnight. The following examinations were performed for each rat:

Appearance, nitrite, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood and sediment.

(8) **Necropsy:** The rats were sacrificed by decapitation under CO_2 anesthesia, exsanguinated and all were necropsied.

(9) **Organ Weights:** At the terminal sacrifice, the following organs were weighed for all rats on the study: liver, kidneys, adrenal glands and testes.

(10) **Histopathology:** The following tissues were examined for all rats in the control and the 4632 ppm groups:

Adrenal glands	Pancreas
Aorta	Parathyroid glands
Bone marrow (femur)	Pituitary glands
Brain	Prostate, seminal vesicle
Cecum, colon, rectum	Sciatic nerve
Duodenum, jejunum, ileum	Skeletal muscle
Epididymides	Skin
Esophagus	Spinal cord (cervical, thoracic and lumbar)
Eyes	Spleen
Female mammary gland	Sternum (with bone marrow)
Femur with joint	Stomach
Heart	Sublingual gland
Kidneys ■	Testes / ovaries
Liver ■	Thymus
Lungs ■	Thyroid glands
Mandibular gland	Trachea
Mandibular lymph node	Urinary bladder
Mesenteric lymph node	Uterus, vagina

■ Tissues examined for all rats on the study. Gross lesions were also examined for all rats.

The above tissues were embedded in paraffin, sectioned, stained with hematoxylin-eosin and examined by light microscopy.

Other parameters examined in this study were concentration, stability and homogeneity of Mepiquat chloride in the diets, and actual intake of Mepiquat chloride by the rats.

Statistical Analyses:

Mean body weights, food consumption, food efficiency, hematology, clinical chemistry, urinalyses, terminal body weights and terminal organ weights (both absolute and relative) were analyzed statistically as described in Attachment I of this review. In most instances, the ANOVA and Dunnett's tests were used.

RESULTS

Concentration, Stability and Homogeneity of Mepiquat Chloride in Diets

Mepiquat chloride was not detected in samples obtained from the control diet. The concentrations of Mepiquat chloride in the diets of the treated groups were within 2.1-9.5% of the nominal values.

Mepiquat chloride was stable in the rodent feed stored at room temperature for 32 days (length of time tested). In this test, the nominal concentration of Mepiquat chloride in the feed was 499.26 mg/kg. At days 0, 11 and 32, the mean concentration of Mepiquat chloride was 101.2, 97.8 and 94.3% of the nominal concentration, respectively. (In the 90-day feeding study, diets were prepared weekly).

Diets were homogeneous with respect to Mepiquat chloride. In this test, diets containing 500, 2000 and 8000 ppm of Mepiquat chloride (nominal concentrations) were studied before this study was started. The analytical concentrations of Mepiquat chloride in samples obtained from these diets were as follows: 500 ppm: 98.9 ± 3.3% of the nominal value; 2000 ppm: 100.8% of the nominal value; and 8000 ppm: 101.7 ± 1.3% of the nominal value.

Intake of Mepiquat Chloride ●

These data were reported for all treated groups, for each week of the study. The approximate, mean daily intake of Mepiquat chloride was as follows:

Test Group	Concentration (ppm)	Mepiquat Chloride mg/kg body weight/day		
		Males	Females	Both
1	145	10	12	11
2	579	40	47	44
3	2316	163	188	176
4	2632	319	372	346

● Taken from page 0039 of the submitted report (MRID 42337103)

Clinical Observations

Nothing remarkable was observed in any group. Treatment-unrelated findings included alopecia in one Group 3 male, and in

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two Group 4 males and one female. A swelling (not stated where) was also observed on two occasions in one Group 1 female.

Mortality

There were no unscheduled deaths in this study.

Body Weight

Relative to the control values, Mepiquat chloride, at all levels tested, had no effect on body weights of the male and female rats. With the exception of the first two test weeks, the mean body weights of the treated rats were almost the same, the same or grater than those of the controls. During the first two weeks, the male rats in Group 4 weighed slightly less (about 8%) than did the controls, but this was attributed to the decreased food consumption because of its (initial) unpalatability. The body weight data are summarized below.

Group	0	1	2	3	4
Mepiquat chloride (ppm)	0	145	579	2316	4632
Test Day	Mean Body Weights (g) of Male Rats ■				
0	180.9	181.3	181.2	181.4	180.8
7	235.7	234.7	234.6	231.9	216.1**
14	285.2	285.1	285.1	278.3	261.2**
35	373.4	381.0	375.1	364.4	350.5
70	462.5	473.9	461.8	453.9	436.5
91	485.4	495.5	487.1	478.0	460.2
Test Day	Mean Body Weights (g) of Female Rats ■■				
0	144.7	144.7	144.5	145.5	145.2
7	167.6	165.0	165.9	167.7	164.2
14	186.6	186.6	186.6	186.8	182.7
35	227.2	229.0	228.8	228.7	219.7
70	265.6	268.7	265.0	266.6	254.0
91	275.5	276.4	276.0	282.4	263.9

This table is based on Tables 005 and 006, pages 0055-0056 (■), and Tables 007 and 008, pages 0057-0058 (■■), of the submitted report (MRID 42337103).

** P≤0.01 ANOVA and Dunnett's test (two-sided)

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Food Consumption

Relative to the control values, Mepiquat chloride, at all levels tested, had no effect on food consumption of the male and female rats. With the exception of the first test week, the mean food consumption of the treated rats was similar to or greater than that of the controls. During the first test week, the rats in Group 4 consumed less food than did the controls (males, 24% less and females, 15% less). However, these decreases in the food consumption were not statistically significant and were attributed to the (initial) unpalatability of the food. The food consumption data are summarized below.

Group	0	1	2	3	4
Mepiquat chloride (ppm)	0	145	579	2316	4632
Test Day	Mean Food Intake (g/rat/day) of Male Rats ♥				
7	25.2	24.9	25.3	24.1	19.1
14	27.1	26.9	27.2	26.4	24.6
35	26.8	27.9	26.4	26.4	25.3
70	26.7	27.3	26.3	26.6	25.6
91	25.9	26.6	26.0	26.5	25.3
Test Day	Mean Food Intake (g/rat/day) of Female Rats ♥♥				
7	18.2	18.1	18.4	17.3	15.4
14	18.4	18.8	18.9	18.5	18.4
35	19.0	19.5	19.4	19.1	18.2
70	18.9	19.3	18.2	19.3	18.4
91	18.3	18.7	18.3	19.1	18.0

This table is based on Tables 001 and 002, pages 0051-0052 (♥), and Tables 003 and 004, pages 0053-0054 (♥♥), of the submitted report (MRID 42337103).

Food Efficiency

Food efficiency was calculated as is shown in Attachment II of this review. Relative to the control values, Mepiquat chloride, at all levels tested, had no effect on food efficiency of the male and female rats. In some weeks, the food efficiency was lower for the Group 4 male and female rats than for the controls, but in other weeks the reverse was true. All of the observed differences from controls were statistically insignificant. The food efficiency data are summarized below.

Group	0	1	2	3	4
Mepiquat chloride (ppm)	0	145	579	2316	4632
Test Day		Food Efficiency of Male Rats ●			
7	30.9	30.7	30.1	29.9	26.2
14	26.1	26.8	26.5	25.0	26.0
35	14.2	14.6	14.5	13.0	13.6
70	6.2	6.3	5.2	7.2	7.2
91	3.0	3.0	4.8	4.3	3.1
Test Day		Food Efficiency of Female Rats ●●			
7	17.9	15.8	16.5	18.2	17.6
14	14.7	16.2	15.5	14.6	14.2
35	10.1	7.4	10.7	8.6	6.6
70	2.2	4.3	0.6	2.0	2.9
91	1.2	1.3	1.4	3.7	1.3

This table is based on Tables 009 and 010, pages 0059-0060 (●), and Tables 011 and 012, pages 0061-0062 (●●), of the submitted report (MRID 42337103).

Ophthalmoscopy

These data were reported only as individual data for the male and female rats in Group 0 (controls) and Group 4 (4632 ppm), the only groups examined. Treatment-related abnormalities were not observed in Group 4. Remainders of the papillary membrane, observed in some rats in Group 4 (and Group 0) one day before the start of the study were also observed in the same rats on test day 85, when the ophthalmological examination was performed.

Hematology

Compared with the controls, Mepiquat chloride, at all levels tested, had no effect on any of the parameters examined.

Clinical Chemistry

Relative to the control values, Mepiquat chloride, at all levels tested, had no effect on any of the parameters examined.

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Urinalyses

Relative to the control values, Mepiquat chloride, at all levels tested, had no effect on any of the parameters examined.

Necropsy

Treatment-related effects were not observed in any of the groups. Treatment-unrelated effects were noted in one male rat from Group 3 and four male and one female rats from Group 4. One male in Group 3 had alopecia on the front leg. The following findings were reported for Group 4: alopecia on the front legs in two males and one female; small testes and epididymides in one (the same) male; adipose tissue necrosis in the region of the epididymides, in one male; and ovarian cyst in one female.

Organ Weights

These data were reported for liver, kidneys, testes and adrenal glands, as group mean values and as individual values. The absolute mean organ weights were also tabulated, whereas the relative mean organ weights (organ weight x 100 / body weight) were reported only with the individual values.

Relative to the control values, Mepiquat chloride, at all levels tested, had no effect on the absolute and relative organ weights. Data concerned with the absolute organ weights are in Attachment III of this review, whereas data concerned with the relative organ weights are summarized below.

Group		0	1	2	3	4
Mepiqut chloride (ppm)		0	145	579	2316	4632
Organ		Relative Weight (organ wgt. x 100 / body wgt.)				
Liver:	M	3.32	3.55	3.40	3.47	3.25
	F	2.94	2.99	3.01	3.03	3.03
Kidneys:	M	0.67	0.68	0.69	0.68	0.68
	F	0.74	0.73	0.75	0.73	0.77
Adrenals:	M	0.018	0.017	0.017	0.017	0.018
	F	0.038	0.037	0.038	0.036	0.039
Testes		0.078	0.077	0.080	0.080	0.081

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■ Group mean values. The above table is based on data reported on pages 0254-0263 of the submitted report (MRID 42337103).

Histopathology

Data summarized on pages 0249-0243 of the submitted report (MRID 42337103), **INCIDENCE OF MICROSCOPIC FINDINGS**, as well as individual data, support the testing facility's statement that " Apart from spontaneous changes, there were no histopathological findings in any of the examined organs which were attributable to the compound administered ". All of the findings observed are shown in the table below.

Group	0	1	2	3	4
Mepiquat chloride (ppm)	0	145	579	2316	4632
Finding	Number of Male Rats Affected				
Liver: Vacuolization	3	2	0	3	1
Lungs: Calcification	1	0	0	0	0
Kidneys: Infiltrates	0	0	0	0	1
Hyperplasia, tubular	0	1	0	0	0
Testes: Atrophy	0	0	0	0	1
Spermatogenesis, absent	0	0	0	0	1
Leydig cell hyperplasia	0	0	0	0	1
Adipose tissue: Necrosis	0	0	0	0	1
Skin: Hyperkeratosis	0	0	0	1	0
Finding	Number of Female Rats Affected				
Liver: Vacuolization	0	0	0	1	0
Kidneys: Calcification	10	9	10	10	9
Tubular hyaline cast	0	1	0	0	0
Uterus: Hydrometra	3	0	0	0	2
Spleen: Hematopoiesis	0	0	0	0	1
Pituitary gland: Cyst	0	0	0	0	1
Skin: Hyperkeratosis	0	0	0	0	1

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COMMENTS

This study does not meet the December 24, 1989, EPA ACCEPTANCE CRITERIA because a NOEL was not determined. Based on the results of the 4-week range-finding study (MRID 42412102), the dose levels of Mepiquat chloride selected for this 90-day feeding study were 250, 1000, 4000 and 8000 ppm. Adjusting for the 57.9% purity of Mepiqut chloride, these levels were to be achieved by using diets containing 432, 1727, 6908 and 13817 mg of Mepiquat chloride /kg of food, respectively. However, after the life part of the 90-day study was completed, it was discovered that the technician, who prepared the food mixtures, made no adjustment for the purity of Mepiquat chloride. Therefore, the actual dose levels used in this study were 145, 579, 2316 or 4639 ppm, and not 250, 1000, 4000 or 8000 ppm, as planned. To determine a dose level with adverse effects in a subchronic study and to aid the dose selection for a subsequent 2-year rat feeding study, a supplementary 3-month feeding study with one dose level was conducted. In that study, rats were fed diets containing 0 and 12000 ppm of Mepiquat chloride for 3 months (Project No. 31S0112/89077; MRID 42337102).

The following statements were included in the submission:

(1) Statement of No Data Confidentiality Claim.

(2) Statement that this study was conducted in accordance with OECD Principles of Good Laboratory Practice, Paris, 1981 and did not meet the requirements for 40 CFR 160, Good Laboratory Practice Standards.

(3) Data Flagging Statement (there are no 6a-2 data in this study).

(4) Quality Assurance Statement. This study was inspected as follows: protocol, on January 17, 1990; and in life, three times during February 7, 1990 and April 27, 1990; The final report was audited on April 30, 1992. The inspections were performed by the BASF Quality Assurance Unit.

The procedures used in the assignment of rats to groups, diet preparation and anlyses, hematology, clinical chemistry, urinalyses and histopathology have been described and/or referenced. The statistical procedures used have also been referenced.

This study was submitted in response to the FIFRA '88 data requirements for the reregistration of Mepiquat chloride.

ATTACHMENT I

RIN-0825-06

DER FOR MRID NO. 42337103

Page_____ is not included in this copy.

Pages 14 through 22 are not included.

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.

X 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.
