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

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OCT 11 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **Mepiquat Chloride:** Evaluation of the Following Studies: **83-3** Teratology/Developmental With Rats; **82-1** Subchronic (90 Days, 4 Dose Levels) With Rats; **82-1** Subchronic (90 Days, 1 Dose Level) With Rats; **Nonguideline** *In Vitro* Neuropharmacological With Mouse Feet Muscles; and **Nonguideline** *In Vitro* Neuropharmacological With Membranes of Animal Origin.

DP Barcode No. D182069	Submission No. S424471
EPA ID No. 109101	P.C. Code No. 109101
Rereg. Case No. 2375	Case No. 819426
CAS Registry No. 24307-26-4	Tox. Chem. No. 380 AB

FROM: Krystyna K. Locke, Toxicologist
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Krystyna K. Locke 9/23/94

TO: Linda Deluise/Ruby Whiters, PM Team No. 52
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for **THRU:** Roger Gardner, Section Head
Section I, Toxicology Branch I
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Pamela M. Hurley 10/3/94
KB 10/3/94

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

83-3 Study of the Prenatal Toxicity of Mepiquat Chloride in Rats After Oral Administration (Gavage); J. Hellwig, DVM; BASF, Germany; Report Nos. 30RO112/89102 and 92/10331; Study Completion Date: April 7, 1992. **MRID No.** 42337101

82-1 Study on the Oral Toxicity of Mepiquat Chloride in Wistar Rats - Administration in the Diet Over 3 Months; K. Schilling, DVM; BASF, Germany; Report Nos. 31SO112/89053 and 92/10433; Study Completion Date: May 15, 1992. **MRID No.** 42337103

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82-1 Supplementary Study on the Oral Toxicity of Mepiquat Chloride in Wistar Rats - Administration in the Diet Over 3 Months; K. Schilling, DVM; BASF, Germany; Report Nos. 31SO112/89077 and 92/10434; Study Completion Date: May 18, 1992. MRID No. 42337102

Nonguideline (Neuropharmacological) Report on the in Vitro Test of the Action of Mepiquat Chloride at Nicotinic Acetylcholine Receptors of Adult Mouse Muscle; C. Franke; Technical University, Munich, Germany; Report Nos. 99PO697/909016 and 91/11204; Study Completion Date: December 12, 1991. MRID No. 42337104

Nonguideline (Neuropharmacological) Study on the Affinity of Mepiquat Chloride for Muscarinic Receptors; H. Weifenbach; Knoll Research and Development, Ludwigshafen, Germany; Report Nos. 99PO697/909018 and 91/11206; Study Completion Date: September 19, 1991. MRID No. 42337105

Studies 82-1 and 83-3 were submitted in response to the FIFRA '88 toxicity data review. The nonquideline special (neuropharmacological) studies were not requested by the Agency.

In the teratology/developmental toxicity study (83-3), pregnant Wistar strain rats were administered aqueous solutions of Mepiquat chloride by gavage during gestation days 6 through 15. The doses used were 0, 50, 150 or 300 mg/kg/day. Treatment-related maternal effects were observed only in the 300 mg/kg group and included clinical signs of toxicity (tremors, unsteady gait, indrawn flanks, hypersensitivity and ataxia) and decreases in the food consumption and weight gain. These effects were not observed when dosing was discontinued and there were no unscheduled mortalities. Mepiquat chloride, at the three levels tested, had no effect on any of the developmental toxicity parameters examined. Based on these findings, the **Maternal Toxicity NOEL and LOEL are 150 mg/kg/day and 300 mg/kg/day, respectively.** The **Developmental Toxicity NOEL is 300 mg/kg/day** (HDT). This study is classified as **Core-Guideline.**

In the subchronic feeding study (82-1), Wistar strain rats were first fed diets containing 0, 145, 579, 2316 or 4632 ppm (about 11, 44, 176 or 346 mg/kg/day, respectively) of Mepiquat chloride for 90 days, but toxic signs were not observed. Another 90-day feeding study was then initiated with 0 and 12000 ppm (about 889 mg/kg/day) of Mepiquat chloride in order to demonstrate a dose level with an adverse or toxic effect. The following treatment-related effects were observed at the 12000 ppm level in males and females: (1) Clinical/neurological signs (tremors, long legged gait, abdominal position and unsteady gait); (2) Decreased food consumption, food efficiency and body weight gain; (3) Increase in the clotting (thromboplastin) time and de-

crease in the serum calcium, creatinine, glucose, total protein, albumin, globulin and triglycerides; (4) Reduced grip strength of the forelimbs and hindlimbs, and prolonged reaction on the hot-plate test (in males only); (5) Decreased absolute weights of liver and adrenals, and of kidneys (in males only); and (6) Decreased relative weight (organ/body weight ratio) of liver (in the males) and increased relative weights of kidneys and testes. Considered individually, each study is classified as Core-Supplementary and does not satisfy the requirement, 82-1, for a subchronic feeding study in the rat. **Considered together as one study, the study is classified as Core-Minimum and meets the requirement for a subchronic feeding study. The NOEL for males and females in this study is 4632 ppm (about 346 mg/kg/day) and the LOEL is 12000 ppm (about 889 mg/kg/day).**

In one of the nonguideline *in vitro* neuropharmacological studies, Mepiquat chloride activated the nicotinic acetylcholine receptors in the cells of the mouse feet muscles. However, the activation was slower than that observed with acetylcholine and ceased with the removal of Mepiquat chloride fron the reaction mixture. **This study is acceptable as a special study.**

In another nonguideline *in vitro* neuropharmacological study, Mepiquat chloride had low and unselective affinity for the muscarinic acetylcholine receptors in membranes of animal origin, when compared with the reference compounds. The membranes studied were isolated from bovine cerebral cortex, rat heart and rat submaxillary gland. **This study is acceptable as a special study.**

Krystyna K. Locke 9/23/94 011283

Primary Review by: Krystyna K. Locke, Toxicologist
Section I, Toxicology Branch I/HED

for **Secondary Review by:** Roger L. Gardner, Section Head
Section I, Toxicology Branch I/HED

Pamela M. Hurley 10/3/94

DATA EVALUATION RECORD

STUDY TYPE: Teratology - Developmental Toxicity (83-3)
Species: Rat

EPA IDENTIFICATION NUMBERS:

MRID No. 42337101
DP Barcode No. D182069
P.C. Code No. 109101
Tox. Chem. No. 380 AB

Rereg. Case No. 2375
Submission No. S424471
Case No. 819426

TEST MATERIAL: Mepiquat chloride

SYNONYMS: Pix

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

STUDY NUMBER: 30RO112/89102 and 92/10331

TESTING FACILITY: BASF Aktiengesellshaft, Department of Toxicology, Ludwingshafen/Rhein, Germany

TITLE OF REPORT: Study of the Prenatal Toxicity of Mepiquat Chloride in Rats After Oral Administration (Gavage)

AUTHOR: J. Hellwig, DVM

STUDY COMPLETED ON: April 7, 1992

EXECUTIVE SUMMARY:

In this developmental toxicity study, pregnant Wistar strain rats, 25/group, were administered aqueous solutions of Mepiquat chloride by gavage during gestation days 6 through 15. The doses used were 0, 50, 150 or 300 mg/kg b.w./day and were based on the results of the range finding study in which 100, 300 or 600 mg of Mepiquat chloride/kg b.w./day were tested. The purity (active ingredient content) of Mepiquat chloride was 57.9%.

Treatment-related **maternal effects** were observed only in the high-dose (300 mg/kg) group and incl uded clinical signs of toxicity and decreases in the food consumption and body weight gain. These effects were not observed when dosing with Mepiquat chloride was discontinued. There were no unscheduled mortalities.

Most dams (22-24/25) in the high-dose group showed pronounced but reversible tremors, unsteady gait, indrawn flanks and hypersensitivity, whereas 4/25 dams also had ataxia. All of these findings were noted at approximately 1.5-2.0 hours after dosing, lasted for about 4 hours and, with the exception of ataxia, were less frequent during the second half of the treatment period. Ataxia was observed in 2 dams during gestation day (g.d.) 7 only, in one dam during g.d. 8 and in another dam during g.d. 9.

Compared with the control values, food consumption of the high-dose dams was reduced by 10.4 to 19.1% ($P<0.01$) during the greater part of the dosing period (g.d. 6-13), but not thereafter. Mean body weight gains were also reduced during the same period by 16.2-65.1% ($P<0.01$), when compared with the control values. However, when mean body weights on g.d. 20 were corrected for uterine weights, the high-dose dams weighed only 13% less than did the controls and this difference was statistically insignificant.

Mepiquat chloride, at the three levels tested, had no effect on all of the **developmental toxicity** parameters examined. No embryotoxicity, fetotoxicity and no indications of any teratogenic effects were observed in this study.

Based on the clinical signs of toxicity and decreases in the food consumption and body weight gains, the **Maternal Toxicity LOEL is 300 mg/kg/day** and the **Maternal Toxicity NOEL is 150 mg/kg /day.**

Since developmental toxicity was not observed in this study, the **Developmental Toxicity NOEL is ≥ 300 mg/kg/day** (HDT).

This study is classified as **Core-Guideline (Acceptable)** and satisfies the requirement, § 83-3, for a developmental toxicity (teratology) study in rats.

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A. MATERIALS

Test Compound:

Purity: 57.9 % Mepiquat chloride (active ingredient, w/w)
44.3 % Doubly distilled water (w/w)

Description: Yellowish liquid

Batch No.: WW 262/CP 1490

Test Substance No.: 89/112

Contaminants: CBI appendix was not submitted, but analytical data are available from the sponsor.

Vehicle: Doubly distilled water

Test Animals:

Species: Rat

Strain: Wistar [Chbb:THOM (SPF)]

Source: Karl Thomae, Biberach an der Riss, Germany.

Age (females): 60 Days at receipt and 69-71 days at the beginning of the study (day 0, detection of sperm). Age of the male rats was not reported.

Weight (females): 230 g, at the beginning of the study. Weight of the male rats was not reported.

B. STUDY DESIGN

This study was designed to assess the effects of Mepiquat chloride on embryonic and fetal development when it was administered by gavage as an aqueous solution to pregnant rats during the period of organogenesis (gestation days 6 through 15). Also, information about effects of the test material on the maternal organism was to be obtained. During the study, the rats were housed singly in stainless steel wire mesh cages at temperatures of 20-24°C, relative humidity of 30-70% and 12-hour light/dark cycle. The food, used in unrestricted amounts, was ground Kliba 343 feed for rats, mice and hamsters, supplied by Klingentalmuhle Ag., Switzerland.

Mating:

Following the acclimation period of at least 5 days, 4 untreated female rats were mated with one untreated fertile male rat of the same breed. Mating took place " from about

16.00 hours to about 7.30 hours on the following day ". If sperm were detected microscopically in the vaginal smear, the rats were considered to be fertilized. This day was designated day 0 (beginning of the study) and the following day, gestation day 1.

Group Arrangement:

Test Group	Mepiquat chloride (mg/kg b.w./day*)	Number of Rats
0 (I)	0	25
1 (II)	50	25
2 (III)	150	25
3 (IV)	300	25

* Based on nominal concentrations of Mepiquat chloride, adjusted for purity, in dosing solutions. The analytical concentrations of Mepiquat chloride in dosing solutions ranged from 97% to 101% of the nominal concentrations. The dose levels used in this study were based on the results of the range-finding study (1) in which three doses of Mepiquat chloride (100, 300 or 600 mg/kg/day) were tested. A full report on the range-finding study was submitted.

(1) Results of a range-finding study of the prenatal toxicity of Mepiquat-chloride in rats after oral administration (gavage) - Proj. No. 10RO112/89097; BASF Aktiengesellschaft (1991)

Dosing:

All doses were in a volume of 10 mL/kg of body weight/day. Dosing was based on the individual body weights determined at the beginning of the administration period (gestation day 6). Dosing solutions were prepared daily just before use. All dosing solutions were analyzed for the concentration of Mepiquat chloride twice during the treatment period. Because the test substance itself was an aqueous solution, stability and homogeneity analyses of the aqueous solutions administered to the rats were, according to the submitted report, unnecessary.

Maternal Examinations

The animals were checked for mortality or abnormal condition once or twice daily, from the initiation of dosing to the termination of the study. Other parameters examined were food consumption and body weights, each determined on gestation days 0, 1, 3, 6, 8, 10, 13, 15, 17 and 20. Dams were sacrificed on gestation day 20. Examination at sacrifice consisted of gross necropsy, excising and weighing the

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intact gravid uterus, number of corpora lutea, number and distribution of implantations, early and late resorptions, and live and dead fetuses. Early resorptions were diagnosed by Salewski test (2). The conception rate and the preimplantation and postimplantation losses were calculated according to the formulae shown in Attachment I of this review.

(2) Salewski, E. Farbemethode zum makroskopischen Nachweis von Implantations-stellen am Uterus der Ratte; Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak. 247, 367-368 (1964).

Fetal Examinations

At necropsy, each fetus was weighed, sexed and examined macroscopically for external alterations. Also, the viability of the fetuses and the condition of the placentae, the umbilical cords, the fetal membranes and fluids were examined. Individual placental weights were recorded. After these examinations, about one-half of the fetuses per dam was placed in ethyl alcohol and examined for skeletal alterations according to a modified (not specified how) method of Dawson (3). The remaining half was fixed in Bouin's solution and examined for soft tissue alterations by the procedure of Barrow and Taylor (4). The evaluation criteria for skeletons and soft tissues (organs) were submitted (Attachment II in this review).

(3) Dawson, A.B. A note on the staining of the skeleton of cleared specimens with Alizarin red S.; Stain Technol. 1, 123 (1926)

(4) Barrow, M.V. et al. A rapid method for detecting malformations in rat fetuses; J. Morph. 127, 291-306 (1969)

Historical Control Data

Historical control data for the Wistar strain of rats were provided to allow comparison with concurrent controls. These data were obtained from the breeder (Dr. K. Thomae), but the number of studies used to compile the historical data base was not reported. The submitted data (one set was dated March 29, 1990 and another, March 6, 1992) inclued the following endpoints: (a) mean maternal body weights during gestation days 0, 1, 3, 6, 8, 9, 10, 12, 13, 15, 17, 18, 19 and 20; (b) reproduction data; (c) placental weights; (d) mean fetal weights; (e) fetal external malformations; (f) fetal external variations; (g) fetal external unclassified findings; (h) fetal soft tissue malformations; (i) fetal soft tissue variations; (j) fetal soft tissue unclassified findings; (k) fetal skeletal malformations; (l) fetal skel-

etal variations; and (m) fetal skeletal retardations.

Statistical Analysis

Dunnett's Test (5, 6) was used for statistical evaluation of food consumption, body weight, body weight change and corrected body weight gain (net maternal body weight change); weight of the intact uterus, fetuses and placentae; and corpora lutea, implantations, pre- and postimplantation losses, resorption and live fetuses. Fisher's Exact Test (7) was used for statistical evaluation of conception rate and of all fetal findings.

(5) Dunnett, C.W. A multiple comparison procedure for comparing several treatments with a control; J. Amer. Statistical Assoc. 50, 1096-1121 (1955)

(6) Dunnett, C.W. New tables for multiple comparisons with a control; Biometrics 20, 482-491 (1964)

(7) Dixon, W.J. In: BMDP Statistical Software, p. 663; University of California Press; Berkely, Los Angeles, London (1981)

Compliance

A signed statement of Confidentiality Claim was not provided.

A sign statement of compliance with "OECD Principles of Good Laboratory Practice" (Paris, 1981) was provided.

A signed Quality Assurance Statement was provided.

C. RESULTS

Maternal Toxicity

Clinical Observations

Treatment-related clinical symptoms were observed only in the high-dose group (300 mg/kg/day). Nearly all dams in this group (22-24/25) showed tremor, unsteady gait, piloerection, indrawn flanks and hypersensitivity, whereas 4/25 dams also had ataxia. All of these symptoms were pronounced but reversible, occurring approximately 1.5-2.0 hours after dosing in most dams and lasting for about 4 hours. These findings (all but ataxia) were observed less frequently during the second half of the treatment period. Ataxia was observed in 2 dams during gestation day (g.d.) 7 only, in 1 dam during g.d. 8 and in another dam during g.d. 9. The

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other symptoms were observed in 9-21 dams during g.d. 6-10, in 7-8 dams during g.d. 11 and in 1-3 dams during g.d. 2-14.

Mortality

There were no unscheduled mortalities during this study.

Food Consumption

Compared with the controls, Mepiquat chloride had no effect on food consumption of the low-dose (50 mg/kg/day) and mid-dose (150 mg/kg/day) dams. Food consumption of the high-dose dams (300 mg/kg/day) was reduced up to 19% during the greater part of the dosing period [gestation days (g.d.) 6-13]. Thereafter, (g.d. 14-20), food consumption of the high-dose dams reached or even exceeded control values. These data are summarized below.

Table I: Mean Maternal Food Consumption (g/dam/day) #

Group ##	I	II	III	IV
Predosing Period:				
Days 0-6	21.3	31.8	21.1	21.4
Dosing Period:				
Days 6-15	25.1	25.9	25.0	22.4
6-8	24.1	24.6	22.9	19.5 **b**
8-10	24.4	25.0	24.4	20.0 **b**
10-13	26.0	26.6	26.0	23.3 **b**
Postdosing Period:				
Days 15-20	29.9	31.2	31.3	30.7
Entire Gestation Period:				
Days 0-20	24.9	25.7	25.1	23.9

Data extracted from BASF Project No. 30RO112/89102, Table 001, page 45 and Table 002, page 46.

Groups I, II, III and IV received 0, 50, 150 or 300 mg of Mepiquat chloride/kg b.w./day, respectively.

b Significantly different from control: $P<0.01$. Relative

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to the control values, the food consumption of 19.5, 20.0 and 23.3 g/dam/day represented decreases of 19.1, 18.0 and 10.4%, respectively.

Body Weight

Compared with the controls, Mepiquat chloride had no effect on body weight gains of the low-dose (50 mg/kg/day) and mid-dose (150 mg/kg/day) dams. Mean body weight gains of the dams in the high-dose group (300 mg/kg/day) were significantly reduced (up to 65%) during most days of the treatment period (g.d. 6-13). However, in the subsequent time (g.d. 14-20), weight gain of the high-dose dams reached or even exceeded control values. When mean body weights on g.d. 20 were corrected for uterine weights, the high-dose dams weighed only 13% less than did the controls and this difference was statistically insignificant. The body weight data are summarized below.

Table II: Mean Maternal Body Weight Gains (grams) #

Group ##	I	II	III	IV
Predosing Period:				
Days 0-6	28.1	28.2	28.5	27.8
Dosing Period:				
Days 6-15	45.1	48.5	45.1	37.8 **b**
6-8	6.3	7.2	4.6	2.2 **b**
8-10	10.9	9.5	10.9	7.3 **b**
10-13	18.0	18.3	17.9	14.3 **b**
13-15	9.9	13.4 **a**	11.8	14.0 **b**
Postdosing Period:				
Days 15-20	72.7	77.2	76.5	75.5
Entire Gestation Period:				
Days 0-20	145.8	153.9	150.1	141.1
Corrected Body Weight Gains ###	42.5	46.6	44.6	37.1

Data extracted from BASF Project No. 30RO112/89102, Table

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004, page 48; Table 005, page 49 and Table 006, page 50.

Groups I, II, III and IV received 0, 50, 150 or 300 mg of Mepiquat chloride/kg b.w./day, respectively.

Reported as net weight changes from day 6 (start of treatment) or carcass weight minus day 6 body weight. Carcass weight = terminal body weight minus uterine weight. The mean gravid uterine weights were similar among the four test groups (75.3, 79.1, 77.0 and 76.3 grams for Groups I, II, III and IV, respectively).

Significantly different from control: a = P<0.05 and b = P<0.01. Relative to the control values, the mean body weight gains of 37.8, 2.2, 7.3 and 14.3 g in Group IV represented decreases of 16.2, 65.1, 33.0 and 20.6%, respectively.

Gross Pathological Observations

The following data were submitted: 1) Summary of necropsy findings and 2) Individual observations at scheduled sacrifices. There were no treatment-related findings in any of the dams. Only one (nonpregnant) dam from the low-dose group (50 mg/kg/day) had hydronephrosis, which was regarded by the testing laboratory as a spontaneous finding.

Cesarean Section Observations

Cesarean section observations were based on the 20, 23, 23 and 24 pregnant rats that were sacrificed on gestation day 20 in Groups I, II, III and IV, respectively. Mepiquat chloride, at all levels tested, had no effect on any of the parameters examined. These data are summarized below.

Table III: Cesarean Section Observations #

Group ##	I	II	III	IV
Number of Rats Assigned	25	25	25	25
Number of rats Mated	25	25	25	25
Pregnancy Rate (%)	80	92	92	96

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Table III - continued

Group ##	I	II	III	IV
Maternal Wastage				
Number died	0	0	0	0
Nonpregnant	5	2	2	1
Aborted	0	0	0	0
Premature births	0	0	0	0
Dams with viable fetuses	20	23	23	24
Dams with all resorptions	0	0	0	0
Corpora Lutea				
Total	304	362	343	362
Per dam	15.2	15.7	14.9	15.1
Implantation Sites				
Total	286	324	319	332
Per dam	14.3	14.1	13.9	13.8
Preimplantation loss (%)	5.8	12.6	6.7	9.1
S.D.	12.27	25.61	9.58	19.67
Postimplantation loss (%)	6.9	3.0	6.7	5.3
S.D.	8.58	4.97	7.22	6.20
Resorptions				
Total	20	11	22	18
Per dam	1.0	0.5	1.0	0.8
Early	18	11	17	17
Late	2	0	5	1

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Table III - continued

Group ##	I	II	III	IV
Fetuses				
Live (total)	266	313	297	314
Per dam	13.3	13.6	12.9	13.1
Dead	0	0	0	0
Weight (g)				
Males	4.0	4.0	4.1	4.1
Females	3.8	3.9	3.9	3.9
Sex Ratio				
Males (%)	45.1	49.5	49.8	44.3
Females (%)	54.9	50.5	50.2	55.7
Placental Weight (g)				
Male fetuses	4.0	4.0	4.1	4.1
Females	3.8	3.9	3.9	3.9

Data extracted from BASF Project No. 30RO112/89102, Table 010, page 54; Table 011, page 55; Table 012, page 56 and Table 013, page 57.

Groups I, II, III and IV received 0, 50, 150 or 300 mg of Mepiquat chloride/kg b.w./day, respectively.

Developmental Toxicity

Mepiquat chloride, at all levels tested, had no effect on any of the parameters examined. No embryotoxicity, fetotoxicity and no indications of any teratogenic effects were noted up to and including the high dose of 300 mg/kg b.w./day. The developmental toxicity data are summarized below.

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Table IV: External Examinations #

Group ##	I	II	III	IV
Number of litters examined	20	23	23	24
Number of fetuses examined	266	313	297	314
Live	266	313	297	314
Dead	0	0	0	0
Malformations				
Fetal incidence *				
Number affected	0	0	1 ♦	1 ♦
Percent	0	0	0.3	0.3
Litter incidence				
Number affected	0	0	1	1
Percent	0	0	4.3	4.2
Variations				
Fetal incidence	0	0	0	0
Unclassified Observations				
Fetal incidence				
Number affected	3 ♦♦	0	0	1 ♦♦
Percent	1.1	0	0	0.3
Litter incidence				
Number affected	2	0	0	1
Percent	10.0	0	0	4.2

Data extracted from BASF Project No. 30RO112/89102, Table 0.14, page 58; Table 015, page 59; Table 016, page 60; and Table 016, page 61.

Groups I, II, III and IV received 0, 50, 150 or 300 mg of Mepiquat chloride/kg b.w./day, respectively.

♦ One fetus in the mid-dose group had anophthalmia and another fetus in the high-dose group had microglossia.

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♦♦ Three fetuses in the control group had fused placentae and one fetus in the high-dose group had blood coagulum around placenta. The fetus with microglossia came from dam No. 96 and that with blood coagulum came from dam No. 100.

Table V: Soft Tissue (Visceral) Examinations #

Group ##	I	II	III	IV
Number of litters examined	20	22	23	23
Number of fetuses examined	129	150	141	151
Live	129	150	141	151
Dead	0	0	0	0
Malformations				
Hydrocephaly				
Fetal incidence *	2	1	0	2
Percent	1.6	0.7	0	1.3
Litter incidence *	2	1	0	2
Percent	10.0	4.5	0	8.7
Malformation of Great Vessels				
Fetal incidence	0	0	1	0
Percent	0	0	0.7	0
Litter incidence	0	0	1	0
Percent	0	0	4.3	0
Heart: Dilatation of Right Ventricle				
Fetal incidence	1	1	0	0
Percent	0.8	0.7	0	0
Litter incidence	1	1	0	0
Percent	5.0	4.5	0	0

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Table V - continued

Group ##	I	II	III	IV
TOTAL VISCERAL MALFORMATIONS				
Fetal incidence	2	1	1	2
Percent	1.6	0.7	0.7	1.3
Litter incidence	2	1	1	2
Percent	10.0	4.5	4.3	8.7
Variations				
Dilated renal pelvis				
Fetal incidence	14	17	18	21
Percent	11	11	13	14
Litter incidence	10	11	13	14
Percent	50	50	57	61
Hydroureter				
Fetal incidence	8	7	4	10
Percent	6.2	4.7	2.8	6.6
Litter incidence	7	5	4	8
Percent	35	23	17	35
TOTAL VISCERAL VARIATIONS				
Fetal incidence	14	17	18	21
Percent	11	11	13	14
Litter incidence	10	11	13	14
Percent	50	50	57	61

Data extracted from BASF Project No. 30RO112/89102, Table 019, page 63 and Table 020, page 64.

Groups I, II, II and IV received 0, 50, 150 or 300 mg of Mepiquat chloride/kg b.w./day, respectively.

* Incidence = Number of fetuses or litters affected.

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Table VI: Skeletal Examinations #

Group ##	I	II	III	IV
Number of litters examined	20	23	23	24
Number of fetuses examined	137	163	156	163
Live	137	163	156	163
Dead	0	0	0	0
Malformations				
Thoracic Vertebral Body/ Bodies Dumbbell-Shaped				
Fetal incidence *	2	5	8	4
Percent	1.5	3.1	5.1	2.5
Litter incidence *	2	4	4	3
Percent	10	17	17	13
Sternebra(e) Bipartite, Ossification Centers Dislocated				
Fetal incidence	0	1	3	2
Percent	0	0.6	1.9	1.2
Litter incidence	0	1	2	2
Percent	0	4.3	8.7	8.3
TOTAL SKELETAL MALFORMATIONS				
Fetal incidence	3	7	11	7
Percent	2.2	4.3	7.1	4.3
Litter incidence	3	6	6	6
Percent	15	26	26	25
Variations				
Sternebra(e) of Irregular Shape				
Fetal incidence	55	55	51	45 a
Percent	40	34	33	28
Litter incidence	20	21	22	17 a
Percent	100	91	96	71

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Table VI - continued

Group ##	I	II	III	IV
Sternebra(e) Bipartite				
Fetal incidence	5	3	0 a	3
Percent	3.6	1.8	0	1.8
Litter incidence	4	2	0 a	3
Percent	20	8.7	0	13
Rudimentary Cervical Ribs				
Fetal incidence	8	6	3	3
Percent	5.8	3.7	1.9	1.8
Litter incidence	5	6	3	2
Percent	25	26	13	8.3
13th Rib(s) Shortened				
Fetal incidence	9	12	3	8
Percent	6.6	7.4	1.9	4.9
Litter incidence	6	10	3	5
Percent	30	43	13	21
TOTAL SKELETAL VARIATIONS				
Fetal incidence	68	69	57 a	57 a
Percent	50	42	37	35
Litter incidence	20	22	22	20
Percent	100	96	96	83
Retardations				
Thoracic Vertebral Body/ Bodies Dumbbell-Shaped				
Fetal incidence	28	46	35	34
Percent	20	28	22	21
Litter incidence	14	16	17	18
Percent	70	70	74	75

Continued on next page

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Table VI - continued

Group ##	I	II	III	IV
Sternebra(e) Incompletely Ossified or Reduced in Size				
Fetal incidence	32	35	35	34
Percent	23	21	22	21
Litter incidence	13	19	16	16
Percent	65	83	70	67
Sternebra(e) not Ossified				
Fetal incidence	13	15	5 a	10
Percent	9.5	9.2	3.2	6.1
Litter incidence	10	9	4 a	9
Percent incidence	50	39	17	38
Sternebra(e) - Only One Ossification Center				
Fetal incidence	22	26	17	14
Percent	16	16	11	8.6
Litter incidence	11	17	11	8
Percent	55	74	48	33
TOTAL SKELETAL RETARDATIONS				
Fetal incidence	71	98	75	76
Percent	52	60	48	47
Litter incidence	18	23	23	22
Percent	90	100	100	92

Data extracted from BASF Project No. 30RO112/89102, Tables 023-027, pages 67-71.

Groups I, II, III and IV received 0, 50, 150 or 300 mg of Mepiquat chloride/kg b.w./day, respectively.

* Incidence = Number of fetuses or litters affected.

Significantly different from control: a = P<0.05 and b = P<0.01.

The above **Table VI** contains the predominant skeletal findings. For all of the skeletal findings, see Attachment III of this review. A summary of all classified fetal external, soft tissue and skeletal observations is in Attachment IV.

D. COMMENTS

This rat developmental study was carried out from April 3, 1991 (beginning of study) to April 25, 1991 (last scheduled sacrifice of the animals).

This study is well planned and, in general, clearly reported, and it meets the December 24, 1989 EPA Acceptance Criteria. All analytical precedures used have been referenced. However, the following points should be noted for future submissions:

1. Food consumption was reported as grams/animal/day. A more meaningful way to report food consumption would have been as grams/kilogram of body weight/day. The latter manner of reporting also allows the calculation of food efficiency.

2. It was reported that mating took place " from about 16.00 hours to about 7.30 hours on the following day ". If that is correct, " from about 4.00 p.m. to about 7.30 a.m. ....." would have been clearer to the American (non-military) readers.

3. Historical control data, obtained from the breeder who supplied the rats for this study, were submitted in the tabular form only. One set of tables was dated March 29, 1990 and another, March 6, 1992. However, it was not reported how many studies were used to compile these data, the dates of these studies, and whether the the experimental conditions were similar to those used in the current study.

Although it was stated in the FLAGGING CRITERIA STATEMENT that this study neither met nor exceeded any of the applicable criteria, this study was submitted as the 6(a)(2) data. According to the correspondence from BASF Corporation to Office of Pesticide Programs (May 27, 1992; MRID 423371-00), the adverse effects (tremor, unsteady gait, piloerection and hypersensitivity) were observed only at the highest dose level of Mepiquat chloride tested (300 mg/kg of body weight). As was already noted in the **Maternal Toxicity** section of this review (page 6), these effects occurred in most dams at approximately 1.5 to 2 hours after gavaging, were no longer observed after approximately 4 hours and were less frequent during the second half of the treatment period.

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Section I, Toxicology Branch I/HED did not regard these findings as the 6(a)(2) data.

The range finding study (1991; Project No. 10RO112/89097), which was used to select doses for the currently reviewed rat developmental study, had been summarized in Attachment V. The range finding study was not a separate submission, but was included in the report on the rat developmental study.

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RIN 0186-06

Mepiquat Chloride MRID 42337101-05

Page _____ is not included in this copy.

Pages 23 through 35 are not included in this copy.

The material not included contains the following type of information:

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_____ The product confidential statement of formula.

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✓ FIFRA registration data.

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Attachment V

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SUMMARY OF THE RANGE-FINDING STUDY, entitled:

" **Results of a range-finding study of the prenatal toxicity of Mepiquat-chloride in rats after oral administration (gavage) - Proj. No. 10RO112/89097 BASF Aktiengesellschaft (1991)** "

The purpose of this study was to determine dose levels for the main rat developmental toxicity study (No. 30RO112/89102 and 92/10331; MRID 42337101). The range-finding study was not submitted separately, but was included in the report on the main rat developmental study.

Experimental Procedures: In the range-finding study, pregnant Wistar strain rats, 10/group, were administered aqueous solutions of Mepiquat chloride by gavage during gestation days (g.d.) 6 through 15. The rats were sacrificed on g.d. 16. The doses used were 0, 100, 300 and 600 mg/kg b.w./day. The experimental conditions used in the range-finding study were the same as those in the main study. The following parameters were examined for the dams in the range-finding study: (1) clinical observations; (2) food consumption, body weights and body weight changes; (3) hematology, clinical chemistry and urinalysis; (4) absolute and relative organ (kidneys and liver) weights; (5) necropsy; and (6) reproductive data. The following parameters were examined for the fetuses: (a) placental and body weights; (b) number of live and dead fetuses; and (c) external observations, characterized as " rough and very limited ".

Results: Parameters (1) and (5) were examined for all rats on the study, whereas the remaining parameters, including (a), (b) and (c), were examined only for rats in the control, low-dose and mid-dose groups.

In the low-dose group **(100 mg/kg/day)**, no treatment-related findings were observed in the dams and fetuses.

In the mid-dose group **(300 mg/kg/day)**, the following treatment-related findings were noted in the dams: (1) reduced food consumption during g.d. 6-13 (8.4% less than the controls, but statistically insignificant); (2) decreased body weight gain during g.d. 6-15 (14% less than the controls, but statistically insignificant); (3) decreased body weight gain, corrected for uterine weight (1.8 ± 6.31 g, compared with 7.1 ± 2.75 g for the controls, but statistically insignificant); (4) tremor and hypersensitivity at 1-2 hours after dosing, in all rats, during g.d. 6-12; and (5) ataxia, unsteady gait, piloerection and indrawn flanks in 5-6/10 rats during g.d. 6-12. The duration of the toxic effects listed in (4) and (5) was not reported, but in the main study these effects lasted for 4 hours after dosing. Nothing abnormal was observed at necropsy in the dams. There were no dead or abnormal-looking fetuses, and placental and fetal weights were unaffected by treatment.

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In the high-dose group **(600 mg/kg/day)**, 5 out 10 dams died after the first or second dosing and the dosing of the survivors was discontinued on g.d. 8. Because the dosing of the survivors was discontinued, the food consumption and body weight data collected for the dams in this group were discarded; blood and urine samples were not collected; and the animals were discarded after sacrifice on g.d. 16 without any further examination. However, the five non-surviving dams were necropsied and the findings reported. Also, the results of the maternal clinical observations for all (10) dams in this group were reported.

Toxic effects in the high-dose group were very severe, occurred in almost all dams (9/10) within 1-2 hours after dosing and included tremor, unsteady gait, ataxia, hypersensitivity and piloerection. The duration of these effects after each dosing was not reported. However, it was reported that these effects were reversible because none of the surviving dams had any toxic symptoms on or after g.d. 8, when dosing was discontinued. Necropsy of the five non-surviving dams revealed edema of the lungs in 3 dams ($P<0.05$) and " particular find. on implants " in all dams ($P<0.01$).

Considering the results of this range-finding study, the following doses of Mepiquat chloride were selected for the subsequent (main) developmental toxicity study with rats: 0, 50, 100 or 300 mg/kg b.w./day (MRID 42337101).

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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)

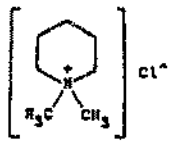
DATA EVALUATION RECORD

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. 3180 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. 3180112/89053; MRID 42337103) is classified as Core-Minimum and satisfies the requirement, § 82-1, for a subchronic feeding study in the rat.**

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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.

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(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.

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(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 homogenaity 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.

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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.

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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. 31SO112/89077; 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.

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ATTACHMENT I

RIN 0186-06

Mepiquat Chloride MRID 42337101-05

Page _____ is not included in this copy.

Pages 52 through 60 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.

Krystyna K. Locke 9/23/94 011283

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)

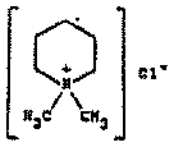
DATA EVALUATION RECORD

STUDY TYPE: 82-1 Subchronic Feeding in the Rodent

EPA IDENTIFICATION NUMBERS:

MRID No. 42337102	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: 31SO112/89077 and 92/10434

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

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

TITLE OF REPORT: Supplementary 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 18, 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 the 4-week range-finding study (MRID 42412102) and the earlier 90-day feeding study (MRID 42337103), the dose of Mepiquat chloride selected for this study was 12000 ppm (about 889 mg/kg of body weight). In the earlier 90-day feeding study, four levels of Mepiquat chloride tested (145, 579, 2316 and 4632 ppm or about 11, 44, 176 and 346 mg/kg of body weight, respectively) produced no toxic effects. Therefore, the objective of the current study was to demonstrate a dose level with a toxic or adverse effect and to aid in the dose selection for the following long-term feeding study. In the current study, the serum, erythrocyte and brain cholinesterase activities and the neurotoxic potential of Mepiquat chloride (grip strength forelimbs and hindlimbs, and hot plate tests) were also examined.

The following toxic or adverse effects were attributed to the 12000 ppm dose of Mepiquat chloride: **(1)** Tremor in all rats; long legged gait in 7 males and all females; abdominal position in 6 males and 5 females; and unsteady gait in 4 males and 2 females; **(2)** Decreased body weight gain throughout the study in the males (23-34%) and the females (15-19%), P≤0.001; **(3)** Decreased food consumption in the males (33-68% during the first 3 weeks and 19-31% during weeks 4-91) and in the females (55% during the first week and 8-27% during weeks 2-91); **(4)** Decreased food efficiency in the males only during the first 2 test weeks and in the females only during the first test week; **(5)** Increase in the thromboplastin time in the males (9-12%, P≤0.01 or 0.001) and in the females (8%, P≤0.0.05); **(6)** Decrease, in both sexes, in the serum calcium (2-6%, P≤0.05 or 0.001); creatinine (11-14%, P≤0.001); glucose (12-18%, P≤0.05 or 0.01); total protein (7-11%, P≤0.01 or 0.001); albumin (5-8%, P≤0.05 or 0.001); globulin (8-17%, P≤0.05, 0.01 or 0.001); and the triglycerides (38-66%, P≤0.01 or 0.001); **(7)** Reduced grip strength of forelimbs and hindlimbs in the males (15-33%, P≤0.05 or 0.001) and the females (15-29%, P≤0.05, 0.01 or 0.001); **(8)** Prolonged reaction time in the hot-plate test on day 93 in the males (57%, P≤0.05); **(9)** Decreased absolute weights of liver (45%, P≤0.01), kidneys (17%, P≤0.01) and adrenals (25%, P≤0.01) in the males, and of liver (15%, P≤0.01) and adrenals (15%, P≤0.05) in the females; **(10)** Decreased relative weight (organ/body weight ratio) of liver (18%, P≤0.05) in the males; and **(11)** Increased relative weight of kidneys (24%, P≤0.01) and testes (46%, P≤0.01) in the males, and of kidneys (11%, P≤0.01) in the females. Mepiquat chloride, at the 12000 ppm dose, had no effect on the macroscopic and microscopic pathology. (The terms "Decreased, Reduced, Increased, Increase" or "Decrease" mean "in relation to the control values").

Because the NOEL has not been determined, this study is clas-

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sified 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 (No. 3180112/89053; MRID 42337103) in which rats were fed four dose levels of Mepiquat chloride for 3 months, the current study (No. 3180112/89077; MRID 42337102) is classified as Core-Minimum and satisfies the requirement, § 82-1, for a subchronic feeding study in the rat.

Considering both 3-month rat feeding studies (MRID 42337102 and MRID 42337103) as one study, the NOEL for males and females is 4633 ppm (about 346 mg/kg body weight/day) and the LOEL for males and females is 12000 ppm (about 889 mg/kg body weight/day).

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EXPERIMENTAL PROCEDURES

The rats were received in the testing facility on July 16, 1990 and dosing was carried out from July 24, 1990 to October 26, 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 and 12000 ppm (about 889 mg/kg body weight). The 12000 ppm dose was based on the results of the 4-week range-finding study with rats (MRID 42412102) and the 90-day rat feeding study (MRID 42337103) in which a NOEL was not determined. In the range finding study, Mepiquat chloride was tested at dose levels of 500, 2000 or 8000 ppm (about 46, 183 or 661 mg/kg of body weight, respectively). In the 90-day feeding study, the dose levels of Mepiquat chloride used were 145, 579, 2316 or 4632 ppm (about 11, 44, 176 or 346 mg/kg of body weight, respectively). As in the previous studies, the diets in the current study were prepared weekly and were not pelleted. **The rats were:**

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

(2) Acclimated for 8 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 183 (177-192) g and of females 150 (140-160) 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.

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(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 90, the eyes of all rats were examined with a hand-held slit lamp.

(6) **Hematology and Clinical Chemistry:** On study days 41 and 91 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. For brain cholinesterase (acylcholinacylhydrolase) examination, tissue samples were obtained at necropsy (study day 94). 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, serum gamma glutamyl transferase, and serum and erythrocyte cholinesterases (acylcholinacylhydrolases).

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

Brain cholinesterase (EC 3.1.1.8.) was determined by the procedure of G.L. Ellman et al., Biochem. Pharmacol. 7, 88-95 (1961)

Erythrocyte cholinesterase (EC 3.1.1.8.) was determined according to the method of K.B. Augustinsson et al., Clin. Chim. Acta 89, 239-252 (1978)

Serum cholinesterase (EC 3.1.1.7.) was analyzed as described by M. Knedel and R. Rottger, Klin. Wschr. 45, 325-327 (1967)

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(7) **Urinalyses:** On study days 38 and 85, the rats were transferred to metabolism cages and urine was collected overnight. The following examinations were performed for each rat:

Volume, appearance, nitrite, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood, specific gravity and sediment.

(8) **Neurofunctional Observations:** Each rat in this study was evaluated as is described in Attachment I of this review. The examination of the neural functions was performed on study days 34, 69 and 93.

(9) **Necropsy:** In the accompanying 90-day feeding study (MRID 43237103), the rats were sacrificed by decapitation under CO_2 anesthesia, exsanguinated and all were necropsied. It is assumed that the same was done in the current study (page 2 of the pathology report, volume III, describing materials and methods, is missing).

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

(11) **Histopathology:** The following tissues were examined for all rats on the study:

Adrenal glands
Aorta
Bone marrow (femur)
Brain
Cecum, colon, rectum
Duodenum, jejunum, ileum
Epididymides
Esophagus
Eyes
Female mammary gland
Femur with joint
Heart
Kidneys
Liver
Lungs
Mandibular gland
Mandibular lymph node
Mesenteric lymph node

Gross lesions

Pancres
Parathyroid glands
Pituitary glands
Prostate, seminal vesicle
Sciatic nerve
Skeletal muscle
Skin
Spinal cord (cervical, thoracic and lumbar)
Spleen
Sternum (with bone marrow)
Stomach
Sublingual gland
Testes / ovaries
Thymus
Thyroid glands
Trachea
Urinary bladder
Uterus, vagina

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

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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, hematology, clinical chemistry, urinalyses, results of the neurological tests (grip strength forelimbs, grip strength hindlimbs and hot plate test), terminal body weights and terminal organ weights (both absolute and relative) were analyzed statistically as described in Attachment II of this review. Food consumption and food efficiency were not analyzed statistically.

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 diet of the treated group were within 0.2-7.0% of the nominal value.

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, 99.2 and 95.5% 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.

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Intake of Mepiquat Chloride ●

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

Test Group	Concentration (ppm)	Mepiquat Chloride mg/kg body weight/day		
		Males	Females	Both
1	12000	826	951	889

● Taken from page 0043 of the submitted report (MRID 42337102)

Clinical Observations

Toxic signs were not observed in the control group, but were observed throughout the study in the treated group. The treated rats were also vocal when handled. The vocalizations were more frequent and more pronounced in the males than in the females. All of the toxic signs reported and their frequency of occurence are shown below.

Observation	Frequency / Number or Rats Affected	
	Males	Females
Tremor	111 / 10	99 / 10
Long legged gait	63 / 7	71 / 10
Abdominal position	48 / 6	36 / 5
Unsteady gait	37 / 4	14 / 2
Nervousness *	9 / 1	15 / 2
Ataxia	0 / 0	9 / 1
Lateral position	4 / 1	0 / 0
Reduced general state *	4 / 1	0 / 0
Corneal clouding	0 / 0	1 / 1

The above table is based on TABLE 017, page 0077, and TABLE 018, page 0076, of the submitted report (MRID 42337102).

* Descriptive details were not provided.

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Mortality

There were no unscheduled deaths in this study.

Body Weight

Relative to the control values, body weight gains were reduced in the treated rats throughout the study. At the terminal sacrifice, males and females weighed 32% and 17%, respectively, less than did the controls. The effect of Mepiquat chloride on body weight is shown below.

Test Day	Body Weights of the Treated Group (Percent of Control Values)	
	Male Rats	Female Rats
7	72.2 ***	81.5 ***
21	66.4 ***	84.2 ***
35	66.2 ***	82.8 ***
49	67.6 ***	85.0 ***
63	66.7 ***	82.1 ***
77	67.1 ***	83.0 ***
91	67.9 ***	82.8 ***

This table is based on TABLES 005-008, pages 0064-0067, of the submitted report (MRID 42337102). The actual mean body weights of the control and Mepiquat chloride-treated (12000 ppm) groups are in Attachment III of this review.

Student's t-test (two-sided): * P≤0.05 ** P≤0.01 *** P≤0.001

Food Consumption

These data were reported as grams of food consumed / animal / day (group mean and individual values). Relative to the control values, the Mepiquat chloride-treated rats ingested significantly less food only during the first test week (females, 55% less) or during the first three test weeks (males, 36-68% less). Thereafter, until the termination of the study, male and female rats ingested 19-31% and 8-27%, respectively, less food than did the controls. The food consumption data are summarized below.

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Test Day	Food Consumption of the Treated Group (Percent of Control Values)	
	Male Rats	Female Rats
7	31.6	45.1
14	55.7	73.4
21	63.9	85.5
35	68.8	78.8
49	75.4	88.9
63	74.1	82.0
77	77.9	87.4
91	81.5	92.0

This table is based on TABLES 001-004, pages 0060-0063, of the submitted report (MRID 42337102). The actual mean food consumption of the control and the Mepiquat chloride-treated (12000 ppm) groups are in Attachment IV of this review. These data were not analyzed statistically.

Food Efficiency

Food efficiency was calculated as is shown in Attachment V of this review. Relative to the control values, food efficiency was impaired in the male rats only during the first two test weeks and in the female rats only during the first test week. Thereafter, until the termination of the study, Mepiquat chloride had no efffect on the food efficiency of both males and females. The food efficiency data and standard deviations (±) are summarized below.

	Food Efficiency			
Group	0	1	0	1
Test Day	Males		Females	
7	31.2 ± 1.5	-18.8 ± 10.1	18.9 ± 2.5	-13.1 ± 15.2
14	27.3 ± 2.5	17.2 ± 9.2	12.8 ± 4.4	13.7 ± 4.6
21	19.9 ± 2.4	21.6 ± 7.1	7.7 ± 4.3	13.0 ± 1.7
49	14.9 ± 2.3	13.8 ± 3.8	8.6 ± 6.3	9.0 ± 3.1
77	5.9 ± 4.4	7.5 ± 2.3	2.9 ± 4.4	5.1 ± 3.6
91	2.3 ± 2.1	2.0 ± 2.6	1.8 ± 4.4	0.6 ± 3.7

This table is based on TABLES 009-012, pages 0068-0071, of the submitted report (MRID 42337102). The dose level for Group 1 was 12000 ppm of Mepiquat chloride and Group 0 was the control group. These data were not analyzed statistically.

Ophthalmoscopy

These data were reported only as individual data for all rats on the study. Treatment-related abnormalities were not observed.

Hematology

Compared with the control values, there were statistically significant increases in the thromboplastin times in the serum of the treated males at both time intervals tested (study weeks 6 and 13) and in the females after test week 6. The testing facility considered these increases to be treatment-related and attributed them to the reduced general state of health of the animals. These findings are summarized below.

Mepiquat chloride (ppm)	0	12000
Thromboplastin Time, in Seconds, and (Percent of Control Value)		
Test Week 6 (Day 41):		
Males	31.7	35.6 (112) ***
Females	29.0	31.4 (108) *
Test Week 13 (Day 91):		
Males	34.4	37.4 (109) **
Females	31.3	31.0 (99)

This table is based on TABLES 041-044, pages 0099-0103, of the submitted report (MRID 42337102).

Student's t-test (two-sided): * P≤0.05 ** P≤0.01 *** P≤0.001

Clinical Chemistry: Enzymes

Relative to the control values, Mepiquat chloride, at the 12000 ppm level, had no effect on all of the enzyme activities examined. Although cholinesterase (ChE) activities were statistically significantly inhibited in three instances in the treated group, these inhibitions were regarded by the testing facility as treatment-unrelated because each inhibition was observed only at one sampling interval and only in one sex. At the test week 6 (day 41), the first time ChE activities were determined, serum ChE activity was inhibited in the treated females (32.9 %; P≤ 0.001). At the test week 13 (day 91), the second and last time ChE activities were determined, erythrocyte ChE activity was inhibited in the treated males (12.3%; P≤0.05). At the termination of the study

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(day 94), brain ChE activity was increased only in the treated males 40.4%; P≤0.001). The ChE activity data are summarized below.

Mepiquat chloride (ppm)	0		12000	
	Males	Females	Males	Females
Test Week 6:				
Serum ChE ■	5.60 ‡	30.43	5.26 ‡	20.41 ***
% of control	-	-	99.3	67.1
Erythrocyte ChE ■	24.61 ‡	22.87	22.20 ‡	25.27
% of control	-	-	90.2	110.5
Test Week 13:				
Serum ChE ■	5.35	25.73	4.85	22.16
% of control	-	-	90.6	86.1
Erythrocyte ChE ■	24.56	22.31	21.54 *	22.86
% of control	-	-	87.7	102.5
Brain ChE ■	1083	1248	1521 ***	1382
% of control	-	-	140.4	110.7

This table is based on TABLES 046-050, pages 0101 and 0104-0107, of the submitted report (MRID 42337102).

■ Serum ChE activity was reported as MYKAT/L (microkatal/liter); erythrocyte ChE activity, as MYKAT/L E. (microkatal/liter erythrocytes); and brain ChE activity, as NAKAT/G P. (nanokatal/gram protein). No other definitions were provided.

‡ These values were obtained from TABLE B 021, page 0189 (Individual Data Listing With Means) because TABLE 045 (Group Means), containing these values was missing from the submitted report that was available to Toxicology Branch I/HED.

Student's t-test (two-sided): * P≤0.05 ** P≤0.01 *** P≤0.001

Clinical Chemistry: Other Determinations

Treatment-related, statistically significant decreases were observed in the serum calcium, creatinine, glucose, total protein, albumin, globulin and triglycerides. These decreases occurred in the male rats at both sampling times and in the female rats mostly at the test weeks 6. According to the submitted report, the observed changes were probably caused by the reduced body weight gain

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and food consumption of these animals. These findings are summarized below.

Parameter	Test Week 6		Test Week 13	
	Males	Females	Males	Females
	Percent of Control Value			
Calcium	94.9 ***	96.8 *	97.8 *	98.9
Creatinine	89.1 ***	87.7 ***	85.9 ***	87.9 ***
Glucose	82.2 **	87.7 *	86.5 **	97.7
Total protein	89.3 ***	91.4 ***	91.0 ***	93.1 **
Albumin	95.4 *	91.8 ***	95.5 *	94.4 *
Globulin	83.0 ***	90.9 **	86.8 ***	91.6 *
Triglycerides	33.7 ***	62.4 **	36.1 ***	70.8

This table is based on TABLES 051-058, pages 0108-0115, of the submitted report (MRID 42337102).

Student's t-test (two-sided): * P≤0.05 ** P≤0.01 *** P≤0.001

Urinalyses

The only treatment-related and statistically significant finding was an increased incidence of crystals (mostly triphosphates) in the urinary sediment of the male rats at the end of the study (day 85). In the male group, 2/10 controls and 10/10 ** treated rats had crystals in the urinary sediment. The corresponding values for the female group were 0/10 and 2/10, respectively.

** P<0.01 Fisher's exact test (two-sided)

Neurofunctional Observations

Three neurofunctional tests were performed on all rats during the study days 34, 69 and 93: grip strength forelimbs, grip strength hindlimbs and hot plate. During these tests, tremors, ataxia, and posture and respiration abnormalities were observed in some treated rats. Compared with the controls, Mepiquat chloride also reduced the grip strength of forelimbs and hindlimbs in both sexes at each test interval, but not always in a statistically significant manner. In the hot plate test, relative to the control values, Mepiquat chloride prolonged reaction time on day 93 in the male rats. The results of the neurofunctional observations are summarized below.

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Findings Observed in the 12000 ppm Mepiquat Chloride Group During the Neurofunctional Tests

Finding		Day 34	Day 69	Day 93
		Number of Rats Affected ♦		
Tremors:	Males	6	6	2
	Females	7	6	3
Ataxia:	Males	5	0	4
	Females	6	3	7
Posture Abnormality:	Males	5	6	3
	Females	5	2	4
Respiration Abnormality:	Males	0	4	3
	Females	0	1	1

This table is based on the data (Fig. 1) reported on page 0045 of the submitted report (MRID 42337102).

♦ Out of 10 rats examined in each instance. These findings were not observed in the male and female control groups.

Results of the Neurofunctional Tests

Test	Day 34	Day 69	Day 93
	Percent of Control Value		
Grip Strength Forelimbs:			
Males	85.3 *	89.0	93.7
Females	81.5 **	70.8 ***	81.4 *
Grip Strength Hindlimbs:			
Males	67.4 ***	81.2 *	94.3
Females	85.3	85.4 *	82.0
Hot Plate:			
Males	125.0	111.1	157.1 *
Females	109.1	100.0	112.5

The above table is based on TABLES 019-024, pages 0078-0083, of the

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submitted report (MRID 42337102). In the submission, the results of the grip strenth tests were reported in terms of newtons and of the hot plate test, in terms of seconds.

Student's t-test (two-sided): * P≤0.05 ** P≤0.01 *** P≤0.001

Necropsy

Based on the data on page 0222 (INCIDENCE OF GROSS LESIONS) and the individual animal data, pages 0231-0271 of the submitted report (MRID 42337102), a light-yellow focus was observed in the liver (right medial lobe) of two Mepiquat chloride-treated rats, one male and one female. No other gross lesions were observed in the treated rats and no gross lesions were found in the controls.

Organ Weights

These data were reported for the liver, kidneys, testes and adrenal glands, as group mean absolute and relative weights and as individual weights.

Relative to the control values, the absolute weights of the liver and adrenal glands in the treated male and female groups, and the absolute weight of the kidneys in the male group, were statistically significantly decreased. However, the relative weights of the kidneys in the treated male and female groups, and of the testes, were statistically significantly increased. Because these abnormalities in organ weights could not be explained histologically, they were attributed by the testing facility to the decreases in body weights. The organ weight data are summarized below.

Organ Weights of the Treated Rats as Percent of Control Values

Organ	Absolute Weights		Relative Weights	
	Males	Females	Males	Females
Liver	55.5 **	84.8 **	82.2 *	103.9
Kidneys	83.1 **	90.9	124.2 **	111.1 **
Adrenals	74.7 **	85.4 *	110.5	105.2
Testes	97.4	-	145.7 **	-

This table is based on the group mean data reported on pages 0217-0220 of the submission (MRID 42337102). Relative weight = Organ

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weight x 100 / Final body weight.

Student's t-test (two-sided): * P≤0.05 ** P≤0.01

Histopathology

Mepiquat chloride had no effect on the microscopic findings observed in this study. The predominant findings in the male and female rats were minimal-to-slight vacuolization in the liver and small cysts in the pituitary gland, but the incidences in the control group were the same or higher than those in the treated group. In the females, calcification in the kidneys was also a predominant finding, but, again, the incidence was higher in the control group than in the treated group. All of the microscopic findings reported are shown below.

Mepiquat chloride (ppm)	0		12000	
	Males	Females	Males	Females
Organ Affected	Number of Rats Affected			
Liver: Vacuolization	6	3	2	1
Cholangiofibrosis	0	1	0	0
Pituitary: Cyst(s)	3	2	3	1
Kidneys: Calcification	0	10	0	9
Uterus: Hydrometra	-	1	-	2
Eyes: Keratitis	0	0	0	2
Heart: Infiltrates #	0	1	0	0
Pancreas: Hyperplasia	0	0	1	0
Skel. muscle: Infiltrates #	0	0	1	0
Stomach: Dilatation ##	1	0	0	0
Testes: ↓ Spermatogenesis	1	-	0	-

This table is based on the data (INCIDENCE OF MICROSCOPIC FINDINGS) reported on pages 0221 and 0224 of the submitted report (MRID 42337 102), and on the individual data (PATHOLOGY REPORT), pages 0231-0271 of the submitted report. All rats (10/sex/group) were examined. # Mononuclear ## Glandular ↓ = Decreased

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COMMENTS

This study, in which one dose of Mepiquat chloride was tested (12000 ppm), does not meet the December 24, 1989, EPA ACCEPTANCE CRITERIA because a NOEL was not determined. This study should, therefore, be considered with another 3-month feeding study (MRID 42337103) in which the highest dose of Mepiquat chloride tested (4639 ppm) did not produce toxic effects. Together the studies satisfy the EPA ACCEPTANCE CRITERIA for a subchronic feeding study in the rat, § 82-1.

Although Mepiquat chloride was positive for neurotoxicity in this study, the testing facility concluded that Mepiquat chloride had no neurotoxic potential. This was based on the results of the *in vitro* studies with muscles from the feet of the adult mice (MRID 42337104) and with membranes of bovine cerebral cortex, rat heart and rat submaxillary gland (MRID 42337105). In these studies, the effects of Mepiquat chloride on the acetylcholine receptors were investigated. It was concluded that the activation of the receptors, observed in these studies, would eventually lead to muscle weakness and impaired neuropharmacological functions. Because the neuropharmacological effects observed in the *in vitro* experiments were reversible, it was concluded that the neurological effects observed *in vivo* (current study) were also reversible upon withdrawal of Mepiquat chloride, without subsequent morphological changes. Therefore, according to the testing facility, as long as the neurological effects were reversible under any experimental conditions, Mepiquat chloride had no neurotoxic potential. Section I/Toxicology Branch I/HED regards this conclusion as superficial and disagrees with it. A continuation of the current study and of the neurofunctional testing in the absence of Mepiquat chloride would have shown whether or not the existing neurological effects disappeared. Furthermore, even with the disappearance of the neurological effects, Mepiquat chloride (12000 ppm) would still be regarded as having a neurotoxic potential in the current study.

The following statements were included in this 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 July 19, 1990; and in life, two times: during August 14, 1990 and October 23/24, 1990.

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The final report was audited on April 29, 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, neurofunctional testing 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.

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ATTACHMENT I

RIN 0186-06
Mepiquat Chloride MRID 42337101

Page _____ is not included in this copy.

Pages 80 through 96 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.

Krystyna K. Locke 9/23/94 011283

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 (abbreviated)

STUDY TYPE: Nonguideline: *In Vitro* Neuropharmacological

EPA IDENTIFICATION NUMBERS:

MRID No. 42337104	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 MATERIALS: Mepiquat chloride (Purity: 99%; Batch No. WW 285) and Acetylcholine chloride (Purity: 99%; Batch No. 109F006).

REPORT NUMBER: 99PO697/909016 and 91/11204

SPONSOR: BASF Aktiengesellschaft, Germany.

TESTING FACILITY: Institute of Physiology, Technical University, Munich, Germany.

TITLE OF REPORT: Report on the in vitro Test of the Action of Mepiquat Chloride at Nicotinic Acetylcholine Receptors of Adult Mouse Muscle.

AUTHOR: Dr. C. Franke

STUDY COMPLETION DATE: December 12, 1991

EXECUTIVE SUMMARY

The purpose of this study was to demonstrate the *in vitro* activity (channel openings) of Mepiquat chloride at the nicotinic acetylcholine (ACh) receptors of adult mouse muscle.

Interosseal muscles from the feet of the wildtype NMRI mice were dissociated enzymatically (collagenase and trypsin) into cells 1 mm long and 50-100 um wide, which were then suspended in the solution (pH 7.4) containing NaCl, KCl, $CaCl_2$, NaH_2PO_4, KH_2PO_4, 4-(2-hydroxyethyl)-1-piperazineethansulphonic acid (HEPES) and glucose. The electrophysiological measurements were made at 20°C using the in the cell-attached and in the outside-out patch clamp technique (four papers were referenced in the submission). The test materials were Mepiquat chloride (10, 100 or 1000 uM) and ACh (10 and 100 uM ; reference compound). Single channel openings were elicited by binding the test materials to the nicotinic

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receptors.

The mean frequencies of the channel openings, which were concentration-dependent, with the 10, 100 and 1000 uM of Mepiquat chloride were (number of openings/second) 0.8 ± 0.3, 13.2 ± 5.0 and 74.7 ± 17.0, respectively. No values were reported for ACh, but it was stated that the frequencies of the channel openings for 10 uM ACh were about the same as those for 1000 uM Mepiquat chloride. Irrespective of the concentration of Mepiquat chloride, the duration of the single channel opening was about 0.32 millisecond or about 3 times lower than the value for ACh (the actual value for ACh was not reported).

The nicotinic ACh receptor channels were activated immediately after the application of Mepiquat chloride, but the activation ceased with the removal of Mepiquat chloride. It was stated in the submission that the activation of the receptor channels would cause depolarization of the muscle fibers, excitation of the muscle and muscle weakness, and that Mepiquat chloride must, therefore, be regarded as a partial agonist of the nicotinic ACh receptors.

Classification of Study: Acceptable as a Nonguideline, special (neuropharmacological) *in vitro* study.

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Krystyna K. Locke 9/23/94 011283

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

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

DATA EVALUATION RECORD (abbreviated)

STUDY TYPE: Nonguideline: *In Vitro* Neuropharmacological

EPA IDENTIFICATION NUMBERS:

MRID No. 42337105	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 (Purity: 99%: Batch No. WW 285)

REPORT NUMBER: 99PO697/909018 and 91/11206

SPONSOR: BASF Aktiengesellschaft, Germany.

TESTING FACILITY: Knoll AG Research and Development, Ludwigshafen, Germany.

TITLE OF REPORT: Study on the Affinity of Mepiquat Chloride for Muscarinic Receptors.

AUTHOR: Dr. H. Weifenbach

STUDY COMPLETION DATE: September 19, 1991

EXECUTIVE SUMMARY

The purpose of this *in vitro* study was to investigate the affinity of Mepiquat chloride for subtypes of muscarinic acetylcholine receptors (M_{1-3}) in membranes of animal origin and to compare these findings with those obtained with standard muscarinic reference compounds.

The membranes, prepared in the testing facility and stored at -196°C until needed, were those of bovine cerebral cortex (M_{1+2}), rat heart (M_2) and rat submaxillary gland (M_3). The subtype-specific substances with high affinity for muscarinic acetylcholine receptors (reference compounds) were pirenzepine (M_{1+2}), methoctramine (M_2), 4-diphenylacetoxy-N-methylpiperidine methiodide [4-DAMP] (M_3) and atropine (a high affinity compound without subtype specificity). The radioligand was [N-Methyl-^{3}H]-N-methylscopolamine (^{3}H-NMS). The rats (males of Sprague-Dawley strain, weighing about 200 g) were obtained from the local supplier

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(Charles River) and the bovine brains were obtaine from the local slaughterhouse. The affinity of Mepiquat chloride for the receptors was studied by incubating membranes with ^{3}H-NMS and Mepiquat chloride (10^{-5}, 10^{-4} or 10^{-3} moles/liter) or with ^{3}H-NMS and reference compounds (10^{-10}-10^{-3} moles/liter) for 1 hour at 37°C. After incubation, the membranes were washed, the radioactivity measured and the K_i values (inhibition constants of Mepiquat chloride and reference compounds) calculated.

Compared with the reference compounds, Mepiquat chloride had very low and unselective affinity for the muscarinic receptors (^{3}H-NMS binding sites). The inhibition constants (K_i) of Mepiquat chloride were 88, 160 and 200 micromoles/liter for the receptors (M_1), (M_2) and (M_3), respectively. The K_i values for the reference compounds were in the nanomolar range. According to this submission, " there is only a small risk of side effects with muscarinic receptors with high concentrations of mepiquat chloride in animals and man ", meaning, apparently, that Mepiquat chloride is not a serious health hazard to humans and animals.

Classification of Study: Acceptable as a Nonguideline, special (neuropharmacological) *in vitro* study.

END