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

012023

OPP OFFICIAL RECORD AUG 20 1996
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

July 19, 1996

MENORANDUM

SUBJECT: Mepiquat Chloride: Review of the Following Studies: Six Acutes (81-1 Through 81-6), Two Developmental With Rabbits (83-3b), Three Mutagenicity (Ames, Structural Chromosome Abberation and Other Genotoxic Effects; 84-2) and General Metabolism (85-1).

Rereg. Case No. 2375 Chemical Code No. 109101
CAS Reg. No.24307-26-4 Tox. Chem. No. 380 AB

Sponsors: BASF Corporation, Agricultural Chemicals Group, Research Triangle Park, NC for studies 81-1, 81-2, 81-3, 81-6 and 84-2; BASF Wyandotte Corporation, Parcippany, NJ for studies 81-4, 81-5 and 83-3b; and BASF Aktiengesellschaft, Limburgerhof, Federal Republic of Germany for study 85-1.

FROM: Krystyna K. Locke, Toxicologist
Section I, Toxicology Branch I
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THRU: Roger 1. Gardner, Section Head Roger Gardner 8/2/96
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TO: Kathleen Depukat/Patrick Dobak, PM Team 51
Accelerated Reregistration Branch
Special Review and Reregistration Division (7508W)

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

Guideline No.	MRID No.	DP Barcode	Acceptability
81-1	41488101	D221662 for	Yes
81-2	41488102	all studies	Yes
81-3	41954101	listed in this	Yes
81-4	00071942 #	table.	Yes ■
	92091006 ##		-
81-5	41488103 #		Yes ■
	92091007 ##		-
81-6	41488104 #		Yes ■
	92091008 ##		-
83-3b	00148090		No ■■
	00148089		No ■■
84-2	41488106		Yes
	41488107		Yes
	41488108		Yes
85-1	40299001		Yes

Main study submitted under DP Barcode D221662.

Phase 3 Summary (not submitted under D221662, but retained by Tox. Branch I [KKL] from the FIFRA '88 Review).

■ Study is acceptable when considered together with Phase 3 Summary which supplies data (such as purity of the test material) missing in the original study.

■■ Developmental effects were not observed in the low-dose group (50 mg/kg). Because of high abortion rate in the mid-dose group (100 mg/kg), and high death and abortion rates in the high-dose group (150 mg/kg), inadequate numbers of fetuses were available for a meaningful evaluation of developmental toxicity in this study (MRID 00148090), conducted in 1979. Therefore, this study was to be considered with another study, conducted in 1981, in which two doses of Mepiquat chloride (75 and 100 mg/kg) were tested (MRID 00148089). However, because the results were reported only in the form of a brief summmary, the second study could not be evaluated. Also, only x-rays (no staining techniques) were used for the evaluation of fetuses in both studies.

Although, at the time of the review, Toxicology Branch I/HED classified the main study (MRID 00148090) as Supplementary but upgradable, the HED Reference Dose (RfD)/Peer Review Committee concluded on May 2, 1996 that a new developmental toxicity study with rabbits (83-3b) is required.

The one-year dog feeding supplementary study (MRID 43264403), in which dogs received 6000 ppm (170 mg/kg) of Mepiquat chloride and

which was submitted with the above studies for review, was already reviewed with the main study (MRID 41488105) under the DP Barcode D205089.

Results obtained in the above studies are summarized below.

MRID 41488101 (Acute Oral LD_{50}; Rat) - Dyspnea, apathy, staggering, twitching, compulsary gnawing, abdominal position, cyanosis and poor general health in the last three male and female groups; and general congestion in the nonsurvivors. Doses tested: 100, 200, 464, 1470 and 2150 mg/kg, in Wistar rats.

MRID 41488102 (Acute Dermal LD_{50}; Rat) - Dermal reaction, clinical signs, mortalities and abnormal macroscopic findings were not observed. Dose tested: 2000 mg/kg (limit dose) in male and female Wistar rats. 24-Hour exposure and intact skin rinsed after exposure.

MRID 41954101 (Acute Inhalation LC_{50}; Rat) - Irregular respiration in both groups during and after exposure; respiratory sounds, abdominal position and convulsions in Group 2 after exposure; and general congestion and pulmonary focal hyperemia in the nonsurvivors. Doses tested: 2.59 and 4.89 mg/L in male and female Wistar rats. Nose-only exposure (4 hours) to an aerosol of respirable particles.

MRID 00071942 (Primary Eye Irritation; Rabbit) - Slight erythema (score 1.4/110; Draize method) in 4/6 rabbits at 24 hours after treatment. All other readings were negative. Dose tested: 0.1 g in young adult New Zealand white male and female rabbits. Unwashed eyes.

MRID 41488103 (Primary Dermal Irritation; Rabbit) - The scores for erythema and edema were 0 (on a scale 0-4; Draize method) after 24 hours of exposure and 48 hours later. Other parameters examined (behavior, general condition, food intake and body weight gain) were unaffected. Dose tested: 0.5 g in young adult New Zealand white male and female rabbits. Intact or abraded skin unwashed after exposure, and patch-test technique were used.

MRID 41488104 (Dermal Sensitization; Guinea Pig) - Not a skin sensitizer after 10 intracutaneous induction treatments (one 0.05 mL and nine 0.1 mL on Mondays, Wednesdays and Fridays) and, 2 weeks later, one intracutaneous challenge treatment (0.1 mL). Test material: 10% solution of Mepiquat chloride in physiological saline, based on the results of a preliminary study. The test was conducted according to the "Proposed Rules", EPA, August 22, 1978 in Pirbright White Guinea Pigs (intact skin). Positive control was not used.

MRID 00148090 and 00148089 (Developmental Toxicity; Rabbit) - In these studies, Mepiquat chloride was administered (gavage) to the artificially inseminated Himalayan rabbits at dose levels of 0, 50, 100 and 150 mg/kg/day (MRID 00148090), and at dose levels of 0, 75 and 100 mg/kg/day (MRID 00148089), during gestation days 6-18. The following treatment-related findings were observed in the first study: (1) Weight loss, decreased food consumption and amber-colored liquid in the abdomen, in all treated groups; (2) Increased abortion rate in the mid-dose and high-dose groups; (3) High mortality in the high-dose group: and (4) Heart dilatation and hyperemia of organs in the nonsurvivors. Based on these findings, **the maternal NOEL is 50 mg/kg/day (borderline value) and the maternal LOEL is 100 mg/kg/day.** As was already noted on page 2 of this memorandum (■■), **the second study could not be reviewed and the developmental toxicity could not be evaluated in these studies. Therefore, these studies, do not satisfy the guideline requirements 83-3b and a new study is required.**

MRID 41488106 through 41488108 (Mutagenicity Studies) - Each study is negative and satisfies the 84-2 guideline requirements as follows: (1) Bacterial reverse gene mutation data (Ames test; MRID 41488106); (2) *In vitro* cytogenetic data (chromosome aberration in CHO cells; MRID 41488107); and (3) Other genotoxic data (unscheduled DNA synthesis in primary rat hepatocyte cultures; MRID 41488108).

MRID 40299001 (General Metabolism) - Mepiquat chloride, labeled in with ^{14}C in the 2,6-carbon atoms of the ring structure, was administered to young adult Sprague-Dawley male and female rats as follows: (1) Low or high single dose by intravenous (i.v.) route; (2) Low or high single dose by oral (gavage) route; (3) Single low dose after pretreatment (14 unlabeled low doses) by oral route; and (4) Seven consecutive high doses by oral route. The high dose was approximately 12 mg/kg of body weight (b.w.) and the low dose, 1.2 mg/kg b.w.

Mepiquat chloride was absorbed rapidly from the stomach, was excreted mostly in urine as unchanged Mepiquat chloride, and did not accumulate in tissues. The excretion patterns (% of the administered radioactivity) were: urine, 52-84 and 77-99 in 12 hours and 168 hours after dosing, respectively; feces, 2-15; exhaled air ($^{14}CO_2$), 0.20; and bile, 0.23-0.31. Only unchanged Mepiquat chloride was also detected in feces, exhaled air and bile. By 96 hours after the last dose, all organs and tissues examined contained < 0.1 ppm of Mepiquat chloride. The following organs/tissues were examined: gastrointestinal tract with contents, bone, brain, fat, uterus, testes, heart, kidneys, liver, lungs, muscle, spleen, skin with fur, adrenals, urinary bladder, thyroid, carcass, blood and plasma.

This study satisfies the guideline requirements for a metabolism study (85-1) in the rat.

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Mepiquat chloride Acute Oral Study (81-1)

Primary Review by: Krystyna K. Locke K.K. Locke **Date:** 7/23/96
Review Section I, Toxicology Branch I (7509C)

Secondary Review by: Roger Gardner Roger Gardner **Date:** 8/2/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity - Rat
OPPTS 870.1100 (81-1)

EPA IDENTIFICATION NUMBERS:

DP Barcode: D221662 Rereg. Case No. 2375
Submission No. S498213 Case No. 819426
P.C. Code No. 109101 Tox. Chem. No. 380 AB

TEST MATERIAL: Mepiquat chloride (1,1-Dimethyl-piperidinium chloride); technical grade; purity: 60% a.i. (w/w) in water.

SYNONYMS: Reg. No. 85 559

CITATION: Kirsch [no first name]. (1989) Report on the Study of the Acute Oral Toxicity of Mepiquat Chloride in Rats. BASF, Germany; Report No. 89/0462 and 10AO112/891188; Study Completion Date: December 19, 1989; Unpublished. **MRID 41488101**

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

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 414881-01), fasted Wistar rats, 5/sex/group, were given (by gavage) single doses of Mepiquat chloride (tech. grade; purity: 60% a.i.) as follows: 100, 200, 464, 1470 and 2150 mg/kg. The test material was administered in aqua dest, an aqueous formulation similar to the physiological medium. Before treatment, the mean weight of males was 179-190 g and of females, 178-189 g (age was not reported). The observation period was 14 days.

Oral LD_{50} = 464 mg/kg (males and females)

Toxicity Category: II

Toxic signs, observed only in the last three male and female groups, included: **(1)** Poor general health, dyspnea, apathy, staggering, twitching, compulsary gnawing and cyanosis; and **(2)** General congestion in the nonsurvivors.

This study is classified as **Acceptable** and satisfies the guideline requirement for an acute oral study (81-1) in the rat.

Mepiquat chloride Acute Oral Study (81-1)

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. This study was conducted according to the OECD Guidelines, Paris, 1981. Flagging Statement was not submitted.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:

   Description: Yellowish liquid
   Lot number: WW 262/CP 1490
   Purity: 60% a.i.
   CAS Registry No. 24307-26-4
   Verification of concentration in dosing solutions: Yes

2. Vehicle: The preparation of the test solutions has been done by serial dilution.

3. Test Animals:

   Species: Rat
   Strain: Wistar
   Weight at dosing: 178-190 g (both sexes)
   Source: Dr. K. Thomae, animal breeder, Germany.
   Acclimation period: At least 1 week.
   Diet: Kliba Laboratory Diet, Switzerland. Ad libitum.
   Water: Tap, ad libitum.
   Housing: Singly, in stainless steel wire mesh cages.
   Environmental conditions: Temperature, 20-24°C; humidity, 30-70%; and photoperiod, 12 hours light/12 hours dark. Air changes were not reported.

B. STUDY DESIGN AND METHODS:

1. In Life Dates - Start: 10/17/89 End: 11/07/89

2. Animal Assignment and Treatment - Rats were assigned to test groups as noted in TABLE 1. Following a 16-hour fast, they were given a single dose of Mepiquat chloride by gavage and then were observed (several times on dosing day and twice daily thereafter) for 14 days. The rats were weighed before dosing and during the observation days 7 and 13. Survivors were sacrificed with CO_2 and necropsy was performed on all rats in the study.

TABLE 1 Doses and Mortality/Animals Treated

Dose (mg/kg)	Males	Females	Combined
100	0/5	0/5	0/10
200	0/5	0/5	0/10
464	2/5	3/5	5/10
1470	5/5	5/5	10/10
2150	5/5	5/5	10/10

3. Statistics - No reference was made in the report to any procedure used to calculate the LD_{50}. Apparently, since 50% of the rats died in the 464 mg/kg group, 464 mg/kg became the LD_{50} value.

II. RESULTS AND DISCUSSION

A. Mortality is given in TABLE 1. Rats in the 464 mg/kg group died within 1 day after dosing. In the 1470 and 2150 mg/kg groups, 4/10 and 5/10 rats, respectively, died within 1 hour after dosing, whereas the remaining rats died within 1 day after dosing.

The oral LD_{50} for males and females is 464 mg/kg.

B. Clinical Observations - Dyspnea, apathy, staggering, twitching and compulsory gnawing occurred at 1 hour after dosing in the 464 mg/kg group. These toxic signs, as well as cyanosis and abdominal position, occurred immediately-to-within 1 hour after dosing in the 1470 and 2150 mg/kg groups. The duration of the clinical signs and the numbers of rats affected were not reported.

C. Body Weight - All of the surviving rats gained weight in this study.

D. Necropsy - General congestion was observed in all nonsurvivors.

E. Deficiencies - Individual clinical observations and duration of toxic signs were not reported. However, these are minor deficiencies and will not affect the classification of this study.

Mepiquat chloride Acute Dermal Toxicity (81-2)

Primary Review by: Krystyna K. Locke K. K. Locke **Date:** 7/23/96
Review Section I, Toxicology Branch I (7509C)

Secondary Review by: Roger Gardner Roger Gardner **Date:** 8/2/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Dermal Toxicity - Rat
OPPTS 870.1200 (81-2)

EPA IDENTIFICATION NUMBERS:

DP Barcode: D221662 Rereg. Case No. 2375
Submission No. S498213 Case No. 819426
P.C. Code No. 109101 Tox. Chem. No. 380 AB

TEST MATERIAL: Technical Mepiquat chloride; 60% a.i. in water.

SYNONYMS: Reg. No. 85 559

CITATION: Kirsch (no first name). (1989) Report on the Study of the Acute Dermal Toxicity of Mepiquat Chloride in Rats. BASF, Germany; Report No. 89/0461 and 11AO112/891190; Study Completion Date: December 19, 1989; Unpublished. **MRID 41488102**

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

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID 41488102), Wistar strain rats, 5/sex/dose, were exposed to Mepiquat chloride (60% a.i. in water) for 24 hours. The dose used was 2000 mg/kg (limit dose) and the observation time was 14 days.

Dermal LD_{50} = > 2000 mg/kg (males and females)

Toxicity Category: III

Dermal reaction, clinical signs, mortalities and abnormal macroscopic findings were not observed.

This study is classified as **Acceptable** and satisfies the guideline requirement for an acute dermal study (81-2) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. This study was conducted according to the OECD Guidelines, Paris, 1981. Flagging statement was not submitted.

Mepiquat chloride Acute Dermal Toxicity (81-2)

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:

Description: Yellowish liquid
Lot number: WW 262/CP 1490
Purity: 60% a.i.
CAS Registry No. 24307-26-4
Verification of concentration in dosing solution: Yes

2. Vehicle: Mepiquat chloride was applied without dilution.

3. Test Animals:

Species: Rat
Strain: Wistar
Weight at dosing (means): 280 g (M) and 241 g (F)
Source: Dr. K. Thomae, animal breeder, Germany.
Acclimation period: At least 1 week.
Diet: Kliba Laboratory Diet 343, Switzerland. Ad libitum.
Water: Tap, ad libitum.
Housing: Singly, in stainless steel wire mesh cages.
Environmental conditions: Temperature, 20-24°C; humidity, 30-70%, and photoperiod, 12 hours light/12 hours dark. Air changes were not reported.

B. STUDY DESIGN AND METHODS:

1. In Life Dates - Date of application: October 12, 1989

2. Animal Assignment and Treatment - Rats (Wistar strain) were assigned to the test groups as noted in TABLE 1. Single dose of undiluted Mepiquat chloride was applied to the clipped dorsal/dorsolateral parts of the trunk and the application site was covered with a semi-occlusive dressing for 24 hours. After removal of the dressing, the site was rinsed with warm water and the rats were observed (several times on treatment day and twice daily thereafter) for 14 days. The application area was apparently 5 cm x 1 cm [reported : 50 cm (???) x 1 cm]. The skin findings were scored at 30-60 minutes after removal of the dressing, one week later and before the termination of the study. The rats were weighed before dosing and during the observation days 7 and 13. Survivors were sacrificed with CO_2 and necropsy was performed on all rats in the study.

Mepiquat chloride Acute Dermal Toxicity (81-2)

TABLE 1. Doses and Mortality/Animals Treated

Dose (mg/kg)	Males	Females	Combined
2000 #	0/5	0/5	0/10

Limit dose

3. Statistics - Not applicable

II. RESULTS AND DISCUSSION

A. Mortality - None

The dermal LD_{50} for males and females is > 2000 mg/kg

B. Clinical Observations - No clinical symptoms were observed

C. Body Weight - All rats gained weight.

D. Necropsy - No abnormalities were noted.

E. Deficiencies - Other than stating that the application site was covered with a semi-occlusive dressing, no details concerning the dressing were provided. Also, there must be an error in reporting the dimensions of the application area, 50 cm x 1 cm, However, these are minor deficiencies and will not affect the classification of this study.

Mepiquat chloride Acute Inhalation Study (81-3)

Primary Review by: Krystyna K. Locke K.K. Locke **Date:** 7/23/96
Review Section I, Toxicology Branch I (7509C)

Secondary Review by: Roger Gardner Roger Gardner **Date:** 8/2/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Inhalation Toxicity - Rat
OPPTS 870.1300 (81-3)

EPA IDENTIFICATION NUMBERS:

DP Barcode: D221662 Rereg. Case No. 2375
Submission No. S498213 Case No. 819426
P.C. Code No. 109101 Tox. Chem. No. 380 AB

TEST MATERIAL: Technical Mepiquat chloride (1,1-Dimethyl-piperidinium chloride); purity: 60% a.i. (w/w) in water.

SYNONYMS: Reg. No. 55 559

CITATION: Gamer,A.O. (1991) The Acute Inhalation Toxicity LC_{50} of Mepiquat Chloride as a Liquid Aerosol in Rats, 4-Hour Exposure BASF, Germany; Report No. 91/10505 and 13IO112/897082; Study Completion Date: June 21, 1991; Unpublished. **MRID 41954101**

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

EXECUTIVE SUMMARY: In an acute inhalation toxicity study (MRID 41954101), young adult Wistar strain rats, 5/sex/group, were exposed (nose-only) to an earosol of Technical Mepiquat chloride (60% a.i., respirable particles) for 4 hours and then were observed for 14 days. The analytical concentrations of Mepiquat chloride used were 2.59 mg/L (Group 1) and 4.89 mg/L (Group 2).

LC_{50} = > 4.89 mg/L (males)
Approx. 4.89 mg/L (females)
> 4.89 mg/L (combined)

Toxicity Category: IV

Clinical signs, observed in most rats during exposure, were irregular and accelerated respiration (Group 1) and irregular, accelerated, intermittent and gasping respiration (Group 2). These signs occurred after 15-30 min. of exposure and persisted throughout the entire exposure.

After termination of the exposure, accelerated respiration and ruffled fur were observed in both groups, and intermittent res-

piration, respiratory sounds, abdominal position and tonic-clonic convulsions in Group 2. Accelerated respiration persisted through the observation day 1 in Group 1 and through day 4 in Group 2. The remaining clinical signs occurred only during the exposure day (observation day 0).

Mortalities occurred during exposure and shortly after termination of the exposure. Necropsy revealed general congestion and pulmonary focal hyperemia in the nonsurvivors. No pathological findings were noted in the survivors. Body weights were not affected by treatment.

This study is classified as **Acceptable** and satisfies the guideline requirement for an acute inhalation toxicity study (81-3) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statement were provided. This study was conducted according to the OECD Guidelines, Paris, 1981. Flagging Statement was not submitted.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:

   Description: Yellowish liquid
   Lot number: WW 262/CP 1490
   Purity: 60% a.i.
   CAS Registry No. 24307-26-4
   Verification of concentration in dosing solutions: Yes

2. Vehicle: Water was the vehicle. The test material, 60% (a.i.) aqueous Mepiquat chloride, was diluted to 50% with water and the latter solution was aerosolized.

3. Test Animals:

   Species: Rat
   Strain: Wistar
   Age and weight at dosing: 8-9 weeks; 275 ± 19 g (males) and 194 ± 4.7 g (females).
   Source: Dr. K. Thomae, animal breeder, Germany.
   Acclimation period: Not reported.
   Diet: Kliba rat/mouse laboratory diet 24-343-4, 10 mm pellets, Switzerland. Ad libitum.
   Water: Tap, ad libitum.
   Housing: In groups of 5, in Becker's cages.

Environmental conditions: Temperature, 20-24°C; humidity, 30-70% and photoperiod, 12 hours light/12 hours dark. Air changes were not reported.

B. STUDY DESIGN AND METHODS:

1. In Life Dates - Start: 2/7/91 End: 2/25/91

2. Exposure Conditions - Head-nose inhalation system was used. The rats were restrained in tubes and their snouts projected into the inhalation chamber.

3. Animal Assignment and Treatment - Rats were assigned to the test groups as noted in TABLE 1 and exposed (nose-only) to Mepiquat chloride for 4 hours. They were observed (several times during exposure and daily thereafter) for 14 days, and weighed before treatment and during observation days 7 and 14. Survivors were sacrificed with CO_2 and necropsy was performed on all rats in the study.

TABLE 1 Concentrations, Exposure Conditions and Mortality/ Animals Treated

Concentrations (mg/L) Nominal	Analytical	MMAD um	GSD um	Males	Females	Combined
17.23	2.59	2.9	4.0	0/5	0/5	0/10
119.30 #	4.89	2.7	4.5	1/5	2/5	3/10

The selection of the concentration of Mepiquat chloride for Group 2 was based on the limit test, OECD Guidelines, method 403.

4. Generation of the Test Atmosphere and Description of the Chamber - Using a 50% aqueous solution of Mepiquat chloride, liquid aerosol was generated by means of a piston metering pump, a two-component atomizer and compressed air. The air flow was 2000 L/hour. The exposure system (glass-steel construction; volume, 55 L) was placed in an air-conditioned laboratory. The temperatures inside the system were 19-25°C.

Time to equilibrium was not reported.

Analytical chemistry:

a. Test atmosphere concentration - The nominal con-

centration was calculated from the amount of substance consumed and the air flow. The sampling frequency was once/concentration group/hour. The results are in TABLE 1 above.

For the quantitative determination of aerosol concentration, an ion-selective method was used. The results are in TABLE 1 above.

b. **Particle size determination** - Before the sampling, the impactor was equipped with metal collecting discs and a backup particle filter. The impactor was connected to the pump and the test apparatus, and one sample (9-24 L) per group was taken 30 minutes after the beginning of the test for the particle size analysis. The obtained samples were analyzed by the ion-selective method. The results are in TABLE 1 above.

5. Statistics - According to page 17 of the submitted report (MRID 41954101), the statistical evaluation of the dose-response relationship was carried out using FORTRAN program AKPROZ. Depending on the data of the dose-relationship obtained, this program is used to estimate the LC_{50}. Estimation of the LC_{50} will produce types of LC_{50} - LC_{50} greater, LC_{50} about or LC_{50} smaller. If the results are Type LC_{50} greater or LC_{50} smaller, an additional binominal test will be carried out (■) to verify these statements statistically, if necessary.

(■) Whiting, H. (1974): "Mathematical Statistik" B.G. Teubner, Stuttgart, pp. 32-35

II. RESULTS AND DISCUSSION

A. Mortality is given in TABLE 1. Two rats (not stated which ones) died during exposure (after 3.0-3.5 hours) and the third rat died shortly after the termination of the exposure.

The LC_{50} for males is > 4.89 mg/L
for females is about 4.89 mg/L
for males and females is >4.89 mg/L

B. Clinical Observations - Toxic signs observed in most rats during exposure were irregular and accelerated respiration (2.59 mg/L Group), and irregular, accelerated, intermittent and gasping respiration (4.89 mg/L Group). Also, all rats in both groups had their eyes closed during the enti-

re exposure. Toxic signs occurred at 15-30 minutes after start of the exposure and persisted throughout the 4-hour exposure.

After termination of the exposure, accelerated respiration and ruffled fur were observed in both groups, and intermittent respiration, respiratory sounds, abdominal position, squatting position and tonic-clonic convulsions in the 4.89 mg/L Group. Accelerated respiration persisted through the observation day 1 in the 2.59 mg/L Group and through the observation day 4 in the 4.89 mg/L Group. The remaining clinical signs occurred only during the exposure day (observation day 0).

C. Body Weight - All of the surviving rats gained weight in this study.

D. Necropsy - General congestion and pulmonary focal hyperemia were observed in the 3 nonsurvivors. No pathologic findings were noted in the survivors.

E. Deficiencies - There are no deficiencies that would affect the classification of this study. Only one air sample was obtained for the particle size determination. However, this study was conducted according to the OECD Guidelines which do not require the determination of particle size.

Mepiquat chloride Primary Eye Irritation Study (81-4)

Primary Review by: Krystyna K. Locke K.K. Locke **Date:** 7/23/96
Review Section I, Toxicology Branch I (7509C)

Secondary Review by: Roger Gardner Roger Gardner **Date:** 8/2/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Primary Eye Irritation - Rabbit
OPPTS 870.2400 (81-4)

EPA IDENTIFICATION NUMBERS:

DP Barcode: D221662 Rereg. Case No. 2375
Submission No. S498213 Case No. 819426
P.C. Code No. 109101 Tox. Chem. No. 380 AB

TEST MATERIAL: Mepiquat chloride (1,1-Dimethyl-piperidinium chloride; technical grade; purity (a.i.): > 99%

SYNONYMS: DMP; Reg. No. 85 559; BAS 083 W Technical

CITATION: Leuschner, F. (1977) Tolerance of the Rabbit Ocular Mucosa to 1,1-Dimethylpiperidinium Chloride, Reg. No. 85 559, Techn. - Called for Short "DMP": Single Treatment; BASF, Germany; Report No.: None; Study Date: January 20, 1977; Unpublished. **MRID 00071942** (main study) and Kieczka, H. (1990) Phase 3 Summary of MRID 00071942; BASF Reg. Doc. No. 90/6228. **MRID 92091006** (additional data).

SPONSOR: BASF Wyandotte Corporation, Parsippany, NJ (main study) and BASF Corporation, Agricultural Chemicals Group, Research Triangle Park, NC (Phase 3 Summary).

EXECUTIVE SUMMARY: In a primary eye irritation study (MRID 00071942 and 92091006, Phase 3 Summary), 0.1 g of Mepiquat chloride (> 99% a.i.), was instilled into the conjunctival sac of the left eye of 6 young adult New Zealand White rabbits (3 males and 3 females). The unwashed eyes were then examined at 24, 48 and 72 hours after treatment and the ophthalmic reactions were scored by the method of Draize.

Mepiquat chloride was not an eye irritant in this study. Slight erythema (score 1.4/110) was observed in 4/6 rabbits only at 24 hours after treatment. All other readings were negative. Based on these findings, Mepiquat chloride is in **Toxicity Category IV** for primary eye irritation.

This study is classified as **Acceptable** and satisfies the guideline requirement for a primary eye irritation study (81-4) in the rabbit.

Mepiquat chloride Primary Eye Irritation Study (81-4)

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided in the Phase 3 Summary of this study (MRID 92091006). This study does not meet all of the GLP requirements because it was conducted prior to November, 1983 when GLP guidelines became effective. However, these deviations are minor (not grading the eyes before treatment and at 1 hour after treatment, and not reporting that the test material was ground to a fine powder) and do not affect the scientific validity of this study.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:

   Description: White solid
   Lot/Batch No.: Not reported
   Purity: > 99% a.i.
   CAS Registry No. 24307-26-4
   Verification of concentration: Yes

2. Vehicle: It is not clear if Mepiquat chloride was instilled in the left eye as a dry powder or in physiological sodium chloride solution because the latter was placed in the right eye serving as a control.

3. Test Animals:

   Species: Rabbit
   Strain: New Zealand White
   Weight at dosing: 2.3-2.8 kg
   Source: BASF breeding colony
   Acclimation period: Not reported
   Diet: Artificially composed ALTROMIN 2023. Ad libitum.
   Water: Tap, ad libitum.
   Housing: Singly, in steel cages with a base of 0.4 m^2.
   Environmental conditions: Temperature, 21 ± 2°C; humidity, 60 ± 3%; and photoperiod, 12 hours light/12 hours dark. Air changes were not reported.

B. STUDY DESIGN AND METHODS:

1. In Life Dates - July to August, 1976

2. Animal Assignment and Treatment - Rabbits without eye diseases were treated as described in HAZARDOUS SUBSTANCES, Part 191, Section 12, FDA, Washington 1965. This

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procedure is also detailed in the submitted report (MRID 00071942).

Mepiquat chloride, 0.1 g, was instilled singly into the conjunctival sac of the left eye of 6 rabbits (3 males and 3 females). After instillation, the lid was kept closed briefly and then the animal was released. The right eye served as a control and was treated with physiological sodium chloride solution (volume was not reported). Twenty four hours after the instillation, the unwashed eyes were examined without and with fluorescein treatment, and then examined again 48 and 72 hours later. The ophthalmic reactions were evaluated by two independent "experts" according to the procedure of Draize (details are in APPENDIX of this review).

II. RESULTS AND DISCUSSION

A. The only finding observed was a slight erythema, in 4 rabbits, at 24 hours after treatment. There was no redness after 48 and 72 hours. No abnormalities were observed in the cornea, anterior chamber, iris, vitreous body and fundus. The possible changes in the eyes were classified as follows:

Maximun Score	Evaluation
0 - 10	Non-irritant
11 - 25	Slight irritant
26 - 56	Moderate irritant
57 - 110	Severe irritant

In this study, the Draize scores were as follows:

At 24 hours = 1.4/110
At 48 hours = 0.0/110
At 72 hours = 0.0/110

Mepiquat chloride was, therefore, non-irritant.

B. Deficiencies - There are no major deficiencies in this study (see **COMPLIANCE**).

procedure is also detailed in the submitted report (MRID 00071942).

Mepiquat chloride, 0.1 g, was instilled singly into the conjunctival sac of the left eye of 6 rabbits (3 males and 3 females). After instillation, the lid was kept closed briefly and then the animal was released. The right eye served as a control and was treated with physiological sodium chloride solution (volume was not reported). Twenty four hours after the instillation, the unwashed eyes were examined without and with fluorescein treatment, and then examined again 48 and 72 hours later. The ophthalmic reactions were evaluated by two independent "experts" according to the procedure of Draize (details are in APPENDIX of this review).

II. RESULTS AND DISCUSSION

A. The only finding observed was a slight erythema, in 4 rabbits, at 24 hours after treatment. There was no redness after 48 and 72 hours. No abnormalities were observed in the cornea, anterior chamber, iris, vitreous body and fundus. The possible changes in the eyes were classified as follows:

Maximun Score	Evaluation
0 - 10	Non-irritant
11 - 25	Slight irritant
26 - 56	Moderate irritant
57 - 110	Severe irritant

In this study, the maximum score was 1.4 (or 1.4/110). Mepiquat chloride was, therefore, non-irritant.

B. Deficiencies - There are no major deficiencies in this study (see **COMPLIANCE**).

Mepiquat chloride Primary Eye Irritation Study (81-4)

APPENDIX

The evaluation of the ophthalmic reactions is based on the following scheme of DRAIZE: ■

1. Cornea

A. Opacity - degree of density

0 No opacity

1 Scattered or diffuse area, details of iris clearly visible

2 Easily discernible translucent areas, details of iris slightly obscured

3 Opalescent areas, no details of iris visible, size of pupil barely discernible

4 Opaque, iris invisible

B. Area of cornea involved

0 No involvement
1 One quarter or less, but not 0
2 Greater than one quarter, but less than half
3 Greater than half, but less than three quarters
4 Greater than three quarters, up to whole area

Evaluation: A x B x 5 Maximun Score: 60

2. Iris

0 Normal

1 Folds above normal, congestion, swelling, iris still reacting to light

2 No reaction to light, hemorrhage, gross destruction

Evaluation: A x 5 Maximum Score: 10

3. Conjunctivae

A. Redness

0 Vessels normal
1 Vessels definitely injected above normal
2 More diffuse, deeper crimson red, individual

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vessels not easily discernible
3 Diffuse beefy red

B. Chemosis

0 No swelling
1 Any swelling above normal
2 Obvious swelling with partial eversions of lids
3 Swelling with lids about half closed
4 Swelling with lids about half closed to completely closed

C. Discharge

0 No discharge
1 Any amount different from normal
2 Discharge with moistening of the lids and hairs just adjacent to lids
3 Discharge with moistening of the lids and hairs, and considerable area around the eye

Evaluation: (A + B + C) x 2
Maximum Score: 20

■ Copied verbatim from the submitted report (MRID 00071942).

Mepiquat chloride Primary Dermal Irritation (81-5)

Primary Review by: Krystyna K. Locke K.K. Locke **Date:** 7/23/96
Review Section I, Toxicology Branch I (7509C)

Secondary Review by: Roger Gardner Roger Gardner **Date:** 8/2/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Primary Dermal Irritation - Rabbit
OPPTS 870.2500 (81-5)

EPA IDENTIFICATION NUMBERS:

DP Barcode D221662	Rereg. Case No. 2375
Submission No. S498213	Case No. 819426
P.C. Code No. 109101	Tox. Chem. No. 380 AB

TEST MATERIAL: Mepiquat chloride (1,1-Dimethylpiperidinium chloride); technical grade; purity: 94% a.i.

SYNONYMS: Reg. No. 85 559; DMP; BAS 083 W Technical

CITATION: Leuschner, F. (1976) Tolerance of the Rabbit Skin to 1,1-Dimethylpiperidinium Chloride, Reg. No. 85 559 (Patch Test); BASF, Germany; Report No. 76/0011; Study Completion Date: September 28, 1976; Unpublished. **MRID 41488103** (main study) and Kieczka, H. (1990) Phase 3 Summary of MRID 41488103; BASF Reg. Doc. No. 90/6229. **MRID 92091007** (additional data).

SPONSOR: BASF Wyandotte Corporation, Parsippany, NJ (main study) and BASF Corporation, Agricultural Chemicals Group, Research Triangle Park, NC (Phase 3 Summary).

EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID 41488103 and 92091007, Phase 3 Summary), young adult New Zealand White rabbits, 3/sex/concentration, were exposed to 0.5 g of technical Mepiquat chloride (94% a.i.) for 24 hours and then were observed for 14 days. The test material, undiluted or 50% diluted (not stated with what), was applied on linen patches measuring 2.5 x 2.5 cm and these patches were then placed on the shaved intact or abraded skin (between the fore and hind legs). Each patch was covered with a plastic foil of the same size and fixed by rubberized cloth. The application sites were examined and graded for irritation (Draize method) after removal of the patches and 48 hours thereafter. It could not be determined from the raw data if the application sites were washed at the end of the esposure. Behavior, general condition, food intake and body weight gain were also examined during the observation period.

Mepiquat chloride was not a dermal irritant in this study. The scores for erythema and edema were 0 (on a scale of 0-4) after 24

hours of exposure and 48 hours later. Other parameters examined were also unaffected by treatment. Based on these findings, Mepiquat chloride is in **Toxicity Category IV** for primary dermal irritation.

This study is classified as **Acceptable** and satisfies the guideline requirement for a primary dermal irritation study (81-5) in the rabbit.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. This study does not meet all of the GLP requirements because it was conducted prior to November, 1983 when GLP guidelines became effective. However, these deviations are minor (24-hour instead of 4-hour dosing; no documentation when the application sites were shaved or if they were washed after dosing; no individual observations for the entire day of dosing; and no individual daily observations after dosing) and do not affect the scientific validity of this study.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:

Description: Not provided, but apparently not solid, since it is stated in the submitted report (MRID 41488103) that the "soaked patches" were applied on 6 rabbits with intact skin and 6 rabbits with abraded skin.

Lot/Batch No.: Not reported
Purity: 94% a.i.
CAS Registry No. 24307-26-4
Verification of concentration: Yes

2. Vehicle: Not reported

3. Test Animals:

Species: Rabbit
Strain: New Zealand White
Weight at dosing: 2.3-2.8 kg
Source: Not reported, but in a primary eye irritation study, conducted also in 1976, rabbits from BASF breeding colony were used.

Acclimation period: Not reported
Diet: Artificially composed ALTROMIN 2023. Ad libitum.

Water: Tap, ad libitum.
Housing: Singly, in steel cages with a base of 0.4 m^2.
Environmental conditions: Temperature, 21 ± 2°C; humidity, 60 ± 3%; and photoperiod, 12 hours light/12 hours dark. Air changes were not reported.

B. STUDY DESIGN AND METHODS:

1. In Life Dates - This study was carried out during July, 1976.

2. Animal Assignment and Treatment - This study was conducted as described in HAZARDOUS SUBSTANCES, Part 191, Section 11, FDA, Washington 1965. This procedure is also detailed in the submitted report (MRID 41488103).

The primary dermal irritation of Mepiquat chloride was evaluated by a patch-test technique. Young adult New Zealand White rabbits, 3/sex/concentration, were exposed to 0.5 g of Mepiquat chloride for 24 hours and then were observed for 14 days. The test material, undiluted or 50% diluted (not stated with what) was applied on linen patches measuring 2.5 x 2.5 cm and these patches were then placed on the shaved intact or abraded skin (between the fore and front legs). Each patch was subsequently covered with a plastic foil of the same size and fixed by rubberized cloth. During exposure, the rabbits were kept in cages which allowed some movement but did not permit turning around the whole body. The application sites were examined and graded for irritation (Draize method) after removal of the patches and 48 hours thereafter. This examination and grading were performed independently by two "experts". It could not be determined from the raw data if the application sites were washed at the end of the exposure. Behavior, general condition, food intake and body weight gain were also examined during the observation period.

II. **RESULTS AND DISCUSSION**

A. Mepiquat chloride was not a dermal irritant in this study. The scores for erythema and edema were 0 (on a scale of 0-4) after 24 hours of exposure and 48 hours later. Other parameters examined were also unaffected by treatment.

B. Deficiencies - There are no major deficiencies in this study (see **COMPLIANCE**).

Mepiquat chloride Dermal Sensitization Study (81-6)

Primary Review by: Krystyna K. Locke K.K. Locke **Date:** 7/23/96
Review Section I, Toxicology Branch I (7509C)

Secondary Review by: Roger Gardner Roger Gardner **Date:** 8/2/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Dermal Sensitization - Guinea Pig
OPPTS 870.2600 (81-6)

EPA IDENTIFICATION NUMBERS:

DP Barcode: D221662 Rereg. Case No. 2375
Submission No. S498213 Case No. 819426
P.C. Code No. 109101 Tox. Chem. No. 380 AB

TEST MATERIAL: Mepiquat chloride (1,1-Dimethylpiperidinium chloride); technical grade; purity: 99 % a.i.

SYNONYMS: Reg. No. 85 559

CITATION: Grundler, O.J. (1979) Report on the Study of the Sensitizing Effect of Mepiquat Chloride (Reg. No. 85559) in the Guinea Pig According to "Proposed Rules" of EPA, Aug. 22, 1978, Federal Register, Vol. 43, No. 163, Page 37361, 163.81-6. BASF, Germany; Report Nos. 85/0080 and 79/162; Study Completion Date: November 13, 1979; Unpublished. **MRID 41488104** (main study) and Kieczka, H. (1990) Phase 3 Summary of MRID 41488104; BASF Reg. Doc. No. 90/6230. **MRID 92091008** (additional data).

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

EXECUTIVE SUMMARY: In a dermal sensitization study (MRID 414881 04, main study and MRID 92091008, Phase 3 Summary), with technical grade Mepiquat chloride (99% a.i.), young adult male Pirbright White Guinea Pigs were used, 6 in the concurrent control group and 12 in the treated group. The test was conducted according to the "Proposed Rules", EPA, August 22, 1978. The principle of this test is based on a comparison of the skin fold thickness between the concurrent control and the treated animals.

Based on the results from a preliminary study, a 10% solution of technical Mepiquat chloride in physiological saline was used in the induction and challenge periods in the current study. During the induction phase, each animal in the treated group received a total of 10 intracutaneous injections of Mepiquat chloride (one 0.05 mL and nine 0.1 mL) on Mondays, Wednesdays and Fridays (the application sites were not identified). The concurrent control

group was not induced. During the challenge phase, guinea pigs in the control and the treated groups received one (0.1 mL) intracutaneous injection of Mepiquat chloride in the clipped right flank and one (0.1 mL) injection of physiological saline in the left flank. The treated group was challenged 2 weeks after the last induction application. The dermal reactions were examined 24, 48 and 72 hours after the challenge and induction applications. Positive control was not used.

Mepiquat chloride was not a dermal sensitizer in this study. Very slight to well defined erythema and slight to severe edema were observed in all animals during the induction phase. Slight erythema and edema were observed in the control and the treated animals during the challenge phase. The skinfold thickness (edema) was lower in the treated group than in the control group.

This study is classified as **Acceptable** and satisfies the guideline requirement for a dermal sensitization study (81-6) in the guinea pig.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. This study was conducted prior to November, 1983 when the GLP guidelines became effective. Flagging statement was not submitted.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:

   Description: 10% solution in physiological saline
   Test substance number: 79/162
   Purity: 99% a.i.
   CAS Registry No. 24307-26-4
   Verification of concentration in dosing solutions: Not reported.

2. Vehicle: Physiological saline. Since the principle of this test is based on a comparison of the skin fold thickness between the control and experimental animals and a statistical evaluation, positive control was not used.

3. Test Animals:

   Species: Guinea pig
   Strain: Pilbright White

Weight at start of treatment: 546-635 g
Source: Breeder Hagemann, Germany
Acclimation period: Not reported
Diet: Saniff K, standard diet for rabbits and guinea pigs, supplied by Intermast GmbH, Soest, Germany. Ad libitum.
Water: Tap, ad libitum
Housing: Not reported
Environmental conditions: Not reported

B. STUDY DESIGN AND METHODS:

1. In Life Dates - Induction phase: 9/10 - 10/1/79;
Challenge treatment: 10/15/79

2. Animal Assignment and Treatment - This test was conducted according to the "Proposed Rules", EPA, August 22, 1978. The principle of this test is based on a comparison of the skin fold thickness between the control and the treated animals. There were 6 guinea pigs in the concurrent control group and 12 in the treated group.

Based on the results from a preliminary study, a 10% solution of technical Mepiquat chloride in physiological saline was selected for the induction and challenge applications in this study. During the induction phase, each animal in the treated group received a total of 10 intracutaneous injections of Mepiquat chloride (one 0.05 mL and nine 0.1 mL) on Mondays, Wednesdays and Fridays. The dermal reactions (erythema and edema) were assessed 24 and 48 or 72 hours after each application, as is detailed in Attachment I of this review. The application sites were not identified. The concurrent control group was not induced.

During the challenge phase, guinea pigs in the control and the treated groups received one (0.1 mL) intracutaneous injection of Mepiquat chloride in the clipped right flank and one (0.1 mL) injection of physiological saline in the left flank. The treated group was challenged 2 weeks after the last induction application. The skin reactions and skin fold thickness were evaluated 24, 48 and 72 hours after the challenge dosing.

II. RESULTS AND DISCUSSION

A. Induction Reactions and Duration - Very slight to well-defined erythema (score 1-2 on a scale of 0-4) and slight

to severe edema (score 2-4 on a scale of 0-4) were observed in all animals. Necrosis was also found at the injection site in various animals. These reactions were present at all times and, in most instances, did not intensify with repeated applications.

B. Challenge Reactions and Duration - Slight erythema and edema were observed in both the control and the experimental animals. The skinfold thickness (edema), shown below, was lower in the treated group than in the control group.

Group	Reading Times #	Skinfold Thickness (mm) ■	Scatter (mm)
Control	48	4.35	0.432
Treated	48	3.78	0.374
Control	72	3.98	0.371
Treated	72	3.57	0.293

Hours after treatment with 10 % Mepiquat chloride.

■ Mean values.

The above table is on page 17 of the submitted report (MRID 41488104). The statistical evaluation of the skin reactions is in Attachment II of this review. **Based on the above data, Mepiquat chloride was not a sensitizer in this study.**

C. Positive Control - As noted on page 2 of this review (under Vehicle), positive control was not used.

D. Need for Additional Series 81-6 Dermal Sensitization Studies - This test was conducted according to the procedure proposed by EPA and appears to be adequate. Another dermal sensitization test is, therefore, not required.

E. Deficiencies - Verificatin of concentration in dosing solutions, acclimation period, and housing and environmental conditions were not reported. Also, the application sites during the induction phase were not identified. However, these deficiencies are relatively minor and will not affect the classification of this study.

As noted above, positive control was not used in this study. This study was conducted according to the EPA

Mepiquat chloride Dermal Sensitization Study (81-6)

Guidelines dated August 22, 1978 in which a positive control was recommended but not required. Since the skin treated with diluted technical Mepiquat chloride (10% a.i.) was compared with that treated with saline (solvent for Mepiquat chloride), this study should be accepted as valid. Since Mepiquat chloride (a formulation containing 22.5% a.i.) was also not a dermal sensitizer in a study conducted in 1986 (maximization test; MRID 40308319), it appears that nothing will be gained from repeating the current study (MRID 41488104).

Attachment I

0186-06

Mepiquat Chloride MRID 41488101-02, 41954101, 41488103, 148090, 148089, 14888108, -06, -07 40299001, 92091014

Page _____ is not included in this copy.

Pages 31 through 37 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.

Mepiquat chloride Developmental Toxicity (83-3b)

Primary Review by: Krystyna K. Locke R.K. Locke **Date:** 7/23/96
Review Section I, Toxicology Branch I (7509C)

Secondary Review by: Roger Gardner Roger Gardner **Date:** 8/2/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Developmental - Rabbit
OPPTS 870.3700 (83-3b)

EPA IDENTIFICATION NUMBERS:

DP Barcode No. D221662 Rereg. Case No. 2375
Submission No. S498213 Case No. 819426
P.C. Code No. 109101 Tox. Chem. No. 380 AB

TEST MATERIAL: Mepiquat chloride (1,1-Dimethylpiperidinium chloride); technical grade; purity: 99% a.i.

SYNONYMS: Reg No. 85559; Compound No. 78/642

CITATION: Hofmann, H.T. and Merkle, J. (1979) Study of the prenatal toxicity of 1,1-dimethyl-piperidinium-chloride (Reg. No. 85559) on rabbits. BASF, Germany; No report number; Date: February 14, 1979; Unpublished. **MRID 00148090** (main study) and O'Reilly, I. (1990) Phase 3 Summary of MRID 00148090 and 00148089 BASF Reg. Doc. No. 90/6235. **MRID 92011910** (additional data).

SPONSOR: BASF Wyandotte Corp. Parsippany, N.J. (main study) and BASF Corporation, Agricultural Chemicals Group, Research Triangle Park, N.C. (Phase 3 Summary).

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 00148090, main study and MRID 92011910, Phase 3 Summary), technical Mepiqaut chloride (1,1-dimethylpiperidinium chloride; 99% a.i.) was administered to the artificially inseminated Himalayan rabbits (21-22/group) in aqua bidest (twice distilled water) at dose levels of 0 (untreated control), 0 (vehicle control), 50, 100 and 150 mg/kg/day. The dosing was done by gavage (in a volume of 5 mL/kg of body weight) during gestation days 6-18 and the animals were sacrificed on day 28. The animals received 130 g of dry food per day and water ad libitum during the study.

In the 50 mg/kg group, there was 1 abortion on day 26, weight loss and decreased food consumption during dosing days 6-12, and various amounts of amber-colored liquid in the abdomens of 5 rabbits. These findings were not observed in the control groups.

The following findings were reported for the 100 mg/kg group: weight loss during days 6-12 and decreased body weight gain during days 12-28; decreased food consumption during days 6-18; amber-colored liquid in the abdomens of 2 rabbits; diarrhea, trembling and apathy in one rabbit; and 6 abortions during days 18-28.

The following findings were reported for the 150 mg/kg group: 7 deaths during days 6-18; 4 abortions during days 18-21; weight loss during days 6-18; decreased food consumption during days 6-28; amber-colored liquid in the abdomens of 3 rabbits; and heart dilatation and hyperemia of organs in the nonsurvivors.

Based on the above findings, **the maternal NOEL is 50 mg/kg/day (borderline value) and the LOEL is 100 mg/kg/day.**

Developmental effects were not observed in the 50 mg/kg group. Because of high abortion rate in the 100 mg/kg group (6/16 pregnant = 37.5%), only 8 litters and 26 fetuses were available for evaluation. Because of high death rate and abortion rate in the 150 mg/kg group (total 10/17 pregnant = 58.8%), only 7 litters and 36 fetuses were available for evaluation. **The inadequate numbers of fetuses in the mid-dose and high-dose groups preclude the meaningful evaluation of developmental toxicity in this study.**

In order to evaluate developmental toxicity in the rabbit, the current study (MRID 00148090) was to be considered with another study (MRID 00148089) in which two doses of Mepiquat chloride (75 and 100 mg/kg) were tested. However, because the results were reported only in the form of a brief summary, the second study cannot be presently evaluated.

The developmental toxicity study in the rabbit (MRID 00148090) is classified as **Supplementary/Unacceptable** and does not satisfy the guideline requirement 83-3b (OPPTS 870.3700). Toxicology Branch I concluded that the study is upgradable following the review and acceptance of the second study (MRID 00148089). However, considering the data gaps and the finding that only x-rays (no staining techniques) were used for the evaluation of fetuses in both studies, the HED Reference Dose (RfD)/Peer Review Committee concluded on May 2, 1996 that a new developmental toxicity study with rabbits is required.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided in the Phase 3 Summary of this study (MRID 92011910). This study does not meet all of the GLP requirements because it was conducted prior to November, 1983 when GLP guidelines became effective. However, these deviations (analyses for test material stability, homogeneity and con-

centration in dosing medium were not performed), although important, are not critical to the acceptance of this study.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: 1,1-Dimethylpiperidinium chloride (Reg. No. 855599); Technical; BASF's I.D. No. 78/642; Solid, 99% a.i.; CAS No. 24307-26-4.

2. Vehicle: Aqua bidest (twice distilled water).

3. Test Animals:

Species: Rabbit (females).
Strain: Himalayan, Chbb:HM strain.
Age at mating: 23-29 weeks.
Weight at mating: 2.321 (1.712 - 2.5760 kg.
Source: The breeding facilities of Messrs. Thomae, Biderach.
Housing: Singly, in wire cages.
Diet: Ssniff-K ("sole diet for rabbits"), 130 g daily.
Water: Lukewarm tap water in bottles, ad libitum.
Environmental conditions: Temperature, 22 ± 2°C; Humidity, 55 ± 5%; Photoperiod, 12 hours light/12 hours dark.
Acclimation period: 10 days.

B. PROCEDURES AND STUDY DESIGN:

1. In Life Dates - From October to December, 1978.

2. Mating: - Described in the report as follows: " ---- the rabbits were fertilized by artificial insemination. One hour prior to insemination the animals were injected intravenously in the marginal ear vein with 40 I.U. Primogonyl in a volume of 1 ml. The day of insemination was designated day 0 of pregnancy and the following day as the first day post insemination (p.i.)".

3. Animal Assignment - Inseminated animals were assigned randomly to five groups as shown in TABLE 1 below.

TABLE 1. Animal Assignment

Test Group	Dose (mg/kg/day)	No. of Females
Untreated control	0	22
Aqua bidest *	0	22
Low dose	50	21
Mid dose	100	21
High dose	150	22

* Vehicle control.

4. Dose Selection Rationale - Not reported.

5. Dose Preparation and Analysis - The test material was dissolved in aqua bidest, but the solutions were not analyzed for concentration or stability. Also, it was not reported if the solutions were prepared daily.

6. Dose Administration - All doses were administered once daily by gavage, on gestation days 6 through 18, in a volume of 5 mL/kg of body weight. Dosing was based on the body weight on gestation day 0.

C. OBSERVATIONS:

1. Maternal Observations and Evaluations - The animals were checked daily for mortality and clinical signs. Food consumption was determined daily. Body weight was determined three times a week (Mondays, Wednesdays and Fridays), as well as on the day of insemination and on the 6th, 12th, 18th and 28th days post insemination. Dams were sacrificed on day 28 of gestation and the body cavity opened, and the uterus was removed in toto. Examination at sacrifice consisted of macroscopic examination of the internal organs and determination of the conception rate, corpora lutea, implantation sites, viable and dead fetuses, and early, intermediate and late resorptions. Salewski's procedure was also used to determine early resorptions.

2. Fetal Evaluations - Each fetus was grossly examined for alterations, weighed and measured for length. The placentae were also weighed. The heads of all fetuses were x-rayed, fixed in Bouin's solution, sectioned and examined " under a magnifying glass or a stereomicroscope ".

Skeletal and organ assessment of the fetuses were done as follows: An autopsy was performed on all fetuses, the organs were examined macroscopically and the animals x-rayed in two planes, laterally and " anteriorly - posteriorly ". The assessment of the skeleton was based on the x-rays and covered anomalies, variations and retardations. The following criteria were used:

Anomalies were considered to be detectable changes that went beyond the degree of retardations and variations.

Variations were considered to be regularly occurring changes that did not impair the functions of the animal.

Retardations were delays in development compared with the controls at the time of the examinations.

D. DATA ANALYSES:

1. Statistical Analyses - Statistical analyses were described in the submitted report (MRID 00148090) as follows:

A trend analysis in the sense of the generalization of the t-test indicated by Williams (#) was carried out for the variables body weight, body weight gain, (fetal) weight, length and placenta weight.

If, in a parameter, a stronger effect is found in the case of a low dose than with the next higher dose, the figures for the two groups are combined for the statistical evaluation of this parameter. Each dose is then assigned the effect observed in the combined group. This reduces the probability of incidental differences (= not induced by the compound) and an experiment-related control of the probability error is achieved.

Differences between relative frequencies were tested for significance using Fisher's exact test. The individual variables were as follows:

Conception rate
Viable fetuses per pregnant animal
Dead implantations per pregnant animal
Dead animals
Litters with anomalous fetuses per litters overall
Litters which have fetuses with variations and retardations per litters overall

Fisher's exact test was carried out in the sense of a trend analysis in the same way as the mean figure comparison described above.

The U test was carried out with the figures for implants per pregnant animal, viable and dead embryos in per cent per pregnant animal, as well as anomalies, variations and retardations as a percentage of viable fetuses per litter.

Mann-Whitney's asymptotic U test was used with Walter's binding correction and, if necessary, the continuity correction according to Yates.

Williams, D.A. (1971) A test for differences between treatment means when several dose levels are compared with a zero dose control. Biometrics 27, 103-117 and Williams, D.A. (1972) The comparison of several dose levels with a zero dose control. Biometrics 28, 519-531.

2. Historical Control Data - These data were provided in another study (MRID 00148089) which should be considered together with the current study.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations - The following observations were reported: In the untreated control group, "individual animals had diarrhoea temporarily". In the mid-dose group (100 mg/kg), one rabbit had diarrhea during gestation days 11-14; on day 18, the animal began to tremble 1 1/2 hours after dosing, became apathetic and traces of blood were discovered in the wood shavings. In the high-dose dose group (150 mg/kg), 11 rabbits exhibited trembling and spasms, followed by apathy, on various days after the treatment. The symptoms varied in intensity.

One rabbit died in the untreated control group and 7 died during the administration of the test material in the high-dose group ($P<0.05$ with reference to the untreated controls and $P<0.01$ with reference to the vehicle controls).

2. Body Weight - Body weight data are summarized in TABLE 2 below.

TABLE 2. Maternal Body Weight Gain (g/dam)

Dose in mg/kg/day #	0	0	50	100	150
Group	1	2	3	4	5
		Pretreatment: Days 0-6			
No. of animals	16	19	17	16	17
Weight gain	0.13	10.05	15.06	23.05	14.35
		Treatment: Days 6-12			
No. of animals	16	19	17	16	14
Weight gain	-0.13	25.50	-32.59	-33.13	-130.14**
		Treatment: Days 12-18			
No. of animals	16	19	17	15	13
Weight gain	37.50	53.89	66.71	0.20	-44.62*
		Posttreatment: Days 18-28			
No. of animals	16	19	16	10	7
Weight gain	169.13	118.84	141.13	91.00	204.86

The above table is based on data reported in TABLE 005 of the submitted report (MRID 00148090). # Mepiquat chloride Statistical significance: * 95% and ** 99%, relative to Group 2 (vehicle control). Group 1 = Untreated control

3. Food consumption - Relative to Group 2 (vehicle control), food consumption was decreased in Group 3 during days 6-12; in Group 4, during days 6-18; and in Group 5 during days 6-28. These data are summarized in TABLE 3 below.

Mepiquat chloride Developmental Toxicity (83-3b)

TABLE 3. Food Consumption (g/dam/day) #

Dose in mg/kg/day ##	0	0	50	100	150
Group	1	2	3	4	5
		Pretreatment: Days 0-6			
No. of animals	16	19	17	16	17
Food consumption	86	81	89	89	83
		Treatment: Days 6-12			
No. of animals	16	19	17	16	14
Food consumption	79	74	62*	57**	43**
		Treatment: Days 12-18			
No. of animals	16	19	17	16	14
Food consumption	68	75	65	42**	25**
		Posttreatment: Days 18-28			
No. of animals	16	19	16	11	7
Food consumption	92	88	89	84	83*
		Entire Study: Days 1-28			
No. of animals	16	19	16	11	7
Food consumption	83	81	77	71*	66**

This table is based on data reported in TABLE 002 of the submitted report (MRID 00148090). ## Mepiqut chloride Statistical significance: * 95% and ** 99%, relative to the vehicle control group.

4. Gross Pathology - Emaciation was observed in one rabbit from the untreated control group and deformed uterus in one rabbit from the vehicle control group; both animals were not pregnant. In the 50 mg/kg group, various amounts of an amber-colored liquid were found in the abdomens of 5 rabbits. Macroscopic findings in the 100 mg/kg group included amber-colored liquid in 2 rabbits (about 10 mL in one that aborted) and deformed uterus in another rabbit. In the 150 mg/kg group, 3 rabbits had amber-colored liquid in the abdomen and one rabbit had deformed uterus. The animals that died had heart dilatation and hyperemia of the organs. Focal pneumonia that was healing was detected in the lungs of one animal.

5. Cesarean Section Data are summarized in TABLE 4.

Mepiquat chloride Developmental Toxicity (83-3b)

TABLE 4. Cesarean Section Observations #

Dose (mg/kg/day) ##	0	0	50	100	150
Group	1	2	3	4	5
No. of Animals Assigned (Mated)	22	22	21	21	22
No. of Animals Pregnant	16	19	17	16	17
Pregnancy Rate (%)	72.7	86.4	80.9	76.2	77.3
No. Nonpregnant	6	3	4	5	5
Maternal Wastage					
No. Died	1	0	0	0	** 7
Pregnant	0	0	0	0	6
Nonpregnant	1	0	0	0	1
No. Aborted ■	0	0	1	6	4
No. Premat.Delivery ■	0	0	0	0	0
Total No. of Corpora Lutea	132	142	150	88	64
Corpora Lutea/Dam ■■	8.2	7.5	9.4	8.8	9.1
Total No. of Implantations	91	107	98	37	41
Implantations/Dam ■■	5.7	5.6	6.1	3.7	5.8
Total No. of Litters	16	19	16	8	7
Total No. of Live Fetuses	82	84	81	26	36
Live Fetuses/Dam	5.1	4.4	5.1	* 2.6	* 5.1
Total No. of Dead Fetuses	0	0	0	0	0
Dead Fetuses/Dam	0	0	0	0	0
Total No. of Resorptions ♥	9	23	17	11	5
Early	5	14	10	7	3
Early (Salewski)	0	0	0	0	0
Intermediate	3	7	6	3	2
Late	1	2	1	1	0
Resorptions/Dam	0.6	1.2	1.1	* 1.4	* 0.7
Early	0.31	0.74	0.62	0.90	0.43
Interm. and Late	0.25	0.47	0.44	0.50	0.29

Mepiquat chloride Developmental Toxicity (83-3b)

Litters with Total Resorptions	0	0	0	2	0
Mean Fetal Wgt. (g)	35.0	36.0	34.5	36.5	* 34.3
Males	35.3	34.8	34.5	38.4	* 34.3
Females	34.1	36.3	35.0	33.7	* 31.9
Sex Ratio (% Male)	57.3	39.3	53.1	61.5	69.4
Preimplantation Loss (%)	31.1	24.7	34.7	58.0	35.9
Postimplantation Loss (%)	9.9	21.5	17.4	29.7	12.2

This table is based on TABLES 016-023, pages 62-69, of the submitted report (MRID 00148090) and on the unnumbered table on page 27 of the same report.

Mepiquat chloride; Statistical Significance: * 95% and ** 99%, relative to the untreated and/or the vehicle control group (Fisher Test).

▪ There seems to be no distinction between abortions and premature deliveries in the submitted report (Attachment I in this review).

▪▪ Pregnant animals that died and those that aborted were excluded from the calculations.

▼ In the submitted report (MRID 00148090, pages 16-17), resorptions (early, intermediate and late) were identified as dead implants. Resorptions were defined as follows:

Early - These are recognizable to the naked eye as yellowish brown points.

Early (Salewski) - Following staining with 10% ammonium sulfide solution, early resorptions are recognizable as brown points on the mucosa of the uterus. This test was performed on rabbits that were apparently not pregnant.

Intermediate - These are dead embryos and embryos undergoing resorption in which no parts of the body were recognizable macroscopically.

Late - These are dead embryos and embryos undergoing resorptions in which individual parts of the body are

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recognizable macroscopically.

Relative to the untreated and/or vehicle control group, there was an increased number of abortions in Groups 4 and 5, and of deaths in Group 5. Because 2 animals in Group 4 also had total resorptions, only 8 litters were available for evaluation in Group 4 and 7 litters in Group 5. Other findings observed in Groups 4 and 5 were: Decreased total number of corpora lutea (but not corpora lutea/dam); Decreased total number of implantations and implantations/dam (only in Group 4); Decreased total number of live fetuses and live fetuses/dam; Decreased fetal body weight in Group 5; and Increased preimplantation and postimplantation losses in Group 4.

B. DEVELOPMENTAL TOXICITY

1. External Examination - Only 3 fetuses in the whole study were affected: One in the vehicle control group had multiple anomalies; one in the mid-dose group had umbilical hernia; and one in the high-dose group had pseudo-ankylosis.

2. Visceral Examination - One fetus in the low-dose group had hypoplasia of gallbladder. Other visceral abnormalities were not observed in this study.

3. Skeletal Examination - Variations and retardations involving sternum and ribs were observed in the control and the treated groups. These findings and the external and visceral observations are summarized in TABLE 5 (a,b,c) below.

TABLE 5a. External Examinations #

Dose (mg/kg/day) ##	0	0	50	100	150
Group	1	3	3	4	5
No. of Fetuses Examined	82	84	81	26	36
No. Affected	0	1	0	1	1
No. of Litters Examined	16	19	16	8	7
No. Affected	0	1	0	1	1
Finding:					
Pseudo-Ankylosis Fetal Incidence (%)	0	0	0	0	2.8

Mepiquat chloride			Developmental Toxicity (83-3b)		
Litter Incidence (%)	0	0	0	0	** 14.3
Multiple Anomalies					
Fetal Incidence (%)	0	1.2	0	0	0
Litter Incidence (%)	0	5.3	0	0	0
Umbilical Hernia					
Fetal Incicence (%)	0	0	0	3.8	0
Litter Incidence (%)	0	0	0	** 12.5	0

This table is based on TABLE 037, page 83, of the submitted report (MRID 00148090). ## Mepiquat chloride

** 99 % statistical significance, relative to both control groups (Fisher Test).

TABLE 5b. Visceral Examinations #

Dose (mg/kg/day) ##	0	0	50	100	150
Group	1	2	3	4	5
No. of Fetuses Examined	82	84	81	26	36
No. Affected	0	0	1	0	0
No. of Litters Examined	16	19	16	8	7
No. Affected	0	0	1	0	0
Finding:					
Hypoplasia of Gallbladder					
Fetal Incidence (%)	0	0	1.2	0	0
Litter Incidence (%)	0	0	6.2	0	0

This table is based on TABLE 038, page 84, of the submitted report (MRID 00148090). ## Mepiquat chloride

Mepiquat chloride Developmental Toxicity (83-3b)

TABLE 5c. Skeletal Examinations #

Dose (mg/kg/day) ##	0	0	50	100	150
Group	1	2	3	4	5
No. of Fetuses Exam.	82	84	81	26	36
No. Affected	61	60	58	19	28
Percent	74.4	71.4	71.6	73.1	77.8
No. of Litters Exam.	16	19	16	8	7
No. Affected	16	19	16	8	7
Finding:					
STERNUM					
Absent Sternebrae					
Fetuses Affected	33	26	21	7	13
Incidence (%)	40.2	30.9	25.9	26.9	36.1
Litters Affected	12	10	13	5	4
Incidence (%)	75.0	52.6	81.2	62.5	80.0
Partially Ossified Sternebrae					
Fetuses Affected	27	25	30	10	12
Incidence (%)	32.9	29.8	37.0	38.5	33.3
Litters Affected	12	15	13	6	6
Incidence (%)	75.0	78.9	81.2	75.0	85.7
Asymmetrical Sternebrae					
Fetuses Affected	1	1	0	1	1
Incidence (%)	1.2	1.2	0	3.8	2.8
Litters Affected	1	1	0	1	1
Incicence (%)	6.2	5.3	0	12.5	14.3
RIBS					
Accessory, Bilateral					
Fetuses Affected	0	5	4	0	2
Incidence (%)	0	5.9	4.9	0	5.6
Litters Affected	0	3	3	0	1
Incidence (%)	0	15.8	18.7	0	14.3
Accessory, Unilateral					
Fetuses Affected	0	4	3	2	0
Incicence (%)	0	4.8	3.7	7.7	0
Litters Affected	0	4	3	2	0
Incidence (%)	0	25.0	15.8	25.0	0

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Mepiquat chloride Developmental Toxicity (83-3b)

This table is based on TABLES 036-060, pages 82-106, of the submitted report (MRID 00148090). ## Mepiquat chloride

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

According to the report (MRID 00148090), the toxic dose for dams is between 50 and 100 mg/kg, and for fetuses, "..... from/above 150 mg/kg." According to the Phase 3 Summary of this study (MRID 92011910), which was submitted during the FIFRA '88 Data Review, the maternal NOEL is 100 mg/kg b.w./day and the NOEL for developmental toxicity (embryotoxicity and teratogenicity) is 150 mg/kg b.w./day. It was also stated in the Summary that the required number of litters was not obtained in the 100 and 150 mg/kg groups, and that the current study should be considered together with another study, conducted in 1980, in which 2 doses of Mepiquat chloride (75 and 100 mg/kg) were tested (MRID 00148089). In the 1980 study, 11 litters were obtained in the 100 mg/kg group.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY:

In the 50 mg/kg group, there was 1 abortion on day 26, weight loss and decreased food consumption during dosing days 6-12, and various amounts of amber-colored liquid# in the abdomens of 5 rabbits.

The following findings were reported for the 100 mg/kg group: weight loss during days 6-12 and decreased body weight gain during days 12-28; decreased food consumption during days 6-18; amber-colored liquid in the abdomens of 2 rabbits; diarrhea, trembling and apathy in one rabbit; and 6 abortions during days 18-28.

The following findings were reported for the 150 mg/kg group: 7 deaths during days 6-18; 4 abortions during 18-21; weight loss during days 6-18; decreased food consumption during days 6-28; amber-colored liquid in the abdomens of 3 rabbits; and heart dilatation and hyperemia of organs in the nonsurvivors.

Because of high abortion rate in the 100 mg/kg group (6/16 pregnant = 37.5%), and high death rate and abortion rate in the 150 mg/kg group (total 10/17 pregnant

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= 58.8%), the cesarean section data cannot be meaningfully evaluated.

No comment was made about an amber-colored liquid observed in some treated animals in the current study. According to another (supplementary) study (MRID 00148089), which is to be considered together with the current study, " The various amounts of an amber-colored liquid ---- were also detected in this study in all test groups. Since it is a low-protein liquid (transsudate) and there were no signs of any cardiac insufficiency or of any congestion, no importance was attached to this finding. "

2. DEVELOPMENTAL TOXICITY:

a. Deaths/Resorptions: As is shown in TABLE 4 of this review, there were no dead fetuses in the control and the treated groups. Relative to the untreated controls, there were more total resorptions per dam in the mid-dose group (1.4 vs 0.6; $P<0.5$). Relative to the vehicle control group, there were fewer total resorptions per dam in the high-dose group (0.7 vs 1.2; $P<0.05$). However, as noted above, the cesarean section data cannot be evaluated meaningfully in this study.

b. Altered Growth: Male and female fetuses in the high-dose group weighed less than those in the control groups (TABLE 4 in this review).

c. Developmental Variations and Retardations: These involved sternum and ribs, and were observed in every litter of the control and treated groups.

Based on the above findings, **the maternal NOEL is 50 mg/kg (borderline value) and the LOEL is 100 mg/kg.** Because too few fetuses were available for evaluation in the 100 mg/kg and 150 mg/kg groups (26 and 36, respectively), **the developmental NOEL cannot be determined in this study.**

Classification of study: Supplementary (upgradable)

C. STUDY DEFICIENCIES

Because of a major deficiency (inadequate data to determine a developmental NOEL), this study (MRID 00148090) is not acceptable. The registrant was aware of

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determine a developmental NOEL), this study (MRID 00148090) is not acceptable. The registrant was aware of this problem and another study with 2 doses of Mepiquat chloride (75 and 100 mg/kg) and 15 Himalayan rabbits/dose was, therefore, undertaken in June, 1980. That study (MRID 00148089) was to be considered together with the current study. However, the report submitted to the Agency consists only of the following data: experimental precedures, brief summary of results, a few graphs and historical control data. Although references to tables are made in the SUMMARY (but not in the CONTENTS), the report contains no tables and cannot, therefore, be reviewed until the missing data (tables) are submitted. Following the review and acceptance of the second (supplemental) study (MRID 00148089), the current study (MRID 00148090) will be upgraded.

NOTE: Considering the data gaps and the finding that only x-rays (no staining techniques) were used for the evaluation of fetuses in both studies, the HED Reference Dose (RfD)/Peer Review Committee concluded on May 2, 1996 that a new developmental toxicity study with rabbits (83-3b) is required.

Mepiquat chloride Developmental Toxicity (83-3b)

ATTACHMENT I

Mepiquat chloride Developmental Toxicity (83-3b)

Dams with abortion

Test Group	Day p.i.	Animal no.	Abortion and detectable implants in the uterus after sacrifice	
3	26th	57	1	7 fetuses
4	18th	74	1	1 early res., 2 late res. 1 dead fetus
	18th	78	1	5 fetuses, 2 dead fetuses, cherry-size blood coagulate around 4 placentae
	21st	81	1	3 fetuses, 1 intermed. res., 1 dead fetus with blood clotting around placenta.
	18th	82	2	4 fetuses, placentae necrobiotic
	28th	83	5 =	3 viable + 2 dead fetuses, no further implants in the uterus
	20th	85	2	2 fetuses, 2 intermed. res., 1 late res. and 1 dead fetus
5	21st	91	2	1 fetus, 1 early res., 3 placentae necrobiotic
	21st	96	6	1 early res. and 1 intermed. res.
	20th	103	1	4 intermed. res. and 1 early res.
	18th	105	1	4 early res. and 6 intermed. res. Placentae necrobiotic

The above table is a verbatim reproduction of one on page 27 of the submitted report (MRID 00148090).

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[MEPIQUAT CHLORIDE] UNSCHEDULED DNA SYNTHESIS

EPA Reviewer: Pamela M. Hurley Pamela M. Hurley, Date 3/27/96
Review Section I, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Roger Gardner Roger Gardner, Date 4/2/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Other Genotoxicity: Unscheduled DNA Synthesis in Primary Rat Hepatocytes/Mammalian Cell Cultures; OPPTS 870.5550 [§84-2]

DP BARCODE: D224363 SUBMISSION CODE: S498213
P.C. CODE: 109101 TOX. CHEM. NO.: 380AB

TEST MATERIAL (PURITY): Mepiquat Chloride (99.86%)

CITATION: Cifone, M. A. (1987) Report on the mutagenicity test on mepiquat chloride in the rat primary hepatocyte unscheduled DNA systhesis assay. Hazleton Laboratories America, Inc., Kensington, MD 20895. Document No. BASF: 87/0393, September 15, 1987. MRID 41488108. Unpublished.

SPONSOR: BASF Corporation Chemicals Division, Agricultural Chemicals, Parsippany, New Jersey

EXECUTIVE SUMMARY:

In an unscheduled DNA synthesis assay (MRID 41488108), primary rat hepatocyte cultures were exposed to mepiquat chloride (99.86% a.i.) at concentrations ranging from 0.026 to 5000 µg/ml for 18-19 hours. Two trials were initiated, one ranging from 0.026 µg/ml to 1020 µg/ml and the other ranging from 0.25 µg/ml to 5000 µg/ml.

Mepiquat chloride was tested up to cytotoxic concentrations. The positive controls induced an appropriate positive response. **Under the conditions of the assay, there was no evidence that mepiquat chloride induces an increase in unscheduled DNA synthesis, as determined by radioactive tracer procedures [nuclear silver grain counts] when compared to the negative control group. In addtion, there was no indication of a dose-response.**

This study is classified as acceptable. It satisfies the requirement for FIFRA Test Guideline 84-2 for other genotoxic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: mepiquat chloride (1,1-dimethyl piperidinium chloride)
Description: white granules
Lot/Batch #: Lot N48; ZNT No. 85/453
Purity: 99.86% a.i.
Stability of compound: Statement in document that says "no decomposition observed at temperatures from room temperature to 50°C."
CAS #: 24307-26-4

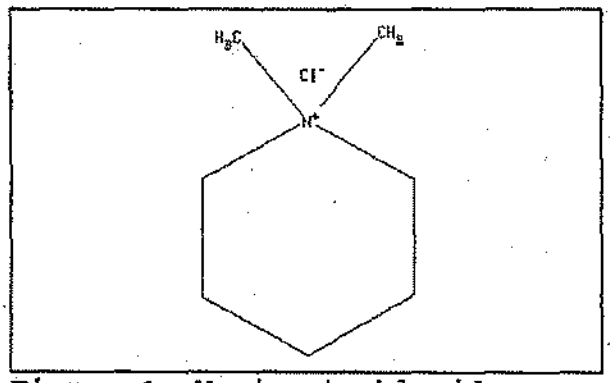

Figure 1 Mepiquat chloride

Solvent used: Williams' Medium E (WME)

2. Control Materials:
Negative: WME
Solvent/final concentration: N/A
Positive: 2-acetyl aminofluorene (2-AAF) at 4.48 x 10^{-7}M (0.10 µg/ml) - solvent not given (assume WME?)

3. Test compound concentrations used: two trials were initiated, one ranging from 0.026 µg/ml to 1020 µg/ml and the other ranging from 0.25 µg/ml to 5000 µg/ml. In order to obtain a good range of toxicities for analysis, 6 treatments from Trial 1 and 5 treatments from Trial 2 were prepared for audioradiographic analysis. The 5000 µg/ml and 4000 µg/ml treatment from Trial 2 could not be analyzed because the nuclei were darkly stained and had an abnormal morphology due to the toxic action of the test material. The dose levels

selected for analysis were: trial 1: 25.6, 51.2, 102, 256, 512 or 1020 µg/ml and trial 2: 1000, 2000 or 3000 µg/ml.

4. Media: The cell cultures were established in Williams' Medium E supplemented with 5% fetal bovine serum, 2mM L-glutamine, 2.4µM dexamethasone, 90 U/ml penicillin, 90 µg/ml streptomycin sulfate and 140 µg/ml gentamicin. The dexamethasone and serum components were removed after the cell cultures were established.

5. Test Cells: The cells for this assay were obtained from an adult male Fischer 344 rat, which had been purchased from the Charles River Breeding Laboratories. The report stated that "the cells were obtained by perfusion of the liver in situ with a collagenase solution. Monolayer cultures were established on plastic coverslips in culture dishes and were used the same day for initiation of the UDS assay. All cultures were maintained as monolayers at about 37°C in a humidified atmosphere containing approximately 5% CO_2."

6. Cell Preparation:

a. Perfusion Technique: The liver was perfused in situ for approximately 4 minutes with Hanks' balanced salts (Ca++ - Mg++ - free) containing 0.5 mM ethyleneglycol-bis (β-aminoethyl ether)-N, N-tetraacetic acid (EGTA) and HEPES buffer at pH 7.2. Then the liver was perfused with WME with 50-100 units/ml of collagenase for about 10 minutes.

b. Hepatocyte Harvest/Culture Preparation: The hepatocytes were obtained by mechanical dispersion of the excised liver tissue in a culture dish containing the WME culture medium and collagenase. The suspension was then either filtered through sterile cheesecloth or allowed to sit while the clumps of cellular tissue and debris settled to the bottom. The filtrate or decantate was centrifuged and the pellet was resuspended in WME containing 5% serum and 2.4 µM dexamethasone. A series of 35-mm culture dishes was inoculated with approximately 0.5×10^6 viable cells in 3 ml WME plus dexamethasone and 5% serum/dish. An attachment period of 1.5 to 2 hours at approximately 37°C in a humidified atmosphere containing about 5% CO_2 was used to establish the cell cultures. Unattached cells were then removed and the cultures were refed with WME.

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B. TEST PERFORMANCE

1. Cytotoxicity Assay: Within 3 hours of the hepatocyte harvest and culture, the media in the culture dishes were replaced with 2.5 ml WME containing 1% fetal bovine serum, 1μCi/ml ^{3}H-thymidine, (20 Ci/mmole) and the test material at the desired concentration. After treatment for 18-19 hours, the the cell monolayers were washed twice with WME. Two of the treated cultures were used for cytotoxicity measurements. These cultures were refed with WME and returned to the incubator. At 20-24 hours after the initiation of the treatments, viable cell counts (trypan blue exclusion) were determined to estimate cell survival.

2. UDS Assay:

a. Treatment: The UDS assay was initiated within 3 hours of the hepatocyte harvest and culture. The media in the culture dishes were replaced with 2.5 ml WME containing 1% fetal bovine serum, 1μCi/ml ^{3}H-thymidine, (20 Ci/mmole) and the test material at the desired concentration. After treatment for 18-19 hours, the the cell monolayers were washed twice with WME. Three of the cultures from each treatment were washed with WME containing 1mM thymidine and were further processed.

b. Preparation of Autoradiographs/Grain Development: The nuclei in the labeled cells were swollen by placement of the coverslips in 1% sodium citrate for 8-10 minutes, fixed in acetic acid:ethanol (1:3) and then dried for 24 hours. The coverslips were mounted on glass slides, dipped in an emulsion of Kodak NTB2 and dried. The coated slides were then stored for 7-10 dyas at 4°C in light-tight boxes containing packets of Drierite. The emulsions were then developed in D19, fixed and stained with Williams' modified hematoxylin and eosin procedure.

c. Grain Counting: The cells were microscopically examined at approximately 1500 x magnification under oil immersion. UDS was measured by counting nuclear grains and subtracting the average number of grains in 3 nuclear-sized areas adjacent to each nucleus. The net nuclear grain count was determined for 50 randomly selected cells on each coverslip. Only normal nuclei were scored. The mean net nuclear grain count was determined from triplicate coverslips (150 total nuclei) for each treatment condition.

e. Evaluation Criteria: The following points taken from the report state the assay acceptance criteria. With careful scientific judgement, all or most of the criteria should be satisfied for an acceptable study.

1. The viability of the hepatocytes collected from the perfusion process cannot be less than 50%.

2. The viability of the monolayer cell cultures used for the assay treatments must be at least 70%.

3. The number of viable cells in the negative (or solvent) control cultures should remain reasonably stable over the experimental time period.

4. The labeling in the negative (or solvent) control cultures must not exceed an average of 2 net grains/nucleus, or 10% of the cells with 6 or more grains, or 1% of the cells with 20 or more grains.

5. The positive controls must provide the appropriate positive response unless the test material induces clear dose-related UDS activity.

6. Grain count data obtained for a given treatment is acceptable as part of the evaluation if obtained from at least 2 replicate cultures and at least 50 cells/culture.

7. A minimum of 6 dose levels will be analyzed for nuclear grain counts. Repeat trials need only augment the number of analyzed dose levels in the first trial to achieve a total of 6 different concentrations.

8. The highest analyzed dose must approach an excessive toxicity or result in test material insolubility, or reach the highest applicable dose of 5000 µg/ml (or 5000 nl/ml).

The following assay evaluation criteria were provided in the report. The test material would be considered active in the UDS assay at applied concentrations that cause:

1. An increase in the mean net nuclear grain count to at least 6 grains/nucleus after subtraction of the concurrent negative control value, and/or

2. An increase in the percent of nuclei having six or more net grains to at least 10% of the analyzed population after subtraction of the concurrent negative control value, and/or

3. The percent of nuclei with 20 or more grains to reach or exceed 2% of the analyzed population.

Satisfaction of any one of the above criteria could indicate UDS activity. A dose-related increase in UDS for at least 2 consecutive applied concentrations is also desirable; however, if there is a positive response at one dose and toxicity at higher doses, then the test material may also be considered positive for UDS activity. The report also stated that "the test material is considered inactive in this assay if none of the above conditions are met and if the assay includes the maximum applied dose or other doses that are shown to be toxic by the survival measurements. If little or no toxicity is demonstrated for any of the applied doses and the test material remains soluble in the culture medium, the assay may be considered inconclusive and may be repeated with higher doses after consultation with the sponsor."

f. Statistical Analysis: The assay evaluation criteria were used in lieu of a statistical analysis.

II. **REPORTED RESULTS**

A. Preliminary Cytotoxicity Assay: Mepiquat chloride was soluble is WME treatment media up to 5000 µg/ml. In trial 1, the test material was weakly toxic at 1020 µg/ml (90.4% survival). None of the other dose levels were toxic. In trial 2, the 5000 µg/ml was highly toxic (32.3% survival) and the cells had a round morphology, unlike the flat morphology of the negative controls. At 4000 µg/ml, the survival increased to 55.4% and the cells were beginning to flatten. At 3000 and 2000 µg/ml the survival rates were 71.6% and 72.8%, respectively and the cells had a flat morphology. At 1000 µg/ml, survival reached 88.8%. The positive control (0.1 µg/ml 2-AAF) induced low to moderate toxicities in the range of the historical data.

B. UDS Assay: The 5000 and 4000 µg/ml treatments from trial 2 could not be analyzed because the nuclei were darkly stained and had an abnormal morphology. The hepatocytes for trials 1 and 2 were collected at a calculated viability of 91.2% and 90.5% respectively. Seventy-one point 2% and 71.3% of the viable cells from the 2 trials, respectively attached to the culture dishes during the 1.5 hour settling period. The

cell monolayers were 92.2% and 88.7% viable for the 2 trials and after the treatment period, the average viable cell counts in the negative control cultures were 97.4 and 104.6% of the viable count at the beginning of the treatments. This stability in cell number of the normal morphological appearance of the cells indicated that the hepatocyte cultures were in good metabolic condition for the UDS assay. In both trials, none of the treatments with the test material induced nuclear labeling that was significantly different from the negative control. In addition, no dose-related trend was evident. The positive control induced large increases in nuclear labeling that greatly exceeded all 3 criteria used to indicate UDS. Only 41 cells (0.21%) among the 19,500 cells screened in the 2 assays were heavily labeled (cells undergoing DNA replication as opposed to DNA repair). The following tables summarize the results of the 2 trials.

Summary of Data From Rat Hepatocyte UD8 Assay - Trial 1					
Test Condition	Concentration	UDS* grains/nucleus	Avg.† % nuclei with > 6 grains	Avg.† % nuclei with > 20 grains	Survival†† at 20 1/2 hrs, %
Negative Control	-	0.60	0.7	0.0	100.0
Positive Control (2-AAF)	0.1 µg/ml	24.73	99.3	73.3	91.6
Mepiquat chloride	1020 µg/ml	0.48	0.0	0.0	90.4
	512 µg/ml	0.29	0.0	0.0	95.4
	256 µg/ml	0.65	0.0	0.0	97.4
	102 µg/ml	0.39	0.0	0.0	100.8
	51.2 µg/ml	0.25	0.0	0.0	96.8
	25.6 µg/ml	0.43	0.0	0.0	ND

*UDS = Average of net nuclear grain counts on triplicate coverslips (150 total cells).
†Average values for triplicate coverslips.
††Survival = Number of viable cells per unit area relative to the negative control x 100%.
2-AAF = 2-acetyl aminofluorene.
ND = Not determined.
Mean cytoplasmic grain count for negative controls = 3.42

Summary of Data From Rat Hepatocyte UDS Assay - Trial 2					
Test Condition	Concentration	UDS* grains/nucleus	Avg.† % nuclei with > 6 grains	Avg.† % nuclei with > 20 grains	Survival†† at 20 1/2 hrs, %
Negative Control	-	0.90	0.7	0.0	100.0
Positive Control (2-AAF)	0.1 µg/ml	10.72	77.3	8.7	75.1
Mepiquat chloride	3000 µg/ml 2000 µg/ml 1000 µg/ml		0.0 0.0 1.3	0.0 0.0 0.0	71.6 72.8 88.8

*UDS = Average of net nuclear grain counts on triplicate coverslips (150 total cells).
†Average values for triplicate coverslips.
††Survival = Number of viable cells per unit area relative to the negative control x 100%.
2-AAF = 2-acetyl aminofluorene.
Mean cytoplasmic grain count for negative controls = 3.51

III. **REVIEWER'S DISCUSSION/CONCLUSIONS:** There are no major deficiencies in this study. Under the conditions of the study, mepiquat chloride did not induce an increase in UDS when compared to the negative controls up to cytotoxic levels.

[MEPIQUAT CHLORIDE] SALMONELLA; GENE MUTATION (84-2)

EPA Toxicologist: Pamela M. Hurley Pamela M. Hurley, Date 3/22/96
Review Section I, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Roger Gardner Roger Gardner, Date 4/2/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Salmonella reverse gene mutation assay; OPPTS 870.5265[1] [§84-2]

DP BARCODE: D224363 SUBMISSION CODE: S498213
P.C. CODE: 109101 TOX. CHEM. NO.: 380AB

TEST MATERIAL (PURITY): Mepiquat Chloride (99.8%)

SYNONYMS: N/A

CITATION: Zeller, H. (1979) Report on the testing of reg. no. 85 559 (mepiquat chloride) in the Ames test. BASF Aktiengesellschaft, Product Safety Crop Protection, Environmental Metabolism, Postfach 220, D-6703 Limburgerhof (FRG). Document No. 79/0035, July 5, 1979. MRID 41488106.

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

EXECUTIVE SUMMARY:

In a reverse gene mutation assay in bacteria (MRID 41488106), strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 of S. typhimurium were exposed to mepiquat chloride (99.8% a.i.) in distilled water at concentrations of 0, 4, 20, 100, 500 or 2500 µg/plate in the presence and absence of mammalian metabolic activation (S-9 mix).

Mepiquat chloride did not induce a significant increase in revertant colonies at any dose level up to 2500 µg/plate under the conditions of the assay, either with or without metabolic activation. However, mepiquat chloride was neither tested up to cytotoxic concentrations nor the limit concentration, 5000 µg/plate. In addition, solubility did not appear to be a problem. The positive controls induced the appropriate responses in the corresponding strains.

This study is classified as acceptable for regulatory purposes. It satisfies the requirement for FIFRA Test Guideline 84-2 for in vitro mutagenicity (bacterial reverse gene mutation) data.

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[1]870.5100 - Reverse mutation *E. coli* WP2 and WP2uvrA
870.5140 - Gene mutation *Aspergillus nidulans*
870.5250 - Gene mutation *Neurospora crassa*

Although mepiquat chloride was not tested at high enough doses for an adequate negative study (the limit dose is 5000 µg/plate), based on the results from the two other mutagenicity studies, the two carcinogenicity studies, the rat reproduction study and the two developmental toxicity studies, each negative for the specific effect being measured (i.e. mutagenic, carcinogenic, reproductive and developmental effects), TB-I has determined that retesting mepiquat chloride in the Salmonella assay would not add any significant knowledge to the current database for this chemical. Therefore, a new study will not be required.

COMPLIANCE: Signed and dated GLP (signed statement that it was conducted prior to GLP's and does not meet requirements) and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: 1,1-dimethyl piperidinium chloride
   Description (e.g. technical, nature, color): not stated
   Lot/Batch #: 79/162
   Purity: 99.8% a.i.
   Stability of compound: not stated, however, it was to be stored at +4°C
   CAS #: 24307-26-4

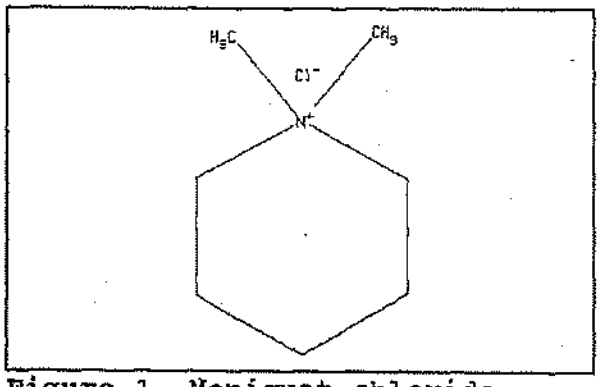

Figure 1 Mepiquat chloride

Solvent used: distilled water

2. Control Materials:
Negative: distilled water
Solvent/final concentration: 0.1 ml test solution was added to each plate; it is assumed that for the negative control, this would be 0.1 ml distilled water.
Positive: Nonactivation:
Sodium azide _____ µg/plate TA100, TA1535
2-Nitrofluorene _____ µg/plate TA98, TA1538
9-Aminoacridine _____ µg/plate TA97, TA1537
Other (list): N-methyl-N'-nitro-N-nitroso-guanidine (MNNG) (5 µg dissolved in DMSO) for strains TA 100, TA 98, TA 1537 and TA 1539

Activation:
2-Aminoanthracene (2-anthramine) 10 µg/plate usually all strains
Other (list): cyclophosphamide (500 µg in distilled water for TA 100 and TA 1535)

3. Activation: S9 derived from
__x Aroclor 1254 __x induced __x rat __x liver
___ phenobarbital ___ non-induced ___ mouse ___ lung
___ none ___ hamster ___ other
___ other

S9 mix composition:

S-9 fraction (3 volumes)
7 volumes of the S-9 supplement (cofactors):

MgCl 8 mM
KCl 33mM
Glucose-6-phosphate 5mM
NADP 4mM
phosphate buffer (pH 7.4) 100mM (prepared from 2 solutions: 25.42 g/l Na_2HPO_4 and 22.28 g/l NaH_2PO_4

4. Test organisms: S. typhimurium strains
___ TA97 __x TA98 __x TA100 ___ TA102 ___ TA104
__x TA1535 __x TA1537 __x TA1538 ; list any others:
Properly maintained? Yes
Checked for appropriate genetic markers (rfa mutation, R factor)? No

5. Test compound concentrations used:
Nonactivated conditions: 0, 4, 20, 100, 500 or 2500 µg/plate
Activated conditions: 0, 4, 20, 100, 500 or 2500 µg/plate

B. TEST PERFORMANCE

1. Type of Salmonella assay:
__x__ standard plate test
____ pre-incubation (___ minutes)
____ "Prival" modification
____ spot test
____ other

2. Protocol: The bacterial cultures were prepared from deep frozen cultures (1 ml in 15 ml glass tubes). These were thawed and 0.1 ml of the bacterial suspension was inoculated into nutrient broth solution (8g Difco Bacto nutrient broth + 5 g NaCl/liter) and incubated for 24 hours at 37°C. The cultures were then kept in iced water to prevent further growth. The soft agar solution was prepared by mixing 100 ml agar (0.6% agar + 0.6% NaCl) and 10 ml amino-acid solution (minimal amino-acid solution for the determination of mutants: 0.5 mM histidine + 0.5 mM biotin). Two milliliters of this mixture were poured into test tubes and kept in water at 45°C while the remaining components were added to each test tube in the following order:

0.1 ml test solution
0.1 ml bacterial suspension
0.5 ml S-9 mix

After mixing, the samples were poured onto the nutrient plates within approximately 30 seconds.

The nutrient medium consisted of the following:

980 ml distilled water
120 ml Vogel-Bonner E Medium
15 g Difco Bacto agar]
20 g D-glucose, monohydrate

Four test plates were used for each concentration for all tester strains, for the vehicle control groups and for the positive control groups.

After incubation for 48 hours at 37°C in the dark, the bacterial colonies (his^+ revertants) were counted.

3. Evaluation Criteria: in order for an assay to be considered as positive, the following criteria had to be met:

- doubling of the spontaneous mutation rate (control)
- dose-effect relationship
- reproducibility of the results

II. **REPORTED RESULTS**

A. Preliminary cytotoxicity assay: A preliminary cytotoxicity assay was not conducted.

B. Mutagenicity assay: No increases in the number of his^+ revertants were observed in any of the strains at any dose level, either with or without metabolic activation. No toxicity was observed at any dose level. The following table summarizes the results.

Number of Revertant Colonies With Mepiquat Chloride in the Ames Test

Dose µg/plate	S-9 Mix	TA 98				TA 100				TA 1535			
0	+	34	33	39	35	127	130	131	120	18	19	13	19
4	+	41	38	34	37	103	108	128	126	17	13	11	19
20	+	-	31	29	28	110	101	113	130	14	14	16	28
100	+	37	36	36	33	114	108	107	129	17	17	18	12
500	+	33	26	31	32	136	112	93	125	14	20	20	15
2500	+	34	38	38	35	119	120	119	135	17	16	14	16
10 2-AA	+	1550	1220	1350	1370	2300	2100	1850	2300	377	397	467	489
500 Cyclophos.	+	-	-	-	-	276	212	258	217	176	198	192	209
0	-	20	19	21	23	134	117	149	119	22	9	19	8
4	-	22	27	18	29	151	110	114	120	14	10	12	5
20	-	18	26	20	25	126	147	146	115	23	18	13	0
100	-	16	22	22	21	132	123	129	148	13	13	21	18
500	-	26	25	23	20	151	138	124	132	17	12	20	23
2500	-	19	23	24	21	148	149	131	120	17	15	13	13
5 MNNG	-	1500	1600	1600	1400	3300	3200	3350	3100	1900	2000	1900	2100

Dose µg/plate	S-9 Mix	TA 1537				TA 1538			
0	+	9	8	11	10	23	26	32	22
4	+	7	6	6	9	23	17	19	24
20	+	7	8	9	12	21	25	26	20
100	+	9	4	8	6	22	26	20	18
500	+	6	7	7	10	28	22	28	26
2500	+	7	5	7	5	23	19	18	24
10 2-AA	+	68	31	64	72	1500	1550	1200	1550
500 Cyclophos.	+	-	-	-	-	-	-	-	-
0	-	5	7	5	4	12	20	14	18
4	-	5	4	6	5	10	11	11	18
20	-	6	6	7	6	17	16	15	11
100	-	7	9	3	4	15	13	12	15
500	-	6	6	9	8	23	14	15	13
2500	-	7	5	5	5	14	13	12	12
5 MNNG	-	21	26	21	20	-	-	-	-

Solvent control - distilled water

III. **REVIEWER'S DISCUSSION/CONCLUSIONS:**

With the exception of the fact that mepiquat chloride was not tested at sufficiently high dose levels for a negative study (maximum dose was 2500 µg/plate), this study was adequately conducted. Based on the results from the other two mutagenicity studies (an in vitro cytogenetic assay in CHO cells and a UDS assay in rat hepatocytes; both negative and tested at sufficiently high dose levels), and on the results of other studies in which mutagenicity may play an important role, the two carcinogenicity studies (both negative for carcinogenic effects), the rat reproduction study (negative for reproductive effects) and the two developmental toxicity studies (both negative for developmental effects), TB-I has determined that retesting mepiquat chloride in the Salmonella assay will not add any significant knowledge to the current database for this chemical. Therefore, a new study will not be required.

[MEPIQUAT CHLORIDE] IN VITRO CHROMOSOMAL ABERRATION (84-2)

EPA Reviewer: Pamela M. Hurley Pamela M Hurley, Date 3/25/96
Review Section I, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Roger Gardner Roger Gardner, Date 4/2/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* mammalian cytogenetics [Chromosome Aberration] in Chinese Hamster Ovary (CHO) Cells OPPTS 870.5375 [§84-2]

DP BARCODE: D224363 SUBMISSION CODE: S498213
P.C. CODE: 109101 TOX. CHEM. NO.: 380AB

TEST MATERIAL (PURITY): Mepiquat Chloride (> 99%)

SYNONYMS: N/A

CITATION: Taalman, R.D.F.M. (1987) Clastogenic evaluation of mepiquat chloride in an *in vitro* cytogenetic assay measuring chromosome aberration frequencies in Chinese Hamster Ovary (CHO) cells. Hazleton Biotechnologies, Landjuweel 11, 3905 PE Veenendaal, The Netherlands. Document No. BASF 87-0450, December 4, 1987. MRID 41488107. Unpublished.

SPONSOR: BASF Corporation Chemicals Division, Agricultural Chemicals, 100 Cherry Hill Road, Parsippany, New Jersey

EXECUTIVE SUMMARY:

In a mammalian cell cytogenetics assay [chromosome aberration in CHO cells] (MRID 41488107), CHO cell cultures were exposed to mepiquat chloride (< 99% a.i.) at concentrations of 2.0, 3.0, 4.0, or 5.0 mg/ml, both with and without metabolic activation.

Mepiquat chloride was tested up to the limit concentration, 5000 µg/ml. Positive controls induced the appropriate response. **There was no evidence of induced increases in chromosomal aberrations over background.**

This study is classified as acceptable. It satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: mepiquat chloride (1,1-dimethylpiperidinium chloride)
Description: white powder
Batch #: N48, ZNT-No. 85/453
Purity: >99% a.i.
Stability of compound: Stable in the carrier (H_2O).
CAS #: 24307-26-4

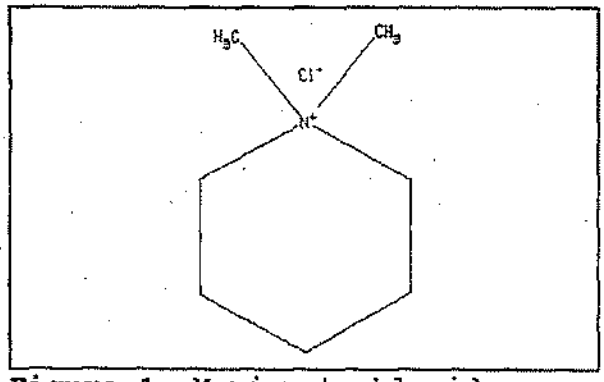

Figure 1 Mepiquat chloride

Solvent used: water

2. Control Materials:
Negative: no treatment
Solvent/final concentration: 10 µl/ml McCoy's 5a culture medium
Positive: Nonactivation (concentrations, solvent): mitomycin C (MMC; 400 ng/ml for range-finding study, 500 ng/ml and 1 µg/ml for main study)

Activation (concentrations, solvent): cyclophosphamide (CP; 20 µg/ml for range-finding study, 25.0 and 50.0 µg/ml for main study); although in main study only one dose was actually analyzed in each of the aberration assays.

3. Activation: S9 derived from

__x__ Aroclor 1254	__x__ induced	__x__ rat	__x__ liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other			___ other

If other, describe below
Describe S9 mix composition (if purchased, give details):

NADP (sodium salt)	1.5 mg
Isocitric acid	2.7 mg
Liver microsomes homogenate (S-9 fraction)	15 µl

4. Test compound concentrations used:
Nonactivated conditions: 166.7 µg/ml, 0.5 mg/ml, 1.7 mg/ml or 5.0 mg/ml for range-finding study and 2.0, 3.0, 4.0 or 5.0 mg/ml for main study.

Activated conditions: 166.7 µg/ml, 0.5 mg/ml, 1.7 mg/ml or 5.0 mg/ml for range-finding study and 2.0, 3.0, 4.0 or 5.0 mg/ml for main study.

5. Test cells: mammalian cells in culture: chinese hamster ovary (CHO-WBI) cells (permanent cell line with an average cycle time of 12 to 14 hours. Obtained from Dr. S. Wolff's laboratory, UCSF and cloned in Dr. A. Bloom's laboratory, Columbia University, New York. Cultures were grown in McCoy's 5a medium supplemented with 10% fetal calf serum, L-glutamine, and antibiotics. Cultures were set up approximately 24 hours prior to treatment by seeding 1.5×10^6 cells per 80 cm^2 plastic flask in 5 ml of fresh medium for the range-finding test.

Properly maintained? Yes

Cell line or strain periodically checked for Mycoplasma contamination? Not stated

Cell line or strain periodically checked for karyotype stability? Not stated

B. TEST PERFORMANCE

1. Preliminary Cytotoxicity Assay: Cultures (5 ml) were exposed to the dose levels stated above. Before fixation, the cultures were examined for degree of confluence and presence of large, rounded mitotic cells. Only those flasks expected to yield at least some dividing cells were fixed.

For the assay without metabolic activation, one day after initiation, cells were treated with mepiquat chloride for 2.25 hours. 5-bromo-2'-deoxyuridine (BrdU) was then added to the cultures to a final concentration of 10 μM. The cultures were incubated in the dark for approximately 23.8 hours. Cell monolayers were washed with phosphate buffered saline and complete medium with BrDU and colcemid (0.1 μg/ml) was added. After a further incubation of 2.5 hours, metaphase cells were collected by mitotic shake-off. Lastly, the cells were swollen with 0.075M KCl hypotonic solution, washed 3 times in fixative (methanol:acetic acid, 3:1), dropped onto slides and air-dried.

For the assay with metabolic activation, the cells were incubated at 37°C for 2 hours in the presence of the test article and the S9 reaction mixture in growth medium without fetal calf serum (FCS). Then the cells were washed with buffered saline and normal growth medium containing 10% FCS and 10 μM BrdU was added (0.1 μg/ml). After 2.5 hours, metaphase cells were collected and fixed as described above.

For estimation of cell cycle delay, the slides were stained for 10 minutes with Hoechst 33258 (5 μg/ml) in phosphate buffer (pH 6.8), mounted in the same buffer and exposed at 55-60°C to "black light" from one 15 Watt tube for approximately 10 minutes and stained with 10% Giemsa for 10 minutes and air-dried.

2. Cytogenetic Assay: Duplicate 10 ml cultures were used for each dose level, in addition to two positive controls and one untreated and one solvent control.

a. Cell treatment:
Cells exposed to test compound, solvent, or positive control for 7.8 hours (nonactivated), 2 hours (activated)

b. Spindle inhibition
Inhibition used/concentration: colcemid at a final concentration of 0.1 μg/ml.

Administration time: 2.3 (nonactivated), 2.25 (activated) hours (before cell harvest)

c. Cell harvest
Cells exposed to test material, solvent or positive control were harvested 2.3 hours after termination of treatment (nonactivated), 8 hours after termination of treatment (activated)

d. Details of slide preparation: Metaphase cells were collected by mitotic shake-off. The cells were then swollen with 0.075M KCl hypotonic solution, washed 3 times in fixative (methanol:acetic acid, 3:1), dropped onto slides and air-dried. The slides were then stained in pH 6.8 buffered 5% Giemsa solution for the analysis of chromosomal aberrations.

e. Metaphase analysis
No. of cells examined per dose: 100 for at least 4 dose levels, the untreated and solvent controls; 25 from one of the positive control cultures.

Scored for structural: Yes

Scored for numerical: N

Coded prior to analysis: Yes

f. Evaluation criteria: the following factors were taken into account in the evaluation of the chromosomal aberration data:

1. The overall chromosomal aberration frequencies
2. The percentage of cells with any aberrations
3. The percentage of cells with more than one aberration
4. Any evidence for increasing amounts of damage with increasing dose, i.e. a positive dose response
5. The estimated number of breaks involved in the production of the different types of aberrations which were observed, i.e., complex aberrations may have more significance than simple breaks.

g. Statistical analysis: Data were evaluated for statistical significance at $p \leq 0.05$, using Chi-square test.

II. REPORTED RESULTS

A. Preliminary cytotoxicity assay: Without metabolic activation a slightly toxic effect was noted at 5.0 mg/ml. Monolayer confluency was reduced by 20%. No toxicity was observed at any of the lower dose levels. An evaluation of cell cycle kinetics indicated that at 5.0 mg/ml there was some inhibition of cell proliferation (66% of the cells were in second mitotic division (M2)). At lower dose levels approximately 100% of the cells were in M2. Based on these results, a regular harvest time was chosen for the nonactivation portion of the study. No toxicity was observed at any dose level with metabolic activation. In addition, no cell cycle delay was observed at any dose

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level. Therefore, a regular harvest time was chosen for the activation portion of the study as well.

B. Cytogenetic assay: Without activation, the report stated that there was a reduction in monolayer confluency of about 20-30% in the cultures treated with mepiquat chloride. The number of rounded mitotic cells was not different from the negative control cultures. There were no increases in cells with aberrations in the treated cultures when compared with the untreated and solvent controls. The positive control compound, Mitomycin-C induced a large amount of chromosome damage. With activation, there was no toxicity at any dose level. Again, there were no increases in cells with aberrations in the treated cultures when compared with the untreated and solvent controls. The positive control, Cyclophosphamide, induced a large statistically significant increase in aberrant cells. The following tables summarize the results.

[MEPIQUAT CHLORIDE] IN VITRO CHROMOSOMAL ABERRATION (84-2)

Chromosome Aberrations in Chinese Hamster Ovary Cells Without Metabolic Activation

Treatment	Cells Scored	Number and Type of Aberration															No. of Aberrations Per Cell	% Cells With Aberrations	% Cells With >1 Aberrations
		Chromatid						Chromosome											
		TB	TD	TF	TR	QR	CR	SB	AF	D	R	MT	PU	E	GT	Other			
Controls Untreated	100																0.00	0.0	0.0
Solvent: McCoy's 5a	100												1				>0.01	1.0	1.0
Positive: Mitomycin-C 500.0 ng/ml	25	4		1	4	2											0.44	28.0	12.0
2.0 mg/ml a	100									1							0.01	1.0	0.0
2.0 mg/ml b	100																0.00	0.0	0.0
3.0 mg/ml a	100																0.00	0.0	0.0
3.0 mg/ml b	100																0.00	0.0	0.0
4.0 mg/ml a	100																0.00	0.0	0.0
4.0 mg/ml b	100																0.00	0.0	0.0
5.0 mg/ml a	100																0.00	0.0	0.0
5.0 mg/ml b	100									1							0.01	1.0	0.0

[MEPIQUAT CHLORIDE] IN VITRO CHROMOSOMAL ABERRATION (84-2)

Chromosome Aberrations in Chinese Hamster Ovary Cells Without Metabolic Activation																			
		Number and Type of Aberration																	
		Chromatid						Chromosome											
Treatment	Cells Scored	TB	TD	TF	TR	QR	CR	SB	AF	D	R	MT	PU	E	GT	Other	No. of Aberrations Per Cell	% Cells With Aberrations	% Cells With >1 Aberrations
Controls: Untreated and Solvent	200												1				>0.005	0.5	0.5
Positive: Mitomycin-C 500.0 ng/ml	25	4		1	4	2											0.440	28.0*	12.0
2.0 mg/ml	200									1							0.005	0.5	0.0
3.0 mg/ml	200																0.000	0.0	0.0
4.0 mg/ml	200																0.000	0.0	0.0
5.0 mg/ml	200[a]																0.005	0.5	0.0

*Significantly greater than solvent and untreated control, $p<0.05$.

[a]Since 0.5% cells have aberrations, there is a missing data point

TB: Chromatid break
TF: Chromatid fragment
D : Dicentric
PU: One or more fragmented chromosomes
TR: Triradial
QR: Quadriradial

[MEPIQUAT CHLORIDE] IN VITRO CHROMOSOMAL ABERRATION (84-2)

Chromosome Aberrations in Chinese Hamster Ovary Cells With Metabolic Activation																			
		Number and Type of Aberration																	
		Chromatid						Chromosome											
Treatment	Cells Scored	TB	TD	TF	TR	QR	CR	SB	AF	D	R	MT	PU	E	GT	Other	No. of Aberrations Per Cell	% Cells With Aberrations	% Cells With >1 Aberrations
Controls Untreated	100																0.00	0.0	0.0
Solvent: McCoy's 5a	100	1															0.01	1.0	0.0
Positive: Cyclophos. 25.0 µg/ml	25	2			2	2		1								TD 3	0.40	24.0	12.0
2.0 mg/ml a	100							1								DM 1	0.02	1.0	1.0
2.0 mg/ml b	100				1											DM 1	0.02	2.0	0.0
30.0 mg/ml a	100									1							0.01	1.0	0.0
30.0 mg/ml b	100																0.00	0.0	0.0
4.0 mg/ml a	100	1															0.01	1.0	0.0
4.0 mg/ml b	100			1													0.01	1.0	0.0
5.0 mg/ml a	100																0.00	0.0	0.0
5.0 mg/ml b	100	1															0.01	1.0	0.0

[MEPIQUAT CHLORIDE] IN VITRO CHROMOSOMAL ABERRATION (84-2)

Chromosome Aberrations in Chinese Hamster Ovary Cells With Metabolic Activation																		
		Number and Type of Aberration																
		Chromatid						Chromosome										
Treatment	Cells Scored	TB	TD	TF	TR	QR	CR	SB	AF	D	R	MT	PU	E	GT	Other	No. of Aberrations Per Cell	% Cells With Aberrations	% Cells With >1 Aberrations
Controls: Untreated and Solvent	200	1															0.005	0.5	0.0
Positive: Cyclophos. 25.0 µg/ml	25	2			2	2		1								ID 3	0.400	24.0*	12.0
2.0 mg/ml	200				1			1								DM 2	0.020	1.5	0.5
3.0 mg/ml	200									1							0.005	0.5	0.0
4.0 mg/ml	200	1		1													0.010	1.0	0.0
5.0 mg/ml	200	1															0.005	0.5	0.0

*Significantly greater than solvent and untreated control, p<0.05.

TB: Chromatid break
TF: Chromatid fragment
D : Dicentric
SB: Chromosome break
PU: One or more fragmented chromosomes
TR: Triradial
QR: Quadriradial
DM: Double minute

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III. REVIEWER'S DISCUSSION/CONCLUSIONS:

This study is adequately conducted. There are no major deficiencies. Mepiquat chloride did not induce a significant increase in aberrant cells under the conditions of the study, either with or without metabolic activation up to a dose of 5.0 mg/ml. The study is acceptable for regulatory purposes and satisfies the guideline requirement for in vitro cytogenetic mutagenicity data.

Primary Review by: Krystyna K. Locke K.K. Locke **Date:** 7/23/96
Review Section I, Toxicology Branch I (7509C)

Secondary Review by: Roger Gardner Roger Gardner **Date:** 8/2/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Rat; OPPTS 870.7485 (85-1)

EPA IDENTIFICATION NUMBERS:

DP Barcode: D221662 Rereg. Case No. 2375
Submission No. S498213 Case No. 819426
P.C. Code No. 109101 Tox. Chem. No. 380 AB

TEST MATERIAL: Mepiquat chloride (1,1-Dimethylpiperidinium chloride), ^{14}C-labeled and unlabeled.

SYNONYMS: Reg. No. 85559; BAS 083 W

CITATION: Holloway, C.J. (1987) Study Report on the Biokinetics and Metabolic Fate of 1,1-Dimethylpiperidinium Chloride in Young Adult Rats. NATEC - Institute for Scientific and Technical Services , Hamburg, West Germany; Report No. 87/0198 and NA 869748; Study Completion Date: June 2, 1987; Unpublished. **MRID 40299001** (Main Study) and **92091014** (Phase 3 Summary).

SPONSOR: BASF Aktiengesellschft, Limburgerhof, Federal Republic of Germany.

EXECUTIVE SUMMARY:

In a metabolism study (MRID 40299001), Mepiquat chloride, labeled with ^{14}C in the 2,6-carbon atoms of the ring structure (radiochemical purity: 98%), was administered to young adult Sprague-Dawley rats (body weight: 200 ± 20 g; 5/sex/group) as follows: **Group A** - single high dose by i.v. route; **Group AA** - single low dose by i.v. route; **Group B** - single low dose by oral (gavage) route; **Group CC** - 14 consecutive daily doses of unlabeled Mepiquat chloride (analytical grade), followed by a single low dose of labeled Mepiquat chloride, all by oral route; and **Group D** - single high dose by oral route. In these five groups, urine and feces were collected at itervals for 7 days in order to determine the excretion pattern of Mepiquat chloride and the form in which it was eliminated. The high dose was approximately 12 mg/kg of body weight (b.w.) and the low dose, 1.2 mg/kg b.w.

The following determinations were made in other groups treated orally with labeled Mepiquat chloride: **Group DX** - exhaled $^{14}CO_2$ in 2 males, for 48 hours following a single high dose; **Group E** - radioactivity in bile, urine and feces collected for 24 hours and then residual radioactivity in carcasses and

G.I. tracts of 3 males and 3 females treated with a single high dose; **Group F** - Like Group E, but using a single low dose; **Group G** - radioactivity in blood (and plasma) obtained at intervals for 4 days from the retroorbital plexus vain of 5 males and 5 females (briefly anesthetized with ether), following treatment with a single low dose; **Group H** - Like Group G, but using a single high dose; **Group J** - radioactivity in tissues (quantitative assay), in 5 males and 5 females, high-dosed consecutively for 7 days and sacrificed (1 male and 1 female) at 8, 24, 48, 96 and 168 hours after the last dose; **Group K** - Like Group J, but qualitative assessment of radioactivity in tissues by whole body autoradiography; **Group L** - Like Group K, but using one male and sacrificing it at 1 hour after the last treatment; and **Group O** - background activity for calculation of detection limits, using 1 male and 1 female after 14 consecutive treatments with unlabeled low dose.

During the study, the rats received a standard diet (pellets) as follows: for body weight ≤ 150 g: 10% of body weight + 3 g; for body weight ≥ 150 g, 10% of body weight + 2 g. On the day of administration of test substance, the rats received a reduced ration of 8 g. Unconsumed food was removed 1 hour before administration and was returned 4 hours after administration of the test substance. Water (bottles) was provided ad libitum.

Mepiquat chloride was absorbed rapidly from the stomach (the highest radioactivity in blood and plasma was detected in less than 1 hour after dosing); was distributed evenly in the intra- and extracellular compartments of the blood; had high bioavailability via the oral route, as revealed by comparisons of radioactivity excreted in urine following oral and intravenous administrations (90-94% at the low dose and 78-89% at the high dose); was excreted mostly in urine (52-84% and 77-99% of the administered radioactivity was detected in 12 hours and 168 hours after dosing, respectively); and did not accumulate in tissues. Other excretions of the administered radioactivity were as follows: feces, 2-15%; exhaled air, ($^{14}CO_2$), 0.20%; and bile, 0.23-0.31%.

The bioavailability of Mepiquat chloride appears to depend on the presence of food in the gastrointestinal tract. In the two male rats used in the study of pulmonary elimination of Mepiquat chloride as ^{14}C-volatiles, which had access to food immediately after dosing, the bioavailability was much lower (58%) than that of a similar treatment group in which food was withheld until 4 hours after dosing (79%). Females were not used in the study of expired radioactivity.

Mepiquat chloride did not accumulate in tissues, as determined by whole body autoradiography and by quantitative determination of radioactivity. By 96 hours after the last dose, all organs and tissues contained < 0.1 ppm of Mepiquat chloride.

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Urine, feces and bile samples from various treatments were used for studies of the metabolic fate of Mepiquat chloride. In all cases, only the unchanged compound could be detected. Therefore, there was no biotransformation of Mepiquat chloride in vivo. The potential metabolites, such as 1-methylpiperidine or piperidine, were not detected.

This study (MRID 40299001) is classified as acceptable and satisfies the guideline requirement for a metabolism study (85-1) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Compound: Mepiquat chloride (1,1-dimethylpiperidinium chloride; BAS 083 W), ^{14}C-labeled in the 2,6-carbon atoms (most stable positions) of the ring structure. Radiochemical purity: 99.08%, as determined by thin layer chromatographic analysis and linear radioscanning. The specific activities of the Mepiquat chloride solutions used in **Groups A, AA, B, CC and D** were 674, 4180, 4245, 4149 and 727 kBq/mg, respectively. The specific activities of the Mepiquat chloride solutions used in the remaining groups ranged from 702 to 4203 kBq/mg (1 uCi = 37 kBq). BASF preparation number and date: 222/4 and 6/24/86, respectively. NATEC (testing facility) storage number: M42/86. Stored at - 20°C until required.

   Nonradioactive Mepiquat chloride: It was reported that non-labeled Mepiquat chloride, NATEC inventory code No. 067/86, was "of BASF quality" and was stored like the labeled Mepiquat chloride. The percentage concentration of the active ingredient (usually 99% in other studies) was not reported in this study.

   Reference Substances: Reference substances for thin-layer chromatographic studies, considered to be possible metabolites of Mepiquat chloride, were 1-methylpiperidine and piperidine, NATEC code numbers 078/86 and 077/86, respectively.

2. Vehicle: Water or saline.

3. Test Animals:

Species: Rat

Strain: Sprague-Dawley

Age and weight at study initiation: Young adult rats (age was not specified) weighing 200 ± 20 g.

Source: Charles River Wiga GmbH, Sandhager Weg 6, D-8741 Sulzfeld 1, Germany.

Housing: During the acclimation period, in communal Macrolon cages; and 5 days before administration of the test substance and during the whole study period, singly, in metabolic cages.

Diet: For body weight ≤ 150 g: 10% of body weight + 3g; for body weight ≥ 150 g: 10% of body weight + 2g. On the day of administration of the test substance, the rats received a reduced ration of 8g. Unconsumed food was removed 1 hour before administration and was returned 4 hours after administration of the test substnce. The actual consumption of food was recorded for each rat throughout the treatment period.

Water: Ad libitum (Macrolon bottles).

Environmental conditions:

Temperature: 22 ± 10°C
Relative humidity: 55 ± 5%
Air changes: 12/hour
Photoperiod: 12 hours (7 am - 7 pm) light.
Acclimation period: At least 1 week.

4. Preparation of dosing solutions: For oral dosing, Mepiquat chloride was dissolved in water and for intravenous dosing, in saline.

B. STUDY DESIGN AND METHODS:

1. Group Arrangements: Animals were assigned randomly to the test groups as shown in TABLE 1.

Mepiquat chloride **Metabolism Study 85-1**

TABLE 1. Dosing Groups for Pharmacokinetic Studies with Mepiquat Chloride

Test Group	Dose of Labeled Material (mg/kg)	Number/ Sex	Remarks/Experimental Aims
Intravenous			
Group A	13.6 ♂ 16.1 ♀	5	Single dose ■
Group AA	1.3 ♂+♀	5	Single dose ■
Low Dose (Gavage)			
Group B	1.2 ♂+♀	5	Single dose ■
Group F	1.3 ♂+♀	3	Single dose ■■
Group G	1.2 ♂+♀	5	Single dose ■■■
Low Dose with Pretreatment (Gavage)			
Group CC	1.2 ♂+♀	5	Doses: 14 unlabeled and 1 labeled ■
High Dose (Gavage)			
Group D	12.5 ♂ 12.0 ♀	5	Single dose ■
Group DX	11.6 ♂	2	Single dose #
Group E	11.8 ♂ 11.3 ♀	3	Single dose ■■
Group H	12.0 ♂ 13.1 ♀	5	Single dose ■■■
Group J	11.7 ♂ 12.0 ♀	5	7 Doses ##
Group K	11.6 ♂ 12.3 ♀	5	7 Doses ###
Group L	8.3 ♂	1	7 Doses ♥
Group O	--	1	14 Unlabeled low (1.2 mg/kg) doses ●

This table is based on Table II, pages 135-158, of the submitted report (MRID 40299001). The experimental aims were as follows:

■ Excretion pattern of Mepiquat chloride and the form in which it was eliminated: ^{14}C was determined in urine and feces for 168 hours (7 days) after dosing, and in tissues on day 7.

■■ Biliary excretion: Determination of ^{14}C in bile, urine and feces for 24 hours after dosing, and in carcass and G.I. tract, at 24 hours.

■■■ Blood profiles: ^{14}C was determined at intervals in blood and plasma for 96 hours (4 days) after dosing.

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Collection of volatiles (CO_2) for 48 hours after dosing.

Quantitation of tissue residues: Rats were dosed for 7 days with ^{14}C-Mepiquat chloride and then 1 male and 1 female were sacrificed at 8, 24, 48, 96 and 168 hours after the last dose for the determination of ^{14}C in tissues.

Like ##, but qualitative assessment of the tissue distribution of ^{14}C by whole body autoradiography.

♥ One male rat was treated with ^{14}C-Mepiquat chloride for 7 days and then was sacrificed at 1 hour after the last dose for whole body autoradiography.

● Background radiactivity determination for calculation of detection limits: 1 male and 1 female were dosed (gavage) for 14 days with unlabeled Mepiquat chloride and then sacrificed for tissue analysis (for ^{14}C).

Note: On the basis of toxicological data provided by the sponsor, dose levels of 12 mg/kg (low dose or no effect level) and 120 mg/kg (high dose) were originally selected for this study. However, because of "mild toxic effects" observed in the 12 mg/kg group and mortalities occurring in the 120 mg group, the original protocol was amended, and the new low dose and high dose selected were 1.2 mg/kg and 12 mg/kg, respectively.

2. Dosing and Sample Collection: Intragastral (gavage) administration was performed using a solution of the appropriate quantities of labeled and/or unlabeled Mepiquat chloride in water. In the case of the unlabeled Mepiquat chloride, the quantities administered were assessed from the volume of solution injected. In the case of the labeled Mepiquat chloride, the syringes with gavage device were weighed before and after administration to allow precise determination of the quantity applied. The radioactivity concentration was determined by weighing out and measuring at least 2 aliquots of the test substance solution.

   For intravenous administration, Mepiquat chloride was dissolved in saline and injected into the tail vain. The radioactivity concentration of these solutions were also determined in at least 2 aliquots. Also, the syringes were weighed before and after injection to determine the quantity of Mepiquat chloride injected.

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Urine and feces were collected into the preweighed glass vessels, attached to the bases of the plexiglass metabolic cages in which the rats were housed singly from the acclimation period until sacrifice. The collection intervals were 0-12, 12-24, 24-48, 48-72, 72-96 and 96-168 hours after dosing. When the rats were removed for terminal sacrifice, the entire apparatus was washed with water and the washes checked for radioactivity.

Rats designated for blood **and plasma ^{14}C profiles** (Groups G and H) were also housed individually in the metabolic cages. At the sampling times, the rats were removed from the cages, briefly anesthetized with ether and at least 0.3 ml of blood was collected from the retroorbital plexus vain into heparinized tubes. About half of the sample was retained for radioactivity determination in whole blood and the other half was centrifuged to obtained plasma. The sampling intervals for both groups were 1, 2, 4, 8, 12, 24, 48 and 96 hours after dosing, and for Group G also at 20 and 40 minutes after dosing.

Catheterization of the bile duct was performed on rats in deep anesthesia. A detailed step-by-step description of how this was done is on page 041 of the submitted report (MRID 40299001). After complete recovery from the anesthetic (4-5 hours after completion of the operation), which was defined as the time when the animals spontaneously began to drink, Mepiquat chloride was administered and the bile was collected continuously with sampling intervals of 1 hour, up to the terminal sacrifice at 24 hours after dosing.

Terminal sacrifice was always performed by exsanguination under ether anesthesia. The rats were then dissected and organ and tissues collected as defined in the aims for each treatment group. The following organs and tissues were collected and weighed for Groups A, AA, B, CC, D, J and O: Gastrointestinal (G.I.) tract (complete with contents), bone, brain, fat, uterus, testes, heart, kidneys, liver, lungs, muscle, spleen, skin with fur, adrenals, urinary bladder with contents, thyroids and residual carcass. For Groups E and F, the entire G.I. tract was removed and the remainder was treated as residual carcass.

The procedures for **whole body autoradiography** (freezing in dry ice-ethanol, embedding in sodium carboxymethylcellulose, sectioning, freeze-drying of the sec-

tions, exposure to film under deep-freeze conditions for 120-480 hours and developing of the film) are detailed on pages 044-049 of the submitted report (MRID 40299001).

^{14}C-radioactivity was determined in all samples by liquid scintillation counting. In the case of urine, plasma and bile, direct measurements in scintillation cocktail were made. Feces were combusted prior to determination of radioactivity as $^{14}CO_2$. Tissues and blood were solubilized before counting.

3. Metabolite Characterization: Urine, feces and bile from various treatments (Groups A, AA, B, CC, D, F and E) were employed for studies of the metabolic fate of Mepiquat chloride. Urine and bile samples were applied directly (without pretreatment) on Silicagel G thin-layer plates but, in the case of fecal samples, 2N HCl:aqueous methanol extracts (concentrated) were used. The plates were then developed (one- or two-dimensional separations) in one or two of the following solvent systems (volume proportions): methanol/water/HCl 50:50:0.5; methanol/chloroform/HCl 60:70:0.5; and methanol/acetone/HCl 90:10:4. After development and drying of the plates, one-dimensional separations were visualized by radiometric scanning and autoradiography, whereas two-dimensional separations were visualized only by autoradiography. Unlabeled Mepiquat chloride and reference compounds (potential metabolites), 1-methylpiperidine and piperidine, were located by staining the plates with Dragendorf's reagent and/or Ninhydrin spray reagent.

4. Statistics: With the exception of means and standard deviations, no reference is made to statistical analyses in this study.

II. RESULTS

A. PHARMACOKINETIC STUDIES:

1. Toxic Signs: Mild toxic symptoms were observed in most of the rats at the high dose level of Mepiquat chloride (12 mg/kg). These symptoms (twitching of the ears and head, and lethargy) appeared within about 30 min. after dosing and lasted for 2-3 hours. No such symptoms were observed in the low dose (1.2 mg/kg)

groups. No macroscopic abnormalities were observed in any group at necropsy.

2. Absorption: Mepiquat chloride was rapidly absorbed from the stomach. The highest radioactivity in blood and plasma was detected in less than 1 hour after dosing, and 61-74% (males) and 52-66% (females) of the administered radioactivity appeared in urine within 12 hours after dosing. These data are summarized in TABLES 2 and 3.

TABLE 2. Radioactivity Concentrations in Whole Blood and Plasma as (ppm) Equivalents of Mepiquat Chloride at Various Sampling Times After Dosing

Test Group	Dose (mg/kg)	Hours After Dosing: 0.33	0.67	1	2	4	8
		Blood					
G ♂	1.2	0.086	0.155	0.155	0.115	0.047	0.004
G ♀	1.2	0.138	0.197	0.194	0.131	0.044	0.007
H ♂	12.0	-	-	1.865	1.210	0.391	0.048
H ♀	13.1	-	-	1.817	1.129	0.333	0.040
		Plasma					
G ♂	1.2	0.103	0.207	0.191	0.117	0.042	0.005
G ♀	1.2	0.212	0.245	0.235	0.132	0.036	0.006
H ♂	12.0	-	-	2.370	1.227	0.369	0.053
H ♀	13.1	-	-	2.167	1.172	0.309	0.035

This table is based on Table VIII, pages 198-205, of the submitted report (MRID 40299001). 0.33 hour = about 20 minutes and 0.67 hour = about 40 minutes. - Not reported.

Mepiquat chloride concentrations in blood and plasma approached the detection limit of 0.0004 ppm at 24 hours after dosing for Group G, and the detection limit of 0.002 ppm at 48 hours after dosing for Group H.

TABLE 3. Radioactivity (Percent of Administered Dose and Standard Deviations) Recovered in Urine During the First 12 Hours After Dosing

Test Group	Dose (mg/kg) Males	Females	Recoveries Males	Females
A	13.6	16.1	84.36 ± 2.78	73.12 ± 16.17
AA	1.3	1.3	81.53 ± 3.66	72.54 ± 19.44
B	1.2	1.2	62.96 ± 19.38	51.95 ± 14.83
CC	1.2	1.2	74.22 ± 6.95	62.84 ± 8.24
D	12.5	12.0	60.84 ± 10.53	66.45 ± 10.82

This table is based on Table III, pages 159-168, of the submitted report (MRID 40299001). ^{14}C-Mepiquat chloride was administered intravenously in Groups A and AA, and orally (gavage) in the remaining groups. Additional information about dosing is on page 5 of this review.

The finding that the autoradiographs showed little residual radioactivity in the stomach also supported the rapid absorption of Mepiquat chloride.

3. Tissue Distribution: Mepiquat chloride did not accumulate in tissues, as determined by whole body autoradiography (Groups K and L) and by quantitative determination of radioactivity (liquid scintillation counting after homogenization and solubilization of tissues; Groups J, A, AA, B, CC and D). The following tissues were examined: G.I. tract with contents, bone, brain, fat, uterus, testes, heart, liver, kidneys, lungs, muscle, spleen, skin, adrenals, urinary bladder, thyroid and carcass. Blood and plasma were also examined in each of these groups. In Group J, radioactivity was determined at 8, 24, 48, 96 and 168 hours after dosing and in the remaining groups, at 168 hours after dosing.

   After 7 consecutive treatments with high dose of Mepiquat chloride (Group J), the concentrations of Mepiquat chloride (ppm) in tissues at 8 hours after the final dose were as follows: G.I. tract (17-25); heart, kidneys, liver, lungs, adrenals and thyroid (0.3-1.8); and the remaining tissues (0.02-0.88). By 48 hours after the last dose, most tissues contained < 0.1 ppm of Mepiquat chloride. These data are summarized in Attachment I (this review).

In Group A, most tissues contained < 0.1 ppm of Mepiquat chloride, and in Groups AA, B, CC and D, < 0.01 ppm. These data are summarized in Attachment II (this review).

4. Excretion: The major route of excretion of Mepiquat chloride when administered parenterally (i.v.) or orally (gavage) was in urine. The excretion was rapid (half-life = < 12 hours) and the elimination characteristics (percent of the administered radioactivity excreted at the following time intervals after dosing: 0-12, 12-24, 24-48, 48-72, 72-96 and 96-168 hours) were similar for the low dose and high dose male and female groups.

   The fecal excretions in Groups A, AA, B, CC and D ranged from 1.85% to 14.73% of the administered dose and most of that radioactivity (69-98%) was recovered during the first 48 hours after dosing. Since defecation was less regular than urination, considerable individual and group mean differences were observed.

   Pulmonary excretion ($^{14}CO_2$) and billiary excretion were minor routes of elimination of Mepiquat chloride. After one high oral dose of Mepiquat chloride, 0.20 ± 0.21% of the administered radioactivity was recovered as "volatiles" during the 48-hour collection period (Group DX). The recoveries in bile were also very low during the 24-hour collection period: 0.24 ± 0.05% and 0.23 ± 0.08% of the administered radioactivity for males and females, respectively, after one high oral dose; and 0.27 ± 0.02% and 0.31 ± 0.09% of the administered radioactivity for males and females, respectively, after one low oral dose. The excretion data are summarized in TABLES 4 AND 5.

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TABLE 4. Recovery of Radioactivity in Tissues and Excreta of Rats After Parenteral (I.V.) Administration of ^{14}C-Labeled Mepiquat Chloride

Test Group		A	AA
Dose (mg/kg):	Males	13.6	1.3
	Females	16.1	1.3
		Percent of Radioactive Dose Recovered	
Urine *	Males	98.83 ± 2.01	95.87 ± 1.59
	Females	95.20 ± 6.40	93.36 ± 4.70
Feces	Males	1.85 ± 0.92	2.36 ± 1.60
	Females	5.85 ± 6.74	4.12 ± 3.45
Cage Washes	Males	4.26 ± 1.22	2.11 ± 0.90
	Females	6.54 ± 3.39	3.29 ± 2.51
Tissues **	Males	0.11 ± 0.02	0.07 ± 0.04
	Females	0.28 ± 0.17	0.16 ± 0.14
Total	Males	100.78 ± 2.13	98.30 ± 1.52
	Females	101.32 ± 2.86	97.64 ± 1.40

This table is based on Table VI, pages 180-183, of the submitted report (MRID 40299001). Rats in each group received a single dose of Mepiquat chloride as noted in the table and were sacrificed on day 7 (168 hours)) after dosing.

* Recoveries of radioactivity include cage washes.
** Carcass, blood and plasma are included in these recoveries.

TABLE 5. Recovery of Radioactivity in Tissues and Excreta of Rats After Oral (Gavage) Administration of ^{14}C-Labeled Mepiquat Chloride

Test Group		B	CC	D
Dose (mg/kg):	M	1.2	1.2	12.5
	F	1.2	1.2	12.0
		Percent of Radioactivity Recovered		
Urine *	M	85.91 ± 7.93	90.08 ± 4.28	77.28 ± 12.58
	F	87.53 ± 3.90	88.42 ± 6.20	84.26 ± 6.59
Feces	M	14.98 ± 7.98	8.16 ± 3.92	14.73 ± 7.20
	F	10.57 ± 3.49	8.74 ± 6.48	13.27 ± 5.38
Cage Washes	M	3.24 ± 2.75	2.21 ± 2.02	3.09 ± 1.92
	F	7.71 ± 5.76	7.13 ± 2.91	2.30 ± 0.94
Tissues **	M	0.05 ± 0.01	0.08 ± 0.08	0.04 ± 0.01
	F	0.16 ± 0.12	0.08 ± 0.04	0.10 ± 0.04
Total	M	100.93 ± 1.54	98.32 ± 1.03	92.05 ± 6.81
	F	98.25 ± 1.11	97.25 ± 2.09	97.62 ± 3.60

This table is based on Table VI, pages 184-189, of the submitted report (MRID 40299001). Rats in Group CC received 15 doses of Mepiquat chloride (14 labeled and 1 unlabeled), and those in Groups B and D, single doses as noted in the table. Rats in these three groups were sacrificed on day 7 (168 hours) after dosing.

* Recoveries of radioactivity include cage washes.
** Carcass, blood and plasma are included in these recoveries.

5. Bioavailability: Mepiquat chloride had high bioavailability via the intragastral (oral) route, as revealed by comparisons of radioactivity excreted in urine following oral and intravenous administration. In the low dose Group B, the bioavailability was 90-94% and in the high dose Group D, 78-89%. The bioavailability at the low dose level was slightly higher than at the high dose level, and female rats gave slightly higher bioavailabilities than male rats. In these groups, and in the intravenously-treated Groups A and AA, the food was withheld for one hour before dosing and was returned 4 hours after dosing.

The bioavailability of Mepiquat chloride appears to be highly dependent on the presence of food in the gastrointestinal tract. In the two male rats used in the study of excretion of ^{14}C-volatiles (Group DX), which had access to food immediately after dosing, the bioavailability was much lower (58%) than that of Group D (79%), a similar treatment group, in which food was withheld until 4 hours after dosing. The bioavailability data are summarized in TABLE 6.

TABLE 6. Bioavailability Data

Test Group	Dose mg/kg		Percent of Radioactivity Excreted in Urine #	Bioavailability Percent
A ♂	13.6	i.v.	98.83	-
A ♀	16.1	i.v.	95.20	-
D ♂	12.5	i.g.	77.28	78.19
D ♀	12.0	i.g	84.26	88.51
AA ♂	1.3	i.v	95.87	-
AA ♀	1.3	i.v.	93.36	-
B ♂	1.2	i.g.	85.91	89.61
B ♀	1.2	i.g	87.53	93.76
A ♂	13.6	i.v.	91.09 ##	-
DX ♂	11.6	i.g.	52.77 ##	57.93
D ♂	12.5	i.g	71.75 ##	78.77

This table is based on data from pages 86-87, 160, 168 and 179 of the submitted report (MRID 40299001). # During 168 hours and ## 48 hours, respectively, after dosing. i.v. = intravenous and i.g. = intragastral (gavage).

B. METABOLITE CHARACTERIZATION STUDIES:

Metabolite characterization is described on page 8 of this review (METHODS section). Only unchanged Mepiquat chloride was identified in the urine and feces of rats in Groups A, AA, B, CC, D, F and E; and in the bile of rats in Groups F and E. Mepiquat chloride was, therefore, not metabolized by the rats. The residual levels of radioactivity in organs and tissues were too low for metabolite characterization.

III. **DISCUSSION**

Using ^{14}C-labeled and unlabeled Mepiquat chloride, two doses (approximately 1.2 and 12.0 mg/kg), two types of dosing (oral and intravenous), two treatment regimens (single and multiple dosing) and 13 test groups of Sprague-Dawley rats, the following findings were obtained: **(1)** Mepiquat chloride was rapidly absorbed from the stomach (the highest radioactivity in blood and plasma was detected in less than 1 hour after dosing); **(2)** Mepiquat chloride was not metabolized *in vivo*, but was excreted unchanged mostly in urine (77-99% of the administered radioactivity); **(3)** The excretion was rapid and most of the administered radioactivity (52-84%) was detected in urine, in males and females, within the first 12 hours after dosing; **(4)** Fecal excretions of the administered radioactivity were smaller with intravenous dosing (1.8-5.8%) than with oral dosing (8.2-15.0%); **(5)** Exhaled air, bile and tissues contained very little radioactivity (0.20, 0.23-0.31 and 0.04-0.28% of the dose, respectively); **(6)** Mepiquat chloride had high bioavailability (78-94 %), as determined by comparisons of radioactivity excreted in urine following oral and intravenous administration, and withholding of food for 4 hours after dosing; and **(7)** The presence of food in the gastrointestinal tract immediately after dosing decreased bioavailability to 58%.

This study is scientifically sound and is reported in a very detailed manner, but having 13 groups instead of the required 3-5, makes it very involved and very much time-consuming to review. There are no major deficiencies, only one omission and two errors, as follows:

a. It was reported that the nonradioactive Mepiquat chloride was "of BASF quality", but the purity was not reported.

b. It was reported in the METHODS section (page 34) that the rats in Group J were sacrificed at 8, 24, 48, 96 and 168 hours after dosing. However, in the RESULTS section (page 93), it was reported that these rats were sacrificed at 8, 24, 48 and 96 hours after dosing.

c. Table III of the submitted report (MRID 40299001), pages 202-203, shows radioactivity concentrations in plasma of the rats in Group H. These concentrations should be for whole blood (and not plasma).

The above deficiencies are not serious enough to affect the classification of this study as Acceptable.

ATTACHMENT I

Concentration (ppm) of Mepiquat Chloride in Tissues After 7 Consecutive Treatments with High Dose (Group J)

Tissue		Time Interval After Last Dose 8 hrs	24 hrs	48 hrs	96 hrs	168 hrs
G.I.	♂ #	17.10	1.46	0.12	0.05	0.01
	♀ #	25.41	0.97	0.08	0.40	0.09
Bone	♂	0.04	0.01	0.01	*	*
	♀	0.10	0.01	*	*	*
Brain	♂	0.02	0.01	0.01	0.01	0.01
	♀	0.04	0.01	0.01	0.01	0.01
Fat	♂	0.20	0.04	0.04	0.02	0.02
	♀	0.38	0.05	0.02	0.02	0.02
Uterus	♂	-	-	-	-	-
	♀	0.54	0.05	0.02	0.03	0.02
Testes	♂	0.33	0.19	0.12	0.05	0.02
	♀	-	-	-	-	-
Heart	♂	0.44	0.10	0.04	0.02	0.09
	♀	1.44	0.08	0.02	0.03	0.01
Kidney	♂	1.11	0.55	0.24	0.06	0.07
	♀	1.75	0.19	0.05	0.10	0.02
Liver	♂	0.65	0.18	0.09	0.03	0.03
	♀	1.61	0.10	0.04	0.05	0.02
Lungs	♂	0.26	0.25	0.30	0.02	0.04
	♀	1.17	0.07	0.03	0.03	0.01
Muscle	♂	0.78	0.56	0.33	0.15	0.05
	♀	0.76	0.53	0.22	0.10	0.03
Spleen	♂	0.43	0.06	0.03	0.02	0.02
	♀	0.55	0.06	0.02	0.02	0.01
Skin	♂	0.51	0.13	0.06	0.03	0.02
	♀	0.67	0.39	0.06	0.43	0.11
Adrenals	♂	0.94	0.14	0.06	0.03	0.02
	♀	1.75	0.12	0.04	0.06	0.02
Bladder	♂ ##	0.59	1.79	0.27	0.03	0.04
	♀ ##	0.75	0.09	0.03	0.03	0.02
Thyroid	♂	0.91	0.27	0.12	0.03	0.04
	♀	1.85	0.27	0.10	0.08	0.02
Carcass	♂	0.81	0.44	0.28	0.12	0.04
	♀	0.88	0.65	0.13	0.11	0.03

This table is based on Table IX, pages 206-207, of the submitted report (MRID 40299001). * Values at or below the detection limit. # Gastrointestinal tract with contents. ## Urinary.

High dose = 11.7 and 12.0 mg of Mepiquat chloride/kg of body weight/day for males and females, respectively.

ATTACHMENT II

Concentration (ppm) of Mepiquat Chloride in Tissues at 168 Hours (7 Days) After Dosing

Tissue		Test Group A	AA	B	CC	D
G.I.	♂ #	0.012	0.002	0.001	0.001	0.006
	♀ #	0.124	0.006	0.002	0.001	0.034
Bone	♂	*	*	*	*	*
	♀	0.003	*	*	*	*
Brain	♂	0.003	*	*	*	*
	♀	0.004	*	*	*	*
Fat	♂	0.004	*	*	*	0.003
	♀	0.008	*	*	*	*
Uterus	♂	-	-	-	-	-
	♀	0.027	0.001	*	0.001	0.012
Testes	♂	0.009	0.001	0.001	0.001	0.006
	♀	-	-	-	-	-
Heart	♂	0.006	0.001	*	*	*
	♀	0.030	0.001	*	*	0.004
Kidney	♂	0.057	0.006	0.001	0.001	0.008
	♀	0.109	0.007	0.001	0.001	0.009
Liver	♂	0.026	0.004	0.001	0.001	0.004
	♀	0.034	0.002	*	*	0.005
Lungs	♂	0.015	0.001	*	*	*
	♀	0.012	0.001	*	*	0.003
Muscle	♂	0.021	0.001	0.001	0.002	0.011
	♀	0.026	0.002	0.002	0.001	0.014
Spleen	♂	0.011	0.001	*	*	0.003
	♀	0.019	0.002	*	0.001	0.004
Skin	♂	0.004	*	*	*	*
	♀	0.020	0.002	0.005	0.001	0.008
Adrenals	♂	0.008	0.001	0.001	*	0.004
	♀	0.023	0.001	0.001	0.001	0.007
Bladder	♂ ##	0.007	0.001	0.001	0.001	0.008
	♀ ##	0.024	0.001	*	*	0.005
Thyroid	♂	0.007	0.001	*	0.001	*
	♀	0.015	0.001	*	0.001	0.006
Carcass	♂	0.014	0.001	0.001	0.001	0.006
	♀	0.035	0.001	0.001	0.001	0.008

This table is based on Table IX, pages 208-217, of the submitted report (MRID 40299001). * Values at or below the detection limit. # Gastrointestinal tract with contents. ## Urinary. Mepiquat chloride (mg/kg/day): A 13.3 ♂ and 16.1 ♀; AA 1.3 ♂+♀; B and CC 1.2 ♂+♀; D 12.5 ♂ and 12.0 ♀. Groups A and AA were dosed

by i.v. and the remaining groups, orally. Multiple dosing was used in Group CC and single dosing in other groups.

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