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

JAN 22 1996

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

February 1, 1996

MEMORANDUM

SUBJECT: Mepiquat Chloride - Review of a 2-Generation Rat Reproduction Study

DP Barcode No. D208108 Rereg. Case No. 2375
Chemical Code No. 109101 Tox. Chem. No. 380 AB
CAS Registry No. 24307-26-4
Sponsor: BASF Corporation, Agricultural Products Group, Research Triangle Park, NC

FROM: Krystyna K. Locke, Toxicologist
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THRU: Roger L. Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (7509C) Roger Gardner 2/14/96

Karl P. Baetcke, Branch Chief
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Health Effects Division (7509C) KPB 2/20/96

TO: Kathryn Davis/Ruby Whiters, PM 52
Accelerated Reregistration Branch
Special Review and Reregistration Division (7508W)

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

Guideline No.	MRID No.	Acceptability
83-4	43378601	Yes

In this 2-generation reproduction study, Wistar strain rats, 25/sex/dose, were fed Mepiquat Chloride in their diets at concentrations of 0, 500, 1500 or 5000 ppm for 10 weeks (F_0) or 14 weeks (F_1) before mating, and during mating, gestation and lactation. The doses corresponding to the dietary concentrations were 51.2/48.6, 153.1/146.6 and 499.3/574.5 mg/kg/day, respectively, for the F_0/F_1 males and 54.0/53.3, 163.6/162.0 and 530.0/626.5 mg/kg/

day, respectively, for the F_0/F_1 females.

No treatment-related systemic effects occurred in male and female rats receiving 500 or 1500 ppm of Mepiquat Chloride. The following effects were observed in the 5000 ppm group: **(1)** Impaired neurological functions (tremors, hypersensitivity upon handling and decreased forelimb strength in the F_0 and F_1 dams; and decreased hindlimb grip strength in the F_0 and F_1 dams and F_1 males; **(2)** Decreased body weights of the F_0 and F_1 males and females; **(3)** Decreased food consumption of the F_1 males and F_0 and F_1 females; and **(4)** Retarded growth of the F_1 and F_2 pups. Plasma, erythrocyte and brain cholinesterase activities were not affected by treatment with the test material.

Based on the above findings (neurological impairment, decreased body weight and body weight gain in the adults, and retarded growth of the pups), **the systemic NOEL is 1500 ppm (mg/kg/day: 150 for males and 163 for females) and the systemic LOEL is 5000 ppm (mg/kg/day: 537 for males and 578 for females).** The mg/kg/day values are averages of the F_0 and F_1 values noted above.

There were no treatment-related effects on reproductive parameters. Therefore, **the NOEL for reproductive toxicity is > 5000 ppm.**

Although this study did not establish a reproductive NOEL, it is classified as **Acceptable (Core-Guideline)** and satisfies the guideline requirements for a multigeneration reproduction study (83-4). Because of the severe toxic effects observed at the 5000 ppm dose, the rats could not have tolorated a higher dose.

DATA EVALUATION REPORT

MEPIQUAT CHLORIDE

STUDY TYPE: MULTIGENERATION FEEDING - RAT (83-4)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Health Sciences Research Division
Oak Ridge National Laboratory*
Oak Ridge, TN 37831
Task Order No. 95-07H

Primary Reviewer:
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Disclaimer

This final DER may have been altered by the Health Effects Division subsequent to signing by Oak Ridge National Laboratory personnel.

*Managed by Lockheed Martin Marietta Energy Systems for the U.S. Department of Energy under Contract No. DE-AC05-84OR21400.

[MEPIQUAT CHLORIDE]

Reproduction Study (83-4)

EPA Reviewer: W. Greear, M.P.H., D.A.B.T.
Review Section IV, Toxicology Branch I (7509C)
EPA Section Head: M. Copley, D.V.M., D.A.B.T.
Review Section IV, Toxicology Branch I (7509C)

M Copley, Date 1/4/95

Ron Gardner, Date 12/14/95

DATA EVALUATION REPORT

STUDY TYPE: Multigeneration Reproduction - Rat (83-4)

TOX. CHEM. NO: 380AB

P.C. CODE.: 109101

D.B. BAR CODE: D [redacted] 208108

MRID NO.: 433786-01

TEST MATERIAL: Mepiquat chloride

SYNONYMS: 1,1-Dimethylpiperidinium chloride, BAS-083, BAS 85559X, Pix

STUDY NUMBER: 93/10983

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

TESTING FACILITY: BASF Aktiengesellschaft, Department of Toxicology, D-67056 Ludwigshafen/Rhine, FRG

TITLE OF REPORT: Reproduction Toxicity Study With Mepiquat Chloride in Rats - Continuous Dietary Administration Over 2 Generations

AUTHOR: J. Hellwig

REPORT ISSUED: September 2, 1993 (study completion date)

EXECUTIVE SUMMARY: Groups of 25 male and 25 female Wistar rats were fed mepiquat chloride in their diets at concentrations of 0, 500, 1500, or 5000 ppm for 10 weeks (F_0) or 14 weeks (F_1) before mating, and during mating, gestation, and lactation. The F_0 parents were mated a second time 2 weeks after weaning the first litter. The doses corresponding to the dietary concentrations are 51.2 and 48.6, 153.1 and 146.6, and 499.3 and 574.5 mg/kg/day, respectively for F_0 and F_1 males and 54.0 and 53.3, 163.6 and 162.0, and 530.0 and 626.5 mg/kg/day, respectively for F_0 and F_1 females.

No treatment-related systemic effects occurred in male or female rats receiving 500 or 1500 ppm of the test material. In animals receiving 5000 ppm (high-dose), effects indicative of impaired neurological function included tremors and hypersensitivity upon handling in 70-85% of F_0 and F_1 dams; decreased forelimb strength (7 to 17%, $p<0.01$) and hindlimb grip strength (11 to 16%, $p<0.01$) in F_0 and F_1 dams before mating, during lactation or after weaning; and decreased hindlimb grip strength (15%, $p<0.01$) in high-dose F_1 males. Mean body weights of the high-dose F_0 males were reduced by 10-11% ($p<0.01$) relative to controls during the entire 29-week treatment period. Mean body weights of high-dose F_1 males were reduced by about 50% relative to controls at the start of the premating period; thereafter the animals steadily gained weight such that at the end of the premating period overall body weight gain was only slightly reduced (15%). Food consumption in high-dose F_1 males was reduced by 15% ($p<0.01$) compared with controls. Mean body weight of high-dose F_0 females was reduced by 7% ($p<0.05$) and body weight gain by 11% ($p<0.05$) during the premating period. Gestation body weight of F_0 females was reduced by 10% or less and body weight gain by 12% or less. High-dose F_0 dams loss weight during lactation, and weight gain of their F_1 pups was reduced by as much as 39% during the same period. High-dose F_1 dams lost weight during lactation, and weight gain of the F_2 pups was reduced by 36%. Food consumption by the high-dose dams was reduced by 31 to 33% during lactation. Changes in hematologic, clinical chemistry, and urinalysis parameters in the adult high-dose rats were unrelated to dose, biologically insignificant, or were due to the reduced body weight. Plasma, erythrocyte, and brain cholinesterase activities were not affected by treatment with the test material. Decreased liver and kidney weights and decreased incidence of lipid storage in the liver in observed in high-dose males and females were consistent with the decreased terminal body weights and are unlikely to be due to toxicity of the test material. A significant number of high-dose F_1 and F_2 pups were slow in reaching developmental milestones (pinna unfolding, opening of the auditory canal and eyes, and gripping reflex), but these effects are attributed to retarded growth of the pups.

The LOEL for systemic toxicity is 5000 ppm for male and female rats based on neurological impairment, decreased body weight and body weigh gain in the adults, and retarded growth of F_1 and F_2 pups. The corresponding NOEL is 1500 ppm.

There were no treatment-related effects on reproductive parameters.

The NOEL for reproductive toxicity is >5000 ppm.

This study is classified as core - guideline and it satisfies the requirements for a multigeneration reproduction feeding study (83-4). This study did not establish a reproductive NOEL; however, because of the impairment of neurological function and the severe effects on body weight and body weight gain, the animals could not have tolerated a higher dose. Therefore, this study is considered acceptable and receives a core - guideline rather than supplementary - upgradable classification.

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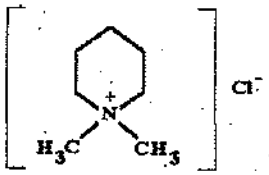
Special Review Criteria (40 CFR 154.7) None

I. MATERIALS AND METHODS

A. MATERIAL

1. Test material: Mepiquat chloride
   Description: liquid
   Lot/Batch No.: WW 262/CP 1490
   Purity: 57.9% a.i. (w/w)
   Stability of compound: >2 years
   CAS No.: 24307-26-4
   Structure:



2. Vehicle and/or positive control

   The test material was mixed with the food (Kliba 343 meal); no additional vehicle was required. a positive control was not included.

3. Test animals

   Species: rat
   Strain: Wistar (Chbb = THOM (SPF))
   Age and weight at start of study: 34 ± 1 days old, 102 - 119 g (males), 93 - 115 g (females)
   Source: Karl THOMAE, Biberach an der Riss, FRG
   Housing: individually housed except during mating and lactation; they were housed in DK III stainless steel wire mesh cages except the males during mating and females between gestation day (gd) 18 and lactation day 14 were housed in Makrolon type III cages.
   Environmental conditions:
   Temperature: 20 - 24°C
   Humidity: 30 - 70%
   Air Changes: not reported
   Photoperiod: 12 hours light/12 hours dark
   Acclimation period: 6 days

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4. Diet preparation and analysis

Diet was prepared at intervals not exceeding 32 days. The test substance was weighed and appropriate amounts (equivalent to 100% a.i.) were mixed with a small amount Kliba 343 meal using a household mixer; an appropriate amount of Kliba 343 meal was then added to obtain the target concentration and the preparation mixed for 10 min in a laboratory mixer. Homogeneity and stability in the diet were determined before start of the study. Samples of treated food were analyzed four times during the study for verification of concentration.

Results -

a. Homogeneity analysis - According to the study authors, homogeneity was confirmed. No data were presented.

b. Stability analysis - According to the study authors, the diet was stable 32 days. No data were presented.

c. Concentration analysis - The concentration of mepiquat chloride ranged from 82.5 to 95% of target in the 500-ppm diet, 89.7 to 97.9% of target in the 1500-ppm diet, and 95.3 to 99.5% of target in the 5000-ppm diet.

5. Diet

Animals received food (ground Kliba maintenance diet rat/mouse/hamster GLP 343 meal) and water *ad libitum*.

B. PROCEDURES AND STUDY DESIGN

1. Animal assignment

F_0 animals were randomly assigned to test groups as seen in Table 1. The 25 F_1 parents of each sex were randomly selected from the F_1 litters before weaning.

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TABLE 1. ANIMAL ASSIGNMENT

Dose Group	Conc. in diet[a,b] (ppm)	No. of Animals per Group[c]	
		Male	Female
0 (Control)	0	25	25
1 (Low)	500	25	25
2 (Mid)	1500	25	25
3 (High)	5000	25	25

Data taken from page 47, MRID No. 433786-01.
[a]Concentration based on active ingredient content of 57.9%; the concentration of test material was 864, 2591, and 8636 ppm.
[b]Diets were administered from the beginning of the study until the animals were sacrificed
[c]The same number of animals were selected from the F_1 litters as parents for the F_2 geoeration

Starting at 5 weeks of age, F_0 male and female rats received the control or test diet for 70 days (premating period), during mating, gestation, lactation (F_{1a} litters), and a 10-day rest period, and during a second mating, gestation, and lactation period (F_{1b} litters). Selected F_{1a} offspring were fed the same diets as their F_0 parents starting at weaning and continuing through mating, gestation, and lactation (F_2 litters). The F_{1b} and F_2 weanlings were given the test diet until sacrificed.

2. Dose selection rationale

The selection of doses was based on five studies in rats: (1) In a reproduction toxicity study, mepiquat chloride (0, 319.1, 1063.8, or 3191.5 ppm) was administered in the diet to F_0, F_1, and F_2 parental generations. No toxic effects were observed. (2) In prenatal, perinatal, and postnatal toxicity studies, rats were given dietary concentrations of 0, 100, 300, 1000, or 3000 ppm from day 0 to 20 post coitum (p.c.) or day 0 p.c. to day 21 postpartum (p.p.). Increased relative spleen weight occurred at 3000 ppm in the dams, and slight increases in body weight of female pups occurred on day 14 p.p. at 100 and 300 ppm and on days 7 and 14 p.p. at 1000 and 3000 ppm. (3) In a range-finding reproduction study, F_0 animals were given diets containing 0, 2000, 4000, or 6000 ppm of the test material starting 6 weeks before mating and continuing until the F_1 litters were weaned. At 6000 ppm, decreased food and water consumption, decreased body weight and body weight gain, tremors and hypersensitivity upon handling, neurofunctional effects, clinical chemistry effects, and hematologic effects were observed in F_0 animals; the number and weight of the pups were reduced. Similar, but less severe, effects were observed at 4000 ppm and no treatment-related effects were observed at 2000 ppm. (4) Mepiquat chloride given to male and female rats at concentrations of 145, 579, 2316, or 4632 ppm for 3 months caused no treatment-related toxic effects; a transient reduction in food consumption and a corresponding reduction in body

weight gain was observed. (5) A dietary concentration of 12,000 ppm administered for 3 months caused reduction in food consumption, body weight and body weight gain, clinical toxicity (tremors, reduced general state, altered behavior, impaired gait, ataxia, neurofunctional deficits, and abnormal posture, respiration, vocalization, and feces), and clinical chemistry effects. The study authors selected 5000 ppm as the top dose (expect clear toxicity), 1500 ppm as the mid dose (expect marginal or no toxicity), and 500 ppm as the low dose (expect no toxicity).

3. Mating procedure

The F_0 animals were mated (1 male:1 female) after they had received the test diets for 10 weeks. Each female and male pair was placed together overnight for 3 weeks or until sperm was detected in the vaginal smear. Males and females were housed separately during the day. The day sperm was detected or day 21 of mating was designated as day 0 post coitum (p.c.). Ten days after weaning the first litter, F_0 animals were mated again to produce a second litter (F_{1b}). The F_{1a} offspring were randomly selected before weaning, given mepiquat chloride for 14 weeks starting with the time of weaning (premating period), and mated as described for F_0 animals to produce the F_2 generation. Brother:sister matings were avoided. Fertility was reevaluated for mating pairs that did not produce offspring after the delivery of F_{1b} and F_2 litters; each animal was mated for 3 weeks or less to a fertile male or female from the control group.

C. METHODS

1. Observation schedule

a. Parental animals –

1) Clinical observations – The observation schedule for F_0 and F_1 parental animals is presented in Table 2. Treated animals reevaluated for fertility did received the control diet during overnight mating with the control animals; therefore, their food consumption was not measured. Body weights were not measured in females that showed no evidence of sperm or that did not produce litters. The dams were weighed weekly between the time their first litter were weaned and the second mating started. Clinical observations included clinical signs of toxicity, nesting, littering, and lactation behavior. The dams were checked twice daily for littering. F_0 and F_1 parental animals were subjected to neurofunctional tests to measure sensory and motor functions. The tests were conducted on all F_0 and F_1 parental animals before the first mating, all F_0 and F_1 dams with litters between days 13 and 21 p.p. and after weaning their litters (between day 30 and 35 p.p.), and in all F_0 and F_1 males at the same times the dams were tested. During the tests, the animals were examined for a variety of functional parameters.

TABLE 2. OBSERVATION SCHEDULE FOR F_0 AND F_1 PARENTAL ANIMALS

Type of observation	No. Animals per Sex per Group	Time of Observation	Frequency of observation
Mortality, moribundity, and clinical signs of toxicity	All	Throughout study	Daily
Detailed clinical observations	Not done	Not done	Not done
Neurofunctional tests	All parental animals	Premating, lactation, postlactation	Once for each period
Body weight			
Females	All	Premating	Weekly intervals
	All with evidence of sperm	Gestation	Days 0, 7, 14, and 20 p.c.
	All with litters	Lactation	Days 0, 4, 7, 14, and 21 p.p.
Males	All	Throughout study	Weekly intervals
Food consumption			
Females	All	Premating	Once a week for each 7-day period
	All with evidence of sperm	Gestation	Days 0-7, 7-14, and 14-20 p.c.
	All with litters	Lactation	Days 0-4, 4-7, and 7-14 p.p.
Males	All	Premating	Once a week for each 7-day period

Data taken from pages 57-59 and 64, MRID No. 433786-01.

2) Hematology, clinical chemistry, and urinalysis of nonpregnant animals – Blood was taken from the retroorbital venous plexus of 12 F_0 animals of each sex and dose group after the F_{1b} litters were weaned and from 12 F_1 animals of each sex and dose group after the F_2 litters were weaned. The animals were bled at least 28 days p.p. (F_0 animals after weaning F_{2b} litters). Urine samples were collected before or after blood samples were taken. The following CHECKED (X) hematological, clinical chemistry, and urinalysis parameters were evaluated:

Hematology

X	Hematocrit(HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc. (MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count	X	Reticulocyte count
X	Blood clotting measurements		
X	(Thromboplastin time)		

Clinical chemistry

Blood Chemistry

X	Calcium	X	Blood urea nitrogen
X	Chloride	X	Cholesterol
X	Magnesium	X	Globulins
X	Phosphorus (inorganic)	X	Glucose
X	Potassium	X	Total bilirubin
X	Sodium	X	Total serum protein (TP)
X	Albumin	X	Triglycerides
X	Blood creatinine		

Enzymes

X	Alkaline phosphatase	X	Serum cholinesterase
X	Serum alanine aminotransferase	×	Erythrocyte cholinesterase
X	Serum aspartate aminotransferase	X	Brain Cholinesterase
X	Gamma glutamyl transferase		

Urinalysis

X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood pigments
X	Sediment (microscopic)	X	Nitrite
X	Protein	X	Urobilinogen

b. Reproductive performance – Mating of the animals was considered successful if sperm were detected in a vaginal smear, the females delivered a litter, or pups/fetuses were observed *in utero*. A female was considered pregnant if she gave birth to a litter or had pup/fetuses *in utero*. The fertility of male animals was considered proven if the mated female was pregnant. Parental reproductive performance was assessed from breeding and parturition records of animals in

the study. The following reproductive indices, as described by the study authors, were calculated for parental animals producing the F_{1a}, F_{1b}, and F_2 litters:

Female mating index = (No. of females mated/No. of females placed with males) × 100

Male mating index = (No. males with confirmed mating/
No. of males placed with females) × 100

Female fertility index = (No. females pregnant/Total no. females mated) × 100

Male fertility index = (No. males with proven fertility/
Total no. males placed with females) × 100

Gestation index = (No. females with live litters on day of birth/No. females pregnant) × 100.

c. Litter observations – Litter observations are described in Table 3. In addition, the pups were examined on days 4 (pinna unfolding), 13 (auditory canal opening), and 15 (eye opening) for assessment of developmental milestones. Behavioral tests (gripping reflex, hearing test, and pupillary reflex) were conducted on days 13, 21, and 20, respectively.

TABLE 3. F_1/F_2 Litter Observations

Observations	Time of Observation (Lactation Day)					
	Birth (Day 0)	Day 1	Day 4	Day 7	Day 14	Day 21
No. of live pups/litter	X		X	X	X	X
Pup weight		X	X	X	X	X
External alterations	Daily					
No. of dead pups	Daily					
Sex of each pup (m/f)	X					X
Clinical signs	Daily					

The following litter indices were calculated:

Live birth index = (No. live pups born/Total No. (live + dead) pups born) × 100

Viability index = (No. live pups at day 4 (precull)/No. pups born alive) × 100

Lactation index = (No. alive pups on day 21/No. pups alive on day 4 (postcull)) × 100.

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2. Postmortem studies

a. Sacrifice – Adult F_0 and F_1 animals were anesthetized with carbon dioxide and exsanguinated by decapitation. Pups were sacrificed by carbon dioxide asphyxiation.

b. Necropsy –

1) Parental animals – All surviving F_0 parental animals were sacrificed after the F_{1b} generation was weaned. The F_1 parental animals were sacrificed "some weeks" after the F_2 generation was weaned. F_0 and F_1 animals reevaluated for fertility were sacrificed on schedule with other animals (males), before littering (fertile females), or 10 days after the last mating (infertile females). Animals that died before termination or sacrificed at termination were subjected to *post mortem* examinations consisting of gross and microscopic evaluations.

2) Offspring – The F_1 and F_2 offspring that were culled or not used as parents were sacrificed at 21 days of age or later. These animals were examined externally, eviscerated, and the internal organs examined grossly. Additional examinations (e.g. by skeletal staining) were conducted if clinical examinations indicated an abnormality. Stillborn pups and pups dying before weaning were examined similarly. Pups without notable findings or abnormalities were discarded.

3) Necropsy observations – The following tissues (X) from F_0 and F_1 parents were prepared for microscopic examination and weighed (XX):

X	Ovaries	XX	Epididymides
X	Uterus	X	Prostate
X	Vagina/cervix	X	Seminal vesicle
X	Gross lesions	XX	Testes

Additional tissues prepared for microscopic examination included the coagulation gland, pituitary gland, liver and kidneys; the liver and kidneys were also weighed. All excised tissues were examined microscopically in F_0 and F_1 parental animals from the control and high-dose groups. In addition, liver, kidney, and gross lesions from the low- and intermediate-dose groups were examined, and the reproductive organs from infertile animals were examined in the low- and intermediate-dose groups.

D. STATISTICAL ANALYSIS

Statistical analysis of clinical observations were performed using (1) Dunnett's test for all parametric data generated from clinical examinations (such as food and water consumption, body weights, body weight gain, grip strength, and hot plate test); and (2) the Fisher exact test for quantitative data such as developmental milestones, number of live and dead pups, reproductive performance indices, and litter indices. Clinical chemistry and hematologic data were evaluated using parametric one-way analysis of variance (ANOVA) via the F-test; Dunnett's test was performed simultaneously. Urinalysis data were converted on a scale of 0, 1, 2, and 3 and analyzed using the Fisher exact test. Except for organ weights, which were analyzed using Dunnett's test, pathology data were not analyzed statistically. Statistical significance was indicated by a p-value ≤ 0.05.

E. Signed and dated GLP and Quality Assurance statements were provided.

II. RESULTS

A. SYSTEMIC (PARENTAL) TOXICITY

1. Mortality and clinical signs

a. F_0 parents – No deaths occurred in the control group or the groups receiving the test material (mepiquat chloride). No clinical signs of toxicity attributable to the test material were observed in F_0 male or female rats during the premating period or in F_0 females during gestation. During the lactation period, tremors were noted in 17 of 20 (85%) high-dose dams; hypersensitivity upon handling was observed in 16 of 20 (80%) high-dose dams between day 10 and 24 of lactation of F_{1a} litters and in 20 of 25 (80%) high-dose dams between day 4 and 26 of lactation of F_{1b} litters. These signs were not observed at other dose levels. The neurofunction tests in male rats showed no treatment-related effect for the hot plate test, forelimb grip strength, and hindlimb grip strength measured before mating and after weaning of F_{1a} and F_{1b} litters. A significant increase in the mean hindlimb grip strength was observed in 500-ppm males (130%, $p<0.01$ compared with controls) after the F_{1a} litters were weaned, but not at the higher doses. The forelimb grip strength was significantly reduced in high-dose dams during lactation (7%, $p<0.05$ compared with controls) and after weaning the F_{1a} pups (9%, $p<0.01$ compared with controls). An increase was observed in the forelimb grip strength in 500-ppm dams during lactation for F_{1b} pups (107%, $p<0.05$ compared with controls) but not at higher doses. No effect on hindlimb grip strength was observed in the treated dams. The lack of a dose-response suggest that the neurofunctional responses are not treatment-related in the F_0 males. In the high-dose group, tremors also occurred in two dams when they were handled for the tests during lactation of F_{1a} litters and in

four dams during lactation F_{1b} litters; ataxia occurred in three dams during lactation of F_{1a} litters and in two dams during lactation of F_{1b} litters.

b. F_1 parents – No treatment-related clinical signs of toxicity were observed in males during the study or in females during the premating period. Except for insufficient or no nesting activity, which occurred in all dose groups including controls, there were no notable clinical findings during gestation. During lactation, tremors and hypersensitivity occurred in 14 of 20 (70%) dams and a reddish nasal discharge was observed in 4 high-dose dams. These findings did not occur in the other dose groups including controls. The neurofunction test was conducted on F_1 parents before mating and after weaning in both sexes and during lactation in females. The results of the hotplate test in treated animals of both sexes were similar to that of controls. Forelimb grip strength was significantly decreased in high-dose F_1 females before mating (10%, $p<0.05$ compared with controls) and during lactation (17%, $p<0.01$ compared with controls). Hindlimb grip strength was significantly decreased in high-dose males before mating (15%, $p<0.01$ compared with controls) and in high-dose females before mating (11%, $p<0.05$) and during lactation (16%, $p<0.05$). Tremors were observed in six high-dose females during neurofunctional testing.

2. Body weight and food consumption

a. Premating – Selected body weights, body weight gain, and food consumption values are summarized in Table 4. Treatment-related reduction in mean body weights were noted in F_0 and F_1 male and female rats receiving 5000 ppm of mepiquat chloride. In high-dose F_0 males, mean body weights were reduced throughout the study starting at week 1 (90% of control, $p<0.01$), continuing through the premating period (90%, $p<0.01$) and termination at week 29 (89% of control, $p<0.01$). Overall body weight gain during the premating period was only 86% of the control value in high-dose F_0 males, with individual weekly values being significantly reduced ($P<0.05$ or 0.01 compared with controls) during six of the 10 weekly intervals. Body weight gain over the entire 29-week treatment period for F_0 male rats was reduced by 13% ($p<0.01$). In high-dose F_1 males, mean body weights were reduced ($p<0.01$ compared with controls) throughout the treatment period (weeks 0-25). They were reduced by 49% at week 0 compared with controls, 22% at week 14 (end of the premating period), and 20% at termination. Body weight gain of high-dose F_1 males was reduced by 14% (compared with the control value) during the premating period, with most of the reduction occurring during the first three weeks of treatment (33 to 38%, $p<0.01$). Overall body weight gain (weeks 0-25) was reduced by 13% ($p<0.01$). Mean body weights and body weight gain in F_0 and F_1 male rats receiving 500 and 1500 ppm of mepiquat chloride were similar to that of the corresponding controls.

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In F_0 females rats receiving 5000 ppm of the test material, mean body weights were reduced throughout the premating period; statistical significance ($p < 0.05$ or 0.01 compared with controls) was achieved for seven of the ten weekly intervals. Overall body weight gain during the premating period was reduced by 11% ($p < 0.01$) in high-dose F_0 females compared with that controls. In high-dose F_1 females, mean body weights were also reduced throughout the 14-week premating period. They were reduced by 48% ($p < 0.01$) at week 0 and 16% ($p < 0.01$) at week 14 (end of the premating period). During the first week of the premating period, high-dose F_1 females gained 26% less weight than controls ($p < 0.01$), but thereafter, weekly body weight gain was similar to or sometimes exceeded that of the controls. At the end of the premating period, F_1 high-dose females gained slightly more weight than controls. There were no treatment-related effects on body weights or body weight gain in either F_0 or F_1 females receiving 500 or 1500 ppm of mepiquat chloride.

Statistically significant ($p < 0.01$) reductions occurred in food consumption in high-dose F_0 male rats: 24% during week 0-1, 11% during week 1-2, and 6-9% during other weekly intervals of the premating period (not presented in Table 4). Overall food consumption during the premating period was reduced by 9% ($p < 0.01$) (Table 4). In high-dose F_1 males, food consumption steadily increased from 55% ($p < 0.01$) during the first week to 92% ($p < 0.01$) of the control value after week 9. Food consumption in high-dose F_0 female rats was significantly reduced by 16% ($p < 0.01$) of the controls during the first week, but was similar to the control values thereafter. In high-dose F_1 females, food consumption was also significantly ($p < 0.01$ or 0.05) reduced especially during the early premating period, with values ranging from a low of 56% at week 0-1 to 88% at week 2-3 and fluctuated between 91 and 95% of the control values for the remainder of the premating period. In high-dose animals, food efficiency was slightly reduced in F_0 male and female rats, similar to that of controls in F_1 males, and slightly higher than that of controls in F_1 females.

TABLE 4. MEAN BODY WEIGHTS, BODY WEIGHT GAIN, AND FOOD CONSUMPTION DURING THE PREMATING OR POSTMATING PERIODS IN RATS FED MEPIQUAT CHLORIDE FOR TWO GENERATIONS

Observation	Control	500 ppm	1,500 ppm	5,000 ppm
F_0 Generation – Males				
Body weight (g) - Week 0	110.6	110.8 (100)	110.4 (100)	109.9 (99)
Body weight (g) - Week 5	326.6	332.8 (102)	321.2 (98)	296.3** (91)
Body weight (g) - Week 10	427.7	432.9 (101)	418.7 (98)	383.0** (90)
Body weight (g) - Week 15	473.1	483.8 (102)	470.4 (99)	429.9** (91)
Body weight (g) - Week 20	496.7	499.9 (101)	490.0 (99)	444.6** (90)
Body weight (g) - Week 25	552.1	557.3 (101)	543.9 (99)	492.4** (89)
Body weight (g) - Week 29	562.2	566.3 (101)	555.7 (99)	502.8** (89)
Weight gain (g)[b] - Week 0-10	317.1	322.1 (102)	308.3 (97)	273.1 (86)
Weight gain (g) - Week 0-29	453.6	455.5 (100)	445.4 (98)	392.9** (87)
Food consumption (g/rat/day)[c] - Week 1-10	25.9	26.2 (101)	25.5 (98)	23.6* (91)
Food efficiency ((g bw/g food) × 100)[d]	17.5	17.6	17.3	16.5
F_0 Generation – Females				
Body weight (g) –Week 0	103.1	102.8 (100)	102.9 (100)	102.7 (100)
Body weight (g) –Week 5	201.1	205.8 (102)	210.3 (105)	191.9 (95)
Body weight (g) - Week 10	246.5	247.3 (100)	255.2 (104)	229.6* (93)
Weight gain (g) - Week 0-10	143.3	144.6 (101)	152.4 (106)	126.9* (89)
Food consumption (g/rat/day) - Week 0-10	18.9	19.1 (101)	19.7* (104)	18.0* (95)
Food efficiency ((g bw/g food) × 100)[d]	10.8	10.8	11.1	10.1

TABLE 4. CONTINUED

Observation	Control	500 ppm	1,500 ppm	5,000 ppm
F_1 Generation – Males				
Body weight (g) – Week 0	108.5	104.5 (96)	101.9 (94)	55.7** (51)
Body weight (g) –Week 5	347.2	345.5 (100)	338.8 (98)	244.3** (70)
Body weight (g) – Week 10	440.5	443.9 (101)	432.7 (98)	333.5** (76)
Body weight (g) –Week 14	476.9	485.1(102)	473.4 (99)	371.1** (78)
Body weight (g) –Week 20	525.3	535.0 (102)	521.0 (99)	418.6** (80)
Body weight (g) –Week 25	544.6	556.5 (102)	547.6 (101)	437.1** (80)
Weight gain (g)[b] – Week 0-14	368.4	380.6 (103)	371.5 (101)	315.4 (86)
Weight gain (g) – Week 0-25	436.1	452.0 (104)	445.7 (102)	381.3** (87)
Food consumption (g/rat/day) – Week 0-14	28.0	28.5 (102)	28.1 (100)	23.8** (85)
Food efficiency ((g bw/g food) × 100)[d]	13.4	13.6	13.5	13.5
F_1 Generation – Females				
Body weight (g) – Week 0	97.0	95.6 (99)	93.3 (96)	50.6** (52)
Body weight (g) – Week 5	211.5	213.8 (101)	214.5 (101)	165.5** (78)
Body weight (g) – Week 10	255.6	260.5 (102)	259.5 (102)	210.9** (83)
Body weight (g) – Week 14	270.3	273.2 (101)	274.7 (102)	227.7** (84)
Weight gain (g) – Week 0-14	173.3	177.7 (103)	181.4 (105)	177.1 (102)
Food consumption (g/rat/day)[c] – Week 0-14	20.9	21.1 (101)	21.2 (101)	18.5** (89)
Food efficiency ((g bw/g food) × 100)[d]	8.5	8.6	8.7	9.8

Data taken from pages 145, 146, 153-160, 220-223, 230-239; MRID No. 433786-01.
[a]The numbers in parentheses are the percents of the control values.
[b]Calculated by the reviewer using body weights on page 153.
[c]Average of the weekly mean values.
[d]((g body weight gain/g food consumed) × 100), calculated by the reviewer using data on body weight gain and food consumption for the premating period.
*$p<0.05$, **$p<0.01$

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b. Gestation and lactation – Selected mean body weights and food consumption values are summarized in Table 5. Treatment-related decreases were observed for mean body weights in high-dose F_0 and F_1 dams. Mean body weights in F_0 dams ranged from 90 to 93% of the control value ($p<0.05$ or <0.01) during the gestation period and from 83 to 89% ($p<0.01$) during lactation of both litters. Body weight gain was significantly reduced in high-dose F_0 dams during the day 14-20 interval of the gestation period of F_{1a} litters and during the entire gestation period for F_{1b} litters. There was no net weight gain for the entire lactation period of both F_{1a} and F_{1b} litters, due primarily to weight loss during the first 4 days. High-dose F_1 dams showed a similar pattern, with body weights ranging from 83 to 87% ($p<0.01$) of the control values during gestation and 79 to 81% ($p<0.01$) during lactation of the F_2 litters. Body weight gain of the high-dose F_1 dams was also significantly depressed during gestation, and weight loss occurred during the first 4 days of lactation resulting in a net weight loss over the 21-day lactation period.

Overall food consumption (g/rat/day) was reduced by 6 to 10% (N.S. compared with controls) during gestation and by 21 to 23% (N.S. compared with controls) during lactation (F_{1a}, F_{1b}, and F_2 litters). However, during the individual 7-day interval of gestation, food consumption was reduced by ≤10% of the control values, with statistical significance being achieved for the day 14- to 20-interval for F_{1a} and F_{1b} litters and all three intervals for F_2 litters. During lactation, food consumption was significantly reduced by 17 to 26% for each 4- or 7-day interval, with statistical significance ($p<0.01$) being achieved for each interval.

3. Test substance intake

Based on food consumption, body weights, and nominal concentration of the test substance in feed, the doses expressed as mg of test substance/kg body weight during the premating period are presented in Table 6. During gestation F_0 and F_1 dams receiving 5000 ppm, consumption of test substance averaged 460.2 (F_{1a} litters), 433.3 (F_{1b} litters), and 485.8 mg/kg/day (F_2 litters). During lactation, consumption of test substance averaged 662.3, 625.1, and 715.2 mg/kg/day, respectively in dams receiving 5000 ppm.

TABLE 5. SELECTED MEAN BODY WEIGHTS, BODY WEIGHT GAIN, AND FOOD CONSUMPTION VALUES FOR PREGNANT AND NURSING RATS FED MEPIQUAT CHLORIDE FOR TWO GENERATIONS

Observation	Control	500 ppm	1,500 ppm	5,000 ppm
F_0 Generation – F_1 Litter a				
Mean body weight (g)				
Day 0 of gestation	253.6	256.1 (101)[a]	263.9 (104)	236.5* (93)
Day 20 of gestation	391.0	390.4 (100)	401.8 (103)	358.2** (92)
Day 0 of lactation	304.8	303.7 (100)	310.3 (102)	272.4** (89)
Day 21 of lactation	325.9	328.9 (101)	328.6 (101)	272.1** (83)
Mean body weight gain (g)				
Day 0-20 of gestation	137.4	134.3 (98)	137.9 (100)	121.7* (89)
Day 0-21 of lactation	21.0	25.2 (120)	18.3 (87)	-0.2** (-1)
Mean food consumption (g/rat/day)				
Day 0-20 of gestation	25.8	26.0 (101)	26.8 (104)	24.3 (94)[b]
Day 0-14 of lactation	46.2	48.7 (105)	47.5 (103)	35.7 (77)[c]
F_0 Generation – F_1 Litter b				
Mean body weight (g)				
Day 0 of gestation	306.5	308.5 (101)	310.1 (101)	278.0** (91)
Day 20 of gestation	450.6	444.4 (99)	449.4 (100)	404.3** (90)
Day 0 of lactation	347.5	347.4 (100)	353.3 (102)	308.2** (89)
Day 21 of lactation	358.2	359.9 (100)	363.2 (101)	306.2** (85)
Mean body weight gain (g)				
Day 0-20 of gestation	144.1	136.0 (94)	139.5 (97)	126.2** (88)
Day 0-21 of lactation	10.7	12.5 (117)	9.8 (92)	-2.0* (-19)
Mean food consumption (g/rat/day)				
Day 0-20 of gestation	28.0	28.1 (100)	28.8 (103)	26.2 (94)[b]
Day 0-14 of lactation	48.5	49.8 (103)	47.3 (98)	38.3 (79)[c]

Zo

TABLE 5. CONTINUED

Observation	Control	500 ppm	1,500 ppm	5,000 ppm
F_1 Generation – F_2 Litter				
Mean body weight (g)				
Day 0 of gestation	274.8	275.5 (100)	279.6 (102)	229.7** (84)
Day 20 of gestation	400.3	400.6 (100)	407.6 (102)	334.0** (83)
Day 0 of lactation	317.9	318.8 (100)	322.5 (101)	257.2** (81)
Day 21 of lactation	325.8	325.9 (100)	325.0 (100)	253.9** (78)
Mean body weight gain (g)				
Day 0-20 of gestation	125.5	125.1 (100)	128.0 (102)	104.2** (83)
Day 0-21 of lactation	7.9	7.1 (90)	2.5 (32)	-3.3* (-42)
Mean food consumption (g/rat/day)				
Day 0-20 of gestation	27.2	27.5 (101)	27.6 (101)	24.6 (90)[b]
Day 0-14 of lactation	46.2	46.0 (100)	48.1 (104)	36.6 (79)[c]

Data taken from pages 147-150, 161-164, 167-170, 224, 225, and 240-243, MRID No. 433786-01.
[a]Percent of control value.
[b]$p<0.01$, gd 14-20 (F_{1a} and F_{1b} litters); $p<0.05$, gd 0-7 (F_2 litters); $p<0.01$, gd 7-14 and 14-20 (F_2 litters)
[c]$p<0.01$ for lactation days 0-4, 4-7, and 7-14 (F_{1a}, F_{1b}, and F_2 litters)
*$p<0.05$, **$p<0.01$

TABLE 6. TEST SUBSTANCE INTAKE (mg/kg/day) IN RAT FED MEPIQUAT CHLORIDE DURING THE PREMATING PERIOD

	Male			Female		
	500 ppm	1,500 ppm	5,000 ppm	500 ppm	1,500 ppm	5,000 ppm
F_0 Generation						
Range	31.8 - 98.5	96.5 - 287.7	332.7 - 754.2	38.2 - 91.7	114.9 - 272.5	401.8 - 749.4
Grand Mean	51.2	153.1	499.3	54.0	163.6	530.0
F_1 Generation						
Range	29.4 - 106.3	88.1 - 319.1	345.8 - 1093.7	37.8 - 102.4	114.7 - 310.7	433.0 - 1045.5
Grand Mean	48.6	146.6	574.5	53.3	162.0	626.5

Data taken from 171, 172, 244-249, MRID No. 433786-01.

4. Hematology, clinical chemistry, and urinalysis

No statistically significant changes were observed in hematology, clinical chemistry, or urinalysis parameters of F_0 or F_1 rats except those described below. Serum creatinine levels were decreased (93% of control, $p<0.05$) in high-dose F_0 females, serum alanine aminotransferase activity was decreased in low-dose F_0 females (85% of control, $p<0.05$) and in high-dose F_1 males (82% of control, $p<0.05$), and serum globulin levels were decreased (92% of control, $p<0.05$) in high-dose F_1 females. There were no statistically significant effects on cholinesterase activity in the brain, erythrocytes, or plasma.

5. Reproductive performance

Results for the parental animals are summarized in Tables 7a,b,c. No treatment-related effects were observed for reproductive performance of either F_0 or F_1 animals. Evidence of mating was confirmed in all F_0 males and females (all dose groups) placed together for production of F_{1a} and F_{1b} litters. Except for one control (14 days of cohabitation) and one 5000-ppm second-mating pair (14 days of cohabitation), all animals mated within 4 days; the mean duration ranged from 2.5 to 2.8 days for the first mating and 2.5 to 3.4 days for the second mating. There was a significant reduction (20 vs 25 controls (80%), $p<0.05$) in the number of high-dose F_0 males whose fertility was confirmed during the first mating interval (F_{1a} litters); however, fertility was confirmed in all 25 high-dose males during the second mating (F_{1b} litters), indicating that the effect was not treatment related. In addition the fertility index was within the range of historical controls (80-100%, MRID No. 433786-01, page 1694). Evidence of pregnancy was obtained for 20 of 25 high-dose females ($p<0.05$ compared with controls) and 23 of 25 low-dose females for the first mating and 24 of 25 low-dose females in the second mating. Only one F_0 female (500-ppm group) did not prove to be fertile during the two mating cycles; fertility was not proven when this female was mated with a control male with proven fertility. The duration of gestation was similar in all treated F_0 and F_1 groups and controls except for a statistically significant decrease in high-dose animals during the first (22 vs 21.6 days for controls, $p<0.01$) and second mating (21.9 vs 21.6 days for controls, $p<0.05$) . The gestation length for this group was within range of historical controls and is not considered to be treatment related.

All surviving F_1 male and female rats mated, resulting in a mating index of 100% for both sexes. Fertility was confirmed in all but two controls, three low-, two mid-, and four high-dose mating pairs, resulting in fertility indices ranging from 83-92%; all within range of historical controls. Fertility was reevaluated for the nonfertile males and females separately; fertility was confirmed for all except one female in the 500-ppm group. The gestation length was similar in all groups.

TABLE 7a. REPRODUCTIVE PERFORMANCE IN RATS FED MEPIQUAT CHLORIDE FOR TWO GENERATIONS

Observation	Treatment Groups			
	Control	500 ppm	1,500 ppm	5,000 ppm
F_0 Generation – Litter a				
Males				
No. at start of study	25	25	25	25
No. placed with females	25	25	25	25
No. with confirmed mating[a]	25	25	25	25
No. with proven fertility[b]	25	23	25	20*
Females				
No. at start of study	25	25	25	25
No. mated	25	25	25	25
No. pregnant	25	23	25	20
No. with live born pups	25	23	25	20
Indices (%)				
Mating index - males	100	100	100	100
Mating index - females	100	100	100	100
Fertility index - males	100	92	100	80
Fertility index - females	100	92	100	80
Duration of gestation (days)	22.0 ± 0.35	22.0 ± 0.00	22.0± 0.20	21.6 ± 0.49**

Data taken from pages 195 and 197, MRID No. 433786-01.
[a]Females had vaginal sperm or gave birth to litter.
[b]The females gave birth to a litter or fetuses were detected *in utero*.
*$p < 0.05$, **$p < 0.01$

TABLE 7b. REPRODUCTIVE PERFORMANCE IN RATS FED MEPIQUAT CHLORIDE FOR TWO GENERATIONS

Observation	Treatment Groups			
	Control	500 ppm	1,500 ppm	5,000 ppm
F_0 Generation - Litter b				
Males				
No. at start of study	25	25	25	25
No. placed with females	25	25	25	25
No. with confirmed mating[a]	25	25	25	25
No. with proven fertility[b]	25	24	25	25
Females				
No. at start of study	25	25	25	25
No. mated	25	25	25	25
No. pregnant	25	24	25	25
No. with live born pups	25	24	25	25
Indices (%)				
Mating index - males	100	100	100	100
Mating index - females	100	100	100	100
Fertility index - males	100	96	100	100
Fertility index - females	100	96	100	100
Duration of gestation (days)	21.9 ± 0.44	21.9 ± 0.34	21.8 ± 0.44	21.6 ± 0.51*

Data taken from pages 196 and 198 MRID No. 433786-01.
[a]Females had vaginal sperm or gave birth to litter.
[b]The females gave birth to a litter or fetuses were detected *in utero*.
*$p<0.05$, **$p<0.01$

TABLE 7c. REPRODUCTIVE PERFORMANCE IN RATS FED MEPIQUAT CHLORIDE FOR TWO GENERATIONS

Observation	Treatment Groups			
	Control	500 ppm	1,500 ppm	5,000 ppm
F_1 Generation				
Males				
No. at start of study	25	25	25	24[a]
No. placed with females	25	25	25	24[a]
No. with confirmed mating[b]	25	25	25	24
No. with proven fertility[c]	23	22	23	20
Females				
No. at start of study	25	25	25	24[a]
No. mated	25	25	25	24
No. pregnant	23	22	23	20
No. with live born pups	23	22	23	20
Indices (%)				
Mating index - males	100	100	100	100
Mating index - females	100	100	100	100
Fertility index - males	92	88	92	83
Fertility index - females	92	88	92	83
Duration of gestation (days)	22.0 ± 0.00	22.0 ± 0.21	21.9 ± 0.29	22.0 ± 0.00

Data taken from 263 and 264, MRID No. 433786-01.
[a]One male and one female died before premating period started.
[b]Females had vaginal sperm or gave birth to litter.
[c]The females gave birth to a litter or fetuses were detected *in utero*.
*$p < 0.05$, **$p < 0.01$

6. Necropsy results

a. Organ weights - The liver (84 and 75%) and kidneys (92 and 81%) of high-dose F_0 and F_1 males, respectively, weighed significantly ($p<0.01$) less than that of controls. The testes (89%) and epididymides (92%) of high-dose F_1 males also weighed significantly ($p<0.01$) less than that of controls. The relative liver weights were significantly reduced, and the relative weights of the testes and epididymides were significantly elevated in both F_0 and F_1 males receiving the high dose. At the high-dose, the livers of F_0 and F_1 females weighed significantly less than those of controls (90 and 84%, $p<0.01$) as did the kidneys of F_1 females (77%, $p<0.01$). The relative kidney weight was significantly elevated in the high-dose F_0 females (110%, $p<0.01$), but it was reduced (90%, N.S.) in high-dose F_1 females. The changes in organ weights are probably a consequence of the significantly reduced ($p<0.01$) terminal body weights of high-dose F_0 and F_1 animals.

b. Pathology -

1) Macroscopic examination - There were no gross lesions attributable to treatment with mepiquat chloride in F_0 or F_1 animals of either sex.

2) Microscopic examination - There were no increased incidences of microscopic lesions attributable to treatment with mepiquat chloride in F_0 or F_1 animals of either sex. The incidence ($p<0.01$ compared with controls) and severity (N.S.) of lipid storage in the liver was decreased in high-dose F_0 male rats and the severity ($p<0.01$) was decreased in high-dose F_1 male rats. The incidence of lipid storage was increased in F_1 males receiving the low- ($p<0.05$) and mid-dose (N.S.); and the severity was significantly decreased ($p<0.01$) in the mid-dose males. The incidence of the same lesion was significantly decreased in F_0 ($p<0.01$) and F_1 female rats ($p<0.05$) compared with the corresponding controls; the severity was not affected. A clear dose-response was not observed for any treated group. These data are summarized in Table 9.

TABLE 8. ABSOLUTE AND RELATIVE ORGAN WEIGHT CHANGES IN RATS FED MEPIQUAT CHLORIDE FOR TWO GENERATIONS

Organs	Control	500 ppm	1,500 ppm	5,000 ppm
Males - F_0 Generation				
Liver (g)	16.66 (3.09)	16.03 (2.98)	15.95 (3.03)	13.97** (2.94)*
Kidneys (g)	3.17 (0.59)	3.21 (0.60)	3.16 (0.60)	2.93** (0.62)
Testes(g)	3.87 (0.72)	3.79 (0.71)	3.82 (0.73)	3.88 (0.82)**
Epididymides (mg)	1343.3 (0.25)	1336.4 (0.25)	1363.5 (0.26)	1326.5 (0.28)**
Males - F_1 generation				
Liver (g)	17.35 (3.30)	17.20 (3.23)	16.62 (3.16)	12.93** (3.09)*
Kidney (g)	3.19 (0.61)	3.27 (0.62)	3.27 (0.63)	2.59** (0.62)
Testes (g)	4.00 (0.77)	3.91 (0.74)	3.97 (0.77)	3.55** (0.85)**
Epididymides (mg)	1363.4 (0.26)	1344.4 (0.26)	1323.7 (0.26)	1260.0** (0.30)**
Females - F_0 generation				
Liver	9.71 (3.15)	9.63 (3.15)	9.83 (3.21)	8.72** (3.19)
Kidney	2.21 (0.72)	2.25 (0.74)	2.30 (0.75)	2.14 (0.79)**
Females - F_1 generation				
Liver (g)	9.44 (3.37)	9.53 (3.30)	10.16 (3.50)	7.95** (3.34)
Kidney (g)	2.30 (0.83)	2.17 (0.75)	2.21 (0.76)	1.78** (0.75)

Data taken from pages 1411-1418, MRID NO. 433786-01.
[a]Numbers in parentheses are the relative organ weights (% of body weight).
*$p<0.05$, **$p<0.01$; Dunnett-test (two-sided)

TABLE 9. MICROSCOPIC FINDINGS IN RATS FED MEPIQUAT CHLORIDE FOR TWO GENERATIONS

Organs/Lesion	Control	500 ppm	1500 ppm	5000 ppm
F_0 Generation				
Males				
Liver Lipid storage	19/25[a] (1.74)	20/25 (1.75)	15/25 (1.47)	10/25** (1.50)
Females				
Liver Lipid storage	16/25 (1.13)	12/25 (1.25)	16/25 (1.13)	3/25** (1.0)
F_1 Generation				
Males				
Liver Lipid Storage	15/25 (1.93)	22/25* (1.68)	20/25 (1.45)**	15/25 (1.27)**
Females				
Liver Lipid storage	9/25 (1.0)	13/25 (1.23)	15/25 (1.07)	2/25* (1.0)

Data taken from pages 1421-1425, MRID NO. 433786-01.
[a]Number of animals affected/number of animals examined; the numbers in parenthesis are the average severity grades based on values of 1, 2, and 3.
*$p<0.05$, **$p<0.01$, pairwise comparison calculated by the reviewer; incidence data analyzed using Fisher exact test (Number Cruncher Statistical System, version 5.03); severity data analyzed using Student's t-test.

B. OFFSPRING TOXICITY

1. Viability and clinical signs

Data concerning viability, clinical observations, and mean litter sizes are summarized in Tables 10a,b,c. There was a significant (89% vs 96% for controls, $p<0.01$) decrease in the survival of high-dose F_{1a} pups during days 0-4 p.p. Survival after day 4 was reduced compared with controls (91 vs 95%), but not significantly. The high-dose F_{1b} and F_2 pups showed no statistically significant decrease in survival at any dose. Survival at the low- and mid-dose was either similar to or increased relative to controls. There was also no statistically significant increase in the number of whole-litter mortality in groups receiving the test material compared with those of controls. There were no clinical signs of toxicity that could be attributed to the test material.

TABLE 10a. VIABILITY AND CLINICAL OBSERVATIONS OF F_{1a} OFFSPRING DURING LACTATION

Observation/study time	Control	500 ppm	1500 ppm	5000 ppm
Total no. of litters	25	23	25	20
Total no. of pups born	350	320	371	265
Total no. born alive	345	314	365	264
Total no. stillborn	5	6	6	1
Mean litter size – day 0	14.0	13.9	14.8	13.3
Mean no. live pups per litter (total pups alive)				
Day 0	13.8 (345)	13.7 (314)	14.6 (365)	13.2 (264)
Day 4 (precull)	13.2 (330)	13.0 (298)	13.7 (343)	11.7 (234)**
Day 4 (post cull)	8.0 (200)	8.0 (184)	7.7 (192)	7.4 (149)
Day 21	7.6 (190)	8.0 (183)*	7.7 (192)*	6.8 (136)
No. litters weaned	24	23	24	18
Survival indices				
Live birth index (%)	99	98	98	100
Viability index (%)	96	95	94	89
Lactation index (%)	95	99	100	91
Sex ratio (% males)– day 0	56.8	51.6	49.9	53.0
Sex ratio (% males) – day 21	52.1	49.7	51.6	52.9

Data taken from pages 200-202 and 707-710, MRID No. 433786-01.

TABLE 10.b VIABILITY AND CLINICAL OBSERVATIONS OF F_{1b} OFFSPRING DURING LACTATION

Observation/study time	Control	500 ppm	1500 ppm	5000 ppm
Total no. of litters	25	24	25	25
Total no. of pups born	385	340	366	348
Total no. born alive	375	332	359	340
Total no. stillborn	10	8	7	8
Mean litter size – day 0	15.4 (385)	14.2 (340)	14.6 (366)	13.9 (348)
Mean no. live pups per litter (total no. of pups alive)				
Day 0	15.0 (375)	13.8 (332)	14.4 (359)	13.6 (340)
Day 4 (precull)	14.2 (355)	13.5 (324)	13.5 (338)	13.0 (326)
Day 4 (postcull)	8.0 (200)	8.0 (192)	7.7 (192)	8.0 (200)
Day 21	8.0 (199)	8.0 (191)	7.6 (190)	7.7 (193)
No. litters weaned	25	24	24	25
Survival indices				
Live birth index (%)	97	98	98	98
Viability index (%)	95	98	94	96
Lactation index (%)	100	99	99	97
Sex ratio (% males) – day 0	50.9	50.3	52.4	49.4
Sex ratio (% males) – day 21	49.7	51.3	50.0	49.2

Data taken from pages 203-205 and 711-714, MRID No. 433786-01.

TABLE 10c VIABILITY AND CLINICAL OBSERVATIONS OF F_2 OFFSPRING DURING LACTATION

Observation/study time	Control	500 ppm	1500 ppm	5000 ppm
Total no. of litters	23	22	23	20
Total no. of pups born	295	275	310	214
Total no. born alive	286	261	299	212
Total no. stillborn	9	14	11	2
Mean litter size - day 0	12.8	12.5	13.5	10.7
Mean no. live pups per litter (total no. of pups alive)				
Day 0	12.4 (286)	11.9 (261)	13.0 (299)	10.6 (212)
Day 4 (precull)	11.9 (274)	11.0 (243)	12.4 (286)	9.9 (197)
Day 4 (post cull)	7.9 (182)	7.4 (163)	7.8 (180)	7.2 (144)
Day 21	7.3 (169)	7.2 (159)	7.8 (180)	6.5 (130)
No. litters weaned	22	22	23	19
Survival indices				
Live birth index (%)	97	95	96	99
Viability index (%)	96	93	96	93
Lactation index (%)	93	98	100	90
Sex ratio (% males) - day 0	52.8	49.8	48.2	51.4
Sex ratio (% males) - day 21	50.3	48.4	48.9	50.0

Data taken from pages 267-269 and 1204-1207, MRID No. 433786-01.

2. Body weight

Selected group mean body weights and body weight gain are summarized in Table 11. The pups from both F_1 litters and the F_2 litters in the high-dose group had significantly decreased body weights and gained significantly less weight during the lactation period than the corresponding controls. Significantly decreased mean body weights were first noted on day 1 p.p. in the F_{1a} (94% of control weight, $p<0.05$) and F_{1b} pups (95% of control weight, $p<0.05$) and on day 7 in F_2 pups (80% of control weight, $p<0.01$) and became progressively more severe up to day 21 p.p. (64, 67, and 68% of control weight, respectively, $p<0.01$). Mean body weight gain followed the same pattern as body weights with F_{1a}, F_{1b}, and F_2 pups gaining only 61 to 64% as much weight as controls between days 4 and 21 p.p. F_{1a} pups in the mid-dose group gained slightly, but significantly ($p<0.05$) less weight than controls between days 14 and 21 p.p.; otherwise, mean body weights and weight gain for pups in the low- and mid-dose groups were similar to those of controls.

3. Developmental milestones

Treatment-related delays in attaining developmental stages were observed in pups exposed to the test material. The pinna unfolded by day 4 in significantly fewer high-dose F_{1a} (67%) and F_{1b} pups (72%), but not in the high-dose F_2 pups, than in the corresponding controls (94 and 93% for F_{1a} and F_{1b} pups, respectively). The auditory canal and the eyes opened on time (day 13 and 15 after birth, respectively) in significantly ($p<0.01$) fewer high-dose F_{1a} (65 and 75%) and F_{1b} pups (73% for both) than in the corresponding controls (95-100%). The eyes opened on time in significantly fewer ($p<0.05$ or 0.01) F_2 pups in all three dose groups than in the control group. A positive response was observed for the gripping reflex in slightly but significantly ($p<0.05$) fewer high-dose F_{1b} and F_2 pups and for pupil constriction in high-dose F_{1b} pups ($p<0.01$) than in the controls. There was no effect on the number of pups showing a positive response in the acoustic startle test.

TABLE 11. GROUP MEAN BODY WEIGHT AND BODY WEIGHT GAIN OF OFFSPRING DURING LACTATION

Observation/study time	Controls	500 ppm	1500 ppm	5000 ppm
F_{1a} Litters				
Mean pup weight per litter (g)				
Day 1	6.4	6.5 (102)[a]	6.3 (98)	6.0* (94)
Day 4 (precull)	9.1	9.2 (101)	9.0 (99)	7.3** (80)
Day 4 (postcull)	9.1	9.2 (101)	9.2 (101)	745** (81)
Day 7	15.1	15.0 (99)	15.0 (99)	10.9** (72)
Day 14	32.7	32.7 (100)	33.0 (101)	23.8** (73)
Day 21	53.5	53.4 (100)	52.0 (97)	34.3** (64)
Mean pup weight gain per litter (g)				
Day 1-4	2.7	2.8 (104)	2.7 (100)	1.3** (48)
Day 4-7	5.9	5.7 (97)	5.9 (100)	3.5** (59)
Day 7-14	17.6	17.8 (101)	17.9 (102)	12.6** (72)
Day 14-21	20.8	20.7 (100)	19.1* (92)	10.5** (50)
Day 4-21	44.3	44.2 (100)	43.0 (97)	26.9** (61)
F_{1b} Litters				
Mean pup weight per litter (g)				
Day 1	6.4	6.6 (103)	6.5 (102)	6.1* (95)
Day 4 (precull)	9.2	9.7 (105)	9.1 (99)	7.7** (84)
Day 4 (postcull)	9.3	9.7 (104)	9.2 (99)	7.6** (82)
Day 7	15.3	15.8 (103)	15.3 (100)	11.6** (76)
Day 14	33.5	34.2 (102)	33.1 (99)	23.9** (71)
Day 21	54.3	55.3 (102)	53.1 (98)	36.2** (67)

TABLE 11. CONTINUED

Observation/study time	Controls	500 ppm	1500 ppm	5000 ppm
F_{1b} Litters (continued)				
Mean pup weight gain per litter (g)				
Day 1-4	2.8	3.1 (111)	2.7 (96)	1.6** (57)
Day 4-7	6.0	6.1 (102)	5.9 (98)	4.0** (67)
Day 7-14	18.1	18.4 (102)	17.8 (98)	12.2** (67)
Day 14-21	20.8	21.1 (101)	20.0 (96)	12.4** (60)
Day 4-21	45.0	45.6 (101)	43.8 (97)	28.6** (64)
F_2 litters				
Mean pup weight per litter (g)				
Day 1	6.3	6.4 (102)	6.3 (100)	6.2 (98)
Day 4 (precull)	8.7	8.8 (101)	8.9 (102)	7.8 (90)
Day 4 (postcull)	8.7	8.8 (101)	8.9 (102)	7.8 (90)
Day 7	14.2	13.7 (96)	14.2 (100)	11.3** (80)
Day 14	31.2	30.3 (97)	30.9 (99)	23.9** (77)
Day 21	50.8	49.2 (97)	49.6 (98)	34.7** (68)
Mean pup weight gain per litter (g)				
Day 1-4	2.4	2,4 (100)	2.6 (108)	1.6* (67)
Day 4-7	5.3	4.9 (92)	5.3 (100)	3.5** (66)
Day 7-14	17.0	16.6 (98)	16.7 (98)	12.3** (72)
Day 14-21	19.6	18.9 (96)	18.7 (95)	10.7** (55)
Day 4-21	42.0	40.4 (96)	40.7 (97)	26.7** (64)

Data taken from pages 206-213 and 270-273, MRID No. 433786-01.
[a]Number in parenthesis is the percent of control calculated by the reviewer.
*$p<0.05$, **$p<0.01$

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TABLE 12. DEVELOPMENTAL MILESTONES IN OFFSPRING DURING LACTATION

Observation/time	Control	500 ppm	1500 ppm	5000 ppm
F_{1a} Litters				
Pinna unfolding/day 4	310/330[a] (94)	286/298 (96)	330/343 (96)	157/234** (67)
Auditory canal opening/day 13	188/190 (99)	174/183* (95)	191/192 (99)	88/136** (65)
Eye opening/day 15	190/190 (100)	182/183 (99)	187/192 (97)	102/136** (75)
Gripping reflex/day 13	190/190 (100)	182/183 (99)	192/192 (100)	135/136 (99)
Acoustic startle/day 21	190/190 (100)	183/183 (100)	192/192 (100)	136/136 (100)
Pupil constriction/day 20	189/190 (99)	182/183 (99)	192/192 (100)	134/136 (99)
F_{1b} Litters				
Pinna unfolding/day 4	329/355 (93)	321/324** (99)	330/338** (98)	236/326** (72)
Auditory canal opening/day 13	194/199 (97)	186/191 (97)	190/189 (99)	141/193** (73)
Eye opening/day 15	189/199 (95)	191/191** (100)	189/190* (99)	141/193** (73)
Gripping reflex/day 13	199/199 (100)	191/191 (100)	190/190 (100)	181/193** (94)
Acoustic startle/day 21	199/199 (100)	191/191 (100)	190/190 (100)	193/193 (100)
Pupil constriction/day 20	199/199 (100)	190/191 (99)	189/190 (99)	184/193** (95)
F_2 Litters				
Pinna unfolding/day 4	255/274 (93)	215/244 (88)	260/286 (91)	182/197 (92)
Auditory canal opening/day 13	162/169 (96)	140/159* (88)	170/180 (94)	105/130** (81)
Eye opening/day 15	168/169 (99)	152/159* (96)	159/180** (88)	116/130** (89)
Gripping reflex/day 13	169/169 (100)	159/159 (100)	180/180 (100)	126/130* (97)
Acoustic startle/day 21	169/169 (100)	159/159 (100)	180/180 (100)	130/130 (100)
Pupil constriction/day 20	169/169 (100)	158/159 (99)	179/180 (99)	128/130 (98)

Data taken pages 214, 215, and 274, MRID No. 433786-01.
[a]Number of pups reaching the stage/number examined; numbers in parenthesis is the percent reaching the stage
*$p<0.05$, **$p<0.01$

4. Necropsy results

a. Organ weight – Organs of the pups were not weighed.

b. Pathology –

Macroscopic examination – There were no statistically significant increases in incidences of gross lesions in the pups exposed to the test material. The pup and litter incidence of dilated renal pelvis was significantly ($p<0.01$) decreased in low- and high-dose F_{1b} litters relative to controls; the incidences were decreased in low- and high-dose F_{1a} and F_2 litters, but not significantly.

III. DISCUSSION

Groups of 25 male and 25 female Wistar rats were fed mepiquat chloride in their diets at concentrations of 0, 500, 1500, or 5000 ppm for 10 weeks (F_0) or 14 weeks (F_1) before mating, and during mating, gestation, and lactation. The F_0 parents were mated a second time 2 weeks after weaning the first litter.

A. SYSTEMIC TOXICITY

Feeding of mepiquat chloride produced no treatment-related effects on mortality, but clinical signs indicative of neurological impairment were observed. Tremors and hypersensitivity occurred in ≥70% of the high-dose F_0 and F_1 dams, but only during lactation showing the transient nature of this effect. In addition, treatment-related decreases in forelimb and/or hindlimb grip strength occurred in high-dose F_0 or F_1 dams during lactation. The transient nature of the effect occurring during lactation could be due to the increased consumption of test material, averaging 625.1 to 715.2 mg/kg/day during lactation days 7-14 compared with 433.3 to 485.8 during the gestation periods. A statistically significant decrease in hindlimb grip strength was observed in high-dose males, but the statistically significant increase in hindlimb grip strength observed only in low-dose males is not considered to be treatment related. There were no statistically significant effects on cholinesterase activity in the plasma, erythrocytes, or brain in male or female rats fed mepiquat chloride.

Mean body weights and body weight gain showed statistically significant decreases in high-dose F_0 and F_1 males and females at different times during the treatment period. Mean body weights in the F_0 males were decreased by 10-11%, compared with controls, throughout the 29-week treatment period, and overall body weight gain was reduced by 13 or 14% during the premating period and the entire 29-week treatment period. During the premating period, the reduced body weights and weight gain in high-dose F_0 female rats did not achieve the levels seen in the males and are not considered to be biologically significant, as body weights were reduced by only 7% or less and weight gain by 11% or less. In addition, there were no biologically significant

females lost weight during this period. The high-dose F_1 pups gained only about 60% as much weight as the corresponding controls during lactation and weighed only about one-half as much as controls at the beginning of the premating period. Compensatory growth in the F_1 adults was apparent by the gradual increase in body weights such that, at the end of the premating period, overall body weight gain in high-dose F_1 males was only 15% less than that of controls and was slightly increased (102%) in high-dose F_1 females relative to controls. The same effect on body weights and weight gain was seen in lactating F_1 dams and the F_2 pups as seen in the F_0 dams and F_1 pups. In addition, the significantly slower growth rate seen in F_1 and F_2 pups maybe due to the weight loss of the dams during lactation; however, direct toxicity of the test material cannot be ruled out.

Decreased food consumption accounted for part of the reductions in body weight gain observed in F_0 and F_1 animals during the premating period, as evidenced by a slight changes in food efficiencies. During the lactation period when F_0 and F_1 dams failed to exhibit a net weight gain, food consumption was reduced by only 21 to 23% compared with that of controls. These results suggest that the reduced body weight gain was due to a toxic effect of the test material as well as the reduction in food consumption.

Mepiquat chloride had a profound effect on body weight gain especially in the lactating adult animals; consequently, the effect on growth of the pups is considered to be a result of the debilitating effect on the dams as well as to direct systemic toxicity (when the pup begin to eat the food) rather than reproductive toxicity. Between day 1 and 21 p.p., body weight gain was so severely affected that the F_1 and F_2 pups weighed only 64 to 68% as much as controls and gained only 61 to 64% as much weight as the controls. Delays observed in the developmental milestones of high-dose F_1 and F_2 pups included unfolding of the pinna, opening of the auditory canal and eyes, and the gripping reflex. Because of the profound effect on weight gain in the high-dose pups, these effects are considered to be due to the retarded growth of the pups rather that to toxicity of the test material.

No treatment-related effects occurred in the urinalysis parameters. Changes in clinical chemistry parameters were not dose-related (decreased serum alanine aminotransferase activity in low-dose F_0 females), were not biologically significant (decreased serum creatinine levels in high-dose F_0 females and decreased serum globulin levels in high-dose F_1 females), or were due to unknown causes or to reduced weight gain (decreased serum alanine aminotransferase activity in high-dose F_1 males).

Effects observed at necropsy included statistically significant reduced absolute or relative weight of the liver and/or kidneys in high-dose males and females. There were no accompanying gross or microscopic lesions in these organs. However, the incidence and/or severity of lipid storage in the liver was significantly ($p<0.01$ or 0.05) decreased in high-dose animals of both sexes and generations. The study authors

attributed the decreased in lipid storage to a "catabolic nutritional situation." The decreased organ weights may be due to the decreased terminal body weights and not to a direct toxic effect of the test material.

The lowest-observed-effect level (LOEL) for systemic effects is 5000 ppm (499.3 mg/kg/day for males; 530.0 mg/kg/day for females) based on impaired neurological function, decreased body weight and body weight gain of the adult males and females, and retarded growth of the F_1 and F_2 pups. The corresponding no-observed-effect level (NOEL) is 1500 ppm (146.6 mg/kg/day for males and 162.0 mg/kg/day for females).

B. REPRODUCTIVE TOXICITY

There were no statistically significant effects on reproductive indices for either F_0 or F_1 generation parents. There was a significant decrease (20/25, $p<0.05$) in the number of high-dose F_0 males with proven fertility during the first mating; however, the male fertility index (80 vs 100% for controls) was not significantly decreased. The fertility of all high-dose F_0 males was proven during the second mating and the decrease noted for F_1 males did not achieve statistical significance, suggesting that the decrease observed during the first mating was not treatment related. Furthermore, the historical control data showed that male fertility indices ranged from 80 to 100% in 31 studies. Fertility was reevaluated and confirmed for all but one F_0 and one F_1 female, both in the 500-ppm group, further showing the lack of a treatment-related effect on the reproductive indices. The duration of gestation was significantly ($p<0.01$ or 0.05) decreased for high-dose F_0 dams during the first and second mating. The range for historical controls was 21.7 to 22.5 days compared with 21.6 and 21.5 days for high-dose dams during gestation for F_{1a} and F_{1b} litters, respectively. Because the values for the treated animals were below the range of historical controls, the shortened gestation period maybe a treatment-related effect, but it is not considered to be biologically significant. There was no effect on the duration of gestation for F_1 dams.

Absolute weights of reproductive organs (testes and epididymides) in F_0 male rats were similar to those of controls, but the relative weights at the high dose were significantly elevated; whereas in high-dose F_1 male rats, the absolute weights of the testes and epididymides were significantly reduced and the relative weights were elevated. The changes in absolute and relative weights were probably due to statistically significant reduced terminal body weights and not to a toxic effect of the test material.

Feeding of mepiquat chloride to male and female rats did not cause reproductive toxicity; therefore, a LOEL cannot be established; the NOEL is >5000 ppm.

C. STUDY DEFICIENCIES

There were no noteworthy deficiencies in this study.

433786-01

83-4 Reproduction

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?:

1. ✓ Technical form of the active ingredient tested.
2. ✓ At least 20 males and sufficient females to yield 20 pregnant /dose group
3. ✓ At least 3 dose groups and a control.
4. ✓ At the high dose, parental toxicity is observed (or a limit dose is given. 1,000 mg/kg/day).
5.* ✓ At the low dose, no reproductive effects are observed.
6.* ✓ Analysis for test material stability, homogeneity and concentration in dosing medium
7. ___ P_1 animals 8 weeks old at the start of the study. 5wks.
8. ✓ Dosing is continuous starting with the P_1 animals until an individual animal is sacrificed.
9. ✓ Mating is 1 male to 1 female.
10. ✓ The mating period is not more than 3 weeks.
11. ✓ At least two generations are bred.
12. ✓ Individual daily observations.
13. ✓ Individual body weights.
14. ✓ Individual food consumption.
15. ✓ Individual litter observations.
16. ✓ Individual litter weights (pup weights) at birth and on days 4, 7 (optional), 14 and 21 .
17.* ✓ Sacrifice schedule, all mating males immediately after last mating, all breeding females immediately after weaning last litter, all animals not used for breeding immediately after weaning.
18.* ✓ Necropsy on all animals
19.* ✓ Histopathology of reproductive organs from all animals on the high dose and control P_1 and F_1 animals selected for mating. Animals from all other dosing groups if histological effects are observed at the high dose.
20.* ✓ Histopathology of all organs with gross lesions.

Criteria marked with a * are supplemental and may not be required for every study.