

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
MEPIQUAT CHLORIDE

Chemical Code # 2075, Tolerance # 384
SB 950 # 303
Original date: 12/18/01
Revision dates: 8/27/02 and 1/17/03

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, no adverse effect.
Chronic toxicity, dog:	No data gap, no adverse effect.
Oncogenicity, rat:	No data gap, no adverse effect.
Oncogenicity, mouse:	No data gap, no adverse effect.
Reproduction, rat:	No data gap, no adverse effect.
Teratology, rat:	No data gap, no adverse effect.
Teratology, rabbit:	No data gap, no adverse effect.
Gene mutation:	No data gap, no adverse effect .
Chromosome effects:	No data gap, no adverse effect.
DNA damage:	No data gap, no adverse effect.
Neurotoxicity:	<u>Not a required study type. Valid supplemental studies are present.</u>

Toxicology one-liners are attached.

All record numbers for the above study types through No. 202111 (Document No. 384-086) were examined. This includes all relevant studies indexed by DPR as of 1/17/03.

In the 1-liners below:

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

File name: T030117

Revised by Aldous, 8/27/02 and Gee, 1/17/03.

NOTE: A series of U.S. EPA reviews of several major current FIFRA studies is found in a folder under DPR tracking ID SBC-162250-E (no Document No. or Record No. assigned to folder).

There were no data in the package. These studies were found acceptable by U.S. EPA and DPR.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may identify additional effects.

NOTE: The following 1-liner addresses issues of the form and stability of test article used in the studies below:

384-045 132715 Hambrick, A. A., "Mepiquat chloride 60% MP - Determination of storage stability," Ricerca, Inc., 12/8/93. BASF Report # 91106. This report and several smaller reports are cited to address appropriate physical form of the test article, and to assess stability of mepiquat chloride in support of several toxicity studies which do not provide internal validations of stability of the a.i. Record No. 132715 found no degradation of mepiquat in a 60% MUP formulation (an aqueous solution) following 97 days of storage at 50°C, nor after 12 months at 25°C. Another report (384-062 144492, MRI Project No. 3334-F) found that a 50% formulation (Batch 23021) was stable for up to 1 year at ambient temperature. A 4.2% liquid formulation was also found to be stable for at least 1 year at room temperature (384-070 150614, MRI Project No. 9941-06). Report 384-045 132681 noted that "the technical grade of the active ingredient (TGAI), is not produced on a commercial scale". Report 384-063 144494 adds that the a.i. is very hygroscopic, and concluded that these physical characteristics would make it irrelevant to designate the dry powder as the appropriate test article for animal studies. Thus, although there do not appear to be stability data on the solid technical a.i., the above data suffice to warrant the use of comparatively concentrated aqueous solutions as test article, and to validate stability of test article as used in chronic animal studies. Aldous, 10/15/01.

Genari, G., "Re-analysis of mepiquat-chloride Batch WW 262 / CP 1490," BASF Aktiengesellschaft, Limburgerhof, 11/93. BASF Document No. 89/112. This batch was used in several studies, including two 2-year rat studies (384-038 130816 and 384-046 132704). A sample received on July 18, 1988 assayed at 616.5 g/l. A sample of the same batch received on Aug. 25, 1993 was found to have a concentration of 614.6 g/l. Thus, no detectable degradation had occurred in over 5 years at room temperature. These data support stability of mepiquat chloride for chronic animal studies. Aldous, 10/23/01.

COMBINED, RAT

(see also "Chronic Toxicity, Rat" and "Oncogenicity, Rat" sections below)

384-002 031127, "Chronic oral toxicity of 1,1-dimethylpiperidinium chloride, Reg.No. 85559, Tech. - called for short 'DMP' - in the Sprague Dawley rat," (F. Leuschner, A. Leuschner, K. Klie, W. Dontenwill and P. von Rogulja, Laboratorium für Pharmakologie und Toxikologie, Report C 20 A, 9/18/79). 1,1-dimethylpiperidinium chloride was administered in diet at concentrations of 0, 100, 300, 1000, 3000 and 9000 ppm to 100, 50, 50, 50, 50 and 30 Sprague-Dawley rats/sex/group, respectively, for 104 weeks. Five additional rats/sex of the control and two highest dose groups were included for sacrifice at 52 weeks. Unacceptable: (Insufficient data for assessment; too few high dose animals). (C. Aldous, 8/23/85).

NOTE: Reports 384-013 040829 and 384-014 040830 are the same study as 384-002 031127. The latter records constitute a more complete report than Record No. 031127. These latter records were re-examined to confirm that there were no indications of tumor effects (Table 24 of the report shows no such increases). No worksheet is needed for these records in the absence of adverse effects, and given the availability of valid rat chronic and oncogenicity studies (below). Aldous, 12/6/01.

CHRONIC TOXICITY, RAT

** 384-038 130816 Mellert, W., "Chronic toxicity study with Mepiquat Chloride in Wistar rats - Administration in the diet for 24 months," BASF Aktiengesellschaft, Ludwigshafen, May 3, 1994. BASF Document No. 94/10285. Twenty rats/sex/group were dosed in the diet with mepiquat chloride (provided as an aqueous solution of purity 57.9%) at 0, 290, 2316, or 5790 ppm for 2 years in a chronic study design. Study included a limited series of neurofunctional observations made pre-study and at 4 intervals during the study. All 31 such measures were negative in all groups at all times, hence these were of limited usefulness. Estimated mean dose levels were 13, 106, and 268 mg/kg/day in treated males, and 18, 146, and 371 mg/kg/day in females. NOEL = 2316 ppm [body weight decrements in both sexes, elevated incidence of urinary crystals (mostly triple phosphate) without evident associated pathology]. Alanine aminotransferase activities were frequently statistically significantly reduced (opposite to the direction of primary diagnostic value) in mid- and high-dose rats. The latter does not appear relevant for further toxicity analysis. There were no gross or histopathology effects. Acceptable as a chronic study. Aldous, 12/18/01.

EPA 1-liner: This study (038 130816) is classified as core-minimum, satisfying the guideline requirements for a chronic feeding study in rats (83-1a).

CHRONIC TOXICITY, DOG

384-022 091025 Mellert, W., "Report on the study of the toxicity of Reg. No. 85 559 in beagle dogs: administration via the diet over 12 months," BASF Aktiengesellschaft, Ludwigshafen, 9/25/89. BASF Document # 89/0357. Six dogs/sex/group were dosed in the diet with 0, 200, 600, or 1800 ppm mepiquat chloride [purity 99.5%, Batch No. N 48, a white-gray powder] for 12 months. Mean achieved dose levels (averaged over sexes) were 6.3, 19.9, and 58.4 mg/kg/day. NOEL = 600 ppm (slight increase in grade of splenic iron storage in males). The increase in the degree of iron pigment storage recorded for spleen in this study was consistent with modest increase in degree of hemosiderin storage in spleens of high dose males in a subsequent high-dose-only study (Record No. 130820). There were no corresponding changes in females in either study. This study is not independently acceptable, since the dose range was insufficient to fully challenge the animals, however these two complementary dog chronic studies are being considered together (see review of Record No. 130820). No adverse effects. Aldous 12/18/01.

**384-042 130820 Mellert, W., "Supplementary study of the toxicity of Mepiquat Chloride in beagle dogs - Administration via the diet for over 12 months," BASF Aktiengesellschaft,

Ludwigshafen, 5/5/94. Laboratory Study # 94/10282. Mepiquat chloride (56.05 % in water), was incorporated into diets of 6 dogs/sex/group at 0 and 6000 ppm. Estimated achieved dosages were 166 (M) and 173 (F) mg/kg/day. Study evaluated parameters of a standard chronic study, and is acceptable as a supplemental study with one notable deficiency (study lacked weekly thorough clinical exams of dogs). No NOEL was achieved (such was not the intent of the study). This study found that 8000 ppm (administered only on the first day of exposure) was excessively toxic, being lethal to 3 of 12 dogs after only one day of dosing. A slight reduction to 6000 ppm dramatically reduced the toxicity, however a death of one female was considered by investigators to be plausibly treatment-related [signs were "Weakness of the hind limbs, lateral position, extension spasms, ataxia of the hind limbs, subnormal body temperature, reduced general state"]. Other common findings at this dose level were increased salivation, slightly increased incidence and degree of kidney vacuolization, and increased degree of splenic hemosiderin storage (the latter in males only). Males had a statistical increase in adrenal gland weights with no associated histopathology. This study together with the primary chronic dog study (Record No. 091025) are **acceptable** to satisfy the dog chronic data requirements, and **do not indicate adverse effects**. The dose levels required to achieve well-characterized chronic toxicity were near to the acutely toxic range, i.e. well above 58 mg/kg/day (the high dose in the primary chronic study) and probably near to 166-173 mg/kg/day (achieved dose levels in this supplemental study in M and F, respectively). Aldous, 10/18/01.

EPA 1-liner: EPA considers the above study (022 091025) along with Supplemental study (042 130820) as ACCEPTABLE, satisfying the guideline requirement for a chronic oral study (83-1) in dogs. The LOEL for males and females is 6000 ppm (170 mg/kg/day) and the NOEL 1800 ppm (58.4 mg/kg/day).

ONCOGENICITY, RAT

**384-046 132704 Mellert, W., "Carcinogenicity study with Mepiquat Chloride in Wistar rats: Administration in the diet for 24 months," BASF Aktiengesellschaft, Ludwigshafen/Rhine, 9/15/94. BASF Document No. 94/10772. Fifty rats/sex/group were dosed in diet with mepiquat chloride (provided as an aqueous solution of purity 57.9%) at 0, 290, 2316, or 5790 ppm for 2 years in an oncogenicity study. Estimated dose levels were 13, 105, and 269 mg/kg/day in treated males, and 17, 141, and 370 mg/kg/day in females. NOEL = 13 mg/kg/day, based on 12% body weight decrement in males. NOEL in females = 141 mg/kg/day (body weight and food consumption decrements, increased mammary secretory activity, dilated secretory activity in ovarian bursa, uterine squamous hyperplasia and stromal hyperplasia, and increased incidence of hemorrhage in thymus). Body weight decrements at termination at the high dose were 18% and 19% in M and F, respectively. No adverse effects: the availability of a substantial mid-dose treatment without histopathology suggests that there was little or no tissue-specific toxicity. There was no oncogenicity. Acceptable. Aldous and Kishiyama, 10/23/01.

384-079 186943 Addendum confirming the purity of the test article used in Record No. 132704, above. No new worksheet. Study was acceptable at original review. Aldous, 8/22/02.

EPA 1-liner: The above study (046 132704) is classified as ACCEPTABLE (core minimum) and satisfies the guideline requirements for an oncogenicity study in rats (83-2a). Systemic

NOEL = 2316 ppm (105 mg/kg/day for males and 141 mg/kg/day for females). Systemic LOEL is 5790 ppm.

ONCOGENICITY, MOUSE

**384-039 130817 Mellert, W., "Carcinogenicity study with mepiquat chloride in B6C7F1 mice: Administration in the diet for 24 months," BASF Aktiengesellschaft, Ludwigshafen, May 4, 1994. BASF Study # 94/10284. Mice were dosed with mepiquat chloride (57.9% a.i.) in diet at 0, 500, 2000, or 7500 ppm of the a.i.. Fifty/sex/group were allocated to the oncogenicity study. Ten/sex/group comprised a 1-yr satellite study involving terminal blood smear, necropsy, and histopathology evaluation. NOEL = 2000 ppm (297 mg/kg/day) in males (modest body weight decrements), and 7500 ppm (1348 mg/kg/day) in females (no changes detected). Acceptable, with no adverse effects. Aldous, 10/25/01.

EPA 1-liner: The above study (039 130817) is classified as core-minimum, satisfying the guideline requirements for an oncogenicity study in mice (83-2a). No MTD, however, the high dose (7500 ppm) exceeded the limit dose of 1000 mg/kg/day.

384-002 031128, "Chronic Oral Toxicity of 1,1-Dimethylpiperidinium Chloride, Reg 85 559, Technical - Called for Short "DMP" - in the NMRI Mouse", (F. Leuschner, A. Leuschner, R/Klie/R. Stehr, W. Dontenwill and P. von Rogulja, Laboratorium für Pharmakologie und Toxikologie, Report C 21 A, July 20, 1979). Reg. 85 559, was fed in diets of NMRI mice at 0, 100, 300, 1000, and 3000 ppm for 104 weeks. There were 100, 50, 50, 50, and 50 mice/sex/group. No apparent toxicity nor oncogenicity up to the HDT of 3000 ppm. Unacceptable (multiple deficiencies). (C. Aldous, 8/23/85).

384-012 040828 This is the same study as the above record (031128) but, is a more complete report. There is no need for a separate worksheet, because no adverse effects were indicated, the study has multiple deficiencies with respect to guidelines, and because dose levels in the more recent and acceptable study were more rigorous than this study. Aldous, 12/6/01.

REPRODUCTION, RAT

**384-040 130818 Hellwig, J., "Reproduction toxicity study with mepiquat chloride in rats: Continuous dietary administration over 2 generations," BASF Aktiengesellschaft, Ludwigshafen, Sept. 2, 1993. BASF Registration Document No. # 93/10983. Wistar rats, 25/sex/group, were dosed in the diet with Mepiquat chloride (57.9% a.i.) at dose levels of 0, 500, 1500, and 5000 ppm (corrected for % a.i.). Treatment was continuous from pre-mating periods (70 and 98 days for F0 and F1 generations, respectively) until sacrifice. There were 2 mating periods for F0 parents, and one for F1 parents. Estimated achieved dose levels were at least 49, 147, and 499 mg/kg/day in males, and at least 53, 162, and 530 mg/kg/day in females during pre-mating periods. Exposures of females during gestation were similar, but lactation intakes averaged at least 70, 198, and 625 mg/kg/day. This study included brief FOB assessments on adult rats prior to mating, during lactation (females), and after weaning periods. Parental NOEL

= 1500 ppm, based on several high dose effects. Food consumption was reduced in both sexes at 5000 ppm throughout the study, being most pronounced during periods of rapid growth of young rats and in lactating females. Body weights followed similar patterns, and were also typically significantly reduced throughout the study (usually $p < 0.01$). Most high dose dams had episodes of tremor and "hypersensitivity" (behavioral), and a few displayed ataxia. These signs were limited to lactation periods. Two rats selected as F1 parents died just before the onset of the pre-mating period [when dietary intake was probably over 1000 mg/kg/day, based on week 0 to 1 estimated compound intakes]. High dose adults had reduced degrees of lipid storage at termination compared to other groups. Pup NOEL = 1500 ppm. High dose pup body weight fell progressively behind other groups over the course of lactation. There were significant delays in 5000 ppm pup developmental benchmarks such as pinna unfolding, auditory canal opening, eye opening, and gripping reflex. Each of these criteria was evident in at least two of the mating periods, corresponding to reduced growth rates. High dose pup viability was reduced in the F1a littering period during the first week of lactation. There was an apparent reduction in 5000 ppm group delivered pups in the F2 littering period (outside historical range). High dose pups frequently had reduced forelimb or hindlimb grip strength. Acceptable, with no adverse effects (no evidence of special toxicity to offspring). Aldous, 12/18/01.

EPA 1-liner: The above study [384-040 130818] is ACCEPTABLE (core guideline). Reproductive NOEL = >5000 ppm. Systemic NOEL = 1500 ppm (150 mg/kg/day for males and 163 mg/kg/day for females). Systemic LOEL is 5000 ppm.

384-002 031129, "Chronic Toxicity of 1,1-Dimethylpiperidinium Chloride, Reg.No. 85559, Tech. - called "DMP" - in Three Succeeding Generations of Sprague Dawley Rats at Oral Administration", (F. Leuschner, A. Leuschner, G. Stehr and W. Dontenwill, Laboratorium für Pharmakologie und Toxikologie, 9/18/79). 1,1-Dimethylpiperidinium Chloride was admixed with the feed at concentrations of 0, 319.1, 1063.8 and 3191.5 ppm and fed to 40 Sprague-Dawley rats/sex/group for 3 generations (2 litters/generation). Twenty dams delivered and 20 were C-sectioned/generation. No maternal toxicity and no reproductive effects observed for any dose level. UNACCEPTABLE. Not upgradeable (dose selection not justified, insufficient data). DPR does not need further information on this study, since an acceptable study at more rigorous dose levels has been evaluated. (C. Aldous, 8/26/85: updated 12/17/01).

384-016 040832 This is the same study as the above record (031129) but, is a more complete report. There is no need for a separate worksheet, because no adverse effects were indicated and because dose levels in the more recent and acceptable study were more rigorous than in this study. Aldous, 12/6/01.

TERATOLOGY, RAT

**384-027 114652 Hellwig, J., "Study of the prenatal toxicity of mepiquat chloride in rats after oral administration (gavage)," BASF Aktiengesellschaft, Ludwigshafen, April 7, 1992. BASF Registration Document No. 92/10331. Wistar rats, 25/group, were dosed by gavage (in 10 ml distilled water per kg b.w.) during gestation days 6-15 at 0, 50, 150, or 300 mg/kg/day in a standard developmental toxicity study. Maternal NOEL = 150 mg/kg/day (decreased food

consumption, decreased body weight gain, and clinical signs in the majority of high dose dams, such as tremor, unsteady gait, hypersensitivity to external stimuli, piloerection, and indrawn flanks). Also, four high dose dams displayed ataxia. No other rats were affected with any of these signs. All clinical signs were limited to about the first 4 hr after daily dosing. There was no evident developmental toxicity. Acceptable, with no adverse effects. Aldous, 12/17/01.

384-004 031119 Hofmann, H. Th. and J. Peh, "Study of the Prenatal, Perinatal and Postnatal Toxicity of 1,1-Dimethylpiperidinium Chloride on Rats" (BASF Gewerbehygiene und Toxikologie, 8/5/77). 1,1-dimethylpiperidinium chloride was administered in diets at 0, 100, 300, 1000 and 3000 ppm to 25 Sprague-Dawley rats from gestation days 0 to 20 in a teratology study. An additional 10 females/group were dosed from days 0 to 21 post partum prior to sacrifices of dams and weanling pups at lactation day 21 to assess effects of post-natal exposure. There were no apparent effects relating to teratogenicity, reproductive effects, nor maternal toxicity at any dose level. UNACCEPTABLE. Not upgradeable. This is not a guideline study design, and dose levels during the period of organogenesis were lower than in the definitive study above. C. Aldous, 8/23/85 (1-liner edited by Aldous on 12/18/01).

384-002 969393: Incomplete duplicate of record # 031119.

TERATOLOGY, RABBIT

**384-076 163195 Bachmann, S., J. Hellwig, and B. Hildebrand, "BAS 083 W - Prenatal developmental toxicity study in Himalayan rabbits: Oral administration (gavage)", BASF Aktiengesellschaft, Ludwigshafen, 5/27/98. Laboratory Project ID: 40R0189/97019. Fifteen does/group were dosed daily by stomach tube on gestation days 7 to 19 with 0, 50, 100, or 150 mg/kg/day of mepiquat chloride (56.7% purity, dosing solutions adjusted for purity in distilled water and administered in 5 ml/kg volume) in a standard developmental toxicity study. Maternal NOEL = 50 mg/kg/day, based on dose-related food consumption decrement during treatment. This diminished food consumption lasted from days 8-20 at 150 mg/kg/day, and from days 11-14 at 100 mg/kg/day. No developmental toxicity was evident at any dose tested. At 150 mg/kg/day the does suffered severe food consumption decrements and a modest reduction in body weight gain. Two high dose does had "blood in bedding." One of these delivered prematurely following 20 days of meager food consumption, and was found with stomach erosions at necropsy. The findings in the latter two high dose does were attributed to maternal toxicity. Study is acceptable, with no adverse effects. Aldous, 12/17/01.

384-017 045515 Merkle, J., "Study to determine the prenatal toxicity of 1,1-dimethylpiperidinium chloride in rabbits", BASF Gewerbehygiene und Toxikologie, May 4, 1981. This appears to be a follow-up study to 384-017 045516 (below), which indicated that 150 mg/kg/day and perhaps 100 mg/kg/day were excessively maternally toxic. Fifteen Himalayan rabbits per group were artificially inseminated, then dosed by gavage with mepiquat chloride (99% purity) at 0, 75, or 100 mg/kg/day on gestation days 6-18 in a basic teratology study. Food consumption was reduced significantly ($p < 0.01$) in both treated groups. Fetal length was significantly

reduced in both treated groups compared to concurrent controls. The latter apparently reflects unusually large concurrent control values, based on historical control data in Appendix 4 of the report. No worksheet: This study is not acceptable or upgradeable by current standards, and there are no essential endpoints unique to this study. No adverse effects. Aldous, 12/18/01.

384-017 045516 Hofmann, H. T. and J. Merkle, "Study of the prenatal toxicity of 1,1-dimethyl-piperidinium chloride in rabbits", BASF Gewerbehygiene und Toxikologie, 2/14/79. Himalayan rabbits, 21-22 per group, were artificially inseminated, then dosed by gavage with mepiquat chloride (99% purity) at 0 (untreated), 0 (5 ml/kg b.w. distilled water), 50, 100, or 150 mg/kg/day on gestation days 6-18 in a basic teratology study. Food consumption was reduced significantly, dose-related, in all treated groups during days 6-12. Food consumption continued to be significantly reduced in the higher two groups during days 12-18. Body weight gain was moderately reduced at 50 to 100 mg/kg/day during days 6-12 (not statistically significant), and was significantly reduced at 150 mg/kg/day during that time period. Body weight gain continued to be reduced during days 12-18 in the 100 to 150 mg/kg/day groups. Excessive maternal toxicity was demonstrated by mortalities and abortions at the higher two doses. Seven 150 mg/kg/day does died, whereas there were no deaths in other treatment groups. Seven 100 mg/kg/day does and four 150 mg/kg/day does aborted, vs. 0 or 1 in other groups. Thus maternal toxicity NOEL = 50 mg/kg/day (considering the transient food consumption decrement at 50 mg/kg/day to most likely be a palatability issue). Fetal length was unaffected by treatment at all dose levels (Table 24), suggesting that an apparent effect in Record No. 045515 was unlikely to be treatment-related. There was no apparent developmental toxicity. No adverse effects. No worksheet as there are no essential endpoints unique to this study, and an acceptable study is available (384-076 163195). Aldous, 12/16/01.

384-015 040831 Same study as 045516. Includes page 2 of the introduction in the first portion of the report and pages 1 and 2 of the summary in the second portion of the report that are missing in 17 045516.

GENE MUTATION

** 384 - 0086 202111 " Mepiquat-chloride: Reverse mutation study on bacteria." (Kanaguchi, Y., Study Director, Toxicology Institute, Environmental Toxicology Laboratory, Nippon Soda Co., BASF # 90/0313, August 6, 1990) Mepiquat chloride (99.6%, lot AG-L-101) was tested with *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 and with *Escherichia coli* WP2 uvrA, with and without rat liver activation. There were triplicate plates per concentration in the preliminary and two subsequent trials. In the two mutation trials, a 20-minute pre-incubation step was used before plating in agar. Concentrations were 0 (distilled water), 156.3, 312.5, 625, 1250, 2500 and 5000 µg/plate in the two definitive trials. There was no evidence of cytotoxicity or of increase in revertants in any test. Positive controls functioned as expected. No adverse effects. ACCEPTABLE. (Gee, 1/16/03)

384 - 023 091026 Zeller, H. "Report on the testing of Reg. No. 85 559 (Mepiquat Chloride) in the Ames test." (BASF Aktiengesellschaft, FRG, 7/5/79) Mepiquat chloride, 99.8%, was tested for genotoxicity with *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98

and TA100, with and without rat liver activation, at 0 (water), 4, 20, 100, 500 or 2500 : g/plate, 48 hours incubation. There were 4 replicate plates per concentration with a single trial. Positive controls with activation were cyclophosphamide and 2-aminoanthracene and without activation, MNNG (not tested with TA1538). Positive controls were functional. There was no evidence of cytotoxicity or induction of reverse mutations under the assay conditions. No adverse effect. UNACCEPTABLE (highest concentration used was not justified.) Possibly upgradeable with adequate justification for the highest concentration of 2500 : g/plate. (Gee, 11/15/01)

384-079 (no record number). A letter from Laura Whatley at BASF notes that although the above study (384 - 023 091026) did not dose to limit test levels and did not elicit cytotoxicity, it was acceptable to U.S. EPA. She noted further that UDS and chromosomal aberration studies were negative, and that there was no evidence of oncogenicity. She requested, therefore, that DPR likewise accept the study. DPR Response: Because the original study did not dose to the limit test levels, did not elicit cytotoxicity, and did not provide analysis of dosing solutions or suspensions, it cannot be documented that cultures actually received the intended treatments. No other studies address the same or equivalent endpoints as this one does. DPR does not have justifiable reason to accept this study. The study should be replaced (or a suitable study addressing the exposures defined by limiting cytotoxicity or the limit test should be provided). Aldous, 8/27/02.

CHROMOSOME EFFECTS

** 384 - 023 091027 Taalman, R. D. F. M. "Clastogenic evaluation of Mepiquat Chloride in an in vitro cytogenetic assay measuring chromosome aberration frequencies in Chinese hamster ovary (CHO) cells." (Hazleton Biotechnologies, The Netherlands, 12/4/87) Mepiquat chloride, batch N48, >99% technical, was tested with CHO-WBI cells for the induction of chromosomal aberrations with and without rat liver activation at concentrations of 0 (untreated), 0 (medium), 2, 3, 4, or 5 mg/ml. Treatment time without activation was 7.8 hours and 2 hours with activation. Total incubation time was approximately 10 hours before harvest. There were duplicate cultures per treatment level with mepiquat chloride, single trial. One hundred metaphases per culture were scored. Concentration selection was based on a range-finding study for mitotic delay and % confluency. Cytotoxicity was seen only at 5 mg/ml without activation. The positive controls, mitomycin C without S9 and cyclophosphamide with activation, were functional. There was no evidence for the formation of chromosomal aberrations with mepiquat chloride under the conditions of the assay. No adverse effects. Although the concentration of the test article in the medium was not determined, the study has been evaluated as ACCEPTABLE. (Gee, 11/15/01)

384-004 031120. "Examination of 1,1-Dimethylpiperidinium Chloride Reg. No 85559, Tech. - Called "DMP" - for Mutagenic Properties (Dominant Lethal Genes) in Mice at Oral Administration", (F. Leuschner, Laboratorium für Pharmakologie and Toxikologie, 5/9/79). DMP, purity not stated, admixed with the feed at concentrations of 0, 100, 300, 1000 and 3000 ppm and fed to 20 NMRI males/group for 5 days prior to breeding. Twenty additional untreated males/group served as controls. Males (treated and untreated) were mated with untreated females. No dominant lethal effects and/or other treatment related effects up to the highest dose tested (3000 ppm). UNACCEPTABLE. Not upgradeable (the high dose selected for this study

too low, considering that higher dose levels were survivable in the oncogenicity study). (C. Aldous, 8/23/85, 1-liner revised 12/17/01).

384-002 969379: Incomplete duplicate of record #031120.

DNA DAMAGE

**384 - 023 091028 [Supplemental Information in 384 - 079 186942, 4/4/02] Cifone, M. A. "Report on the mutagenicity test on mepiquat chloride in the rat primary hepatocyte unscheduled DNA synthesis assay." (Hazleton Laboratories America, MD, BASF No. 87/0393, 9/15/87) Mepiquat chloride, lot N48, 99.9%, was tested with primary hepatocytes from an adult male Fischer 344 rat for the induction of unscheduled DNA synthesis. Two trials were conducted with five replicate coverslips per concentration, with two being used for cytotoxicity by trypan blue dye exclusion. The concentrations in trial 1 were 0 (medium), 25.6, 51.2, 102, 256, 512, and 1020 ug/ml. Survival at 1020 ug/ml was 90.4%. A second trial was conducted at higher concentrations of 0, 1000, 2000, 3000, 4000, or 5000 ug/ml. Survival at 4000 was 55.4% and at 5000 ug/ml, 32.3%. Cells at these concentrations were not evaluated because the morphology was unacceptable. Survival at 3000 ug/ml was 71.6%. The positive control was 2-acetylaminofluorene and was functional. There was no evidence for the induction of UDS at any concentration in either trial. Report was originally unacceptable (lacked data for nuclear counts, cytoplasmic counts and individual results of the triplicate coverslips per concentration). Those data were submitted in Record No. 186942. There were no significant changes in UDS due to treatment. (Gee, 11/16/01: Acceptable, with supplemental data: Gee and Aldous, 8/22/02).

NEUROTOXICITY (including supplemental studies)

Neurotoxicity studies are not required at this time. Supplemental studies to elucidate mechanisms of action relating to clinical signs noted in rat studies are included below.

384-030 115521 Weifenbach, H., "Report on the *in vitro* test of the action of mepiquat chloride at nicotinic acetylcholine receptors of adult mouse muscle", Institute of Physiology of the Technical University, Munich, 12/12/91. BASF Registration Document No. 91/11204. Mepiquat chloride, 99% purity, was tested with the patch-clamp technique to evaluate single channel openings via binding of test article to the nicotinic receptor. Intraosseal foot muscles from adult NMRI mice were dissociated to yield individual muscle cells, and patch-clamp recordings were evidently performed on the external surfaces of endplate portions of cells. Both cell-attached and outside-out modes were tested to expose nicotinic receptors. Frequencies of single channel openings were evaluated by amplifying pulses of current flow, representing channel opening under the influence of a ligand. Acetylcholine was the reference ligand. Under study conditions, mepiquat chloride elicited channel openings with both cell-attached and outside-out modes. Individual data were not provided. Investigators stated that mepiquat chloride possessed about 1/100 of the activity of acetylcholine. Duration of channel opening with mepiquat chloride was about 1/3 the duration of acetylcholine. Investigators concluded that mepiquat chloride is a "partial agonist" of the nicotinic acetylcholine receptor, and that expected

effects would include depolarization of muscle fibers, causing excitation of fibers and eventually muscle weakness. Useful supplemental data. Aldous, 12/12/01.

384-030 115516 Weifenbach, H., "Study on the affinity of mepiquat chloride for muscarinic receptors", Knoll AG, Ludwigshafen, 9/19/91. BASF Registration Document No. 91/11206. Mepiquat chloride, 99% purity, was tested for its affinity toward muscarinic acetylcholine receptors of three subtypes. Test membranes derived from bovine cerebral cortex, rat heart, and rat submaxillary gland. The radioligand was [N-methyl-³H]-N-methylscopolamine. Mepiquat chloride was tested for its capacity to displace the radioligand, compared to known highly specific non-radioactive ligands for each of the three receptor subtypes. Non-specific binding was evaluated by addition of 1 : M atropine. Mepiquat chloride proved to have about 3 to 5 orders of magnitude lower affinity for muscarinic receptors than high affinity ligands. Investigators determined from these results that it is unlikely that mepiquat chloride would have a biologically important effect on muscarinic receptors. Useful supplemental data. No worksheet. Aldous, 12/17/01.

SUBCHRONIC STUDIES

384-028 115514: Schilling, K. "Study on the Oral Toxicity of Mepiquat Chloride in Wistar Rats Administration in the Diet over 3 Months". BASF Aktiengesellschaft, Department of Toxicology, Project No. 31S0112/89053, Document No. 92-10433. May 15, 1992. Mepiquat chloride was administered in diet at 0, 145, 579, 2316, or 4632 ppm to 10 Wistar rats/sex/group for a period of three months. Apparent reduced food palatability was reflected in decreased food consumption and reduced body weights for the high dose group (significant body weight decrement was restricted to males, and limited to the first 2 weeks of treatment). Review of the pathology report summary tables did not reveal treatment effects. Apparent NOEL = 2316 ppm [estimated on p. 039 to be 163 mg/kg/day (M) and 188 mg/kg/day (F)], based upon urinary crystal formation in males only. Supplemental data (treatment levels were too low to demonstrate a meaningful dose-response). Of particular importance with respect to the subsequent high dose supplemental study below (Record No. 115515), there were no clinical signs suggestive of neurological effects at any dose, the highest dose levels being about 319 and 372 mg/kg/day in M and F, respectively. No repeat study is requested at this time. No worksheet (useful data, serving primarily as a range-finding determination for subsequent chronic/oncogenicity studies). Aldous, 12/12/01.

384-029 115515 Schilling, K., "Supplementary study on the oral toxicity of mepiquat chloride in Wistar rats: Administration in the diet over 3 months", BASF AG, Limburgerhof, 5/18/92. BASF Registration Document No. 92-10434. Ten Wistar rats/sex/group were dosed in diet with 0 or 12000 ppm mepiquat chloride (57.9% w/w purity) for 3 months in a supplemental (high dose only) subchronic study. Estimated achieved dose levels were 826 and 951 mg/kg/day in males and females, respectively. In addition to the standard protocol for subchronic studies, design included a limited series of neurofunctional tests on days 34, 69, and 93. No NOEL was sought or achieved. All rats survived, but treated rats reflected great toxicity. Food consumption was sharply reduced, most consistently in males. Body weights were dramatically reduced, with

final body weights lower than controls by 32% in males and by 17% in females. Principal clinical signs were tremors, altered gait, and abnormal posture. Forelimb and hindlimb grip strength was typically reduced. Major clinical chemistry findings were consistent with reduced nutritional status (reduced creatinine, glucose, globulin, and triglycerides). Ionic balance was also disturbed (plasma Cl^- levels were elevated, and Ca^{2+} levels were reduced). Cholinesterase activities were not consistently affected. Urinary triple phosphate crystals were increased, particularly in males (consistent with the high dose males in the primary subchronic study: 384-028 115514). The latter is not diagnostic of toxicity. In the present study, liver weights were sharply reduced, particularly in males. There was no evident histopathology. Investigators suggested that clinical signs may have been due to neurological effects, perhaps mediated by a reversible over-stimulation at nicotinic sites (see study 384-030 115521). The present study should be considered to indicate a possible adverse effect (neurobehavioral signs), noting that the primary subchronic study provided comparatively high NOEL's for findings of toxicological importance. Useful supplemental data, with a possible adverse effect. Aldous, 12/13/01.

384-041 130819 Range-finding study for a supplementary 12-month feeding study (vol. 42, record #130820 831). "The Toxicity of Mepiquat Chloride in Beagle Dogs - Administration Via the Diet over 4 Weeks", (W. Mellert, BASF Aktiengesellschaft, Department of Toxicology, FRG, Project No. 30D112/89109, 5/5/94). Mepiquat chloride, purity 56.05%, admixed with the feed at concentrations of 0, 6000 and 12000 ppm, was fed to 2 beagle dogs/sex/group for 4 weeks. The death of one 12000-ppm female on study day 1 was attributed to treatment. Salivation after feeding was observed in varying degrees at both dose levels. NOEL was not established. No worksheet (this valid, but limited-scope study does not provide any information beyond what is available in chronic dog studies). (Kishiyama and Aldous, 12/17/01).

384-005 969370: Leuschner, F, W. Schwerdtferger, W. Dontenwill, and P. von Rogulja. "3 Months Toxicity of 1,1 -Dimethylpiperidinium Chloride, Reg. No. 85559, Techn. - Called for Short "DMP" in the Sprague-Dawley Rat when Administered in the Food". Laboratorium für Pharmakologie und Toxikologie. October 4, 1977. DMP was admixed with the feed at concentrations of 0, 100, 300, 1000 or 3000 ppm and fed to 25 Sprague-Dawley rats/sex/group for a period of 3 months. Body weight was reduced 8 and 9% for high dose males and females, respectively. Investigators concluded that other parameters, including histopathology, showed no treatment relationships. Apparent NOEL = 1000 ppm. No adverse effects. This study has multiple deficiencies. No further data are requested, since more definitive rat subchronic studies are available. No worksheet (Kishiyama and Aldous 12/17/01).

384-005 065198: Leuschner, F, A. Leuschner, W. Schwerdtferger, W. Dontenwill, and P. von Rogulja. "Oral Toxicity of 1,1 -Dimethylpiperidinium Chloride, Reg. No. 85559, Techn. - Called for Short "DMP" in the Beagle Dog". Laboratorium für Pharmakologie und Toxikologie. September 28, 1977. DMP was fed in diet at 0, 100, 300, 1000 or 3000 ppm to 4 beagle dogs/sex/group for a period of 3 months. Apparent NOEL = 1000 ppm. Reported findings included decreased food consumption and decreased body weight gain, decreased RBC parameters (Hb, HCT, and RBC count), and sedation (usually during the first few weeks of the study). No adverse effects. This study has multiple deficiencies. No further data are requested of this study, since acceptable chronic dog studies are available, and a 4-week range-finding dog subacute study (384-041 130819, above) characterized major subchronic/subacute responses at

higher dose levels than were evaluated in the present study. No worksheet. (Kishiyama and Aldous, 12/17/01). NOTE: The 1994 chronic high dose dog feeding study (see 384-042 130820 in this Summary) found no hematology response at 6000 ppm, suggesting that the red cell findings reported in the present study are incidental.