

Revision of EPA 1-liners pertaining to the EPA Memorandum (2/15/89) was performed (1/4/90) by M. Silva.

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

DIQUAT DIBROMIDE

Chemical Code # 000229, Tolerance # 00226
SB 950 # 017

August 10, 1987

Revised 8/8/88, 7/21/89, 6/28/90, 12/24/90, 9/18/92, 7/29/93, 5/23/95

I. DATA GAP STATUS

Combined, rat:	No Data Gap, possible adverse effects
Chronic toxicity, dog:	No Data Gap, possible adverse effects
Oncogenicity, mouse:	No Data Gap, no adverse effects
Reproduction, rat:	No Data Gap, possible adverse effects ¹
Teratology, rat:	No Data Gap, possible adverse effects

Teratology, rabbit:	No data Gap, possible adverse effects
Gene mutation:	No Data Gap, possible adverse effects
Chromosome effects:	No Data Gap, possible adverse effects
DNA damage:	No Data Gap, possible adverse effects
Neurotoxicity:	Not required at this time.

1 - Not a reproductive effect.

Toxicology one-liners are attached.

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

File name: T950523

Revised by G. Chernoff, 6/28/90; M. Silva, 12/24/90; Kishiyama & Silva, 9/18/92; Gee, 7/29/93;
M. Silva, 5/23/95

Record numbers through 120645, listed by the Pesticides Registration Library have been
rectified with those listed in the Toxicology Summary.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

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

COMBINED, RAT

****025, 026, 027, 028, 029, 030, 051; 037760, 037761, 037762, 037763, 037764, 037765, 058115,** "Diquat Dibromide, Evaluation of Potential Carcinogenicity and Chronic Toxicity by Prolonged Dietary Administration to Rats", (Huntingdon Research Centre plc, Huntingdon, Cambridgeshire, England, # ICI 406/83763, 1/2/85). Diquat dibromide technical (Reglone), 24.6% w/v as cation, diluted with water and fed in the diets of CD rats at 0, 5, 15, 75 and 375 ppm for 104 weeks to 50/sex/group, plus a satellite group of 10/sex/group killed at 52 weeks. Incidence of comparatively rare osteosarcoma was possibly treatment related in males: incidence was 0, 1, 0, 0, and 3 for controls through increasing dosage groups. **Nephrotoxic effect** (decreased clearance and urine concentrating ability) and hematologic changes (reduction in MCV and Hb) seen at 75 and 375 ppm; **cataract development** at 15, 75, and 375 ppm. NOEL = 5 ppm. ACCEPTABLE. (de Vlaming, Carlisle, 7/25/86) Re-reviewed by J Carlisle, 7/30/87; additional data resulted in an additional possible adverse effect (oncogenicity). Re-examined by C. Aldous following receipt of 086:073262, with no status change, 7/17/89.

035 039814, Amended page (table 13f) to replace page 145 of 025:037760.

086 073262, Brief rebuttal against designation of diquat as indicating a "possible adverse [oncogenic] effect", considered in 7/17/89 re-review of 025:037760.

051 058114, "Diquat Dibromide Preliminary Assessment of Toxicity to Rats by Dietary Administration for 4 Weeks, Final Report", (Huntingdon Research Centre, UK, CTL/C/1065, 5/26/81, ICI 383/80935). Diquat dibromide technical diluted in water and fed in the diet for

5 weeks at 0, 75, 200, 350 or 500 ppm diquat ion; 10/sex/dose; NOEL < 75 ppm (gaseous distention of caecum at all doses). Range-finding study for 025:037760. Supplementary data. (Carlisle, 8/5/87).

CHRONIC TOXICITY, RAT

005 916111, "Diquat Dibromide: 2 Year Feeding Study in Rats - (Amended Report)", (Chevron, Pharmaceuticals Division, # CTL/P/253 (A), June, 1978). Diquat dibromide monohydrate, purity 100 %; fed in the diet for 2 years at 0, 15, 25, and 75 ppm diquat ion equivalent, 35/sex/group. This study was conducted to establish the NOEL for cataracts. **Cataract formation** is reported beginning at nine months at the 75 ppm level, with a NOEL of 25 ppm. The report contains useful information but is UNACCEPTABLE to fill the chronic toxicity data gap because it lacks clinical chemistry, hematology, gross necropsy, and histopathology data. (Remsen (Gee), 3/8/85).

CHRONIC TOXICITY, DOG

**** 094 089037** "Diquat: One Year Feeding Study in Dogs," (Hopkins, M.N., ICI Central Toxicology Laboratory, Alderle Park, UK, 5/15/90). Diquat dibromide (26.7% diquat ion, Batch ADH 472434 Bx121, CTL #: Y00895/032) was fed at nominal dose levels of 0 (vehicle = diet), 0.5, 2.5 or 12.5 mg/kg/day for 1 year. NOEL = 0.5 mg/kg/day (Lenticular opacities were observed in both sexes at 12.5 mg/kg/day and in females at 2.5 mg/kg/day. Chronic inflammatory lesions were observed in the large intestine in all animals at 12.5 mg/kg/day. There were significant increases in kidney weights of both sexes but no histopathology was detected. Reductions in adrenal (12.5 mg/kg/day) and epididymal (≥ 2.5 mg//kg/day) weights were noted in males). **Possible adverse effect (lenticular opacities).** ACCEPTABLE. M. Silva, 12/19/90.

005, 031; 916096, "A Long-Term Toxicity Test with Diquat Dichloride Monohydrate in Dogs", (ICI Limited Industrial Hygiene Research Laboratories, England, # IHR/195, May 1966). Diquat dichloride monohydrate, purity 98 %, fed in the diet to 3/sex/group at 0, 1.7, 5, and 15 mg/kg daily for 2 years; one/sex/group was necropsied and the study continued for an additional 2 years with 3 additional dose level groups started at 0, 0.4, and 0.8 mg/kg; **cataracts developed** within 10-11 months at 15 mg/kg/day and after 15-17 months at 5 mg/kg/day. NOEL = 1.7 mg/kg for eyes. This study has major variances from the guidelines, it was designed to assess cataract induction rather than as a full chronic study. It is UNACCEPTABLE and NOT UPGRADEABLE, deficiencies exist in these areas: Number of animals, dose justification, dose analysis, clinical chemistry and hematology, and histopathology. (Schneider, 3/13/85)
EPA one-liner: Not acceptable, 2/7/89. Interim one year report, systemic NOEL = 1.7 mg/kg, systemic LEL = 5 mg/kg (lens opacity); oncogenic NOEL > 15 mg/kg; levels tested = 0, 1.7, 5 and 15 mg/kg.

ONCOGENICITY, RAT

See Combined Rat

ONCOGENICITY, MOUSE

005, 032, 068; 916112, 037767, 063959 (5 parts), "Diquat Dibromide Monohydrate: Evaluation of Potential Carcinogenicity in Dietary Administration to Mice for 80 Weeks, Final Report Revised", (Life Science Research Limited, Revised Report Number 76/ILY001/144, 7/14/76, revised 10/5/87). Diquat dibromide monohydrate, purity at least 98%, batch ADY/15389/B; fed in the diet at 0, 30 or 150 ppm diquat cation, 60/sex/group CD-1 mice; 11 weeks later, two more groups at 0 or 500 ppm were added; subsequently, due to clinical toxicity, the 500 ppm level was reduced to 400 ppm after 3 weeks and again to 300 ppm after a further 2 weeks; all treatment continued for 80 weeks; reduced growth rates at 300 ppm especially in males and at 150 ppm later in study; systemic NOEL = 30 ppm (body weight, liver vacuolation at 150 and 300 ppm in males and at 300 ppm in females); no evidence of oncogenicity. Initially reviewed as unacceptable due to missing appendices (Gee, 3/8/85) and inadequate histopathology (Carlisle, 8/5/87). A revised report, requested by EPA, contains more complete histopathology, however the data (as pointed out in an EPA memorandum of 2/15/89) contain ambiguities and deficiencies overlooked in previous reviews (Gee, 7/28/88). The study, acceptable as of 7/28/88, is downgraded to UNACCEPTABLE and not upgradeable (no oncogenic effect or treatment-related increase in cataracts). Silva, 1/4/90.

EPA one-liner: Unacceptable Study (a new study has been requested, 2/Negative oncogen at 300 ppm (HDT); systemic NOEL = 30 ppm, systemic LEL = 150 ppm (reduced body wt. gain); ocular NOEL = > 300 ppm (highest level tested)

** 098 112959, "DIQUAT: Two Year Feeding Study in Mice", (M.C.E. Hodge, ICI Central Toxicology Laboratory, Laboratory Project I.D. PM0749, 12/23/91). Diquat Dibromide (purity = 26.7%, w/v) was mixed with the feed at 0 (untreated feed), 30, 100 or 300 ppm/day to C57BL/10JfCD-1/Alpk mice (60/sex/group) for 104 weeks. No significant oncogenic effects were observed in this study. Systemic NOEL = 30 ppm/day (Increased eye discharge, primarily in males and increased kidney nephropathies, primarily in females, were observed \geq 100 ppm.) Treatment-related findings at 300 ppm showed significantly decreased body weights and slightly increased kidney weights. ACCEPTABLE. (Kishiyama & Silva, 9/15/92)

REPRODUCTION, RAT

**** 090 091173** "Diquat: Multigeneration Study in the Rat," (M.C.E. Hodge, ICI Central Toxicology Laboratory, Cheshire, UK, 3/5/90). Diquat (1, 1'-ethylene-2-2'-bipyridyldiylum dibromide, diquat ion concentration = 26.7% w/v and < 10 ppm ethylene dibromide, batch #: ADH 472434, Bx. 121) was used in a two generation study (1 litter/generation) on Alpk:APfSD (30/sex/dose) at 0 (deionized water), 16, 80 or 400 ppm diquat for 12 weeks (F0). F1 rats at 9 weeks were reduced from 400 to 240 ppm due to adverse effects. No adverse reproductive effect. Reproductive NOEL = 400 ppm (no significant effects). Systemic Parental NOEL = 80 ppm (decreased bodyweight in both sexes & in F0 & F1 females during pregnancy & gestation at \geq 240 ppm; food consumption decreased in both sexes of F0 & F1 at \geq 240 ppm; kidney weights in both sexes of F0 & in F1 females was decreased at \geq 240 ppm; tongue ulceration was observed in both sexes of F0 and in F1 females at \geq 240 ppm; both sexes of F1 showed hard palate ulceration at \geq 240 ppm) Systemic Pup NOEL = 16 ppm (mean pup weight was reduced in both sexes for F1 & F2 at \geq 240 ppm at day 22; F1 pup weight was reduced in males at \geq 80 ppm & at 400 ppm in females by day 36; F2 pups of both sexes showed lower bodyweight at 240 ppm by day 36 post partum; F1 pup kidneys of both sexes & testes weights were decreased at 400 ppm; F1 pups showed increased caecum distended & hard palate ulceration) **Possible adverse systemic effect in parents and offspring** (cataracts & eye pathologies in both sexes of F0 & F1 at \geq 240 ppm; an increase of hypertrophy & hyperplasia of collecting duct epithelium & tubular dilatation in the renal papilla in both sexes of F1 at 240 ppm; F1 & F2 pups showed hydronephrosis at \geq 240 ppm.) ACCEPTABLE. M. Silva, 11/9/90.

005, 034; 916116, "Diquat Dibromide: Three-Generation Reproduction Study in Rats", (ICI Limited, Industrial Hygiene Research Laboratories, England, # HO/IH/R/334A, April, 1972). Diquat dibromide monohydrate, 100% purity, batch no. ADY 15389/B fed in the diet at 0, 125, and 500 ppm for 3 generations, 2 litters/generation; 12 males/group and 24 females/group; decreased weight gain and food intake at 500 ppm; **cataracts** were observed in the parental generations at the 500 ppm dose level; UNACCEPTABLE and not upgradeable. Deficiencies: no analysis of diet, only two doses - high dose appears close to the MTD based on decreased body weight and food intake, no individual data, no interim body weights for pups at days 4, 7, or

14. Initially reviewed as having an adverse effect for cataract formation. (Schreider 3/11/85). The study is considered UNACCEPTABLE with no effect on reproductive parameters. The cataract formation is addressed in other, long-term studies in the rat and dog. (Gee, 7/29/88)

EPA 1-liner: Core Minimum, 2/7/89.

034 037770, This is an earlier version of 916116. It has less information but does identify some lesions not mentioned in the histopathology of the other report (apparently the slides were re-read or the lesions re-evaluated). UNACCEPTABLE and not upgradeable. (de Vlaming, Carlisle, 7/26/86)

005 916115, "A Study of Reproduction in Rats Treated with Diquat Dichloride Monohydrate in the Diet", (ICI Limited, Industrial Hygiene Research Laboratories, England, #IHR/188, January, 1966. Diquat dichloride monohydrate, purity not stated, incorporated in the diet at 0, 125, or 500 ppm with 10/sex/group or 10 females/group only or 10 males/group only; 5/sex/group was used for the F1 and F2 matings; fed for 3 generations, 3 litters/generation, except from days 12 to 21 of each lactation period; development of **cataracts** and decreased weight gain in the parental stock at the 500 ppm level is reported. This study does not follow the guidelines. Deficiencies are noted in identification and analysis of test article, dosage level justification, number of animals, hematology, clinical chemistry, and histopathology and use of dichloride. It is UNACCEPTABLE and not upgradeable. Initially reviewed as having a possible adverse effect. (Schreider 3/11/85) The effect noted was cataracts and not in reproductive parameters. The cataract formation is addressed in other long-term feeding studies in the rat and dog. There was no adverse effect on reproductive parameters. (Gee, 7/29/88).

002 916123, Summary, insufficient information for assessment.

TERATOLOGY, RAT

****089 075531**, "Teratogenicity Study in the Rat", (Wickramaratne, G. A., ICI Central Toxicology Laboratory, Report No. CTL/P/2331, 2/16/89). Diquat dibromide, 26.2% w/v a.i., Batch #RS44/E, was administered by oral gavage to groups of 24 female Wistar-derived, Alderley Park (Alpk:APfSD) rats at doses of 0 (deionized water), 4, 12, or 40 mg/kg on days 7 thru 16 of gestation. Maternal food consumption and weight gain was significantly reduced at 40 mg/kg/day. Decreased fetal weight, delayed ossification, and hemorrhagic kidneys were observed at 40 mg/kg/day. Nominal maternal NOEL = 4 mg/kg/day (reduced food consumption and body weight gain); nominal NOAEL = 12 mg/kg/day. Nominal developmental NOEL and NOAEL = 12 mg/kg/day (intrauterine growth retardation as measured by decreased weight and delayed skeletal ossification, and hemorrhagic kidneys). The study is **ACCEPTABLE**, and a **POSSIBLE ADVERSE HEALTH EFFECT** (growth retardation and hemorrhagic kidneys) is noted (J. Kishiyama and G. Chernoff, 6/26/90).

033 037768, "Diquat Dibromide: Teratogenicity Studies in the Rat", (ICI Limited, England, #HO/IH/P/82B, June, 1973). Diquat dibromide, 32% w/v diquat ion, fed in the diet throughout pregnancy at 0, 125 and 500 ppm diquat ion to groups of 18, 20, and 20 pregnant rats respectively. Reduced maternal food consumption, reduced maternal and fetal weight gain, and slight incidence of subcutaneous hemorrhage in the fetus were reported at 500 ppm. No developmental effects were reported. Apparent developmental and maternal NOEL = 125 ppm (decreased body weight gain at 500 ppm). This is a resubmission of 018:011325 that includes pages 2 and 3, which were previously missing. It remains **UNACCEPTABLE** and is not upgradeable (dosing schedule does not follow guidelines, no analysis of treated diet). (de Vlaming, Carlisle, 7/26/86). EPA one-liner: teratogenic NOEL > 500 ppm (highest level fed); systemic NOEL = 125 ppm, Systemic LEL = 500 ppm. EPA 1-liner: Core Minimum, 2/7/89.

018 011325, Earlier version of 033:037668 with pages 2 and 3 missing, **UNACCEPTABLE**. (Schreider, 2/27/85)

TERATOLOGY, RABBIT

**** 088, 091 075530, 088957** "Diquat: Teratogenicity Study in the Rabbit", (Hodge, M.C.E., ICI Central Toxicology Laboratory, Study no. RB0404, 1989). Diquat Dibromide, 26.2% w/v of a.i., Batch #RS44/E, was administered by gavage to groups of 20 New Zealand White Rabbits at doses of 0, (deionized water), 1, 3, or 10 mg/kg/day on days 7 thru 19 of gestation. At 10 mg/kg/day, there was an increase in maternal mortality, a decrease in body weight gain, and altered intestinal, hepatic, and vascular histopathology. Fetal ossification was significantly delayed at all doses tested. Fetal malformations were increased at all doses tested, and were statistically significant at 1 and 10 mg/kg/day. Although the phenotype of the individual malformations varied, altered cell migration was the common mechanism of pathogenesis. Based on this observation, the developmental NOEL < 1.0 mg/kg/day (increased rate of malformations resulting from faulty cell migration), and a **POSSIBLE ADVERSE HEALTH EFFECT** is noted. Maternal NOEL = 3 mg/kg/day (excessive maternal death, decreased weight gain, and altered histopathology). Previously reviewed as unacceptable (Chernoff, 6/25/90), upon submission of an explanation of the inconsistencies in reporting fetal malformations in the low dose group, and a new quality assurance check, the study has been upgraded to ACCEPTABLE. M. Silva, 11/14/90.

110 136155, 136157-58, 136160-63, 136165-66 This volume contains rebuttal statements and historical control data in support of the definitive rabbit teratology study (DPR volume/record #: 226/088, 091 075530, 088957). No worksheet performed. M. Silva, 6/1/95.

001, 038; 916113, "Diquat Dibromide: Teratogenic Studies in the Rabbit", (ICI, # HO/CTL/P/114B, July 1974). Diquat dibromide monohydrate, 100% purity dissolved in dispersol and given by gavage to groups of 17, 20, 15, and 19 mated does at 0, 1.25, 2.5, and 5.0 mg/kg/day respectively on days 1 through 28 of gestation. All does were killed on day 29; no adverse effects reported. Developmental NOEL > 5.0 mg/kg, maternal NOEL = 2.5 mg/kg (marginal decrease in body weight gain, not statistically significant). This study has major deficiencies (dosing schedule, histopathology, poor health and too few animals). It is UNACCEPTABLE and not upgradeable. (Schreider, 3/11/85, Parker, 9/3/86).

EPA one-liner: Core Minimum, 2/7/89. Teratogenic NOEL > 5.0 mg/kg (HDT), systemic NOEL = 1.25 mg/kg; levels tested = 1.25, 2.5, and 5.0 mg of diquat ion/kg.

TERATOLOGY, MOUSE

001, 005; 916117, "Effect of Diquat on Pregnancy of the Mouse", (Huntingdon Research Centre, Huntingdon, Cambridgeshire, England, # ICI/167/77642, 2/7/78). Diquat dibromide monohydrate analytical standard, batch no. ADY 15389/B in distilled water given by gavage to groups of 34, 32, 33, and 34 mated females at 0, 1.0, 2.0, and 4.0 mg/kg/day respectively on days 6 through 15 of gestation. Animals were killed on day 17. Initially considered acceptable with a possible adverse effect (Schreider, 3/11/85). In preparation of a rebuttal and revised toxicology summary, the study is now considered as UNACCEPTABLE (clinical observations, necropsy findings and body weights cannot be correlated for maternal animals; fetal individuals cannot be tied to skeletal variants, no historical control data) with no adverse effect. Maternal NOEL = 1.0 mg/kg/day (clinical signs, decreased body weight gain, increased mortality at 2 and 4 mg/kg), developmental NOEL = 1.0 mg/kg/day based on decreased fetal weight at 4 mg/kg/day and skeletal anomalies at 2 and 4 mg/kg/day; exencephaly, open eyes, umbilical hernia, skeletal anomalies occurred at increased incidence at 2.0 and 4.0 mg/kg/day although the effects occurred at a maternally toxic doses with no dose response for major malformations, no similarity in types for major malformations. (Schreider, Parker and Gee, 8/10/88.)

EPA one-liner: teratogenic NOEL = 1.0 mg/kg; systemic LEL = 1.0 mg/kg.

074 066521, "Preliminary Toxicity Studies to Determine the Effect of Diquat on the mouse", (Huntingdon Research Center, UK, CTL/C/330, 10/10/77). Diquat dibromide monohydrate, batch ADY 15389/B, cation to whole molecule 1:1.97, tested in three trials for range finding for full study by oral gavage; trial 1 with CFPL mice from Anglia Laboratory at 0 (water), 20, 30 or 40 mg/kg to 6 females per group, nonpregnant mice; trial 2 with ICI Alderley Park mice, 3/group, at 0, 10, 20 or 40 mg/kg/day; trial 3, same source of mice as trial 2, 6/group at 0, 1.25, 2.5 or 5.0 mg/kg/day; severe reactions at 10 mg/kg/day and above with high mortality; no signs at 1.25 and 2.5; Supplementary data - dose justification for mouse teratology study. (Gee, 7/29/88)

TERATOLOGY, OTHER

074 066519, Review of the teratology studies including interspecies comparisons.

GENE MUTATION

021 022842, "A Simplified Method for the Induction of 8-Azaguanine Resistance in *Salmonella* Typhimurium", (Istituto Superiore di Sanita, Rome, Italy; published in Toxicology Letters 3: (1979) 169-175, Bignami, M. and Crebelli, R.) Publication is a summary of results of a plate incorporation assay for mutagenicity of diquat at 0, 0.1, 0.5, and 1.0 ug/plate with *Salmonella typhimurium* strains His G46, TA92, TA1535, TA1538, and TA100; **mutagenicity reported** in the forward mutation assay for 8-azaguanine resistance in TA1535 and TA92. UNACCEPTABLE and not upgradeable (summary only, insufficient information for evaluation). (Green, Gee, 7/17/87)

021 022844, "A New *Salmonella* Tester Strain (TA102) with AT Base Pairs at the Site of Mutation Detects Oxidative Mutagens", (Biochemistry Department, UC, Berkeley; published in Proc. Natl. Acad. Sci. USA 79: 7445-7449 (1982), Levin et al.) Describes the differences in sensitivity among *Salmonella* tester strains TA102, TA104, TA2638, TA95, and TA96 to a number of chemical oxidants including diquat at 10 ng (toxic to TA102 and TA2638). No mutagenicity was detected for diquat. This article does not follow guidelines. It is UNACCEPTABLE and not upgradeable. Note: TA102 contains multiple copies of the mutant gene (approximately 30) with A-T base pairs instead of C-G base pairs. (Green, Gee, 7/17/87)

035 039758, "Mutational Studies with Diquat and Paraquat in Vitro", (Istituto Superiore di Sanita, Rome and University of Rome, Italy; published in Mutation Research 68: (1979) 183-193, R. Benigni et al.) Diquat, obtained from ICI, in the "Ames test" with Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100, with or without S9 activation, at diquat concentrations ranging from 0.25 to 10 ug/plate, the latter of which was cytotoxic. No mutagenic activity (frameshift or base-pair substitution) expressed. INCOMPLETE and not upgradeable. (Martz, 9/5/86)

035 059147, "Mutational Studies with Diquat and Paraquat in Vitro", (Istituto Superiore di Sanita, Rome and University of Rome, Italy; published in Mutation Research 68: (1979) 183-193, R. Benigni et al.). Diquat, obtained from ICI, caused "forward" mutations (8-azaguanine resistance) in Salmonella tester strains hisG46, TA92, and TA1535 at plate concentrations of 0.1 ug for hisG46 and TA92 or 0.25 ug for TA1535, and Aspergillus nidulans at a plate concentration of 400 ug or liquid (test) concentration of 10 mg/ml. INCOMPLETE and not upgradeable. (Martz, 9/5/86)

**049 057731, "Diquat Dibromide (Technical): An Evaluation of Mutagenic Potential using S. typhimurium and E. coli", (ICI, Central Toxicology Laboratories, U.K.; # CTL/P/1463, 5/8/86). Diquat dibromide technical grade 25.8% w/w purity in water, with/without S9 rat liver (Aroclor-induced) activation, at 0, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10, 50, or 100 ug/plate with Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, or TA100 and Escherichia coli (WP2 uvrA pKM101); 2 trials, 3 replicates/trial. No reverse mutation reported. ACCEPTABLE. (Green, Gee, 7/21/87)

062 062149, "Evaluation of Herbicides for Possible Mutagenic Properties", (Publication by K. Andersen et al. in J. Agricultural and Food Chemistry 20: 649 - 656 (1972)). Diquat (pyrazinedium ion), 35.3%; tested with Salmonella, 8 unspecified strains by the spot test, T₄ mutation with E. coli B as host and two rII mutants of T₄, designated as AP72 and N17 to measure reversions to wild type with E. coli strains B and K as host and determination of differential plaque count; no increase in reversion rate with Salmonella, negative with T₄ for

induction of rII mutants at 20 µg (unit not clear), negative for reversion of AP72 and N17 at 10 µg; UNACCEPTABLE with no adverse effect identified. (Gee, 7/26/88)

062 062152, "Mutagenicity Studies in Salmonella, Streptomyces, Aspergillus and unscheduled DNA synthesis in EUE cell of Paraquat and Diquat (Abstract)", (Published in Mutation Research 64 (2): 81 (1979)). Diquat tested in 4 systems: Forward mutations to 8 AG^r in Salmonella, his⁻ reversion in Salmonella, resistance to streptomycin in Streptomyces coelicolor, mutation and segregation in Aspergillus nidulans and UDS in EUE (human epithelial - like heteroploid) cells; **positive for forward mutation** in Salmonella using a spot test and plate test, negative for reversion at 1 - 1000 µg/plate; negative with Streptomyces in spot and plate test but **positive** in a liquid test with spores with 2 hour incubation (2 - 4 fold), **weakly mutagenic** with Aspergillus (2.5 fold) and positive for induction of unscheduled DNA synthesis when tested up to 1000 µg/ml; no data; UNACCEPTABLE. (Gee, 7/26/88)

062 062158, "The Mutagenicity in Procaryotes of Herbicides", (Publication in "Residue Reviews", 1984). Review article on a number of compounds including diquat. Mixed results reported with the conclusion that diquat is a **weak mutagen** in procaryotes under some circumstances. Organisms include Salmonella, E. coli, Streptomyces. Supplementary information - not a study. (Gee, 7/27/88)

063 062165, "Detection of Oxidative Mutagens with a New Salmonella Tester Strain (TA102)", (Publication in "Methods in Enzymology" 103: 249 (1984) by D. E. Levin et al.). Diquat was tested by plate incorporation and by 20 minute preincubation in liquid at 10 ng; both gave negative results; TA102 has a T:A base pair substitution at the site of reversion rather than a G:C pair in the other strains and is sensitive to chemicals not giving results with the usual strains. No evidence for mutagenicity. UNACCEPTABLE (single concentration, number of replicates not indicated, no individual data), not upgradeable. (Gee, 7/27/88)

063 062168, "Further Mutagenicity Studies on Pesticides in Bacterial Reversion Assay Systems", (Publication in Mutation Research 116: 185 - 216 (1983) by Moriya et al.). Diquat dibromide

was among 228 pesticides tested with Salmonella strains TA100, TA98, TA1535, TA1537, TA1538 and E. coli WP2 ~~nxp~~ with negative results. UNACCEPTABLE (no data). (Gee, 7/27/88)

063 062172, "The Bacterial Mutation Test", (Publication by D. Anderson and J. A. Styles, unknown source and date). Diquat (no further characterization) was negative with four strains of Salmonella at unknown concentrations. UNACCEPTABLE, not upgradeable. (Gee, 7/27/88)

062 062157, "Comparative Mutagenicity Studies with Pesticides", (Publication in IARC Science Publications 10: 161 - 181 (1974) by Fahrig). Diquat dibromide was negative for mutations in E. coli and for mitotic gene conversion in Saccharomyces cerevisiae. UNACCEPTABLE (no data, inadequate protocols). (Gee, 7/26/88)

062 062150, "Mutagenic Activity of Some Pesticides in Drosophila Melanogaster", (MUDr, publication by Benes and Shram in Industrial Med. 38: 50 - 52 (1969)). Diquat tested with Drosophila melanogaster by injection, 0.02% in water, 0.2 µl/fly; recessive lethal test with two broods; negative; UNACCEPTABLE with single concentration, inadequate number of chromosomes tested. Not upgradeable. (Gee, 7/26/88)

049 057729, "Diquat Dibromide (Technical) Assessment of Mutagenic Potential using L5178Y Mouse Lymphoma Cells", (ICI PLC Central Toxicology Laboratories, U.K., # CTL/P/1602, 11/11/86). Diquat dibromide technical grade 25.8% purity batch no. ADH 415276, BX 71; in saline solution at concentrations of 0, 3.125, 6.5, 12.5, 25, or 50 ug/cm³ with S9 rat liver₃ (Aroclor-induced) activation, positive control DMN, and at 0, 6.25, 12.5, 25, 50, or 100 ug/cm³ without activation, positive control EMS. **Gene mutation indicated** with a 2 - 3 fold increase over controls; UNACCEPTABLE (unclear description of data in tables), upgradeable. (Green, Gee, 7/21/87)

049 057730, "Diquat Dibromide: Assessment of Mutagenic Potential using L5178Y Mouse Lymphoma Cells", (ICI PLC, Central Toxicology Laboratories, U.K., # CTL/P/1554, 11/17/86). Diquat dibromide analytical grade 100% purity, in saline solution with S9 rat liver (Aroclor-induced) activation at 0, 3.125, 6.25, 12.5, or 25 ug/ml, benzo(alpha)pyrene and DMN positive control,

and without activation at 0, 6.25, 12.5, 25, or 50 ug/ml, EMS positive control, in the forward mutation plate assay. **Gene mutation indicated** with a 2 - 3 fold increase at moderate cytotoxicity in replicate trials. UNACCEPTABLE (unclear description of data in tables) and not upgradeable (use of purified diquat). (Green, Gee, 7/21/87)

SUMMARY: Diquat dibromide was not mutagenic in the usual strains of Salmonella for reverse mutation in the his⁻ operon but was mutagenic for forward mutations in both Salmonella for 8-azaguanine resistance and gave a 2 - 3 fold increase over the spontaneous rate at moderate cytotoxicity in mouse lymphoma L5178Y cells for thymidine kinase activity in two separate trials with different preparations of diquat dibromide. Note: The authors of the two reports with mouse lymphoma considered diquat to be "non-mutagenic." Overall, there is a possible adverse effect for gene mutation. (Gee, 8/8/88)

CHROMOSOME EFFECTS

**001, 005; 916118, "Dominant Lethal Study in Mice of Diquat", (Inveresk Research International, # 148, 2/20/74). Diquat 28.6% (W/V) of ion, in 0.5% Tween 80; males dosed orally for 5 consecutive days, 30/group at 0 and 15/group at 0.10, 1.00, and 10.0 mg/kg/bodyweight; cyclophosphamide and EMS as positive controls; mated weekly 1 male:2 untreated females for 8 weeks; no dominant lethal effects reported; ACCEPTABLE (Remsen (Gee), 3/8/85).

EPA one-liner: Diquat fed orally to Charles River CD-1 male mice for 5 consecutive days did not produce dominant lethal effects at any of the levels tested (0, 0.1, 1.0, and 10 mg diquat ion/kg bw.). Acceptable.

021 022843, "Dominant Lethal Studies with Paraquat and Diquat in Male CD-1 Mice", (Published in Mutation Research 40: 349 - 358 (1976), Anderson, D. et al.). The diquat data in this article are a summary of those in 001, 005 916118, an acceptable study. (Green, Gee, 7/17/87)

063 062169, "Assessment of the Mutagenic Properties of Diquat and Paraquat in the Murine Dominant Lethal Test", (Publication in Mutation Research 26: 171 - 175 (1974) by Pasi et al.). Diquat dibromide monohydrate given in a single i.p. injection, 76 mmole/kg, to 5 male Swiss-Webster mice; dose approximately the LD₅ i. p.; each male mated with 3 untreated females for 7 days for 8 consecutive weeks; females sacrificed 15 days after caging; pregnancy rates in

diquat groups were lower throughout the 8 weeks and a differential effect among males was noted (no individual data for evaluation); early deaths per pregnancy was elevated in week 3 but not statistically significant at $p < 0.05$; authors consider the results negative; sample size too small for adequate interpretation; UNACCEPTABLE_(number of animals, summary report with no individual data, others), not upgradeable. (Gee, 7/27/88)

035 039760, "Mutational Studies with Diquat and Paraquat in Vitro", (Istituto Superiore di Sanita, Rome, and University of Rome, Italy, published in Mutation Research 68: 183 - 193 (1979). Aspergillus nidulans, diquat obtained from ICI, induced **lethal recessive mutations** in the diploid P3 strain of Aspergillus nidulans, at a concentration of 10 mg/ml. INCOMPLETE and not upgradeable. (Martz, 9/5/86)

**049 057732, "Diquat Dibromide (Technical): An Evaluation in the Mouse Micronucleus Test", (ICI PLC, Central Toxicology Laboratories, U.K.; # CTL/P/1532, 7/25/86). Diquat dibromide (technical), 25.8% diquat ion in water given orally in a single dose at 0, 62.5, or 100 mg/kg; 15/sex/dose; positive control cyclophosphamide; bone marrow sampled 5/sex/dose at 24, 48, and 72 hours. No clastogenic effects reported. ACCEPTABLE. (Green, Gee 7/22/87)

063 062170, "Preliminary Evaluation of the Cytogenetic Activity and Potential Mutagenic Hazard of 22 Pesticides", (Publication in Tsitologiya i Genetika 14: 41 - 47 (1980) by M. A. Pilinskaya et al.). Diquat dibromide one of 22 pesticides tested in noninbred male white mice given one dose of 50 mg/kg by gavage; bone marrow harvested 20 hours later; number per group not given; scored 600 metaphases; negative results; unacceptable (inadequate description of study, single dose, use of males only, insufficient data). (Gee, 7/27/88)

063 062174, "Mutagenic and Embryotoxic Effects of Paraquat and Diquat", (Publication in Bull. Environmental Contamination and Toxicology 25: 513-517 (1980) by A. Selypes et al.). Diquat as Reglone (20% diquat), given i. p. or orally to groups of 20 male mice; i. p. dose was 22 mg/kg and the bone marrow harvested 24 hours later; other groups were given 2 x 7.3 mg/kg, 2 x 3.6 mg/kg or 5 x 0.73 mg/kg with sacrifice 24 hours after last injection; oral dose of 90 mg/kg with bone preparations made 48 hours later; 10 non-treated controls; scored "10-10"

mitoses per animal for a total of "100-100" per group; no effect on bone marrow for aberrations due to treatment. In addition, in a "teratogenic" portion, groups of 20 females were given a single i. p. dose of 11 mg/kg, day 9 as Reglone; a second group received 4 x 2.7 mg/kg days 9 - 12; report states no congenital malformations but a number of findings in the skeleton such as large fontanelles, missing ossification points, wider cerebral sutures, others in animals exposed repeatedly at a low dose and considers it positive for fetotoxicity and "dominant lethal" effect with increased postimplantation loss of 11 % compared with "-" in the controls; the effect on retardation in skeleton occurred in the presence of a 30% lowered fetal weight; in females given a single dose of 11.0 (1/2 LD₅₀), a 9% loss was reported. No information on maternal toxicity, if any. UNACCEPTABLE (protocols, insufficient information), not upgradeable. (Gee, 7/27/88)

049 057733, "Diquat Dibromide: A Cytogenic Study in Human Lymphocytes in Vitro", ICI PLC, Central Toxicology Laboratories, U.K., # CTL/P/1469, 5/1/86). Diquat dibromide, analytical grade 100% w/w purity; treated lymphocytes from 2 donors, 2 trials each, at 0, 13.4, 26.7, 53.5, 107, 267.4, or 534.8 ug/ml with/without S9 rat liver (Aroclor-induced) activation. **Increased chromosomal aberrations** at dose levels with high cytotoxicity. UNACCEPTABLE and not upgradeable (used purified diquat). (Green, Gee, 7/22/87)

****049 057734**, "Diquat Dibromide (Technical): A Cytogenetics Study in Human Lymphocytes in Vitro", (ICI PLC, Central Toxicology Laboratories, U.K.; # CTL/P/1561, 10/30/86). Diquat dibromide technical 25.8% diquat ion in saline, treated lymphocytes from 2 donors, male and female, at 0, 12.9, 25.8, 64.5, or 129 ug/ml with/without S9 rat liver (Aroclor-induced) activation. Decrease in mitotic index for cytotoxicity associated with **increase in chromosomal aberrations**, primarily breaks. ACCEPTABLE. (Green, Gee, 7/22/87)

063 062179, "Induction of Sister-Chromatid Exchange and Chromosomal Aberration in Chinese Hamster Lung Cells by Paraquat and Diquat", (Abstract in Toxicology Letters, page 214, R. Tanaka, date unknown). Diquat (no further characterization), tested with Chinese hamster lung cells for sister chromatid exchanges and chromosomal aberrations with **significant differences**

in the frequencies of SCEs compared with controls" above 0.08µM but not for chromosomal aberrations at 0.08 - 0.4 µM. UNACCEPTABLE (abstract). (Gee, 7/28/88)

001 916119, "Diquat: A Cytogenetic Study in the Rat", (ICI Limited, Central Toxicology Laboratory, # CTL/P/366, 7/5/78). Diquat dibromide monohydrate, purity 100%, batch no. ADY 15389B, in 0.5% Tween 80; dosed by gavage, 8 males/group, for 5 consecutive days at 0, 4.4, 9.5, and 14.0 mg diquat ion/kg body weight/day; EMS at 200 mg/kg/day as positive control; animals were killed at 6 hours; no cytogenetic effects reported. Due to major variances from the guidelines (sampling times, lack of females) this study cannot be evaluated. It is UNACCEPTABLE and not upgradeable. (Remsen (Gee), 3/18/85)

EPA one-liner: Diquat is not mutagenic at all doses tested. Levels of diquat ion fed: 0, 4.4, 9.5, and 14 mg/kg (analytical value). Supplementary.

SUMMARY: Diquat was negative for dominant lethal effects and micronuclei formation in in vivo studies, two of which were evaluated as "acceptable." Positive effects on chromosomes were reported in four in vitro studies, one with Aspergillus nidulans, two with human lymphocytes measuring chromosomal aberrations and one for SCE's in Chinese hamster cells. One of these studies was evaluated as "acceptable." No cytogenetic effects were reported for male rats treated in vivo for 5 consecutive days and sacrificed 6 hours after the final dosing. The weight of evidence suggests that diquat is not genotoxic in the intact animal but induces positive chromosomal effects in vitro with a possible adverse effect. (Gee, 8/8/88.)

DNA DAMAGE

021 022839, "Genetic Effects of Herbicides: Induction of Mitotic Gene Conversion in Saccharomyces cerevisiae", (Universität Freiburg i. Br. and Staatliches Weinbaninstitut, Freiburg i. Br., West Germany, published Mutation Research 22: 111 - 120 (1974) by Siebert, D. and Lemperle, E.). Diquat dibromide (no₇ purity stated) in water at 1000 ppm cultured 16 hours with Saccharomyces cerevisiae D4, 5 x 10⁷ cells/2 ml diquat suspension; 5 plates, 3 trials; induction of mitotic gene conversion reported; unacceptable (lacks test article purity,

concentration justification, individual plate counts or standard error, activation), NOT UPGRADEABLE. (Green, Gee, 7/20/87)

063 062164, "Screening of Environmental Chemical Mutagens by the Rec-Assay System with Bacillus subtilis", (Publication in "Chemical Mutagens", eds. F. J. de Serres and A. Hollaender, vol. 6, 1980, 149-173). Diquat Dibromide; review article listing diquat as negative for rec assay. Primarily describes how the assay is conducted and summarizes results with over 200 chemicals including metals. UNACCEPTABLE. (Gee, 7/27/88)

021 022840, "Effect of Pesticides on Scheduled and Unscheduled DNA Synthesis of Rat Thymocytes and Human Lymphocytes", (Universita di Bologna, Italy and Consorzio Socio Sanitario, Bologna, Italy, published in Arch. Toxicol. 45: 101-108 (1980), Rocchi et al.). Diquat 95% purity dissolved in DMSO at 0, 100, 500, and 1000 ug/ml; tested with rat thymocytes or human lymphocytes in vitro with/without UV irradiation; **inhibition of DNA synthesis (SDS and UDS) reported**; UNACCEPTABLE (missing details of protocol), upgradeable. (Green, Gee, 7/17/87)

062 062146, "Pesticide Induced DNA Damage and Its Repair in Cultured Human Cells", (Ohio State, publication by F. Ahmed et al., in Mutation Research 42: 161-174 (1977)). Diquat dibromide, no purity stated; human fibroblasts transformed with SV-40, VA-4; treated with and without rat liver activation at 1, 10, 100 or 1000 μ M for 1, 3, 5, 8 or 12 hours; unscheduled DNA synthesis by autoradiography, hydroxyurea to suppress semiconservative DNA synthesis; results for 8 hours only **reported as "+" at all concentrations** with and without activation; UNACCEPTABLE with no data. (Gee, 7/26/88)

021 022841, "Chemically-Induced DNA Repair Synthesis in Primary Rat Hepatocytes: A Correlation with Bacterial Mutagenicity", (Lilly Research Laboratories, published in Annals New York Academy of Sciences, 1980, 405-406; Probst, G.S. and Hill, L.E.). Lists results of the unscheduled DNA synthesis assay with a number of chemicals, including diquat dibromide, and compares them with results published (Cancer Res. 1979. 39: 682, McMahon et al.) from the bacterial gradient plate assay. No UDS reported, but mutagenicity reported in the bacterial assay. INCOMPLETE and not upgradeable. (Green, Gee, 7/17/87)

063 062171, "Chemically-induced Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures: A Comparison with Bacterial Mutagenicity Using 218 Compounds", (Publication in Environmental Mutagenesis 3: 11-32 (1981) by G. Probst et al.). Diquat (no further description) was negative at 500 nmoles/ml after 5 or 18-20 hour treatment; single datum; 20 nuclei scored; UNACCEPTABLE (insufficient data), not upgradeable. (Gee, 7/27/88)

035 039759, "Mutational Studies with Diquat and Paraquat in Vitro", (Istituto Superiore di Sanita, Rome and University of Rome, Italy; published Mutation Research 68: 183-193 (1979). Diquat, obtained from ICI, caused **unscheduled DNA synthesis (UDS)** in "epithelial-like human embryo cells" over a concentration range of 20 to 2000 ug/ml; at a diquat concentration of 10 ug/plate in the presence of S9 only, increased lethality was noted in a Salmonella strain deficient in DNA excision repair (TA1538) vs a sister strain stated to have competent repair (TA1978). Results of these 2 tests are suggestive of **DNA damage**. INCOMPLETE and not upgradeable. (Martz, 9/5/86)

**062 062163, "Diquat Dibromide (Technical): Assessment for the Induction of Unscheduled DNA Synthesis in Rat Hepatocytes in vivo", (Imperial Chemical Industries, CTL/P/1814, 4/16/87). Diquat dibromide, technical, 25.8% diquat ion; given by oral gavage to male rats at 0, 225, 450 or 900 mg/kg with rats sacrificed after 4 or 12 hours and hepatocytes isolated; after attachment, cells were incubated for 4 hours with ³H-thymidine followed by an overnight incubation with unlabelled thymidine. Unscheduled DNA synthesis by autoradiography; for 12 hours, a total of 2, 5, 4 and 4 rats were used for control, low-, mid-, and high-doses in two

trials; for 4 hours, 1, 5, 5, 5 rats; scored 100 nuclei per animal from 2 or 3 slides per animal; no evidence of induction of unscheduled DNA synthesis; ACCEPTABLE. (Gee, 7/27/88)

062 062156, "Investigations into the Mechanism of Paraquat Toxicity Utilizing a Cell Culture System", (Publication in Toxicology and Pharmacology 58: 353 - 362 (1981) by Carmines et al. Diquat dibromide, 100 %, in water; cell line P388D, originally isolated from a methylcholanthrene-induced lymphoid neoplasm; effect of diquat on cell proliferation determined from 1×10^{-7} to 1×10^{-3} M by cell count after 24 hour incubation with the IC_{50} at 1.92×10^{-3} M; no effect on lipid peroxidation over this concentration range as measured by effect on malondialdehyde with no stimulation; diquat inhibited DNA and RNA synthesis in a concentration-dependent manner and over time as measured by incorporation of radioactive precursors in acid precipitable material - no effect on protein synthesis; there was no effect on the cell uptake of the precursors; Supplementary data. (Gee, 7/26/88)

062 062157, "Comparative Mutagenicity Studies with Pesticides", (Publication in IARC Science Publications 10: 161 - 181 (1974) by Fahrig). Diquat dibromide was negative for mutations in E. coli and for mitotic gene conversion in Saccharomyces cerevisiae. UNACCEPTABLE (no data, inadequate protocols). Gee, 7/26/88.

SUMMARY: Diquat was reported as inducing unscheduled DNA synthesis in three publications using rat or human cells tested in vitro. Negative results for UDS were reported for rat hepatocytes but adequate data were not provided. Negative results for UDS were reported in an adequate in vivo study. Positive effects were also reported with Saccharomyces cerevisiae for mitotic gene conversion. Diquat represents a potential for adverse genotoxic effects.

CONCLUSION: The data from the three areas of genotoxicity indicate positive effects in a variety of in vitro studies but negative when tested in vivo.

NEUROTOXICITY

102 120645, "Diquat: Acute Neurotoxicity Study in Rats", (J. M. Horner, ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK., Report # CTL/P/3789, 15 December 1992). Technical diquat (1,1'-ethylene-2,2-bipyridyldiylidium dibromide; 20.1 % W/W purity) was gavaged in Alpk:APfSD rats (10/sex/dose) at 0 (deionized water), 25, 75, and 150 mg/kg, followed by 15 days of observation. Increased incidence of diarrhea, piloerection, tiptoe gate, subdued behavior, upward curvature of the spine, and signs of urinary incontinence is noted for females at 150 mg/kg. **Acute neurotoxicity cannot be evaluated until the study is complete.** Systemic NOEL = 75 mg/kg (increased clinical signs in females at 150 mg/kg). A Neurotoxicity NOEL could not be determined in this study since recommended FOB tests were not performed. **Unacceptable**, not upgradeable (The FOB tests were incomplete and there were no acceptable positive controls submitted.) (Green, & Silva, 5/22/95).

101 120427, "Diquat: Subchronic Neurotoxicity Study in Rats", (J. M. Horner, ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK., Report # CTL/P/3751, 23 November 1992). Technical diquat (1,1'-ethylene-2,2-bipyridyldiylidium dibromide) with 20.8% w/w purity was administered in the diet to Alpk:APfSD rats (12/sex/dose) for 13 weeks at 0 (CT1 diet), 20, 100, and 400 ppm. Measurements were made at weeks -1, 5, 9 and 14. Systemic NOEL = 100 ppm (Body weights were reduced 5% to 13% for both sexes at 400 ppm (reduction tended to be greater in males). At 400 ppm, increased eye opacity, eye pallor, cataracts, and posterior lens opacity, and decreased visual placement response was noted for both sexes.) **Unacceptable and not upgradeable** (Complete FOB tests were not performed and an acceptable positive control has not been submitted.) Green & Silva, 5/17/95.