

DIQUAT DIBROMIDE

RISK CHARACTERIZATION DOCUMENT

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Medical Toxicology and Worker Health and Safety Branches

Department of Pesticide Regulation

California Environmental Protection Agency

EXECUTIVE SUMMARY

THE RISK ASSESSMENT PROCESS

Diquat dibromide entered the risk assessment process because of possible adverse effects identified in developmental toxicity studies and chronic toxicity studies. The risk assessment process consists of four aspects: hazard identification, dose response assessment, exposure evaluation, and risk characterization.

Hazard identification entails review and evaluation of the toxicological properties of each pesticide. The dose-response assessment then considers the toxicological properties and estimates the amount which could potentially cause an adverse effect. The amount which will not result in an observable or measurable effect is called the No-Observed-Effect Level, NOEL. A basic premise of toxicology is that at a high enough dose, virtually all substances will cause some toxic manifestation. Chemicals are often referred to as "dangerous" or "safe", as though these concepts were absolutes. In reality, these terms describe chemicals which require low or high dosages, respectively, to cause toxic effects. Toxicological activity is determined in a battery of experimental studies which define the kinds of toxic effects which can be caused, and the exposure levels (doses) at which effects may be seen. State and federal testing requirements mandate that substances be tested at doses high enough to produce toxic effects, even if such testing involves chemical levels many times higher than those to which people might be exposed.

In addition to the intrinsic toxicological activity of the pesticide, the other parameters critical to determining risk are the exposure level, frequency and duration. The purpose of the exposure evaluation is to determine the potential exposure pathways and the amount of pesticide likely to be delivered through those routes.

The risk characterization then integrates the toxic effects observed in laboratory studies conducted with high dosages of pesticide, to potential human exposures at low dosages. The likelihood of potential, non-oncogenic adverse health effects in people is generally expressed as the margin of safety. The margin of safety is a ratio, produced by dividing the human exposure dosage into the dosage which produced no effects in laboratory studies. For oncogenic effects, the excess lifetime risk of cancer is determined by multiplying the cancer potency of the pesticide times the estimated exposure dosage.

INTRODUCTION

Diquat dibromide (Trade names Aquacide® and Dextrone®) is a contact herbicide that damages plant tissues quickly, causing plants to appear frostbitten because of cell membrane destruction. It also reduces plant photosynthetic activity. It is used in aquatic and industrial weed control. It is also used for desiccation of potato vines and seed crops, and to control sugarcane flowering.

TOXICOLOGY

Based on the currently available toxicity information, DPR concluded that diquat dibromide causes cataracts in dogs and rats, and developmental effects in rats and rabbits. DPR has further concluded that, in the absence of additional data to the contrary, diquat dibromide has the potential to cause similar effects in humans.

EXPOSURE ANALYSIS

Diquat monitoring data and surrogate data were used to estimate potential exposure via dermal contact, and inhalation of mixer/loader/applicators utilizing diquat dibromide in aquatic, aerial, and ground application situations. Exposure through the inhalation route was insignificant compared to potential dermal exposure in all but aerial applications. Swimmers had potential short term exposures to diquat through the dermal and oral routes. Based on current use patterns, the potential for annual exposure of swimmers to diquat does not exist.

CONCLUSIONS

Using current toxicity data, estimates from monitoring information on diquat, and surrogate exposure data, the calculated margins of safety (MOSs), based on an estimated no observed effect level in rabbits for developmental defects, for potential short-term exposure were less than the value conventionally recommended to protect people from the toxic effects of diquat for aquatic applicators and boat drivers using handguns, mixer/loaders and flaggers associated with aerial applications, ground applicators (except for hand applications on right-of-ways), and non-occupational exposures to drift at 50 meters. MOSs, based on cataract formation in dogs, for potential annual occupational exposure to diquat for ground applicators using vehicles with normal ground clearance and no cab was also substantially less than the value conventionally recommended to protect people from the toxic effects of diquat. Mitigation measures should be considered to reduce potential exposure.

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I SUMMARY

Diquat dibromide (Trade names Aquacide[®] and Dextrone[®] - 6,7-dihydrodipyrido [1,2-a: 2',1'-c]pyrazinedium ion) is used in aquatic and industrial weed control. It is also used for desiccation of potato vines and seed crops, and to control sugarcane flowering. Diquat dibromide was entered into the risk assessment process because of possible adverse effects identified in developmental toxicity studies and chronic toxicity studies.

Environmental Fate- The half-life for hydrolysis (pH 9) is greater than 9 months, and the half-life for photodegradation in aqueous solution (pH 7) is 74 days. Diquat binds tightly to soil, and it is not degraded under aerobic or anaerobic conditions. The environmental fate data strongly suggest that diquat will persist, bound to the soil, in the area where it is applied.

Illness Reports- Approximately 52 illness reports, including one suicide, concerning diquat dibromide were made between 1982 and 1990. Virtually all of the illnesses reported during agricultural use occurred as the result of equipment failure, accidental exposure, or violations of label requirements. The most common injuries reported were dermal burns, rashes, and eye irritations. Instances of nausea and dizziness were also attributed to diquat exposure.

Pharmacokinetics- Only 0.3% of unoccluded and 1.4% of occluded topically applied diquat dibromide was absorbed through the skin of human volunteers. Intravenously injected diquat had a half life of 4 hours in humans, and 61.7% of the administered dose was excreted in the urine within 5 days. Approximately 10% of orally administered doses of diquat dibromide are absorbed through the gut of rats, goats or cattle. Absorbed diquat is excreted unchanged in the urine of rats in about three to four days. Goats metabolized diquat. Approximately 20% of radiolabel found in goat milk (0.018% of the administered dose) were metabolites. Diquat did not concentrate in any body organs, and only about 0.02% of an orally administered radiolabelled dose appeared in the milk of cattle.

Acute Toxicity- The oral LD₅₀ in rats ranged from 215 to 235 mg/kg for technical grade diquat, and the dermal LD₅₀ in rabbits was 400 mg/kg. Formulations of diquat concentrate (28% diquat) have an oral LD₅₀ in rats greater than 5 g/kg, a dermal LD₅₀ in rabbits greater than 2 g/kg, and an inhalation LC₅₀ greater than 34.1 mg/L. No dermal sensitization responses were observed among guinea pigs induced and challenged with diquat herbicide concentrate.

Subchronic Toxicity- The 4-week No-Observed-Effect-Level (NOEL) for increased relative liver weight in rats from dietary exposure to diquat dibromide was 7.2 mg/kg-day. The NOEL for piloerection in mice caused by gavage with diquat for 10 days was 2.5 mg/kg-day. Inhalation of diquat aerosol at 0.1 µg/L by rats for 3 weeks resulted in statistically significant reductions in blood platelet and reticulocyte counts.

Chronic Toxicity/Oncogenicity- There was no indication of oncogenicity in either mice or rats. The NOEL for decrement in body weight gain and liver vacuolation in mice was 2.5 mg/kg-day. The 1-year NOEL for cataracts was 0.66 mg/kg-day in rats, and 0.5 mg/kg-day in dogs. Other systemic effects, such as inflammatory lesions of the large intestine, shrunken adrenal glands, reduced kidney weights, and reduced epididymal weights were noted in rats and dogs at doses greater than 3.3 mg/kg-day. The NOEL for mild nephropathy in mice was 4.8 mg/kg-day.

Genotoxicity- Diquat dibromide was not mutagenic in *Salmonella* for the reverse mutation in the his⁻ operon. However, it did cause a 2-3 fold increase in mutation frequency at the thymidine kinase locus in mouse lymphoma L5178Y cells in two separate trials. Diquat was negative for dominant lethal effects and micronuclei formation *in vivo*. Positive effects were reported in *in vitro* studies with *Aspergillus nidulans*, with human lymphocytes measuring chromosomal aberrations, and a Chinese hamster cell study of sister chromatid exchange. Diquat induced unscheduled DNA synthesis in rat or human cells tested *in vitro*. However, *in vivo* studies for DNA damage were negative. Thus, the data from gene mutation studies, chromosomal effects, and other genotoxicity studies all indicate positive effects *in vitro*, but no effects *in vivo*. This may suggest that diquat is unable to reach the genome in intact animals.

Reproductive Toxicity- Diquat did not directly affect the reproductive system in rats. The NOEL for parental systemic effects (ulceration of tongue and hard palate, hypertrophy and hyperplasia of the renal collecting duct, and cataracts) was 4 mg/kg-day. The NOEL for pup systemic effects (decrement in weight gain) was approximately 1.6 mg/kg-day.

Developmental Toxicity- The maternal NOEL for rats was 4 mg/kg for reduced weight gain. Delayed skeletal ossification and hemorrhagic kidneys were noted in the developing rat fetuses. The developmental NOEL for delayed ossification in the rat was 12 mg/kg. The maternal NOEL for rabbits was 3 mg/kg (excessive maternal death, decreased body weight gain, and altered histopathology). The rabbit developmental NOEL (increased rate of malformations resulting from faulty cell migration) was less than 1.0 mg/kg. The Estimated-No-Effect-Level in rabbits (delayed ossification) was 0.33 mg/kg.

Neurotoxicity- Diquat dibromide did not cause delayed neuropathies in the rat. The single-dose NOEL for clinical signs in rats was 25 mg/kg. The 14-week NOEL for cataracts and lenticular opacity in rats was 9.5 mg/kg-day.

Hazard Identification- Possible adverse effects, except for carcinogenicity, are indicated in each data category. The most sensitive toxicological endpoint, an ENEL of 0.33 mg/kg for delayed ossification, concomitant with developmental anomalies in the rabbit, was used as the basis for assessing the margins of safety (MOSs) for potential short-term exposure. The MOS calculations presented in this document use the oral NOELs from laboratory animal studies to assess the risk of human exposure to diquat dibromide via dermal contact. Such an assessment should take into account the relative absorption factors. Absorption across the gut in the rat was approximately 10%. The oral ENEL, therefore, was adjusted to 0.033 mg/kg-day to reflect the absorbed dosage which is expected to have no effect. This adjusted ENEL, 0.033 mg/kg-day, was used for the calculation of safety margins for potential short-term exposure to diquat.

Both rats and dogs developed cataracts following chronic exposure to diquat. In rats, the 1-year NOEL for cataracts was 3.3 mg/kg-day. The lowest 1-year NOEL for cataracts was in dogs (0.5 mg/kg-day). This NOEL was adjusted to 0.05 mg/kg-day, as only 10% of the oral dosage of diquat was absorbed in other laboratory animals studies. The adjusted NOEL (0.05 mg/kg-day) was used for the calculation of safety margins for potential annual exposure to diquat.

Occupational and Non-Occupational Exposures- Potential short-term occupational exposures ranged from 0.2 $\mu\text{g}/\text{kg}\text{-day}$ for mixers and applicators injecting diquat into aquatic environments to 106 $\mu\text{g}/\text{kg}\text{-day}$ for ground applicators driving tractors with no cabs and a normal ground clearance. The potential short-term non-occupational exposures for drift were 0.5 $\mu\text{g}/\text{kg}\text{-day}$ at 50 meters and 0.1 $\mu\text{g}/\text{kg}\text{-day}$ at 1600 meters. Potential short term exposures (4 hours) for adult males swimming in treated water 24 hours after application ranged from 0.2 $\mu\text{g}/\text{kg}\text{-day}$ to 1.3 $\mu\text{g}/\text{kg}\text{-day}$. Potential annual occupational exposures ranged from 0.005 $\mu\text{g}/\text{kg}\text{-day}$ for mixers and applicators injecting diquat into aquatic environments to 4.4 $\mu\text{g}/\text{kg}\text{-day}$ for ground applicators. Annual drift exposures were 0.014 $\mu\text{g}/\text{kg}\text{-day}$ at 50 meters and 0.001 $\mu\text{g}/\text{kg}\text{-day}$ at 1600 meters.

Dietary Exposure- Monitoring programs have not found residues of diquat on food commodities; and, based on the physical/chemical properties of diquat and environmental fate studies, residues of diquat are not expected to be found on food commodities.

Risk Characterization- MOSs for short-term occupational exposures, based on the adjusted oral ENEL of 0.033 mg/kg-day for developmental toxicity and maternal clinical signs, ranged from less than 1 (ground applicators) to 165 (aquatic mixers and injectors). The MOSs for potential short-term non-occupational exposure by drift were 67 at 50 meters and 330 at 1600 meters. The MOSs for potential short term exposure of swimmers to diquat dibromide ranged from 26 to 165.

MOSs for annual occupational exposures, based on the adjusted NOEL of 50 $\mu\text{g}/\text{kg}$ for cataracts in dogs, ranged from 11 (ground applicators) to 25,000 (applicators with hand sprayers along right-of-ways). The MOSs for annual non-occupational exposure due to drift were 3,571 and 50,000 at 50 and 1600 meters, respectively.

Conclusions- Using current toxicity data, estimates from monitoring information on diquat, and surrogate exposure data, the calculated margins of safety (MOSs) for potential short-term exposure were less than the value conventionally recommended to protect people from the toxic effects of diquat for aquatic applicators and boat drivers using handguns, mixer/loaders and flaggers associated with aerial applications, ground applicators (except for hand applications on right-of-ways), and non-occupational exposures to drift at 50 meters. MOSs for potential annual occupational exposure to diquat for ground applicators using vehicles with normal clearance and no cab was also substantially less than the value conventionally recommended to protect people from the toxic effects of diquat. Mitigation measures should be considered to reduce potential exposure.

II INTRODUCTION

A. CHEMICAL IDENTIFICATION

Diquat dibromide (Trade names Aquacide[®] and Dextrone[®] - 6,7-dihydrodipyrido [1,2-a: 2',1'-c]pyrazinediium ion) is a contact herbicide that damages plant tissues quickly, causing plants to appear frostbitten because of cell membrane destruction. It also reduces plant photosynthetic activity (Ware, 1978). Diquat dibromide was entered into the risk assessment process because of possible adverse effects identified in developmental toxicity studies and chronic toxicity studies in laboratory animals.

B. TECHNICAL AND PRODUCT FORMULATIONS

Diquat was first registered for use in 1961 (USEPA, 1986a). Currently, there are 26 products containing diquat which are registered in the State of California. Approximately 161,993 pounds of diquat were used in California in 1990, and 133,121 pounds in 1991 (DPR, 1992a, 1993).

All diquat products in California are liquid. Technical diquat contains 35.3% diquat cation/gal (approximately 2 lbs diquat/gal). Formulations of diquat dibromide range from 0.3 to 5.0% diquat. Application rates range from 0.25 to 0.5 lb diquat/acre for agricultural use; 2.0 to 4.0 lb diquat/acre for aquatic use; and 0.5 to 1.0 lb diquat/100 gallons of water for industrial weed control (Appendix B). Diquat may be used alone or in combination with other herbicides, such as paraquat, amitrole, and simazine (Worthing and Hance, 1991).

Ethylene dibromide (EDB), a carcinogen, is an essential starting material in the production of diquat dibromide and remains a process impurity in diquat dibromide formulations (Garbus, 1989). The certified maximum EDB level in the formulating use products is 10 ppm, but the actual levels of EDB average 10 ppb (USEPA, 1986a). EDB dissipates from the formulations with time (Garbus, 1989). Because EDB residues are initially present in the formulations, there is the possibility for exposure to EDB through diquat dibromide use. Assuming the certified maximum EDB level, the USEPA has calculated that the upper bound of lifetime risk of cancer from incidental dietary exposure to EDB from label-approved diquat use is less than 4×10^{-7} ; less than 3×10^{-6} for drinking water treated with diquat dibromide; and 1×10^{-6} for mixer/loader and applicator exposures (USEPA, 1986a).

C. USAGE

Diquat dibromide is a non-selective, contact herbicide used for desiccation of potato vines, seed crops; control of sugarcane flowering; and industrial and aquatic weed control.

Based on data compiled by the USEPA in 1983, of the total amount of diquat dibromide applied in the U.S., approximately 27% was used on highway and railroad rights-of-way, 22% on industrial/manufacturing sites, 21% on golf courses/ornamental plantings, and 18% for aquatic weed control (USEPA, 1986a). The remaining 12% included uses in agriculture, around domestic dwellings, and in parks.

Approximately 199,095 and 161,993 pounds of diquat dibromide were sold in California in 1989 and 1990, respectively (CDFA, 1991).

D. ILLNESS REPORTS

Approximately 52 illness reports, including one suicide, concerning diquat dibromide were made between 1982 and 1990 (DPR, 1992b). Virtually all of the illnesses reported during agricultural use occurred as the result of equipment failure, accidental exposure, or violations of label requirements. The most common injuries reported were dermal burns, rashes, and eye irritations. Instances of nausea and dizziness were also attributed to diquat exposure.

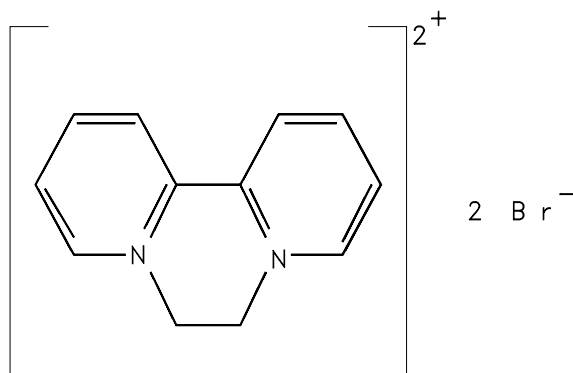
E. PHYSICAL AND CHEMICAL PROPERTIES^a

Chemical Name: 6,7-dihydrodipyrido
(1,2-a: 2',1'-c) pyrazinediium
dibromide

CAS# 85-00-7
Common Name: diquat dibromide

Empirical Formula: $C_{12}H_{12}N_2Br_2$

Chemical Structure:



Molecular Weight: 344 (as the dibromide)

Melting Point: 300°C

Vapor Pressure: not measurable

Henry's Law Constant: not measurable

Solubility (water): 67.7 g/100 ml @ 20°C

(organic solvents) insoluble

Octanol/Water Partition Coefficient: not measurable

^{a/} Chevron, 1985; Pack, 1987a; Worthing and Hance, 1991.

F. ENVIRONMENTAL FATE

Summary- The half-life for hydrolysis (pH 9) is greater than 9 months, and the half-life for photodegradation in aqueous solution (pH 7) is 74 days. Diquat binds tightly to soil, and it is not degraded under aerobic or anaerobic conditions. The environmental fate data strongly suggest that diquat will persist, bound to the soil, in the area where it is applied.

Hydrolysis

Sterile aqueous buffered solutions at pH 5, 7, or 9 containing diquat ion at a concentration of approximately 55 mg/L were incubated at 25°C in the absence of light for 30 days (Upton *et al.*, 1985). The results indicated that no hydrolysis of diquat occurs at pH 5 or 7, and only a limited amount ($\leq 8\%$) hydrolyzed at pH 9 after 30 days. The products of hydrolysis were not identified.

Photodegradation

Layers of sandy loam soil were treated with ^{14}C -bipyridyl-labelled diquat at a rate equivalent to 1.05 kg/ha, and irradiated for 32 days with light from a xenon arc lamp specially filtered to give a spectral distribution closely approximating sunlight (Joseph and Skidmore, 1987). Cycles of irradiation and dark simulated day-night conditions. Irradiated samples lost 5% of the applied diquat. No other radiolabelled components were detected in the soil. Control samples (without irradiation) lost less than 0.2% through volatilization during the same time period.

Aqueous solutions of ^{14}C -bipyridyl-ring-labelled diquat (20.1 $\mu\text{g/ml}$ diquat ion) were irradiated for 32 days at 25°C with light from a xenon arc lamp (filtered to give a natural sunlight spectrum) (Tegala and Skidmore, 1987). The half-life of diquat, in aqueous solution at pH 7, was calculated to be 74 days. No information on photolytic products was reported.

Microbial Degradation

Diquat was stable under the conditions of an aerobic metabolism study (Johnston, 1988a). The study was conducted for 9 months in Stockton sandy loam soil at a dose rate of 2.677 μg diquat ion per gram soil. The concentration of diquat did not vary during the study period.

A study of diquat metabolism was conducted for one month in pond water and sediment at a dose rate of 2.677 μg diquat ion per gram of pond sediment (Johnston, 1988b). No degradation of diquat was observed.

A study of anaerobic metabolism of diquat was conducted for 9 months in pond water and sediment at a dose rate of 2.677 μg diquat ion per gram of pond sediment (Johnston, 1988c). The only products observed were diquat and one minor compound (not identified). The concentration of diquat did not vary throughout the study period.

Mobility

Adsorption and desorption Freundlich coefficients (K_d) were determined in four soils and one pond sediment (Pack, 1987b). The adsorption K_d values ranged from 15 to 10740 and the desorption coefficients ranged from 20 to 10767 (Table 1). These values indicate that diquat binds tightly to the organic matter in soil. The higher the content of organic matter in the soil, the greater the Freundlich coefficient (adsorption or binding coefficient).

Table 1 - Summary of Freundlich coefficients.

Soil Type	Adsorption (Kd)	Desorption (Kd)
Sand	36	39
Sandy Clay Loam	4,895	
Loam	10,740	
Sandy Loam	1,882	10,767
Sediment	15	20

Other studies have also shown that diquat is firmly bound to the surface to which it is initially applied and does not migrate in soil (Riley *et al.*, 1970; Riley, 1986; Helling, 1971a,b,c; Tucker *et al.*, 1967; Prashad and Newby, 1976; Baldwin and Griggs, 1972; Baldwin and Lawson, 1973; Pack, 1984).

Plant Residues/Metabolism

Field plots on a loamy sand with a Strong Adsorption Capacity (SAC) of 120 μg diquat/g soil were treated with diquat at rates of 0, 60, 198 or 720 kg cation/ha incorporated to a depth of 150 mm to achieve potential soil concentrations of 0, 50, 110, and 400% of the SAC value (Wilkinson, 1980). Carrots grown on the soil from the 60 and 198 kg/ha plots did not have residues exceeding 0.07 ppm. Carrots grown in soil from the 720 kg/ha plots, died.

III TOXICOLOGY PROFILE

A. PHARMACOKINETICS

Summary- Only 0.3% of unoccluded and 1.4% of occluded topically applied diquat dibromide was absorbed through the skin of human volunteers. Intravenously injected diquat had a half life of 4 hours in humans, and 61.7% of the administered dose was excreted in the urine within 5 days. Approximately 10% of orally administered doses of diquat dibromide are absorbed through the gut of rats, goats or cattle. Absorbed diquat is excreted unchanged in the urine of rats in about three to four days. Goats metabolized diquat. Approximately 20% of radiolabel found in goat milk (0.018% of the administered dose) were metabolites. Diquat did not concentrate in any body organs, and only about 0.02% of an orally administered radiolabelled dose appeared in the milk of cattle.

Oral and Subcutaneous- rat

Male Wistar rats were given a single oral dose of ¹⁴C-labelled diquat dibromide (100% purity) at 5 or 10 mg/kg, or a subcutaneous dose at 5 or 6 mg/kg (Daniel and Gage, 1966). With oral dosing, 90 to 97% of the label was excreted in the feces, and 4-6% in the urine. After subcutaneous injection, 88 to 98% of the radio-label was excreted in the urine, and only about 2% in the feces.

Male rats (species unknown) were give a single oral dose of diquat dibromide (99% purity) at 45 mg/kg or a subcutaneous dose at 10 mg/kg (Mills, 1976). With oral dosing, 89% was excreted in the feces and 6% in the urine by 96 hours. With subcutaneous injection, 87% was excreted in the urine and 5% in the feces, suggesting poor absorption via the oral route. Diquat was excreted unchanged following subcutaneous injection.

Wistar rats were dosed with 250 ppm diquat dibromide in their diet for 8 weeks (Litchfield *et al.*, 1973). Seven days after being returned to the control diet, no detectable levels of diquat could be found in the rats.

Intravenous- mouse

Male mice were injected intravenously with ¹⁴C-diquat dibromide (purity unspecified) and subjected to whole body autoradiography (Litchfield *et al.*, 1973). Within 10 minutes after injection, diquat dibromide was evenly distributed throughout the body. Only the gastrointestinal region retained radio-labelled material at 72 hours.

Oral- goat

A goat was given a single oral dose of ¹⁴C-labelled diquat (purity unknown, 29 mCi/mmole) at 7 mg/kg (Griggs and Davis, 1973). Most of the dose (94.2%) was excreted in the feces, with 2.2% excreted in the urine by seven days. Only 0.018% of the dose was detected in the milk. In the milk, diquat accounted for 22% of the radiolabel, 1,2,3,4-tetrahydro-1-oxo-pyrido (1,2a)-5-pyrazinium salt represented 13%, and the monopyridone metabolite of diquat constituted 7% of the radiolabel. The remainder of the radiolabel in the milk was not identified.

Oral- cow

A cow was dosed with 5.2 mCi of ¹⁴C-diquat, and its photoproducts, on 794 g of powdered barley straw (Hemingway *et al.*, 1974). Of the radioactive material, only 0.4% was excreted in the urine, the remainder exited in the feces. Less than 4% of the radioactivity in the milk (maximum residue equivalent to 0.0014 μ g radiolabelled material/g) was attributable to diquat or its metabolites.

Four lactating cows were dosed orally with 5 mg/kg ¹⁴C-labelled diquat dibromide (Stevens and Walley, 1966). Less than 5% of the administered radioactivity appeared in the urine and less than 0.02% appeared in the milk. The highest concentration of diquat, or its metabolites, found in muscle tissue was less than 0.01 ppm after 3 days.

Dermal- human

¹⁴C-Diquat dibromide (4 ug/cm²) was applied to the forearms of six male human volunteers and maintained for 24 hours (Feldmann and Maibach, 1974). Urinary excretion of radiolabel was monitored for 120 hours, beginning 4 hours after application of the dose. A total of 0.3% of the applied dose was recovered in the urine.

Occlusion of a dermally applied dose of unlabeled diquat dibromide on humans led to 1.4% being absorbed (Wester and Maibach, 1985). This latter situation most closely simulates work conditions in which pesticides get under and into clothing.

Intravenous- human

Six male human volunteers were injected with a dose of 1 μ Ci (specific activity not given) of diquat dibromide (Feldmann and Maibach, 1974). Urinary excretion of radiolabel was monitored for 120 hours. The half-life of the radio-label in the body was 4 hours, with 61.2% of the label being excreted in the urine.

B. ACUTE TOXICITY

The acute toxicological data for technical grade diquat are summarized in Table 2. Signs of poisoning in the rat included weakness, incoordination, and lethargy. Signs of poisoning in a cow were dullness, inappetence anemia, and increased heart rate. Post-mortem on the cow revealed heart and kidney infarcts and intestinal inflammation (FAO, 1971). No dermal sensitization responses were observed among guinea pigs induced and challenged with diquat herbicide concentrate (Thompson *et al.*, 1985; Robbins, 1987c). Diquat dibromide (20% w/v) was a moderate eye irritant (4 on a scale of 8) in the Draize test (Parkinson, 1974).

Dermal Irritation- rabbit

A dose of 0.5 ml of diquat dibromide herbicide (1.84%) was applied per application site for 4 hours on each of six rabbits (Robbins, 1987d). There was no immediate sign of discomfort. Approximately 45 minutes after patch removal mild irritation was noted, characterized by very slight erythema. The degree of erythema increased to Grade 2 by 48 hours after patch removal. In addition, superficial desquamation was noted. Irritation persisted to day 14 in three test sites.

Table 2 - The Acute Toxicity of Technical Grade Diquat and Diquat Formulations

Species	Sex	Dose (mg/kg)	References ^a
TECHNICAL			
<u>Oral LD₅₀</u>			
Rat	M/F	215-235	1
Mouse	M/F	125	1
Guinea Pig	M/F	100	1
Rabbit	M/F	100	2
Dog	M/F	100-200	1
Cow	F	30	2
<u>Dermal LD₅₀</u>			
Rabbit	M/F	400	2
<u>Inhalation LC₅₀</u>			
Rat	M/F	0.97 mg/L	3
LIQUID FORMULATION (0.23 - 28% A.I.)			
<u>Oral LD₅₀</u>			
Rat	M/F	>5000	4,5
<u>Dermal LD₅₀</u>			
Rabbit	M/F	>2000	6
<u>Inhalation LC₅₀</u>			
Rat	M/F	>34.1 mg/L	7

a/ 1. Clark and Hurst, 1970; 2. FAO, 1971; 3. Bruce, 1985; 4. Karcher, 1987; 5. Robbins, 1987a; 6. Robbins, 1987b; 7. Bradfield and Wong, 1980.

C. SUBCHRONIC TOXICITY

Summary. The 4-week No-Observed-Effect-Level (NOEL) for increased relative liver weight in rats from dietary exposure to diquat dibromide was 7.2 mg/kg-day. The NOEL for piloerection in mice caused by gavage with diquat for 10 days was 2.5 mg/kg-day. Inhalation of diquat aerosol at 0.1 µg/L by rats for 3 weeks resulted in statistically significant reductions in blood platelet and reticulocyte counts.

Oral- rat

Sprague-Dawley rats (10/sex/group) received diquat dibromide (purity unknown) in the diet at 0, 75, 200, 350, or 500 ppm diquat cation (approximately 0, 6.1, 16.1, 27.8, or 37.7 mg/kg-day for males and 0, 7.2, 17.8, 31.4, or 42 mg/kg-day for females) for 4 weeks (Colley *et al.*, 1981). Relative liver weights were significantly ($P < 0.05$) greater for both males (7%) and females (11%) at the highest dose (500 ppm), and for males at 350 ppm (6%) and 200 ppm (15%). No histopathological changes in the liver were observed at any dose which might account for the increased relative liver weight. The NOEL for increased relative liver weight was 7.2 mg/kg-day. High cholesterol (123-133% of control levels) and low SGPT levels (65-73% of control levels) were noted in males at the two high doses, and marginally lower SGPT

levels (85% of controls) were noted in males at 350 ppm. Gaseous distention of the caecum was observed in rats at all doses, but histopathological examination could not establish the basis for the distension.

Oral- mouse

Three separate studies were made on the effect of diquat dibromide administered orally to SPF mice (Palmer *et al.*, 1977). In study one, SPF mice (6/group) were given 0, 20, 30 or 45 mg cation/kg-day for 10 days by gavage. In study two, SPF mice (3/group) were given 0, 10, 20 or 40 mg cation/kg-day for 10 days. In study 3, SPF mice (6/group) were given 0, 1.25, 2.5 or 5 mg cation/kg-day for up to 15 days. In these studies, treatment at 10 mg/kg-day or above elicited severe signs of reaction (including piloerection, ptosis, dyspnea/respiratory noise, hunched posture and death), markedly reduced food consumption (79-100%), and severe weight loss (>28% decrement). At 5 mg/kg-day, the effect of treatment on food consumption (12% depression) and body weight change (3% decrement) was less severe, and piloerection was the only consistent sign of reaction. The NOEL for piloerection was 2.5 mg/kg-day.

Inhalation- rat

Fischer-344 rats (10/sex/group) were exposed 6 hr/day, 5 days/week for three weeks to chamber atmospheres containing room air or an aerosol of diluted diquat concentrate (5% v/v in distilled water)(Bruce and Griffis, 1987). The measured concentration of diquat was 0.097 $\mu\text{g/L}$, with 99% of the aerosol particulates smaller than 10 μm . Reticulocyte counts were significantly ($P<0.05$) reduced in treated males (44%) and females (56%), and platelet counts were significantly ($P<0.01$) reduced in treated males (16%). No other treatment-related, systemic changes were noted.

Dermal- rat

Sprague-Dawley CD rats (6/sex/group) received dermal application of technical diquat (20.64% cation w/w) at dose levels of 0, 5, 20, 40, or 80 mg cation/kg-day, 7 days/week over a 21 day period (Auletta, 1987). Dermal irritation (erythema, edema, atonia, and desquamation) and tissue destruction (necrosis, eschar formation) were observed in several animals in all dose groups. Incidence and severity were generally dose related. Erythema appeared on day 2 in animals dosed with 20, 40, or 80 mg/kg-day, but not until day 8 in animals dosed with 5 mg/kg-day. Microscopic examination of grossly abnormal treated skin revealed an acute necrotizing purulent dermatitis in 6/10, 11/12 and 12/12 rats from the 20, 40 and 80 mg/kg-day groups, respectively. Severe effects were first noted in most animals on day 8 or 11. Systemic toxic effects included mortality (1/12, 5/12, and 11/12, respectively) in groups receiving 20, 40 or 80 mg/kg-day beginning on days 18, 8, and 6, respectively. The single surviving animal in the 80 mg/kg-day group exhibited a variety of hematologic and clinical chemistry abnormalities consistent with dehydration and malnutrition, and suggestive of impaired renal, hepatic and/or cardiac function. One of the surviving females at 40 mg/kg-day had elevated glucose, decreased serum albumin and total protein values. All other rats on doses of 40 mg/kg-day or less had no significant changes in hematology, clinical chemistry or urinalysis values. Organ weight data, and microscopic examination of the liver and kidneys revealed no evidence of a systemic toxic effect. The 2-day NOEL for erythema was 5 mg/kg-day. The study was considered supplemental.

Dermal- rabbit

Rabbits (5/sex/dose) received daily dermal applications of diquat dichloride (purity unknown) at 3.1, 6.3, 12.5 or 25 mg/kg-day for up to 20 days (Swan, 1963). All animals died at the highest dosage (25 mg/kg-day) within 14 days; the first mortality was at four days. One animal survived at 12.5 mg/kg-day (first mortality at day 3); 8/10 survived at 6.3 mg/kg-day (first mortality at day 7); and 9/10 survived at 3.1 mg/kg-day (mortality on day 10). Post mortems indicated the following systemic effects: ulceration of the gastric mucosa, degeneration of the convoluted tubules in the kidneys, areas of hemorrhage in the thymus, and congestion of the lungs and lung blood vessels. The skin application areas developed hyperemic and subcutaneous edema with increasing sloughing of surface layers, followed by scab formation. At the high dosage (25 mg/kg-day), extensive areas of epidermal necrosis developed after 12 applications. The 3-day NOEL for death was 6.3 mg/kg. The study was considered supplemental.

D. CHRONIC TOXICITY/ONCOGENICITY

Summary- There was no indication of oncogenicity in either mice or rats. The NOEL for decrement in body weight gain and liver vacuolation in mice was 2.5 mg/kg-day. The 1-year NOEL for cataracts was 0.66 mg/kg-day in rats, and 0.5 mg/kg-day in dogs. Other systemic effects, such as inflammatory lesions of the large intestine, shrunken adrenal glands, reduced kidney weights, and reduced epididymal weights were noted in rats and dogs at doses greater than 3.3 mg/kg-day. The NOEL for mild nephropathy in mice was 4.8 mg/kg-day.

Dietary- rat

Diquat dibromide technical (24.6% w/v as cation) diluted with water and fed in the diets of CD rats (50/sex/group plus a satellite group 10/sex/dose killed and analyzed at 52 weeks) at 0, 5, 15, 75, or 375 ppm (approximately 0, 0.22, 0.66, 3.3 or 16.5 mg/kg-day of cation, adjusted for purity) for 104 weeks (Colley *et al.*, 1985). At 13 weeks, cataracts were evident in males dosed with 16.5 mg/kg-day (11/49) and 3.3 mg/kg-day (1/50), and in females at 16.5 mg/kg-day (2/50). At 52 weeks the pattern continued, with cataracts evident in males dosed with 16.5 mg/kg-day (45/48) and 3.3 mg/kg-day (1/49), and in females at 16.5 mg/kg-day (43/48). The 1-year NOEL for cataract formation was 0.66 mg/kg-day. At two years, cataract development in males and females was noted at 0.66 (1/22 and 1/20, respectively), 3.3 (3/21 and 3/20, respectively), and 16.5 mg/kg-day (24/24 and 27/27, respectively). The 2-year NOEL for cataracts was 0.22 mg/kg-day (based on the amount of cation consumed). At two years, both males and females exhibited decreased renal clearance and urine concentrating ability at the two high dosages. There was an incidence of comparatively rare osteosarcoma in males- 0, 1, 0, 0, and 3 for the dosages 0, 0.22, 0.66, 3.3, and 16.5 mg/kg-day. However, trend analysis indicated the effect was not dose related. This study was acceptable to DPR under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) guidelines (USEPA, 1984; Appendix A).

Wistar rats (35/sex/group) were fed on a diet containing diquat dibromide monohydrate (100% purity) at 0, 15, 25, or 75 ppm (using a default conversion of 0.05 mg/kg-day= 1 ppm; approximately 0, 0.75, 1.3, or 3.8 mg/kg-day) for two years (Rogerson and Broad, 1978). Cataract formation (76% in males, 65% in females) was reported beginning at 9 months in rats dosed with 75 ppm. The NOEL for cataract formation was 25 ppm (approximately 1.3 mg/kg-day). This study did not meet the FIFRA guidelines as it lacked clinical chemistry, hematology, gross necropsy, and histopathological data.

Dietary- mouse

CD-1 mice (60/sex/group) were fed on a diet containing diquat dibromide monohydrate (98% purity) at 0, 30 or 150 ppm (approximately 0, 4.5, or 22.5 mg/kg-day) for 80 weeks (Ben-Dyke *et al.*, 1975). At 11 weeks, two more groups at 0 or 500 ppm were added. Due to clinical toxicity, the 500 ppm level was reduced to 400 ppm after 3 weeks, and again to 300 ppm (approximately 45 mg/kg-day) after a further 2 weeks. All treatments continued for 80 weeks. Reduced growth rates were seen at 45 (13-24%) and 22.5 mg/kg-day (5-11%), as well as liver vacuolation. There was no evidence of oncogenicity. The systemic NOEL for decrement in body weight gain and liver vacuolation was 4.5 mg/kg-day. This study was not acceptable to DPR under FIFRA guidelines as it lacked adequate histopathology and certain appendices, and contained ambiguities and deficiencies in other aspects of the data.

C57BL/10JfCD-1/Alpk mice (60/sex/group) were fed diets containing 0, 30, 100 or 300 ppm (approximately 3.56, 11.98, and 37.83 mg/kg-day for males, and 4.78, 16.03, and 48.27 mg/kg-day for females from consumption data) diquat dibromide (26.7% w/v purity) for 104 weeks (Hodge, 1991). No evidence of oncogenicity was reported. The kidney weight, adjusted for body weight, exhibited a significant ($p < 0.05$) increase at the two highest dosages (5% at 100 and 7% at 300 ppm) in males. In females, the incidence of tubular dilation and hyaline droplet formation in the kidneys was slightly greater at the two highest dosages (Table 3). Eye discharges occurred with greater frequency in the two highest dosage groups. The NOEL for mild nephropathy was 30 ppm (approximately 4.8 mg/kg-day). This study was acceptable to DPR under FIFRA guidelines.

Table 3. Macroscopic and microscopic effects on mice from long term dietary exposure to diquat dibromide (Hodge, 1991).

Tissue	Male Dosage (mg/kg-day)				Female Dosage (mg/kg-day)			
	0	3.6	12.0	37.8	0	4.8	16.0	48.3
Eye, Discharge	6/60 (10%)	2/60 (3%)	7/60 (12%)	10/60 (17%)	9/60 (15%)	7/60 (12%)	7/60 (12%)	15/60 (25%)
Kidney Tubule Hyaline droplets	6/60 (10%)	13/60 (22%)	4/60 (7%)	9/60 (15%)	3/60 (5%)	3/60 (5%)	10/60 (17%)	11/60 (18%)
Dilation	1/60 (2%)	1/60 (2%)	3/60 (5%)	6/60 (10%)	1/60 (2%)	2/60 (3%)	4/60 (7%)	8/60 (13%)

Dietary- dog

Diquat dibromide (26.7% diquat ion) was fed at dose levels of 0, 0.5, 2.5 or 12.5 mg/kg-day to dogs (5/sex/group) for 1 year (Hopkins, 1990). Lenticular opacities were observed in both sexes at 12.5 mg/kg-day (3/4 males; 3/4 females), and in females (1/4) at 2.5 mg/kg-day. Chronic inflammatory lesions were observed in the large intestine in all animals at 12.5 mg/kg-day. Relative kidney weights increased in both sexes at the high dose (24% for males; 18% for females), but no histopathological changes were detected. Absolute adrenal weights decreased in males at the high dose (14%), and absolute epididymal weight reductions (16%) were noted in the 2.5 mg/kg-day group. The NOEL for lenticular opacities was 0.5 mg/kg-day. This study was acceptable to DPR under the FIFRA guidelines.

Diquat dichloride monohydrate (98% purity) was fed in the diet to dogs (3/sex/group) at 0, 1.7, 5 or 15 mg/kg-day for two years (Hurst, 1966). At the end of 2 years, 1/sex/group was necropsied and the study continued another 2 years. Cataracts developed within 10-11 months at 15 mg/kg-day, and after 15-17 months at 5 mg/kg-day. The NOEL for lens opacity in dogs was 1.7 mg/kg-day. The study was designed to examine the onset of cataracts, and as such omits dose justification, dose analysis, clinical chemistry and hematology, and histopathology. This study not acceptable to DPR under FIFRA guidelines.

E. GENOTOXICITY

Summary. Diquat dibromide was not mutagenic in *Salmonella* for the reverse mutation in the his operon. However, it did cause a 2-3 fold increase in thymidine kinase activity in mouse lymphoma L5178Y cells in two separate trials. Diquat was negative for dominant lethal effects and micronuclei formation *in vivo*. Positive effects were reported in *in vitro* studies with *Aspergillus nidulans*, with human lymphocytes measuring chromosomal aberrations, and a Chinese hamster cell study of sister chromatid exchange. Diquat induced unscheduled DNA synthesis in rat or human cells tested *in vitro*. However, *in vivo* studies for DNA damage were negative. Thus, the data from gene mutation studies, chromosomal effects, and other genotoxicity studies all indicate positive effects *in vitro*, but no effects *in vivo*. This may suggest that diquat is unable to reach the genome in intact animals.

Gene Mutation

Diquat dibromide (25.8% purity) in water, with and without S9 rat liver activation at 0, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10, 50, or 100 $\mu\text{g}/\text{plate}$ was incubated with *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100, and *Escherichia coli* (WP2 *uvrA* pKM101) in two trials with three replicates per trial (Callander, 1986). No reverse mutation was reported. This study was acceptable to DPR. The acceptability of the genotoxicity studies is based on the Toxic Substances Control Act guidelines (Federal Register, 1985).

Diquat dibromide (technical grade, 25.8% purity) was added to mouse lymphoma cells without activation at 0, 6.3, 12.5, 25, 50 or 100 $\mu\text{g}/\text{ml}$; and with rat liver (Aroclor-induced) S-9 activation at 0, 3.1, 6.5, 12.5, 25 or 50 $\mu\text{g}/\text{ml}$ in the forward mutation plate assay [DMN as the positive control] (Cross, 1986a). Gene mutation was indicated with a 2-3 fold increase in revertants over the controls. This study was unacceptable to DPR because the description of the data in the tables was unclear.

The differences in sensitivity among *Salmonella* tester strains TA102, TA104, TA2638, TA95 and TA96 to a variety of chemical oxidants, including diquat at 10 ng, are discussed (Levin *et al.*, 1982). No mutagenicity was detected for diquat, even though it was toxic to TA102 and TA2638. The article did not follow the TSCA guidelines, and was unacceptable to DPR.

Diquat dibromide (analytical grade, 100% purity) in saline solution with S-9 activation at 0, 3.1, 6.3, 12.5, or 25 $\mu\text{g}/\text{ml}$ [benzo(alpha)pyrene and DMN positive control]; and without activation at 0, 6.25, 12.5, 25, or 50 $\mu\text{g}/\text{ml}$ [EMS positive control] was used in the forward mutation plate assay with mouse lymphoma L51784 cells (Cross, 1986b). Gene mutation was indicated with a 2-3 fold increase at moderate cytotoxicity in replicate trials. The study was unacceptable (unclear description of data in tables) to DPR and not upgradeable (use of purified diquat).

A published study reported the results of a plate incorporation assay for mutagenicity of diquat at 0, 0.1, 0.5, and 1 $\mu\text{g}/\text{plate}$ with *Salmonella typhimurium* strains His G46, TA92, TA1535, TA1538, and TA100 (Bignami and Crebelli, 1979). Mutagenicity was reported in the forward mutation assay for 8-azaguanine resistance in TA1535 and TA92. The study was in summary form only, and thus, unacceptable to DPR.

A published study used diquat obtained from ICI Americas in the "Ames test" with *Salmonella* strains TA1535, TA1537, TA1538, TA98 and TA100, with or without S-9 activation, at diquat concentrations ranging from 0.25 to 10 $\mu\text{g}/\text{plate}$ (Benigni *et al.*, 1979). The high concentration was cytotoxic. No mutagenic activity (frameshift or base-pair substitution) was expressed. Diquat did cause forward mutations (8-azaguanine resistance) in *Salmonella* tester strains hisG46, TA92, and TA1535 at plate concentrations of 0.1 μg for hisG46 and TA92, or 0.25 μg for TA1535. Forward mutations were also seen in *Aspergillus nidulans* at a plate concentration of 400 μg , or a liquid concentration of 10 mg/ml. The study was considered incomplete, and unacceptable to DPR.

Diquat (35.3% pyrazinediium ion) was 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 (Andersen *et al.*, 1972). Diquat caused no increase in the reversion rate with *Salmonella*; it was negative with T₄ for induction of rII mutants at 20 μ g; and negative for reversion of AP72 and N17 at 10 μ g. The study was incomplete, and unacceptable to DPR.

Chromosome Effects

Diquat (28.6%, w/v of ion) in 0.5% Tween80 was given to male mice (30/group) at 0 and (15/group) at 0.1, 1, or 10 mg/kg body weight for five consecutive days (McGregor, 1974). Cyclophosphamide and EMS were used as positive controls. Males were mated with untreated females (2 females per male) weekly for 8 weeks. No dominant lethal effects were reported. This study was acceptable to DPR.

Diquat dibromide (25.8% diquat ion in water) was given orally in a single dose at 0, 62.5, or 100 mg/kg to mice (15/sex/dose)(Sheldon *et al.*, 1986). Cyclophosphamide was used as a positive control. No clastogenic effects were reported in body marrow samples (5/sex/dose) at 24, 48, and 72 hours. This study was acceptable to DPR.

Diquat dibromide (technical, 25.8% diquat ion in saline) was used to treat human lymphocytes (2 donors, male and female) at 0, 12.9, 25.8, 64.5 or 129 μ g/ml with and without S-9 rat liver (Aroclor-induced) activation (Richardson *et al.*, 1986). A decrease in the mitotic index for cytotoxicity was associated with an increase in chromosomal aberrations (primarily breaks). This study was acceptable to DPR.

Diquat dibromide (analytical grade 100% w/w purity) was used treat human lymphocytes from 2 donors, two trials each at 0, 13.4, 26.7, 53.5, 107, 267.4 or 534.8 μ g/ml with and without S-9 rat liver (Aroclor-induced) activation (Wildgoose *et al.*, 1986). There were increased chromosomal aberrations at dose levels with high cytotoxicity. The study used analytical grade diquat and was unacceptable to DPR.

Diquat induced lethal recessive mutations in the diploid P3 strain of *Aspergillus nidulans* at a concentration of 10 mg/ml (Benigni *et al.*, 1979). The study lacked individual data and was unacceptable to DPR.

Diquat (no further characterization) was tested with Chinese hamster lung cells for sister chromatid exchanges and chromosomal aberrations (Tanaka, 1988). Significant differences in the frequencies of SCEs compared with controls were found above 0.08 μ M, but not for chromosomal aberrations at 0.08 to 0.4 μ M. The study lacked individual data and was unacceptable to DPR.

Other Genotoxicity

Diquat dibromide (technical, 25.8% diquat ion) was given by oral gavage to male rats at 0, 225, 450 or 900 mg/kg (Trueman, 1986). The rats were killed after 4 or 12 hours, and hepatocytes were isolated and cultured. After the cells attached, they were incubated for 4 hours with ³H-thymidine, followed by an overnight incubation with unlabelled thymidine. Unscheduled DNA synthesis was examined by autoradiography. For the 12 hour time point, a total of 2, 5, 4 and 4 rats were used for control, low-, mid-, and high-doses in two trials. For the 4 hour time point, 1, 5, 5, and 5 rats were used for the doses, respectively. One hundred nuclei were scored per animal from 2 to 3 slides per animal. There was no evidence of induction of unscheduled DNA synthesis. This study was acceptable to DPR.

Diquat (95% purity) was dissolved in DMSO at 0, 100, 500, and 1000 $\mu\text{g}/\text{ml}$, and tested with rat thymocytes or human lymphocytes *in vitro* with and without UV irradiation (Rocchi *et al.*, 1980). Inhibition (56-61%) of DNA synthesis was reported. The study lacked individual data and was unacceptable to DPR.

Diquat dibromide (no purity stated) was used with human fibroblasts (transformed with SV-40, VA-4) *in vitro* with and without rat liver activation at 1, 10, 100, or 1000 μM for 1, 3, 5, 8, or 12 hours (Ahmed *et al.*, 1977). Unscheduled DNA synthesis was determined by autoradiography. Hydroxyurea was used to suppress semi-conservative DNA synthesis. The results for the 8 hour incubation were reported as positive at all concentrations with and without activation. The study lacked individual data and was unacceptable to DPR.

Diquat caused unscheduled DNA synthesis in "epithelial-like human embryo cells" over a concentration range of 20 to 2000 $\mu\text{g}/\text{ml}$ (Benigni *et al.*, 1979). At a diquat concentration of 10 $\mu\text{g}/\text{plate}$ in the presences of S-9 activation only, increased lethality was noted in a Salmonella strain deficient in DNA excision repair (TA1538) versus a sister strain (TA1978) stated to have competent repair. The study lacked individual data and was unacceptable to DPR.

F. REPRODUCTIVE TOXICITY

Summary. Diquat did not directly affect the reproductive system in rats. The NOEL for parental systemic effects (ulceration of tongue and hard palate, hypertrophy and hyperplasia of the renal collecting duct, and cataracts) was 4 mg/kg-day. The NOEL for pup systemic effects (decrement in weight gain) was approximately 1.6 mg/kg-day.

Dietary- rat

Wistar-derived Alpk:APfSD rats (30/sex/dose) were dosed with diquat dibromide (diquat ion concentration = 26.7% w/v) in the diet for 12 weeks at 0, 16, 80 or 400 ppm (Hodge, 1990). The dosage for F₁ rats was reduced from 400 ppm to 240 ppm at 9 weeks because of adverse effects. Cataracts and other eye pathologies (keratitis, iridocyclitis, conjunctivitis) were seen in adults of both sexes of the F₀ and F₁ generations at greater than or equal to 240 ppm. Cataracts were not seen in any F₁ pups. Also at the high dose (greater than or equal to 240 ppm) there was also an increase of hypertrophy and hyperplasia of the collecting duct epithelium and tubular dilation in the renal papilla in both sexes. At greater than or equal to 240 ppm there was: a) decrement in body weight gain in both sexes (6-13%); b) decreased food consumption (10-14%); c) tongue ulceration in both sexes in the F₀, and F₁ females; d) hard palate ulceration in both sexes of the F₁. The systemic, parental NOEL was 80 ppm (approximately 4 mg/kg-day using a conversion factor of 0.05 mg/kg per ppm). No reproductive effects were noted. Pup body weight gain was reduced in both sexes for F₁ and F₂ at the high dose (greater than or equal to 240 ppm), and for the males at 80 ppm. F₁ pup kidney weights (absolute) of both sexes (22-29%), and testes weights (absolute) were decreased (21%) at greater than or equal to 240 ppm. F₁ and F₂ pups also exhibited hydronephrosis at the high dosage. The NOEL for pup systemic effects was 16 ppm (approximately 1.6 mg/kg-day using a conversion of 0.1 mg/kg per ppm). This study was acceptable to DPR under FIFRA guidelines.

Wistar rats (12 males/dosage, 24 females/dosage) were fed on a diet containing diquat dibromide monohydrate (100% purity) at 0, 125 or 500 ppm for 3 generations (2 litters/generation) (Fletcher *et al.*, 1972). Decreased body weight gain (7%) and cataracts were observed in the parental generations at the 500 ppm dose. However, the study was considered unacceptable to DPR under FIFRA guidelines because of lack of analysis of the diet, use of only two doses, no individual data provided, and there were no interim body weights for pups at days 4, 7, or 14.

Wistar rats (10/sex/group or 10 females/group only, or 10 males/group only; 5/sex/group were used for the F₁ and F₂ matings) were fed for 3 generations on a diet containing diquat dichloride monohydrate (purity not stated) at 0, 125, or 500 ppm (Griffiths *et al.*, 1966). The development of cataracts and decreased weight gain (7-13%) in the parental stock were reported at the 500 ppm level. However, the study did not follow FIFRA guidelines regarding identification and analysis of the test article, dosage selection, number of animals, and histopathology. Further it did not use diquat dibromide, which is the compound that is registered.

G. DEVELOPMENTAL TOXICITY

Summary- The maternal NOEL for rats was 4 mg/kg for reduced weight gain. Delayed skeletal ossification and hemorrhagic kidneys were noted in the developing rat fetuses. The developmental NOEL for delayed ossification in the rat was 4 mg/kg. The maternal NOEL for rabbits was 3 mg/kg (excessive maternal death, decreased body weight gain, and altered histopathology). The rabbit developmental NOEL (increased rate of malformations resulting from faulty cell migration) was less than 1.0 mg/kg. The Estimated-No-Effect-Level in rabbits (delayed ossification) was 0.33 mg/kg.

Gavage- rat

Diquat dibromide (diquat ion concentration = 26.2% w/v) was administered by oral gavage to groups of 24 female Wistar derived (Alpk:APfSD) rats at doses of 0, 4, 12 or 40 mg/kg-day on days 7 through 16 of gestation (Wickramaratne, 1989). Maternal weight gain and food consumption were significantly ($P < 0.05$) reduced at 40 mg/kg (22% and 36%, respectively), and slightly reduced at 12 mg/kg (11% and 8%, respectively). The maternal NOEL was 4 mg/kg for reduced weight gain. Intrauterine growth retardation as measured by decreased weight; delayed skeletal ossification; and hemorrhagic kidneys were noted at 40 mg/kg. The NOEL for delayed skeletal ossification was 12 mg/kg. This study was acceptable to DPR under FIFRA guidelines.

Diquat dibromide (32% w/v diquat ion) was fed in the diet through pregnancy at 0, 125, and 500 ppm diquat ion to groups of 18, 20 and 20 pregnant rats, respectively (Moore and Wilson, 1973). Reduced maternal food consumption (20%), decrement in maternal (31%) and fetal (8%) weight gain, and a slight incidence of subcutaneous hemorrhage in the fetus were reported at 500 ppm. No developmental effects were reported. The apparent developmental and maternal NOEL was 125 ppm for decrement in body weight gain. The study was unacceptable to DPR under FIFRA guidelines as it did not follow the guidelines and there was no analysis of the treated diet.

Gavage- rabbit

Diquat dibromide (26.2% w/v) was administered by gavage to New Zealand white rabbits (20/group) at doses of 0, 1, 3, or 10 mg/kg on days 7 through 19 of gestation (Hodge, 1989). There was a statistically significant ($P < 0.05$) increase in maternal mortality at the highest dose (10 mg/kg), as well as a decrement in body weight gain (80%), and histopathological changes in the stomach (ulceration and/or hemorrhage), liver (prominent reticular pattern), and intestinal vasculature (leakage). The maternal NOEL was 3 mg/kg (excessive maternal death, decreased body weight gain, and altered histopathology of the digestive tract). Fetal ossification of the ventral tubercle of the cervical vertebrae was significantly ($P < 0.05$) delayed at all doses tested. Fetal malformations were significantly ($P < 0.05$) increased at 1 and 10 mg/kg compared to the controls (Table 4). In the controls, two fetuses from two different litters exhibited major malformations- one involving craniofacial defects, and the other involving the liver. At 1 mg/kg-day, there were eight malformed fetuses from eight different litters. Four displayed craniofacial defects; three had cardiac defects; two had arthrogryposis; and one had urogenital anomalies. In the 3 mg/kg-day group, four fetuses from four different litters exhibited malformations. One had craniofacial anomalies; one had cardiac anomalies; and two had absent or bilobed gall bladders. At 10 mg/kg-day, seven fetuses from five different litters had malformations. Two involved craniofacial defects; three had gall bladder anomalies; one had an anomalous diaphragm; and one had fused ribs. There was no NOEL for developmental toxicity (fetal malformations and delayed ossification of the ventral tubercle of the cervical vertebrae). The Lowest-Observed-Effect-Level (LOEL) was 1.0 mg/kg-day. This study was acceptable to DPR under FIFRA guidelines.

Table 4. Developmental Effects of Diquat Dibromide in Rabbits

Parameter	Diquat Dose Level (mg/kg-day)			
	0	1	3	10
No. of implants	173	157	153	116
No. of resorptions	26	23	24	20
Percent resorptions	15	15	16	17
No. of live fetuses	147	134	129	96
No. of malformed fetuses	2	8*	4	7*
Percent of malformed fetuses	1.4	6	3.1	7.3
No. delayed ossification fetuses (ventral tubercle of the cervical vertebrae)	1	6*	6*	10*
Percent delayed ossification	0.7	4.5	4.6	10.4

* Significantly different from control at P<0.05 by Fisher's Exact Test

Diquat dibromide monohydrate (100% purity) was dissolved in dispersol and given by gavage to groups of 17, 20, 15, and 19 mated rabbits at 0, 1.25, 2.5, and 5 mg/kg, respectively, on days 1 through 28 of gestation (Palmer and Pratt, 1974). No adverse developmental effects were reported. The developmental NOEL was greater than 5.0 mg/kg, with a maternal NOEL of 2.5 mg/kg (45% decrement in weight gain). This study was not acceptable to DPR under FIFRA guidelines because of the dosing schedule, lack of histopathology, poor health of the animals, and too few animals.

Gavage- mouse

Diquat dibromide monohydrate (100% purity) was given by gavage in distilled water to groups of 34, 32, 33, and 34 mated females at 0, 1, 2, and 4 mg/kg on days 6 through 15 of gestation (Palmer *et al.*, 1978). The maternal NOEL was 1 mg/kg [clinical signs, decreased body weight gain (14%), increased mortality (4/33 at 2 mg/kg-day, 8/34 at 4 mg/kg-day)]. The developmental NOEL was 1 mg/kg based on skeletal anomalies, exencephaly, premature opening of the eyes, and umbilical hernia. This study was not acceptable to DPR under FIFRA guidelines because of the lack of clinical observation, the inability to correlate findings between specific animals and fetuses, and a lack of historical control data.

H. NEUROTOXICITY

Summary- Diquat dibromide did not cause delayed neuropathies in the rat. The single-dose NOEL for clinical signs in rats was 25 mg/kg. The 14-week NOEL for cataracts and lenticular opacity in rats was 9.5 mg/kg-day.

Technical diquat dibromide (25.5% diquat w/v) at 0, 25, 75 or 150 mg/kg was given in a single oral gavage dose to Alpk:APfSD rats (10/sex/dose) (Horner, 1992a). There was no histopathological evidence of delayed neuropathy. One female at 150 mg/kg was terminated *in extremis* on day 6, otherwise there was no mortality. Clinical signs were observed in the 150 mg/kg dose females, including piloerection (7/10), upward curvature of the spine (3/10), stained mouths (3/10), urinary incontinence (3/10), subdued behavior (2/10), and pinched in sides(1/10). At 75 mg/kg, females exhibited staining of the nose (3/10) and diarrhea (2/10). The single-dose NOEL for clinical signs was 25 mg/kg. The study was acceptable to DPR under FIFRA guidelines.

Technical diquat dibromide (26.4% diquat w/v) at 0, 20, 100, or 400 ppm (0, 1.6, 8, or 32.4 mg/kg-day for males; and 0, 1.9, 9.5, or 38.5 mg/kg-day for females- based on consumption data) was administered in the diet to Alpk:APfSD rats (12/sex/dose) for up to 14 weeks (Horner, 1992b). There was no histopathological evidence of neuropathies, nor was there evidence of neurotoxicity in the functional observational battery or motor activity measurement. Toxic systemic effects were noted only at 400 ppm. These effects included posterior lens opacity (6/12 females, 8/12 males), total cataracts (7/12 females, 5/12 males), and a significant ($P<0.01$) decrement in body weight gain (18% in females, 20% in males). The 14-week NOEL for cataracts and lenticular opacity was 9.5 mg/kg-day. The study was acceptable to DPR under FIFRA guidelines.

IV RISK ASSESSMENT

A. HAZARD IDENTIFICATION

Possible adverse effects, except for oncogenicity, are indicated in each data category. Selected studies and results are summarized in Table 5.

Short-Term Exposure

Most occupational and non-occupational exposures to diquat dibromide involve dermal absorption. Consequently, a short-term dermal NOEL would be more applicable as a NOEL for assessing the risks of acute occupational or non-occupational human exposure to diquat. Unfortunately, no such single-dose dermal studies were available in the DPR data base or from a search of the open literature. A subchronic dermal exposure study on the effects of diquat dibromide on rats indicated systemic effects (death) began after 6 days of repetitive dosing (Auletta, 1987). In rabbits, ulceration of the gastric mucosa, degeneration of the convoluted tubules in the kidneys, areas of hemorrhage in the thymus, and congestion of the lungs and lung blood vessels accompanied by death was observed after 3 days of repetitive dermal dosing with diquat dichloride (Swan, 1963). However, deficiencies in the dosing regime, and the difference in the chemical identity of the test material, preclude the use of this study as the basis for regulating short-term exposure to diquat dibromide.

The toxicological basis for assessing the risks associated with potential short-term exposure to diquat was identified in oral dosing studies. A single oral dose of diquat dibromide at 75 mg/kg caused clinical signs (diarrhea and stained noses) in female rats (Horner, 1992a). The NOEL for clinical signs was 25 mg/kg. In a rat developmental study, the developmental NOEL (delayed ossification) was 12 mg/kg-day, while the maternal NOEL (decrement in body weight gain) was 4 mg/kg-day for exposure to diquat by gavage (Wickramaratne, 1989). Mice appeared to be more sensitive to diquat than were rats. The mouse NOELs for maternal toxicity (clinical signs, excessive death) and developmental toxicity (skeletal anomalies, exencephaly, and umbilical hernia) were both 1.0 mg/kg-day (Palmer *et al.*, 1978).

The rabbit appeared to be the most sensitive laboratory animal to diquat. In the rabbit, the maternal NOEL was 3.0 mg/kg-day (histopathological changes in the liver, intestine, and vasculature; excessive mortality), but there was no developmental NOEL (Hodge, 1989). The ossification of the ventral tubercle of the cervical vertebrae was delayed significantly in all treatment groups compared to controls. In addition, the number of fetal malformations was significantly greater in the low dose (1 mg/kg-day) and high dose (10 mg/kg-day) groups compared to the controls. The lack of statistical significance at the mid-dose (even though it represents a 221% increase over controls) may have been a spurious event, and the observed increase may represent a biologically significant finding, supportive of a treatment-related effect. This hypothesis is consistent with the suggested common mechanism (interference with cell migration) for the observed anomalies across the different treatment groups (Appendix A). Although the fetal malformations in the low dose group (1 mg/kg-day) and the high dose group (10 mg/kg-day) were quantitatively different from the controls, the malformations were not qualitatively different from either the concomitant or historical controls (Valent, 1992). Nonetheless, the possibility that diquat caused the significant ($P < 0.05$) increase in the number of malformations found at the low dose (1 mg/kg-day) cannot be ignored. As there was no NOEL, an Estimated-No-Effect-Level (ENEL) was calculated. Because the magnitude (incidence) of the effect was small, and the slope of the dose response was fairly shallow, an uncertainty factor of 3 was used to derive an ENEL of 0.33 mg/kg-day (Dourson and Stara, 1985; USEPA, 1987). As absorption across the gut in the rat was approximately 10% (Daniel and Gage, 1966; Mills, 1976), the ENEL was divided by a factor of 10 to reflect the absorbed dosage which would be expected have no toxic effect. This adjusted ENEL, 0.033 mg/kg-day, based on the observations of delayed ossification and fetal malformations, was used to calculate margins of safety for potential short-term exposure to diquat.

As developmental toxicity may be manifested as the result of a single dose (Schardein, 1985; Ogata *et al.*, 1984; USEPA, 1991), it is assumed, in the absence of data to the contrary, that the observed effects were elicited from a single dose. Although a developmental endpoint for exposure to toxins is only relevant in women of child-bearing age, the assumption that all other population subgroups are as sensitive results in margins of safety (MOSs) that protect the health of these other subgroups for other endpoints that may occur at higher dosages.

Annual Exposure

Diquat was not oncogenic or carcinogenic in either rats or mice. The major toxic effects from chronic oral exposure to diquat were in the liver, large intestine, and eyes. Mice have a NOEL of 4.5 mg/kg-day for liver vacuolation (Ben-Dyke *et al.*, 1975). The NOEL for inflammatory lesions of the large intestine of dogs was 2.5 mg/kg-day (Hopkins, 1990). Both rats and dogs developed cataracts following chronic exposure to diquat. In rats, the 1-year NOEL for cataracts was 0.66 mg/kg-day (Colley *et al.*, 1985). At 2-years, the NOEL for cataracts in rats was 0.22 mg/kg-day. However, the 2-year NOEL represents effects of a lifetime of continuous exposure, and is not appropriate for use in estimating the potential likelihood of adverse effects from annual exposure of workers to diquat. The lowest 1-year NOEL for cataracts was 0.5 mg/kg-day in dogs (Hopkins, 1990). This NOEL was adjusted to 0.05 mg/kg-day, as only 10% of the oral dosage of diquat was absorbed by the rat (Daniel and Gage, 1966; Mills, 1976). The adjusted NOEL (0.05 mg/kg-day) was used for the assessment of safety margins for potential annual exposure to diquat.

The lifetime oral Reference Dose (RfD) used by the USEPA is 0.0022 mg/kg-day, based on minimal lens opacity and cataracts on the eye in the same 2-year feeding study of rats (USEPA, 1991b). The RfD was established using a NOEL of 0.22 mg/kg-day using an uncertainty factor of 100. However, the RfD does not take into account the 10% oral absorption factor. The World Health Organization RfD is 0.008 mg/kg-day (USEPA, 1991b).

Table 5 - Summary of Selected Diquat Dibromide Toxicology Studies

STUDY SPECIES	ROUTE	EFFECT	LOEL	NOEL	GENOTOXIC	REF ^a	
			(mg/kg-day)				
neurotox. (1 dose)	rat	gavage	diarrhea, nose staining	75.0	25.0		1
subchronic	mouse	diet	piloerection	5.0	2.5		1
subchronic (1 day)	rat	dermal	erythema	5.0	-		2
subchronic (3-day)	rabbit	dermal	death	12.5	6.3		3
combined	rat	diet	cataracts	0.66	0.22		4
oncogenicity	mouse	diet	nephropathy	15.0	4.5		5
oncogenicity	mouse	diet	hepatotoxicity	22.5	4.5		*6
chronic	dog	diet	cataracts	2.5	0.5		7
reproduction	rat	diet	parent nephrotox., cataracts	20	4.0		8
reproduction	rat	diet	decr. pup body wt gain	8	1.6		8
developmental	rat	gavage	maternal weight decr.	12	4		9
developmental	rat	gavage	delayed ossification	40	12		9
developmental	rabbit	gavage	maternal death	10	3		10
developmental	rabbit	gavage	delayed ossif., malform.	1	-		10
developmental	rabbit	gavage	maternal weight decr.	5	2.5		*11
developmental	mouse	gavage	clin. signs, death	2	1		*12
developmental	mouse	gavage	skeletal anomalies	2	1		*12
gene mutation	bacteria	<i>in vitro</i>				-	13
gene mutation	mammal	<i>in vitro</i>				+	14
chromosome	mammal	<i>in vivo</i>				-	15
chromosome	mammal	<i>in vitro</i>				+	16,17
chromosome	mammal	<i>in vitro</i>	sister chrom. exchange			+	18
chromosome	bacteria	<i>in vitro</i>				+	19
DNA damage	mammal	<i>in vitro</i>	unscheduled DNA synth.			+	20,21
DNA damage	mammal	<i>in vivo</i>				-	22

a/ 1. Horner, 1992a; 2. Horner, 1992b; 3. Palmer *et al.*, 1977; 4. Auletta, 1987; 5. Swan, 1963; 6. Colley *et al.*, 1985; 7. Hodge, 1991; 8. Ben-Dyke *et al.*, 1975; 9. Hopkins, 1990; 10. Hodge, 1990; 11. Wickramaratne, 1989; 12. Hodge, 1989; 13. Palmer and Pratt, 1974; 14. Palmer *et al.*, 1978; 15. Callander, 1986; 16. Cross, 1986a,b; 17. Sheldon *et al.*, 1986; 18. Wildgoose *et al.*, 1986; 19. Richardson *et al.*, 1986; 20. Tanaka, 1988; 21. Benigni *et al.*, 1979; 22. Ahmed, *et al.*, 1977; 23. Rocchi *et al.*, 1980; 24. Trueman, 1986.

* Unacceptable to DPR under FIFRA guidelines.

B. EXPOSURE ASSESSMENT

Only occupational exposures, exposures due to drift, and non-occupational exposures from swimming in treated water were considered. Based on the physical/chemical properties of diquat, residue monitoring, and environmental fate studies, residues of diquat have not been found, and are not expected to be found on food commodities (Cochran and Silva, 1992). Consequently, exposure through the dietary route is not expected.

The label on technical grade diquat requires workers handling the material to wear face shields or goggles, protective clothing, rubber gloves, rubber apron and shoe coverings impermeable to diquat. Waterproof footwear and clothing are required during spraying, except for subsurface aquatic use. The reentry interval for treated areas is 24 hours, except in aquatic areas where reentry may be effected immediately after application. Swimming is prohibited for 24 hours after application. Some product labels for home garden use warn users to keep children and pets out of the treated areas until the material has dried completely (Appendix B).

The studies and data which form the basis for estimating worker exposure are described in Appendix B. These estimates are based on both monitoring data, and calculations from monitoring data for a surrogate active ingredient (paraquat) with similar application rates and chemical properties. Dermal absorption constituted the principal route of exposure (Appendix B). Monitoring data for both aquatic use and ground spraying indicated that less than 1% of the total exposure came through the inhalation route. Exposure data from aerial application of the surrogate herbicide, paraquat, indicates that pilots and flaggers may be exposed as much through the inhalation route as through the dermal route (Table 3, Appendix B).

The exposure values used for the risk assessment are shown in Table 6. Potential short-term occupational exposures ranged from 0.2 $\mu\text{g}/\text{kg}\text{-day}$ for mixers and applicators injecting diquat into aquatic environments to 106 $\mu\text{g}/\text{kg}\text{-day}$ for ground applicators driving tractors with no cabs and a normal ground clearance. The potential short-term non-occupational exposures for drift was 0.5 $\mu\text{g}/\text{kg}\text{-day}$ at 50 meters and 0.01 $\mu\text{g}/\text{kg}\text{-day}$ at 1600 meters. Potential short term exposures (4 hours) for adult males swimming in treated water 24 hours after application ranged from 0.2 $\mu\text{g}/\text{kg}\text{-day}$ to 1.3 $\mu\text{g}/\text{kg}\text{-day}$.

Potential annual occupational exposures ranged from 0.005 $\mu\text{g}/\text{kg}\text{-day}$ for mixers and applicators injecting diquat into aquatic environments to 4.4 $\mu\text{g}/\text{kg}\text{-day}$ for ground applicators. The estimated annual drift exposures to diquat dibromide were 0.014 $\mu\text{g}/\text{kg}\text{-day}$ at 50 meters and 0.001 $\mu\text{g}/\text{kg}\text{-day}$ at 1600 meters. Repeated exposures of people swimming in water the day after treatment are unlikely to occur. In practice, a body of water is treated with diquat only once or twice a year, and the half-life of diquat is approximately 1.2 days (Appendix B). The measured half-life of diquat in reservoirs seems to be at odds with laboratory studies showing a half-life of 74 days (Tegala and Skidmore, 1987). It is most likely that diquat added to open bodies of water is bound to suspended particulate matter which then settles to the bottom. Once adhered to particulate matter, diquat is unlikely to be desorbed (Pack, 1987b). Two exposure values were derived for swimmers (Appendix B). The high value was based on the calculated theoretical concentration of diquat in a body of water treated at the label approved rate. The low value was based on actual measurements of diquat in the water column.

Table 6. Estimates of Potential Daily and Annual Absorbed Dosages of Diquat Dibromide^a

Work Task	ADD ^b (ug/kg-day)	AADD ^c (ug/kg-day)
Aquatic		
Mixer (injection)	0.2	0.005
Applicator (injection)	0.2	0.005
Applicator (handgun)	3.6	0.1
Boat Driver (handgun)	0.9	0.02
Aerial		
Mixer/Loader	7.8	0.23
Pilot	0.3	0.01
Flagger	8.1	0.22
Ground Application		
Applicator-norm. clearance, no cab	106	4.4
Applicator-norm. clearance, cab	7.4	0.30
Applicator-high clearance, no cab	5.3	0.22
Applicator- hand sprayer (Right-of-Way)	0.35	0.002
Gardener/Landscaper (Ready-to-Use formulation)	0.4	0.02
Gardener/Landscaper (Knapsack sprayer)	11.6	0.48
Non-Occupational		
Aerial Drift (50 m)	0.5	0.14
Aerial Drift (1600 m)	0.01	0.001
Adult, Swimming	0.2-1.3	n.a.

a/ From Tables 3 and 6, Appendix B.

b/ Absorbed Daily Dosage, assumes clothing provides 90% protection, and a dermal absorption rate of 1.4% and a respiratory uptake rate of 50%.

c/ Annual Average Daily Dosage- based on estimated diquat yearly exposure of 15 days for ground workers (including gardeners/landscapers), 10 days for aerial and aquatic workers, and 2.5 days for right-of-way workers (Appendix B).

na not applicable

C. RISK CHARACTERIZATION

The margins of safety (MOS) corresponding to various occupational and non-occupational exposure scenarios are presented in Table 7. A margin of safety is defined as the ratio of the absorbed dosage of diquat dibromide which produced no effect (NOEL or ENEL) in a human or laboratory animal study to the absorbed dosage of diquat dibromide to which a specific population subgroup is potentially exposed. MOSs for short-term occupational exposures, based on the adjusted ENEL of 33 $\mu\text{g}/\text{kg}$ for developmental toxicity and maternal clinical signs, ranged from less than 1 (ground applicators) to 165 (aquatic mixers and injectors). The MOSs for potential short-term non-occupational exposure by drift were 67 at 50 meters and 330 at 1600 meters. The MOSs for potential short term exposure of swimmers to diquat dibromide ranged from 26 (theoretical water concentration) to 165 (measured water concentration).

MOSs for annual occupational exposures, based on the adjusted NOEL of 50 $\mu\text{g}/\text{kg}$ for cataracts in dogs (Hopkins, 1990), ranged from 11 (ground applicators) to 25,000 (applicators with hand sprayers along right-of-ways). The MOSs for annual non-occupational exposure due to drift were 3,571 and 50,000 at 50 and 1600 meters, respectively.

Table 7 - Margins of Safety for Potential Daily and Annual Absorbed Dosages From Occupational and Non-occupational Exposures to Diquat Dibromide.

Work Task	Absorbed Daily Dosage (ug/kg-day)	Short-term Margins of Safety ^a	AADD (ug/kg-day)	Annual Margins of Safety ^b
Aquatic				
Mixer (injection)	0.2	165	0.005	10,000
Applicator (injection)	0.2	165	0.005	10,000
Applicator (handgun)	3.6	9	0.1	500
Boat Driver (handgun)	0.9	37	0.02	2,500
Aerial				
Mixer/Loader	7.8	4	0.23	217
Pilot	0.3	111	0.01	5,000
Flagger	8.1	4	0.22	227
Ground Application				
Applicator-norm. clearance, no cab	106	<1	4.4	11
Applicator-norm. clearance, cab	7.4	5	0.30	167
Applicator-high clearance, no cab	5.3	6	0.22	227
Applicator- hand sprayer (Right -of-way)	0.35	95	0.002	25,000
Gardener/Landscaper (Ready-to-Use formulation)	0.4	83	0.02	2,500
Gardener/Landscaper (Knapsack sprayer)	11.6	3	0.48	104
Non-Occupational				
Aerial Drift (50 m)	0.5	67	0.014	3,571
Aerial Drift (1600 m)	0.01	330	0.001	50,000
Swimmer	0.2-1.3	26 - 165	na	na

a/ Based on an adjusted ENEL of 33 ug/kg-day for developmental toxicity in rabbits (Hodge, 1989).

b/ Based on an adjusted NOEL of 50 ug/kg-day for cataracts in dogs (Hopkins, 1990)

na Not applicable (Appendix B)

V RISK APPRAISAL

Risk assessment is a process used to evaluate the potential for exposure and the likelihood that the toxic effects of a substance, often characterized only in laboratory animals, may occur in humans under the specific exposure conditions. Every risk assessment has inherent limitations on the application of existing data to estimate the potential risk to human health. Therefore, certain assumptions and extrapolations are incorporated into the hazard identification, dose-response assessment, and exposure assessment processes. This, in turn, results in uncertainty in the risk characterization, which integrates all the information from the previous three processes. Qualitatively, risk assessment for all chemicals has similar types of uncertainty. However, the degree or magnitude of the uncertainty varies depending on the quality and availability of the toxicological data and the data for the exposure scenarios being assessed.

A margin of safety calculated to be 100 or greater would generally be considered adequate for protection against the potential toxicity of diquat dibromide. However, the number 100 is only a benchmark. This benchmark of 100 includes an uncertainty factor of 10 for intraspecies variability in responsiveness, and assumes that humans are 10 times more sensitive to diquat dibromide than are laboratory animals (Davidson *et al.*, 1986; Dourson and Stara, 1983, 1985; USEPA, 1986b). In the absence of scientific evidence to the contrary, the effects of diquat dibromide observed in laboratory animals are expected to occur in humans at similar dosages. Specific areas of uncertainty associated with this risk assessment for diquat dibromide are delineated in the following discussion.

Short-term Toxicological Data. It is assumed that the developmental toxicity observed in pregnant rabbits can occur as the result of a single dose. Because of the study design, the LOEL for delayed ossification and fetal malformations in a developmental toxicity study on rabbits was 1 mg/kg-day (Hodge, 1989). An estimated no effect level (ENEL) of 0.33 mg/kg-day was calculated by assuming the NOEL was a factor of three less than the LOEL. The actual NOEL may be somewhat higher or lower. The ENEL was adjusted another order of magnitude lower to 0.033 mg/kg-day because of pharmacokinetic studies demonstrating that only 10% of the oral dosage of diquat was absorbed by the rat (Daniel and Gage, 1966; Mills, 1976). It was assumed that absorption of oral dosages by the rabbit and human would be limited to the same degree as in the rat. It should also be noted that the ENEL used for assessing potential short-term exposures to diquat is based on an oral study, while the principal route of exposure to humans was dermal.

If the developmental ENEL of 0.033 mg/kg-day had not been used as the basis for calculating the MOSs for acute exposure, the next best NOEL was 1 mg/kg-day for clinical signs and death in the mouse (LOEL = 2 mg/kg-day) from a developmental study (Palmer *et al.*, 1978). This NOEL would also have to be adjusted to 0.1 mg/kg-day because of the 10% oral absorption (Daniel and Gage, 1966; Mills, 1976). Using the adjusted NOEL of 0.1 mg/kg-day for clinical signs and death in the mouse, the MOSs for mean acute occupational exposures would range from less than 1 to 500, and the MOSs for mean acute non-occupational exposures would range from 77 to 1,000. Consequently, the conclusions would not change. Margins of safety for some occupational and non-occupational exposures would remain less than 100, the value conventionally recommended to protect people from the toxic effects of diquat dibromide.

Chronic Toxicological Data. The 1-year LOEL for cataracts in the rat was 3,300 $\mu\text{g}/\text{kg}\text{-day}$, with a NOEL of 660 $\mu\text{g}/\text{kg}\text{-day}$ (Colley *et al.*, 1985). The 1-year LOEL in dogs for developing cataracts was 2,500 $\mu\text{g}/\text{kg}\text{-day}$, with a NOEL of 500 $\mu\text{g}/\text{kg}\text{-day}$ (Hopkins, 1990). The actual 1-year NOEL in dogs is probably greater. Again, the NOEL used to assess potential chronic exposures to diquat dibromide was adjusted by a factor of 10 to take into consideration the reduced oral absorption observed in rats (Daniel and Gage, 1966; Mills, 1976). The oral absorption rate in dogs may be greater or less than that of rats.

Exposure Data. A number of factors contribute to the uncertainty of the exposure estimates. Although the default assumptions of 50% retention and 100% diquat absorption by the lungs were overly conservative, less than 1% of the absorbed dose was attributed to the inhalation route. Occupational exposure data associated with aerial and ground applications were derived from surrogate data. Although the application rates, and the chemical nature of the surrogate, paraquat, are similar to diquat, the exposure data carries a high degree of uncertainty because of the small number of workers sampled. The actual work hours and the number of days worked each year using diquat may be different from those in the surrogate study. Only the average of short term exposure values were available in Appendix B. Actual short term exposures may be greater for some occupational and non-occupational exposures.

Finally, the monitoring studies for both diquat and paraquat were conducted at application rates less than the maximum rate on the label. In adjusting the exposures, it was assumed that worker exposure varied directly with application rate.

VI CONCLUSIONS

Using current toxicity data, estimates from monitoring information on diquat, and surrogate exposure data, the calculated margins of safety (MOSs) for potential short-term exposure were less than the value conventionally recommended to protect people from the toxic effects of diquat for aquatic applicators and boat drivers using handguns, mixer/loaders and flaggers associated with aerial applications, ground applicators (except for hand applications on right-of-ways), and non-occupational exposures to drift at 50 meters. MOSs for potential annual occupational exposure to diquat for ground applicators using vehicles with normal clearance and no cab was also substantially less than the value conventionally recommended to protect people from the toxic effects of diquat. Mitigation measures should be considered to reduce potential exposure.

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VIII APPENDICES

APPENDIX A

Toxicology Summaries

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. (Schreider, 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'-bipyridyldiylm dibromide, diquat ion concentration = 26.7% w/v and < 10 ppmethylene 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^r 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 *hcr* 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 µg/cm³ with S9 rat liver (Aroclor-induced) activation, positive control DMN, and at 0, 6.25, 12.5, 25, 50, or 100 µg/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 µg/ml, benzo(alpha)pyrene and DMN positive control, and without activation at 0, 6.25, 12.5, 25, or 50 µg/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. Selyes 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 0.08mM but not for chromosomal aberrations at 0.08 - 0.4 μ M. 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", (Universita"t 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 (no purity stated) in water at 1000 ppm cultured 16 hours with Saccharomyces cerevisiae D4, 5×10^7 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 150mg/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.

APPENDIX B

Occupational Exposure Assessment

ESTIMATION OF EXPOSURE OF PERSONS IN CALIFORNIA
TO THE PESTICIDE PRODUCTS THAT CONTAIN

DIQUAT DIBROMIDE

BY

Tareq A. Formoli, Associate Environmental Research Scientist
HS-1662, May 14, 1993
Revised August 10, 1995

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ABSTRACT

Diquat dibromide is a non-selective, contact herbicide that is used in California for desiccation of seed crops. It is also used for rights-of-way weed control, landscape maintenance, and aquatic weed control. A total of 64 illnesses and injuries associated with the use of diquat dibromide were reported in California from 1984 through 1992. Most of these incidents occurred due to lack of required protective clothing and/or inadequate training. Approximately 60 percent of all illnesses and injuries involved applicators using hand-held equipment. Prolonged dermal exposure to diquat dibromide can cause severe skin damage. Systemically absorbed diquat dibromide does not selectively accumulate in lung tissues. Diquat dibromide is excreted rapidly from the human body, primarily in urine, following an intravenous injection. Its dermal absorption rate is estimated at 1.4 percent in 24 hours in humans. Diquat dibromide exposure monitoring studies and surrogate data were used to estimate workers' absorbed daily dosages.

This report was prepared to be included as Volume 2 in the risk characterization document for diquat dibromide. The risk assessment is being conducted because of chronic, and developmental toxicities observed in toxicity testing in laboratory rats and rabbits.

Department of Pesticide Regulation
Worker Health and Safety Branch

Human Exposure Assessment

Diquat Dibromide

May 14, 1993

Revised August 10, 1995

CHEMICAL/PHYSICAL PROPERTIES

Diquat dibromide is the common name of 6,7-dihydrodipyrido (1,2-a: 2',1'-c) pyrazinediium dibromide. Its chemical formula is $C_{12}H_{12}Br_2N_2$ with a molecular weight of 344.1 daltons. It is completely soluble in water, but insoluble in non-polar organic solvents. The solids melt at 300 °C. The technical material is available only as a liquid. Diquat dibromide is stable in neutral or acidic solutions, but unstable in alkaline solutions and is corrosive to metals. Diquat dibromide is a non-flammable, non-volatile product. Traces of ethylene dibromide are present in the technical material as manufacturing impurities. The word "diquat" used hereafter refers to diquat dibromide.

U.S. EPA STATUS

In June 1986, the United States Environmental Protection Agency (U.S. EPA) issued a reregistration standard based on its assessment of the available data on diquat. The standard identified numerous data gaps, and therefore made conclusions that were subject to change. It concluded that (a) until additional required chronic toxicity data were made available and evaluated, diquat was not considered to cause oncogenic, teratogenic or reproductive effects; (b) the presence of ethylene dibromide as an impurity would not pose a significant risk to human health with the current uses of diquat; and (c) diquat appeared to be acutely toxic through dermal exposure. Consequently the U.S. EPA identified the missing data to be developed and determined certain label restrictions and revisions including a restricted use classification, signal word revision, dermal exposure precaution, crop rotation restriction, reentry interval and protective clothing requirements. As certain data were developed and reviewed, the U.S. EPA changed its position on some label restrictions, including restricted use classification, signal word change, and protective clothing requirements.

USAGE

Diquat is a non-selective, contact herbicide that is used for desiccation of certain seed crops and potatoes. It is also used for non-crop and aquatic weed control. As of June 26, 1995, there were 38 products registered in California that contain diquat. The majority of these products are labeled for non-crop uses, mainly for industrial, home garden, rights-of-way, landscape maintenance and aquatic weed control. The agricultural products are for use as desiccants on seed crops (alfalfa, clover, grain, and soybeans) and potatoes. Based on the pesticide use reports for 1992, a total of 89,000 lb. of diquat were used in California mostly on alfalfa (30% of the total use), rights-of-way (36%) and landscape maintenance (18%) (DPR, 1994). Aquatic use accounted for less than two percent of the total use. Diquat can be used by aerial or ground equipment for agricultural uses. Diquat applications to alfalfa are mainly by air. Diquat right-of-way applications are mostly made by the California Department of Transportation, and county and city employees using vehicle-mounted application equipment. Aquatic application is done by ground equipment.

FORMULATION

All diquat products in California are liquid. The products for agricultural uses are 35.3% diquat formulations containing 2.0 lb. active ingredient (a.i.)/gal. The products for manufacturing uses are also 35.3% formulations. Other formulations are mostly 0.3% to 5.0% diquat. The rate of application is 0.25 to 0.5 lb. a.i. (cation) per acre for agricultural uses, 2.0 to 4.0 lb. a.i. (cation) per surface acre for aquatic uses and 0.5 to 1.0 lb. a.i. (cation) in 100 gal. of water for non-crop terrestrial uses.

LABEL PRECAUTIONS

Products with 35.3% diquat have the toxicity category II signal word of "warning" for their acute oral, dermal and inhalation toxicities. Formulations with lower than 35.3% diquat are either toxicity category II or III. Hazards of ingestion, inhalation and dermal contact have been indicated on these product labels. A statement of practical treatment is given for accidental exposures. The product labels require workers handling diquat to wear the following personal protective equipment (PPE):

- Coveralls over short-sleeved shirt and short pants.
- Waterproof gloves.
- Chemical resistant footwear and socks.
- Protective eyewear.
- Chemical resistant headgear for overhead exposure.
- Chemical resistant apron when cleaning equipment, mixing, or loading.

According to the federal worker protection standards (WPS) for agricultural pesticides [40 CFR 170.240(d)(4-6)], when using closed systems mixing/loading, enclosed cabs, or enclosed cockpits, the PPE requirements for mixer/loaders may be reduced to work clothing (long-sleeved shirt and long pants) plus chemical resistant apron and gloves and for applicators may be reduced to work clothing. The reentry interval to treated terrestrial areas is 24 hours. Entry into treated aquatic areas is prohibited while treatment is in progress. The reentry to treated water for swimming is 24 hours. The use of treated water for domestic purposes, animal consumption, and crop irrigation is prohibited for 14 days after application. Some product labels for home garden uses warn users to keep children and pets out of the treated area until spray mist has completely dried.

WORKER ILLNESSES

A total of 64 illnesses and injuries associated with the use of diquat alone or in combination with other pesticides have been reported in California from 1984 through 1992 (PISP, 1994). The incidents included eye injuries (24 cases), skin injuries (17 cases), eye/skin injuries (two cases), systemic illnesses (20 cases), and respiratory illness (one case). There was one suicidal death from ingestion of diquat in 1989. Illnesses and injuries due to diquat alone accounted for 40 incidents (including three non-occupational), and four required hospitalization ranging from three to 19 days. The longest disability incurred was 74 days which resulted from a prolonged and extensive skin exposure, requiring skin grafting. Most of the worker illnesses and injuries were due to lack of required protective clothing and equipment, or/and inadequate training. Symptoms such as nausea, dyspnea, and dizziness have been reported. Skin or/and eye injuries such as rashes, burns, conjunctivitis as well as loss of toe nails were observed. The majority of incidents occurred to the pesticide applicators. Applicators using hand-held equipment accounted for 60 percent of all illness and injury cases. Other incidents occurred during mixing/loading, foliar contact and incidental activities during handling.

DERMAL TOXICITY

Data collected in 1966 on the handling of diquat indicate incidents of human skin discoloration and nose bleeding (Summary Report, 1966). These data were presented without further details. Severe skin burns as a result of prolonged and extensive exposure to diquat have also been reported (Manoguerra, 1990). Systemically absorbed diquat does not selectively accumulate in lung tissue and pulmonary injury by diquat is less prominent than by paraquat. Diquat has severe toxic effects on the central nervous system (Morgan, 1989). Damage and discoloration of fingernails caused by frequent exposure to concentrated solutions of diquat were also reported. Rashes, blisters and transient skin discoloration were reported as a result of exposure to the concentrated commercial preparation. Accidental ingestion of a small amount of diquat by a person caused diarrhea and oral ulceration (FAO, 1971). Breathing spray mist can cause nasal, throat and respiratory tract irritation (MIB, 1981). Diquat did not cause skin sensitization in guinea pigs tested with formulated products (Thompson, 1985 and Robbins, 1987).

DISLODGEABLE FOLIAR RESIDUE

Most of the work activities following diquat applications to crop and non-crop terrestrial areas are mechanical. Dermal exposure to foliar residues is considered insignificant compared to dermal exposure during handling.

METABOLISM

Male albino Wistar rats were administered ^{14}C -diquat by stomach tube (1.8 uCi, 45 mg/kg) or by subcutaneous injection (5.6 uCi, 10 mg/kg) and kept in metabolism cages for four days (Mills, 1976). Urine and feces were collected daily and analyzed collectively from groups of five rats using a liquid scintillation spectrometer. Rats that were given a single oral dose excreted 6.3% and 89.3% of the administered radioactivity in the urine and feces, respectively, within four days, mainly as diquat. Urine contained 5.1% diquat, and 0.2% diquat monopyridone and 0.1% diquat dipyrindone (diquat metabolites). Feces contained 57% diquat and 4.1% monopyridone. Rats that received a subcutaneous injection excreted 87.1% and 4.8% of the administered radioactivity in urine and feces, respectively, within four days, mainly as diquat.

Tissue distribution of diquat was studied in male and female albino Wistar rats (Litchfield, 1973). Rats were fed diquat (250 ppm diquat cation) in their diet. A group of 10 rats were sacrificed at two, four, and eight weeks. The brain, lungs, liver, kidneys, hind leg muscles, stomach, small and large intestines were analyzed for diquat using colorimetric determination. Recovery of diquat injected into the tissue was 90 - 95%. Diquat presence in tissues was measurable in two weeks. No sex differences were observed. Diquat tissue concentration was generally lower than that of paraquat, particularly in lungs. No diquat was detected in tissues (MDL of 0.05 ug/g) within one week of return to a normal diet.

Male mice were subcutaneously injected with 0.2 mL ^{14}C -diquat, 50 mg cation/kg. Two mice were killed by exposure to diethyl-ether at 10 minutes, one hour, 24 hours, and 72 hours after the injection (Litchfield, 1973). Whole body autoradiography showed that radioactivity was distributed throughout most tissues at 10 minutes. Radioactivity level declined in most tissues but intestinal epithelium and urine radioactivity increased at one hour. At 24 hours, radioactivity was observed only in the small and large intestines and bladder. At 72 hours, radioactivity was observed only in stomach and intestinal contents.

A British Saana goat was administered a single oral dose of 145 mg/kg diquat ion (Griggs, 1970). Milk and excreta were collected daily. Samples were analyzed by scintillation counting using a Packard Tricarb

spectrometer. Approximately 96% of the radioactive dose was recovered in feces (94%), urine (2%), and milk (traces) within seven days, mainly as diquat.

A single oral dose (20 or 5 mg/kg) of ¹⁴C-diquat was given to a cow (Stevens, 1966). Only traces (0.001 to 0.015%) of the administered dose was found in milk and 2.6% in urine in seven days. Tissues and organs of a 120-Kg calf slaughtered 24 hours after dosing with 1.38 g of ethylene bridged-labeled ¹⁴C-diquat were analyzed. The kidney and liver contained 0.66 ppm and 0.21 ppm diquat residues, respectively. Other tissues and organs contained <0.05 ppm diquat residues.

In order to determine the extent of human elimination of diquat in urine, a dose of one uCi ¹⁴C-diquat was administered intravenously (iv) to six male human volunteers (Feldmann, 1974). Urine samples were collected for five days at four-hour intervals followed by a 12-hour interval in the first day and every 24 hours for the remaining four days. Samples were analyzed by wet ashing 5 mL of the urine and trapping all of the carbon as carbon dioxide (CO₂) in ethanolamine. The trapped CO₂ was diluted and counted with a scintillation counter. Total urinary elimination was measured at 61.2 ± 16.0% of the administered dose in five days. Approximately 90% of the excreted dose was eliminated in the first 24 hours following administration.

DERMAL ABSORPTION

In Vitro:

Dermal absorption of diquat has been studied in vitro in humans and animals, using glass diffusion cells (Corrigan, 1989(a)). Human abdominal or rat dorsal whole skin was taken post mortem and mounted in the diffusion cell between the donor and receptor chambers. Different dilutions of diquat (1 mg/mL, 5 mg/mL, and 50 mg/mL) were applied to the skin at the rate of 0.1 mL/cm². ¹⁴C-diquat was diluted in these solutions to a final activity of about 4 uCi/mL. A Betamatic II liquid scintillation spectrometer was used for analysis. A measured volume of 0.9% saline was placed into the receptor chamber. Samples of 50 uL were taken from the receptor chamber at different time intervals. A lag time of about two hours for rat skin and 15 hours for human skin was observed until initial absorption. The initial period of increasing absorption was followed by a steady state. A steady state absorption rate was calculated for each dilution as shown in Table 1.

Table 1. In Vitro Dermal Absorption of Diquat in Human and in Rat

# of reps.	Dilution mg/mL	Application Rate (mg/cm ²)	Dermal Absorption Rate		
			ug/cm ² /hr	ug/cm ² /24 hrs	%/24 hrs
<u>Human:</u>					
7	1	0.1	0.06	1.44	1.44
8	5	0.5	0.18	4.32	0.86
7	50	5.0	0.98	23.54	0.47
<u>Rat:</u>					
6	1	0.1	0.23	5.54	5.52
5	5	0.5	1.01	24.24	4.85
5	50	5.0	9.55	229.20	4.58

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Similar in vitro dermal absorption studies with human skin, using different dilutions of diquat, have resulted in dermal absorption rates that ranged from 0.18 to 1.4% (Corrigan, 1989(b), Scott, 1985).

In Vivo:

Dermal absorption of diquat was studied in rats (Brorby, 1988). ¹⁴C- labeled diquat was dissolved in water and applied (0.05 mg, 0.5 mg, and 5.0 mg) to the shaved dorsal trunk of rats. Urine, feces, and volatiles were collected following the dermal treatment. Approximately 2.5%, 3.6%, and 3.4% of the applied dose were systemically absorbed in 24 hours in rats treated with 0.05 mg, 0.5 mg, and 5.0 mg of ¹⁴C-labeled diquat, respectively.

Human dermal absorption of diquat was also studied in vivo (Feldmann, 1974). ¹⁴C-diquat was applied to the ventral forearm of six normal male human volunteers at four ug/cm². This is equivalent to a thin film of 0.25% diquat solution. The dose was dissolved in a small amount of acetone and applied to the skin. The acetone was evaporated by gentle blowing during application. The application sites remained unoccluded. The volunteers were advised not to wash their forearms for 24 hours. Urine samples were collected for five days at three four-hour intervals followed by a 12-hour interval during the first day and every 24 hours for the remaining four days. Samples were analyzed by wet ashing 5 mL of the urine and trapping all of the carbon as carbon dioxide (CO₂) in ethanolamine. The trapped CO₂ was diluted and counted with a scintillation counter. The results were corrected for incomplete urinary excretion of diquat. Only 0.3 ± 0.1% of the administered dose was recovered in five days.

In evaluating the parameters affecting dermal absorption, it was noted that occluding the application site increased diquat dermal absorption by 3.5 fold to 1.4% (Wester, 1985). Damaged skin absorbed 9.5 fold more diquat (3.8%) than non-occluded normal skin of human volunteers. A dermal absorption of 1.4% will be used in calculating diquat absorbed daily dosages for regulatory purposes.

WORKER EXPOSURE

Aquatic Use

Workers were monitored during normal applications of diquat for aquatic weed control (Wojeck, 1983). Each worker wore a long- or short-sleeved shirt, long trousers, socks and heavy shoes or boots. Two application methods were used. For control of water hyacinths and other floating vegetation, diquat was applied from an airboat by two workers, an applicator using hand-operated spray equipment and a driver. Diquat was used at a rate of 1 qt. (0.5 lb. a.i./acre) formulated product per acre (final spray mixture of 1.76% diquat). Another aquatic herbicide, Komeen[®] (2 qt./acre) was also used as a tank mix with diquat.

For control of hydrilla, diquat was injected into the water at a rate of 2 gal. (4 lb. a.i./acre) formulated product per acre (final spray mixture of 4.41% diquat), using an invert system. The crew for this method consisted of a gloved mixer who prepared the tank mix on the shore and an applicator who drove the airboat and injected the diquat into the water. The applicator also assisted in pumping the spray mixture to the tank on the boat.

Workers applied diquat two to five hours/day, four days a week. There were three handgun spray crews and one invert system crew. The workers were monitored three times over a three-month period. Potential dermal exposure of workers was monitored by placing dermal alpha-cellulose pads at 10 locations on the body outside of the clothing. Hand exposure was estimated from two consecutive hand rinses with 100 mL water or from patches that were cut from palms and backs of cotton sampling gloves worn by each worker. Anderson air samplers with polyurethane foam plugs were used to collect air samples near the breathing zone of workers. Urine samples were also collected, once prior to the monitoring and then each day during the monitoring study.

Diquat recoveries from cotton gloves and alpha-cellulose pads were 94% and 93%, respectively. The recovery from foam plugs was 80%. Samples were analyzed using a Beckman DK-2A spectrophotometer. Urine samples were analyzed separately. Diquat in urine ranged from 0.007 to 0.047 ppm. Respiratory exposure was reported <0.1% of the total body exposure. Workers' potential dermal exposure was estimated from residues found on the alpha-cellulose pads and cotton gloves or hand rinses. Potential dermal exposure in Table 2 was calculated according to the body surface areas and body weight described in the Exposure Assessment

Guidance (Thongsinthusak et al., 1993). Gloves as a medium to assess hand exposure typically overestimate that exposure by up to nine fold when compared with hand washes (Smith, 1991).

Table 2. Estimate of Mixer/Loaders and Applicators' Exposure to Diquat During Application of Aquatic Weeds

	Handgun Application (0.5 lb. a.i./acre)		Invert Application (4.0 lb. a.i./acre)	
	Applicator	Boat Driver	Applicator	Mixer
n	9	9	3	3
	ug/8-hour day	ug/8-hour day	ug/8-hour day	ug/8-hour day
Head	235.20	50.40	184.80	50.40
B. Neck	17.12	4.28	4.28	4.28
F. Neck	46.72	5.84	35.04	5.84
Back	552.64	138.16	138.16	138.16
Chest	1105.28	138.16	828.96	138.16
Upper arms	118.32	59.16	236.64	59.16
Forearm	387.52	96.88	290.64	290.64
Thigh	7032.96	146.52	146.52	586.08
lower leg	1178.40	196.40	98.20	1178.40
Feet	602.88	100.48	50.24	602.88
Hand	3513.60	1098.00	658.80	805.20
Potential Dermal	14790.64	2034.28	2672.28	3859.20
Daily Dermal ^a	6862.36	1600.48	1457.03	3029.80
Daily Dermal ^b	686.24	160.05	145.70	302.98
	(ug/kg/day)	(ug/kg/day)	(ug/kg/day)	(ug/kg/day)
ADD ^c	0.13	0.03	0.03	0.06
ADD ^d	0.51	0.12	0.03	0.06

a - Short-sleeved shirt and short pants, providing 90% protection to covered areas.

b - Coveralls (over short-sleeved shirt and short pants), waterproof gloves, chemical resistant footwear and socks, protective eyewear, and chemical resistant headgear, providing 90% protection.

c - Based on an 8-hour workday, male body weight of 75.9 kg (Thongsinthusak, et al., 1993), and dermal absorption of 1.4% (see dermal absorption section). Samples with non-detected levels were assumed to contain residues at half of MDL, MDL = 0.01 ug/cm².

d - Corrected for the highest label rate (2 lb. a.i./acre) for floating weeds.

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Terrestrial Crop and Non-Crop Uses

Diquat worker exposure data are very limited. In the absence of worker exposure data for diquat, paraquat worker exposure data would be a suitable surrogate. Paraquat is also a bipyridylum herbicide that has chemical and physical properties and use patterns similar to that of diquat. The application equipment is also similar for these two products, except a closed mixing and loading system is required when handling paraquat. A closed mixing and loading system is not a requirement for handling diquat.

Only one worker exposure study of terrestrial use of diquat was available. This study was published in the open literature in German (Sawinsky, 1977). A summary is available in English. The study monitored workers' exposure during aerial application of diquat. It did not describe the rate of application or the type of protective clothing and equipment worn by the workers. The duration of application was approximately four hours. Air samples taken from the cockpit contained a mean value of 4.5 ug/m² diquat and residue samples taken from

pilots' clothing contained a mean value of 61.5 ug/100 cm² diquat during the four hours of monitoring. No residues were detected in the cockpit air when the air filter and ventilation were in operation. Clothing samples of the mixer and the loader contained 3500 ug/100 cm² and 8700 ug/100 cm² diquat, respectively, during the monitoring period. Urine samples were taken only from the ground crew. Urinary diquat residues ranged from non-detectable levels to 30 ug/100 mL sample. The mixer's and the loader's mean urinary diquat were 6.3 ug/100 mL and 19.6 ug/100 mL, respectively. Workers' dermal exposure can not be estimated from residues on clothing samples since it was not clear whether sufficient number of samples were taken from various parts of the clothing. The mixer and the loader exposures were estimated from residues found in their urine corrected for incomplete diquat urinary excretion (Feldmann, 1974), 1400 mL daily urinary output (Guyton, 1969), and 75.9 kg body weight. The mixer and the loader Absorbed Daily Dosages (ADD) were 3.8 ug/kg/day and 11.8 ug/kg/day, respectively. Mixing and loading is normally performed by one worker. The estimate of ADD for diquat aerial mixer/loader spending as much time mixing as loading is 7.8 ug/kg/day. The exposure to a mixer/loader during ground application can be conservatively estimated from the exposure data for mixer/loaders of diquat during aerial application.

Table 3. Diquat Aerial Application Flagger's Estimated Exposure Using Paraquat Data as Surrogate

	Flagger 1 Trial I	Flagger 2 Trial I	Flagger 1 Trial II	Flagger 2 Trial II
	ug/per/day	ug/per/day	ug/per/day	ug/per/day
Head	6384.72	341.64	8074.57	286.54
B. Neck	207.36	29.38	67.39	114.05
F. Neck	732.10	7.08	2432.45	113.36
Back	2994.28	1441.69	1996.19	3659.67
Chest	17189.38	166.35	57113.09	2661.58
Upper arm	3239.04	323.90	5552.64	2059.10
Forearm	2647.68	264.77	4538.88	1683.17
thigh	9696.96	581.82	69818.11	2973.73
lower leg	4235.48	129.66	25499.33	2074.52
feet	2214.64	67.80	13333.06	1084.72
Hand	2064.00	163.20	2544.00	93.60
Potential Dermal	51605.63	3517.29	190970.00	16804.05
Daily Dermal ^a	21797.94	1254.90	69937.68	6585.37
Daily Dermal ^b	2179.79	125.49	6993.77	658.54
Daily Resp.	101.12	0.19	0.19	9.47
ADD ^c	2.14	0.03	1.60	0.30
ADD ^d	5.95	0.09	4.43	0.84

a - Short-sleeved shirt and short pants, providing 90% protection to covered areas.

b - Coveralls (over short-sleeved shirt and short pants), waterproof gloves, chemical resistant footwear and socks, protective eyewear, and chemical resistant headgear, providing 90% protection.

c - Based on an 8-hour workday, female body weight of 61.5 kg (Thongsinthusak, et al., 1993), dermal absorption of 1.4% (see dermal absorption section), and respiratory uptake and absorption of 100%. Samples with non-detected levels were assumed to contain residues at half of MDL, MDL = 0.01 ug/cm².

d - Corrected for the highest label rate (0.5 lb. a.i./acre).

Formoli, WH&S, 1995

A worker exposure study of paraquat during an aerial application to cotton was conducted in the San Joaquin Valley in 1979 to estimate the exposure of pilots, mixer/loaders, and flaggers (Chester and Ward, 1981). This study was used as a surrogate to estimate the exposure of pilots and flaggers to diquat during aerial application. Mixer/loaders' exposure can not be estimated from this study since a closed mixing/loading system was used.

Table 4. Diquat Aerial Application Pilots' Estimated Exposure Using Paraquat Data as Surrogate

	Pilot 1 Trial I	Pilot 2 Trial I	Pilot 1 Trial II	Pilot 2 Trial II
	ug/per/day	ug/per/day	ug/per/day	ug/per/day
Head	12.10	12.10	12.10	11.02
B. Neck	1.03	1.03	1.03	0.86
F. Neck	1.40	1.40	1.40	1.18
Back	33.16	33.16	33.16	27.72
Chest	33.16	33.16	33.16	27.72
Upper arm	14.20	14.20	14.20	11.57
Forearm	11.63	11.63	11.63	9.46
thigh	35.16	35.16	35.16	32.32
lower leg	23.57	381.80	23.57	21.61
feet	12.06	195.33	12.06	11.30
Hand	460.80	249.60	1008.00	139.20
Potential Dermal	638.26	968.57	1185.46	293.97
Daily Dermal^a	491.62	334.57	1038.82	166.44
Daily Resp.	0.34	0.34	0.34	2.02
ADD ^b	0.09	0.06	0.19	0.04
ADD ^c	0.26	0.18	0.54	0.12

a - Long-sleeved shirt, long pants, and footwear providing 90% protection to covered areas and using closed cockpit plane.

b - Based on an 8-hour workday, male body weight of 75.9 kg (Thongsinthusak, et al., 1993), dermal absorption of 1.4% (see dermal absorption section), and respiratory uptake and absorption of 100%. Samples with non-detected levels were assumed to contain residues at half of MDL, MDL = 0.01 ug/cm².

c - Corrected for the highest label rate (0.5 lb. a.i./acre).

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One pint of Ortho Paraquat CL containing 1.4 lb. a.i./gallon was used in 10 gallons of water with one pint of X-77 (as an adjuvant) and three to four lb. of Tumble Leaf[®] (sodium chlorate) as a defoliant per acre on the first trial. Only Ortho Paraquat CL and X-77 were used in the second trial. A total of 1200 acres of cotton were sprayed using a Thrush Commander aircraft. Mixing and loading were done using a closed system. The pilots' clothing consisted of open necked short-sleeved shirt, T-shirt, long trousers, boots and hat. Flaggers wore protective cotton coveralls over normal clothing. Dermal exposure pads consisting of polythene-backed 100 cm² Whatman 542 filter papers were attached to the skin or clothing with adhesive tape at various locations. Hand exposure of the pilots and the flaggers was evaluated using bleached cotton gloves. Penetration through normal work clothing and protective clothing was evaluated by using white cotton T-shirts and by placing cotton Tubigrips (elasticized tubular support bandages) on the left leg (ankle to top of thigh) of each individual. Penetration through normal clothing was 5 percent based on chest and back pad ratio to the T-shirt for flaggers and the mixer/loader. Air samples from the breathing zones of workers were collected using personal air samplers to determine respiratory exposure. Body parts surface area and total dermal exposure were calculated according to methods described in the Exposure Assessment Guidance (Thongsinthusak, et al., 1993). Respiratory exposure was calculated using the instrument sampling rates and duration of exposure. The

instrument sampling rate (three liters/minute) was corrected for human breathing rate during light work of 8 L/minute for females and 14 L/minute for males (Thongsinthusak, et al., 1993). Dermal and inhalation exposure of these workers to paraquat was used in Tables 3, and 4 to estimate dermal and inhalation exposure of flaggers and pilots to diquat.

Ground Applicator Exposure

In the initial exposure assessment document for diquat, the exposure to ground applicators was estimated using a study conducted in Florida where applicators were monitored for dermal and respiratory exposure to paraquat during post-harvest treatment of tomatoes (Wojeck, 1983). Dermal exposure was monitored by attaching alpha-cellulose pads to various parts of the body outside of workers' clothing. Hand exposure was estimated from two consecutive hand washes in water, or from areas cut from the palm and back of a pair of cotton sampling gloves. Respiratory exposure was measured by using Willson "Dustite" respirators fitted with 16-ply gauze backed with filter pads. The estimate of exposure, based on this study, ranged from 5.3 to 106 ug/kg/day for an applicator wearing long sleeved shirt, long pants, and shoes.

In a recent study, a more refined estimate of exposure to handlers of paraquat was made, using the biological monitoring technique (Meier, 1995). The study was conducted in Georgia and Alabama to investigate absorption of paraquat by 17 workers mixing/loading and applying Gramoxone Extra. This study meets the requirements of the EPA's Good Laboratory Practices with a few exceptions, such as using commercially available product and not retaining the test substance containers, that would have no effects on study integrity. A single application of Gramoxone Extra (2.5 lb a.i./gal.) was made to pecan orchards at 0.94 lb a.i./acre. The mixing/loading operation was performed using open pour methods. The application was performed using ground boom spray equipment that was mounted on an open cab tractor. The spray booms of the tractors for two workers were in front of the driver's seat. The amount of product handled during mixing/loading ranged from approximately 7 to 70 lb a.i., averaging 27 lb a.i. per day. All but one worker used a non-ionic surfactant. The duration of exposure ranged from 4 to 11 hours. Mixing/loading lasted 14 to 104 minutes. Wind was generally calm during handling, mostly below 7 MPH.

A complete 24-hour urine sample was collect from each worker one day prior, on the application (exposure) day, and for five days following the exposure day. Collected samples were kept frozen in the field laboratory and during delivery to the analytical laboratory. Field recovery samples were collected each day of monitoring by fortifying the urine samples from non-exposed individuals. Urine samples were analyzed for paraquat and creatinine. The detection limit was 5 ng/mL and the limit of quantitation was 10 ng/mL. The mean recovery for fortifications at 10, 20, and 50.1 ng/mL ranged from 100 to 108 percent.

Paraquat was detected in the urine of only six workers for the exposure day. No paraquat was detected in any samples collected during the two days following the exposure day, therefore, the samples collected on days 3, 4, and 5 after the exposure day were not analyzed. Results were corrected for 59 percent dose recovery in urine (Maibach, 1982). Based on the urine samples with detectable concentrations of paraquat for six workers, the ADD ranged from 0.07 to 0.44 ug/kg/day with an average of 0.21 ± 0.15 ug/kg/day. There is no clear correlation between paraquat absorption and the amount of product handled or the duration of exposure. These six workers handled an average of 34 lb. a.i. during an average 6.4 hours. They wore long- or short-sleeved shirts, long pants, hats, and boots. Only two of them wore eye glasses. No additional PPE was worn during mixing/loading by these six workers. Most (8 out of 11) of the workers with no detectable paraquat in their urine wore additional PPE such as gloves and apron during mixing/loading. The workers with no detectable paraquat in their urine samples were not included in the estimate of ADD so that dose was calculated based on actual measurements rather than lack of detection.

The diquat maximum application rate (0.5 lb. a.i./acre for agricultural uses) is approximately one-half the rate used in this study and diquat dermal absorption rate is approximately three fold greater than that of paraquat. To estimate the ADD for a mixer/loader/applicator of diquat, the average ADD of 0.21ug/kg/day calculated for workers in this study was adjusted for the differences in the application and dermal absorption rates. Using the above study as surrogate, the estimate of ADD for a diquat mixer/loader/applicator wearing a long- sleeved

shirt, long pants, a hat, and boots and using a tractor equipped with a ground boom is 0.32 ug/kg/workday. Additional PPE, as required by the current product label, would further reduce the estimated ADD as evidenced by the non-detects routinely found with those workers using more PPE.

Home Garden and Landscape Use

No human exposure data are available for home garden or landscape uses of diquat. A human exposure study of paraquat during garden and yard application was used as surrogate to estimate the exposure of garden and landscape workers applying diquat (Staiff, 1975). A 0.44% paraquat pressurized product was applied by volunteers as spot treatment to control weeds in gardens and yards. Applicators' potential dermal exposure was monitored by attaching alpha-cellulose pads to various parts of the body or clothing. Hand exposure was measured by rinsing their hands in water. Respiratory exposure was measured by using filter pads (not specified) in the respirators worn by workers. A total of 15 exposure situations were studied. The volunteers wore no gloves or hats. Almost all exposure was on the hands. Only traces ($<1.0 \text{ ug/cm}^2$) of paraquat were found on the lower legs. Respiratory exposure values were below the detection limit except for one sample containing 0.8 ug paraquat. Dermal exposure was calculated based on residues on the hands. Dermal exposure ranged from 0.01 mg/hr to 0.57 mg/hr with a mean of 0.29 mg/hr. Diquat garden and landscape workers' absorbed daily dosage (ADD) was estimated to range from 0.01 to 0.7 ug/kg/7-hour workday, with a mean of 0.4 ug/kg/7-hour workday, assuming 1.4% dermal absorption, body weight of 75.9 kg, and negligible inhalation exposure.

The exposure of applicators using hand-held equipment such as knapsack sprayers was estimated based on the exposure values of workers who applied 2, 4-D (Abbott, 1987). Workers (n=2) loaded premixed 2, 4-D into knapsack tanks and then applied the herbicide using the knapsack sprayers. Dermal exposure was measured by obtaining clothing samples from representative body parts. Gloves were used to measure hand exposure. The mean dermal exposure during loading from a total of eight replicates was 3.6 mg/lb. a.i., assuming the workers wore short-sleeved shirts, short pants, coveralls, waterproof gloves, chemical resistant footwear and socks, protective eyewear, and chemical resistant headgear. The mean dermal exposure during application from a total of 12 replicates was 9.6 mg/lb. a.i., assuming the workers wore short-sleeved shirts, short pants, coveralls, waterproof gloves, chemical resistant footwear and socks, protective eyewear, and chemical resistant headgear. The absorbed daily dosage for a worker loading and applying 1 lb. a.i. diquat during a day was calculated to be 2.4 ug/kg/workday (1.4% dermal absorption, body weight of 75.9 kg, and negligible inhalation exposure).

Chester, *et al.* (1989) monitored the exposure of Sri Lankan tea plantation workers using hand-operated or pressure retaining knapsack sprayers (4 gal. capacity). Paraquat was used at 0.26 lb. a.i./100 gal. at a rate 48 gal./acre. This is equivalent to an application rate of 0.12 lb/acre. Two workers mixed the concentrate formulation with water in large drums and loaded the solution into the knapsack tanks (equipped with double conejet nozzles) with buckets. The applicators (n=10) used the loaded knapsack sprayers to apply paraquat for spot treatment. All workers wore short-sleeved shirts and shorts. No gloves or footwear were worn, but workers exercised high standards of personal hygiene by washing hands, legs, feet, and contaminated skin frequently. Each applicator sprayed 7 to 8 knapsack tanks a day as a spot treatment in hilly and muddy conditions. The average amount of paraquat handled by an applicator was 33 g/day and by a mixer/loader was 164 g/day. The workers were all male with an average body weight of 49.3 kg.

Daily (24-hour) urine samples were collected the day before spraying started, during five days of spraying, and continued for 8 days after the last day of spraying. The workers did not have paraquat exposure at least two weeks prior to the start of this study. Blood samples were taken from workers to monitor serum concentration of paraquat. Blood samples were taken at the end of a workday on day 1, 3, and 5 of spraying days and the day after the last day of spraying. Urine and blood samples were stored frozen until shipment to the central laboratory for analysis. Blood and urine samples were analyzed for paraquat by ICI's radioimmunoassay procedure CT05-085. The limit of detection for serum and urine were 0.006 and 0.03 ug paraquat ion/mL, respectively. Urine samples were also analyzed for creatinine to demonstrate completeness of 24-hour urine collection. The average daily urine volume was 1.94 liters.

No paraquat was detected in any of the serum or urine samples. Assuming that the urine samples contained paraquat at one-half the detection limit and based on the average daily urine volume of 1.94, average body weight of 49.3 kg, and 59 percent paraquat recovery in urine (Maibach, 1982), the ADD is calculated to be 1.0 ug/kg/day, as shown below:

$$[(0.015 \text{ ug/mL} \times 1940 \text{ mL}) (100/59)]/49.3 \text{ kg} = 1.0 \text{ ug/kg/day}$$

In order to use the ADD for Sri Lankan tea plantation workers handling paraquat as surrogate to estimate an ADD for California workers handling diquat, the calculated ADD must be adjusted for the differences in dermal absorption rate, application rate (or dilution rate), number of tanks handled during a workday, and PPE worn during handling. The dermal absorption of diquat is estimated approximately three fold higher than that of paraquat. Diquat application rates of 0.5 lb/acre for agricultural uses and 0.5 to 1.0 lb/100 gal. for non-crop terrestrial uses are at least 4 fold higher than that in this study. Adjusting paraquat estimated ADD for diquat dermal absorption and application rates results in an ADD of 12 ug/kg/day. In addition, this study indicated that the number of tanks sprayed in a workday (7-8) in this hilly condition is two fold lower compared to 13 to 15 tanks handled in a workday in strip spraying in Malaysian plantations. Assuming that in California the number of tanks handled in a workday by a worker is the same as those of Malaysian plantations, the ADD was adjusted to 24 ug/kg/day for a worker wearing only a short-sleeved shirt and short pants.

It is apparent that the work practices conducted and the PPE worn by workers handling diquat in California will provide greater exposure protection compared to those used by the workers in this study. The level of protection provided by work practices can not be quantified. However, based on dermal exposure monitoring using dosimetry (conducted during this study), most of the exposure (99%) occurred to hands, legs, and trunk; therefore, we assume coveralls, gloves, socks, and shoes will provide 90 percent protection and reduce the estimated ADD to 2.4 ug/kg/day. Because of several assumptions and the multiplicative effects of each assumption on the estimate of ADD the inherent uncertainty with each assumption would also be multiplied. However, it is remarkable to note that the estimates of ADD based on these two studies (Chester *et al.*, 1989 and Abbott, 1987) are identical.

Potential dermal exposure was also monitored during this study, but only during two replicates of spraying that took place on the day after the last day of urine monitoring. Each replicate consisted of the application of 4 tanks by each worker which lasted for approximately one hour. Mixer/loaders handled 113 g and applicators sprayed 22.7 g paraquat in a day. All workers wore Tyvek coveralls with hood, cotton gloves, and socks to monitor potential dermal exposure. The coveralls were cut in sections. These samples were stored and shipped at ambient temperature. Spike samples were taken to determine field recoveries of dosimeter samples.

Field recoveries at concentrations ranging between 0.01 and 0.03 mg/sample were 60, 67, and 95% for socks, gloves, and Tyvek, respectively. Field recoveries at concentrations ranging between 1.8 and 3.1 mg/sample were 119 to 122% for the same matrices. No corrections were made for field recoveries since the recoveries of samples spiked at concentrations close to the actual exposure were above 100%. Potential dermal exposure after handling 8 tanks (two replicates of 4 tanks each) was 66.1 mg/person for mixer/loaders and 73.7 ± 22.9 mg/person for applicators. Table 5 shows the estimate of exposure for workers applying diquat based on dermal monitoring of Sri Lankan tea plantation workers handling paraquat. The ADD based on dermal dosimetry exposure monitoring portion of this study overestimates exposure by three to four fold when compared to the estimates derived from the biomonitoring section of this study.

Table 5. Estimate of ADD for Workers Handling Diquat Using Knapsack Sprayers Based on the Exposure of Sri Lankan Tea Plantation Workers to Paraquat

	Mixer/loader exposure (n=2)		Applicator exposure (n=10)	
	mg/person	%	mg/person	%
head	0.1	0.2	0.9	1.2
trunk	2.8	4.2	3.5	4.7
arms	0.6	0.9	1.1	1.5
legs	1.7	2.5	22.3	30.3
feet	4.4	6.7	17.9	24.3
hands	56.5	85.5	27.9	37.9
Total potential	66.1	100	73.7	100
Total dermal ^a	61.5		49.4	
Total dermal ^b	6.2		4.9	
	ug/kg/day		ug/kg/day	
ADD ^c	1.1		0.9	
ADD ^d	9.1		7.2	

a - Short-sleeved shirt and sort pants, providing 90% protection to covered areas.

b - Coveralls (over short-sleeved shirt and short pants), waterproof gloves, chemical resistant footwear and socks, protective eyewear, and chemical resistant headgear, providing 90% protection.

c - Based on dermal absorption of 1.4% (see dermal absorption section), and body weight of 75.9 kg.

d - Adjusted for 4 fold difference in dilution rate and 2 fold difference in the number of tanks handled in a workday.

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Right-of-Way Use

Diquat rights-of-way applications are primarily made by California Department of Transportation (Caltrans), county, and city employees using truck-mounted boom or hand-held sprayers. Worker Health and Safety Branch conducted a study to monitor the exposure to Caltrans employees applying herbicides to rights-of-way (Edmiston *et al.*, 1994). The exposure data from this study for workers handling glyphosate was used as surrogate to estimate the exposure of workers handling diquat. Workers wore Tyvek coveralls and gloves over their normal clothing during mixing/loading and application of glyphosate. Dermal dosimeters (T-shirt and long underwear) were worn under normal clothing to estimate body dermal exposure. Face, neck, and hand wipes were used to estimate face, neck, and hand exposures. Applications were made from a truck using a hand sprayer connected to the spray truck. Table 6 shows glyphosate (anion) dermal exposure to workers performing various work activities. Samples with non-detectable levels were assumed to contain residues at one-half of the detection limit. Glyphosate was at the detectable levels only in two air samples taken from the breathing zone of workers. The method of application for diquat is similar to that of glyphosate. Using glyphosate data as surrogate, the estimates of ADD for workers applying diquat to rights-of-way range between 0.1 to 0.4 ug/kg/day, as shown in Table 6.

Table 6. Estimates of ADD for Workers handling Diquat Based on Dermal Exposure of Rights-of-Way Workers to Glyphosate

Work Task (n)	Head (ug/person)	Hand (ug/person)	body (ug/person)	Total ^a (ug/person)	ADD ^b (ug/kg/day)
Boom application (3)	350	744	134	1229 + 876	0.23
Mix/load/hand wand application (12)	386	1015	846	2247 + 1062	0.41
Driver/handling hose (3)	43	260	414	717 + 310	0.13

n - Number of replicates.

a - Mean (arithmetic) ± standard deviation.

b - Assuming 1.4% dermal absorption, body weight of 75.9 kg, and negligible inhalation exposure.

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Drift

A diquat drift monitoring study, performed in Davis, California, showed residues on the fallout sheet and the air samples at downwind collection stations as far as 1,600 meters from the application site (Akesson, 1986). Diquat was sprayed from a height of five feet using a Weatherly 210 type aircraft at a speed of 100 to 110 miles/hour. The air sampling data were not defined adequately to estimate bystander's inhalation exposure to diquat, but the similarity of drift pattern between diquat and paraquat was evident. Paraquat drift data indicated air residues of 16.7 ug/m³ and 0.5 ug/m³ at 50 meters and 1600 meters respectively downwind of an aerial application at a rate of 0.18 lb. a.i./acre (Chester et al. 1981). Aerial applications move quickly across the fields, thus changing the distance of drift. In addition, a bystander is not expected to remain in a same area for more than two hours. Using paraquat drift data, bystanders' ADDs at various distances downwind from an aerial application of diquat were estimated as shown in Table 7.

Table 7. Estimates of Diquat Exposure to Bystanders Based on Paraquat Downwind Drift Study

Distance meter	Observe d ug/m ³	Observe d ug/m ³	Averag e ug/ m ³	Calculated ^a ug/m ³	Corrected ^b ug/m ³	ADD ^c ug/kg/day
	trial I	trial II				
50	6.40	16.66	11.53	8.60	21.50	0.48
100	2.68	12.91	7.80	8.00	20.00	0.44
200	2.10			7.00	17.50	0.39
400	0.81	5.85	3.33	5.35	13.37	0.30
800	3.44	4.03	3.74	3.13	7.83	0.17
1600	1.70	0.47	1.09	1.07	2.68	0.06

a - from log linear correlation [$\ln y = 2.214 - 0.0013x$] where x is distance and y is the calculated residues in the air, $r^2 = 0.895$

b - corrected for 0.5 lb a.i. (from 0.2 lb/acre) and based on the previous column of calculated values.

c - inhalation rate of 0.84 m³/hour (14 liters/minute) for light activity, body weight of 75.9 kg (Thongsinthusak, et al., 1993), and daily two hours of exposure.

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Aquatic Dissipation

Aquatic dissipation of diquat was studied at two locations in Florida. Diquat was used at a rate of 4 lb. a.i./acre as surface treatment (Fujie, 1988). Four applications were made at each location at 30-day intervals. The ponds had no or very little outflow. Water samples were taken from top, middle and bottom of the pond. Water and sediment samples were taken prior to the first application and continued for about 30 days after each application. After the fourth application, sediment sampling continued for 180 days. Sample analysis indicated that diquat was distributed in all depths one day after application. Diquat dissipated rapidly from the water at both locations. Diquat concentration in water eight hours after the application ranged from 0.06 to 0.12 ug/mL. The concentration dropped to a range of 0.02 to 0.09 ug/mL 24 hours after the application and to a range of <0.004 to 0.02 ug/mL seven days after the application. The half-life at both sites ranged from 0.7 to 2.3 days ($r = 0.96-0.99$) with an arithmetic mean of 1.2 days. Sediment data reflected high variability. Samples taken 180 days after the last application showed little or no degradation from the levels found at seven days after the last application, indicating strong binding of diquat to the clay.

Diquat exposure to a swimmer from treated water is estimated based on a maximum application rate of 4 lb. a.i./acre foot (1.5 ppm). Dermal and ingestion are the primary routes of exposure. The reentry interval to treated water for swimming is 24 hours. In most dermal absorption studies (both *in vivo* and *in vitro*) a volume of 0.1 mL is applied to a skin area of one cm² as an ideal dermal exposure for a period of 24 hours (Corrigan, 1989a, b; Feldmann, 1974). This rate is equivalent to a thin film of the solution covering the skin area. Dermal exposure to diquat during swimming in treated water is comparable to the ideal 0.1 mL/cm² dermal exposure accommodated for dermal absorption studies. At this rate, the dermal exposure to a 75.9-kg male human with a skin surface area of 19,400 cm² (Thongsinthusak et al., 1993) is 1,940 mL of treated water.

Two scenarios were used to estimate diquat exposure to a swimmer spending four hours a day in the treated water. The first scenario assumes the theoretical maximum concentration of 1.5 ppm reached immediately following diquat application and this initial concentration drops to 0.75 ppm at its half-life of 1.2 days after the application. The second scenario is based on the actual measured concentration of 0.09 ppm in the water that was observed in the above dissipation study 24 hours following the application. Absorbed daily dosages (ADD) from dermal and oral routes for the above two scenarios are shown in Table 8.

Table 8. Estimate of Diquat Exposure to Swimmers from Treated Water

Scenario #	Concentration at reentry ug/mL	Water volume available for dermal exposure mL/person	Dermal exposure ug/person	Oral exposure ug/person	ADD ug/kg/day
1	0.75	1940	1455	75	1.26
2	0.09	1940	175	9	0.15

Based on: Dermal absorption rate of 1.4%, ingestion of 100 mL of treated water, body weight of 75.9 kg and four hours of exposure/day.

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Table 9. Diquat Workers Estimated Annual and Lifetime Average Daily Dosage

<u>Work Task</u>	<u>Use</u>	<u>ADD</u> (ug/kg/day)	<u>AADD^a</u> (ug/kg/day)	<u>LADD^b</u> (ug/kg/day)
Mixer/loader/applicator ^c	Ground	0.3	0.01	0.007
Flagger	Aerial	2.8	0.08	0.044
Pilot ^d	Aerial	0.3	0.01	0.005
Mixer/loader ^e	Aerial/Ground	7.8	0.21	0.122
Applicator (Ready-to-use) ^d	Garden/Landscape	0.4	0.02	0.009
Applicator (Knapsack)	Garden/Landscape	2.4	0.10	0.056
Applicator (hand sprayer) ^f	Right-of-way	0.1 - 0.4	0.007	0.004
Applicator (handgun)	Aquatic	0.5	0.01	0.008
Boat Driver (handgun)	Aquatic	0.1	0.003	0.002
Applicator (injection)	Aquatic	0.03	0.001	0.001
Mixer (injection)	Aquatic	0.06	0.002	0.001
Swimmer (theoretical) ^g	Aquatic	1.3	0.007	0.004
Swimmer (actual) ^g	Aquatic	0.2	0.001	0.001
Bystander(50 meters)	Aerial	0.5	0.014	0.008

Except as noted, the ADD values are estimated based on the product label highest rate of application and clothing consisting of short-sleeved shirt and short pants, coveralls, waterproof gloves, chemical resistant footwear and socks, protective eyewear, and chemical resistant headgear. The ADD for the bystanders is from the inhalation route for two hours of exposure/day.

- a) Based on estimated diquat yearly exposure of 15 days for ground workers including garden/landscape, 10 days for aerial and aquatic workers (Ibarra, 1992; Mukai, 1992), 6 days for right-of-way workers (Haskell, 1994), and 2 days for swimmers.
- b) A 40-year work period in a lifetime.
- c) Long-sleeved shirt, long pants, headgear, footwear, and eyewear.
- d) Long-sleeved shirt, long pants, and footwear.
- e) The application rate and clothing protection were not provided.
- f) Normal clothing, Tyvek coveralls, gloves, and footwear were worn during mixing/loading/application.
- g) No clothing.

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ADDENDUM¹

Summary

TOXICOLOGY

Based on a recently submitted, new [December 18, 1996], single-dose dermal toxicity study in rabbits, DPR has re-evaluated the risks associated with the use of diquat dibromide. As most occupational and non-occupational exposures to diquat dibromide involve dermal absorption, the dermal NOEL (100 mg/kg for reduced food consumption and death) was more applicable for assessing the risks of acute human exposure to diquat. Currently available toxicity information indicates that diquat dibromide causes cataracts in dogs and rats, and developmental effects in rats and rabbits. DPR has further concluded that, in the absence of additional data to the contrary, diquat dibromide has the potential to cause similar effects in humans.

EXPOSURE ANALYSIS

Diquat monitoring data and surrogate data were used to estimate potential exposure via dermal contact, and inhalation of mixer/loader/applicators utilizing diquat dibromide in aquatic, aerial, and ground application situations. Exposure through the inhalation route was insignificant compared to potential dermal exposure in all but aerial applications. Swimmers had potential short term exposures to diquat through the dermal and oral routes. Based on current use patterns, the potential for extensive repetitive exposure of swimmers to diquat does not exist. Additionally, new exposure data have been provided to DPR since the completion of the 1994 Risk Characterization Document (RCD), and some of these data have been incorporated into the revised risk assessment.

CONCLUSIONS

Using current toxicity data, estimates from current monitoring information on diquat, and surrogate exposure data, the calculated margins of exposure (MOEs) for potential short-term exposure were all greater than 100, the value conventionally considered to be protective of human health. MOEs for potential annual occupational exposure to diquat were also greater than 100.

¹ Addendum to Risk Characterization Document for diquat dibromide (R.C. Cochran, M.H. Silva, G. Chernoff, E.F. Meierhenry, T.A. Formoli, and K.F. Pfeifer, August 17, 1994.)

Introduction

A 1994 RCD, which detailed the risks associated with various uses of diquat dibromide in California, was completed on August 17, 1994 (Cochran *et al.*, 1994). Several of the occupational and non-occupational scenarios detailed in the RCD resulted in margins of exposure (MOEs) which were less than 100, the value conventionally considered to be protective of human health. Consequently, a mitigation process was initiated in the Department of Pesticide Regulation (DPR) to ascertain whether changing the manner in which diquat was applied would reduce the exposures. Additional exposure studies (Sawinsky and Pasztor, 1977; Meier, 1995) were submitted by the registrant during the mitigation process which resulted in a mitigation document (Formoli, 1995). In January, 1997, a new, acute dermal toxicity study was submitted to DPR. This addendum details how the new toxicological and exposure data were used to re-evaluate the risks associated with the use of diquat dibromide.

Toxicology

New Zealand white rabbits (5/sex/dose) were given a single, 6 hour dermal dose of diquat dibromide (20.5% diquat ion) at 0, 50, 100, or 200 mg/kg, and then were assessed for 14 days (Lees, 1996). Blood levels of diquat were determined 6 hours after dosing, and indicated that diquat was passing through the skin in a generally dose-dependent manner (Table 1). No clinical signs were noted in any of the animals, though one female at the high dose (200 mg/kg) was terminated *in extremis* on day 11. No treatment related lesions were noted in the terminated animal, and no signs of systemic toxicity were noted in the survivors. There was significant ($P < 0.01$) reduction in food consumption (33%-71%) at the high dose for the first 5 days after treatment. Slight to moderate skin irritation was noted with all doses of diquat. The single dose NOEL for death (1/10) and reduced food consumption in the rabbit was 100 mg/kg.

Table 1. Plasma diquat ion concentrations (ng/ml) at 6 hours post-dosing (Lees, 1996).

	Treatment Levels			
	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
Males*	13	170	350	987
Females*	10	144	855	496

* Average concentrations of 5 animals/dose.

As was stated in the Hazard Identification section of the 1994 RCD, "Most occupational and non-occupational exposures to diquat dibromide involve dermal absorption. Consequently, a short-term dermal NOEL would be more applicable as a NOEL for assessing the risks of acute occupational or non-occupational human exposure to diquat than an oral study." At the time the 1994 RCD was written, "no such single-dose dermal studies were available in the DPR data base or from a search of the open literature." Accordingly, the dermal NOEL (100 mg/kg) from the new study was used as the critical NOEL for addressing the occupational and non-occupational risks of acute diquat exposure.

Exposure Assessment

Estimates of occupational and non-occupational exposures from the original exposure assessment (Formoli, 1993) are contained in Table 2. The dermal dose is the amount of diquat estimated to be deposited on the skin daily in each of the work tasks. This represents 99% of the exposure to diquat. However, the exposure estimates for mixer/loader/applicators were updated because a new surrogate biological monitoring study of mixer/loader/applicators using paraquat was submitted (Meier, 1995). As paraquat is nearly chemically identical with diquat, this compound was used as the surrogate for diquat in all exposure categories where specific data for diquat were not available. The new study indicated that the amount of diquat absorbed by workers in this category was 0.3 µg/kg-day. This value was over 300 times less than the previous estimate of absorbed dosage (103 µg/kg-day) for ground applicators, which was based on passive dosimetry and a dermal absorption factor of 1.4% from an earlier worker exposure study involving paraquat (Formoli, 1993).

Risk Characterization

Acute Margins of Exposure (MOEs) were calculated as the ratio of the dermal NOEL (100 mg/kg) divided by the dermal exposure dose, rather than the estimate of the amount absorbed through the skin (Table 2). Only one of the work tasks (ground applicator on a tractor with normal clearance and no cab) indicated an MOE less than 100 (Table 2). However, a new exposure study (Meier, 1995) generated a new estimate of absorbed dose (0.3 µg/kg-day) for this work category. To obtain the MOE for this work category, the adjusted oral Estimated-No-Effect-Level (33 µg/kg-day for developmental toxicity in rabbits- Cochran *et al.*, 1994) which represents the no effect level for an absorbed dose through the oral route, was divided by the estimated absorbed dose of applicators (0.3 µg/kg-day) to obtain the MOE (110).

Chronic (annual) MOEs, as calculated in the 1994 RCD, were all greater than 100, except for the category- ground applicators on a tractor with normal clearance and no cab- which had an MOE of 11 (Cochran *et al.*, 1994). Based on the new exposure study (Meier, 1995), the MOE for these ground applicators is now estimated to be 7,000.

Table 2. Potential Daily Dermal Doses and Margins of Exposure for Occupational and Non-occupational Exposures to Diquat Dibromide

Work Task	Applied Dermal Dose ^a (µg/kg-day)	MOE ^b
Aquatic		
Mixer (Injection)	14.2	7,042
Applicator (injection)*	14.2	7,042
Applicator (handgun)	257	389
Boat Driver (handgun)	64.3	1,555
Aerial		
Mixer/Loader	557	180
Pilot	21.4	4,673
Flagger	579	173
Ground Application		
Applicator-normal clearance, no cab	7,571*	13*
Applicator-normal clearance, cab	529	189
Applicator-high clearance, no cab	379	264
Applicator- hand sprayer (right-of-way)	25	4,000
Gardener/Landscaper (Ready-to-Use formulation)	28.6	3,497
Gardener/Landscaper (Knapsack sprayer)	829	121
Non-Occupational		
Aerial Drift (50 m)	35.7	2,801
Aerial Drift (1600 m)	0.71	140,000
Adult, Swimming	14.2 - 92.9	1,076 - 7,042

a/ Dermal exposure derived from the exposure assessment (Formoli, 1993).

b/ Based on a NOEL of 100 mg/kg for reduced food consumption and death (1/10) in rabbits at 200 mg/kg.

* See text for further explanation.

Risk Appraisal

Most of the uncertainties associated with the assessment of risks related to the use of diquat dibromide have been discussed in the 1994 RCD. The toxicological basis for reconsidering the risk assessment was a new acute dermal toxicity study in rabbits (Lees, 1996). The single dose dermal NOEL was based on reduced food consumption and death in rabbits at 200 mg/kg. On the one hand it is not entirely certain that the death of the animal was substance related. There were no histopathological findings which explained the animal's death. On the other hand, the dermal LD₅₀ for rabbits was 400 mg/kg. It should also be noted that the dermal penetration of diquat through human skin (Feldman and Maibach, 1974; Wester and Maibach, 1985) was 10-fold less than through that of laboratory animals (Brorby, 1988). Thus, an absorbed dose in humans though the dermal route would be substantially less than in rabbits.

The new exposure estimate for mixer/loader/applicators was based on biological monitoring of a surrogate chemical, paraquat (Meier, 1995). Although the exposure data carry a greater degree of uncertainty than if diquat had been used, the application rates and the physical and chemical properties of paraquat are similar to diquat.

Conclusions

Using current toxicity data, estimates from current monitoring information on diquat, and surrogate exposure data, the calculated margins of exposure (MOEs) for potential short-term exposure were all greater than 100, the value conventionally considered to be protective of human health. MOEs for potential annual occupational exposure to diquat were also greater than 100.

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