

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

August 22, 2001

Memorandum

SUBJECT: HED's Review of *Diquat: Worker Exposure During Mixing, Loading, and Application of Reglon with Knapsack Sprayers*; (MRID# 44493001; PC code 032201; DP Barcode D222970).

FROM: Tom Brennan, Chemist
Reregistration Branch 4
Health Effects Division (7509C)

THRU: Susan Hummel, Branch Senior Scientist
Reregistration Branch 4
Health Effects Division (7509C)

TO: Kylie Rothwell, Chemical Review Manager
Special Review and Reregistration Division (7508C)

Attached is a review of a knapsack mixer, loader, applicator biomonitoring study which was submitted by Zeneca to the U.S. EPA (MRID# 444930-01). This review was completed by Versar, Inc. on June 29, 2001 under supervision of HED. It has undergone secondary review and reflects Agency policies.

Executive Summary

This study met most of the OPPTS Harmonized Test Guidelines Group A, 875.1100 (dermal exposure), 875.1300 (inhalation exposure), 875.1500 (biological monitoring) Guidelines and the data presented in the study are of sufficient scientific quality to be used to determine absorbed doses. HED recommends reporting the results of the knapsack mixer, loader, applicator study in the diquat dibromide risk assessment.

Summary

This study was designed to quantify worker exposure to Reglone® (a wettable powder formulation of diquat) at a banana plantation in Guatemala, while mixing/loading and applying using knapsack sprayers. The absorbed dose of diquat was measured by collecting and analysing diquat in urine from 20 workers over a seven day period starting on the day prior to exposure until 5 days after the exposure day.

The field portion of this study was conducted from September 29 to October 3, 1996 on a banana plantation close to the Atlantic coast of Guatemala. The plantation was divided into sections of approximately 0.5 to 0.75 hectare blocks divided by a series of canals. Twenty workers were monitored while spraying approximately one section each. The treated areas were similar with flat areas of dense plantation and deep sided gullies every 100 meters. Each worker mixed, loaded, and applied diquat for approximately 6 hours (275 - 323 minutes). During this time there was a short break for breakfast. Each worker was allocated a different section to spray to ensure there was no overlap. Each worker handled approximately 0.34 kg of diquat (0.29 - 0.38 kg).

The absorbed dose of diquat was determined by measuring diquat in workers' urine. Complete 24-hour urine samples were collected from all test subjects over a seven day period (i.e., one day prior to exposure through the morning of the sixth day after exposure). Two separate collections were made on the day of exposure. The first collection was made during the application and up until the time the workers first washed their hands. The second collection was for the remainder of the application day. In addition to urine sampling: dermal, hand, and inhalation potential exposures were also measured on the same set workers who were wearing label, specified PPE (double layer clothing, as well as a face shield and gloves (when mixing/loading)). Potential dermal exposure was monitored using 100% cotton clothing as the whole body dosimeter. Potential hand exposure was monitored using a handwash procedure. Two handwash samples were taken at times when the workers would normally wash their hands, when they stopped for their breakfast break and upon completion of the day's work. Potential inhalation exposure was monitored using a personal air sampling pump (SAC model 2244-PCX R4) with an SAC 25 mm glass fibre filter (Cat. No. 225-58F) which attached to the workers' belts.

Results

The absorbed dose of diquat based on urinary excretion varied from 0.009 to 0.359 Fg diquat di-cation/kg body weight. The geometric mean absorbed dose of diquat was estimated to be 0.075 Fg/kg body weight/day. In 14 of the 20 test subjects, diquat residues were only found in the samples from the day of exposure. For the remaining six test subjects, diquat residues were only found up to the day after exposure (24-48 hours). One test subject had detectable levels of diquat in the baseline sample (pre-exposure day sample). The data were adjusted for 61 percent of the dose eliminated in urine. The following table summarizes the absorbed dose of diquat from urinary excretion.

Absorbed Dose of Diquat from Urinary Excretion

Parameters			
	#Rep	Arith. Mean	Geo. Mean
Sampling Interval = 0-12 hours			
Diquat di-cation (F g/kg body weight)	20	0.046	0.084
Sampling Interval = 12-24 hours			
Diquat di-cation (F g/kg body weight)	20	0.019	0.059
Sampling Interval = 24-48 hours			
Diquat di-cation (F g/kg body weight)	20	0.023	0.021
Creatinine (g/24 hr)	20	0.990	0.936
Sampling Interval = 48-72 hours			
Diquat di-cation (F g/kg body weight)	20	<DOQ	--
Creatinine (g/24 hr)	20	1.015	0.963
Sampling Interval = 72-96 hours			
Diquat di-cation (F g/kg body weight)	20	<DOQ	--
Creatinine (g/24 hr)	20	1.0615	0.956
Sampling Interval = 96-120 hours			
Diquat di-cation (F g/kg body weight)	20	<DOQ	--
Creatinine (g/24 hr)	20	1.020	0.945
Sampling Interval = 120-144 hours			
Diquat di-cation (F g/kg body weight)	20	<DOQ	--
Creatinine (g/24 hr)	20	1.080	1.030
Total Absorbed Dose	20	0.125	0.075

Arith. Mean = Arithmetic Mean (average)
Std. Dev. = Standard Deviation

Geo. Mean = Geometric Mean

Note: The geometric mean is shown as (--) if a zero was a data point in the calculation. Calculations by the registrant using EXCEL 97 spreadsheet software. The absorbed dose of Diquat from Urinary Excretion is adjusted for 61% urinary excretion.

The potential dermal exposure was calculated as the total amount measured on the clothes and in the handwashes. The registrant calculated the geometric mean total dermal exposure as 78.2 mg/day. The total amount of diquat on the clothing was estimated as 74.5 mg/day, while the total amount of diquat in the handwash was estimated as 3.03 mg/day. The registrant corrected the values for field recovery when they were less than 70 percent. Versar corrected the values for field fortification recovery when they were less than 90 percent and calculated the geometric mean as 80.94 mg/day. The geometric mean potential inhalation exposure was calculated by the registrant as 0.004 mg/day. The registrant did not correct for field recoveries. Versar corrected the values for field fortification recovery when they were less than 90 percent and calculated the geometric mean potential inhalation exposure as 0.006 mg/day.

Conclusions

The absorbed dose of diquat was determined by measuring diquat in worker's urine. Measurement of the urinary excretion of diquat has been confirmed as a suitable means of assessing absorption by the work of Feldman and Maibach (1974) who showed that 61% of an intravenous dose in humans was excreted in the urine as unchanged diquat over 6 days. The data indicate that in 14 of the 20 test subjects diquat residues were only detectable in the samples from 0 to 24 hours after the initial exposure. For the remaining 6 test subjects, diquat residues were only found up to the day after exposure (24-48 hours).

The following items need to be considered with regard the acceptability of the study. This study met most of the OPPTS Harmonized Test Guidelines Group A, 875.1100 (dermal exposure), 875.1300 (inhalation exposure), 875.1500 (biological monitoring). The major issues of concern are: (1) Cotton clothing worn by the study participants was used as the dosimeter rather than a whole-body dosimeter underneath the clothing. Because dermal exposure monitoring was done concurrently with biomonitoring, the appropriateness of measuring dermal exposure via dosimetry is questionable; (2) There was considerable variability in the daily excretion of creatinine, and according to the authors, in some cases the values suggest that the urine collections were incomplete; (3) No information on the limit of quantitation (LOQ) was provided for diquat in clothing, handwash, or inhalation samples. Only a range of LOQs were provided for diquat in urine samples (between 0.50 and 3.77 ng/L); (4) Information on storage stability was not provided for dosimetry, handwash, or inhalation filter samples; and (5) The guidelines specifically state that data should be corrected if any appropriate field fortified, laboratory fortified or storage stability recovery is less than 90 percent. The registrant only corrected data if field recovery was less than 70% or more than 120%.

Recommendations

In Zenica's 90-Day Response to the 1995 Reregistration Eligibility Decision (RED) Document for diquat dibromide, they requested that the EPA reduce the requirements for Personal Protective Equipment (PPE) for groundboom, **knapsack**, and aerial applications. Here is an excerpt of that request: "Based on diquat's low vapor pressure and extremely low skin permeation, and the availability of surrogate biomonitoring data, Zenica requests reductions in the personal protective equipment requirements recommended by EPA in its Reregistration Eligibility Decision (RED) Document." For knapsack mixing, loading and application Zenica has requested the following PPE:

Product Use	PROTECTIVE CLOTHING			
	Body	Head	Hands	Feet
Knapsack mixing/loading and application	Coverall <u>or</u> Long-sleeved shirt and long pants. Chemical-resistant apron when cleaning	None	Waterproof gloves when mixing and loading	Shoes and socks.

HED recommends reporting the results of this knapsack mixer, loader, applicator study in the current diquat dibromide TRED risk assessment. As per the request of Zenica, the data in this study will be used to replace the “backpack mixer/loader/applicator” values from the Pesticide Handlers Exposure Database that were used in the 1995 Diquat RED. Additionally, the data in this study will be used to evaluate Zenica’s request for reduced PPE for backpack mixing, loading and application activities in the current TRED.

Attachment 1

Versar, Inc. Review Memo Dated June 29, 2001

Reviewer: Marit Espevik/Nate Mottl **Date** June 29, 2001

STUDY TYPE: Mixer/loader/applicator passive dosimetry and biological monitoring study

TEST MATERIAL: Diquat (i.e., 9,10-dihydro-8a, 10a-diazoniaphenanthrene; 6,7-dihydrodipyridol [1,2-a:2,1'-c]pyrazine-5,8-dium; 1,1-ethylene-2, d\ -bipyridyldiylum) is the active ingredient in the wettable powder Reglone® formulation

SYNONYMS: Reglone, diquat

CITATION: Study Director/Author: Malcolm.L. Findlay
Title: *Diquat: Worker Exposure During Mixing, Loading, and Application of Reglone® With Knapsack Sprayers.*
Report Date: January 23, 1998
Analytical Laboratories: Zeneca Central Toxicology Laboratory
Alderley Park
Macclesfield
Cheshire SK10 4TJ
UK

CEM Analytical Services Limited
Glendale Park
Fernbank Road
North Ascot
Berkshire SL5 8JB
UK
Identifying Codes: MRID 44493001
Report No. RP-97-004B
Study Number 96JH200
Unpublished

SPONSOR: Zeneca Ag Products.

EXECUTIVE SUMMARY:

This study was designed to quantify worker exposure to Reglone® (a wettable formulation of diquat) at a banana plantation in Guatemala, while mixing/loading and applying using knapsack sprayers. The absorbed dose of diquat was measured by collecting and analyzing diquat in urine from 20 workers over a seven day period starting on the day prior to exposure until 5 days after the exposure day. The absorbed dose of diquat based on urinary excretion varied from 0.009 to 0.359 Fg diquat di-cation/kg body weight. The geometric mean absorbed dose of diquat was estimated to be 0.075 Fg/kg body weight/day. In 14 of the 20 test subjects, diquat residues were only found in the samples from the day of exposure. For the remaining six test subjects, diquat residues were only found up to the day after exposure (24-48 hours). One test subject had detectable levels of diquat in the baseline sample (pre-exposure day sample). The data were adjusted for 61 percent of the dose eliminated in urine.

The potential dermal exposure was calculated as the total amount measured on the clothes and in the handwash. The registrant calculated the geometric mean total dermal exposure as 78.2 mg/day. The total amount of diquat on the clothing was estimated as 74.5 mg/day, while the total amount of diquat in the handwash was estimated as 3.03 mg/day. The registrant corrected the values for field recovery when they were less than 70 percent. Versar corrected the values for field fortification recovery when they were less than 90 percent and calculated the geometric mean as 80.94 mg/day. The geometric mean potential inhalation exposure was calculated by the registrant as 0.004 mg/day. The registrant did not correct for field recoveries. Versar corrected the values for field fortification recovery when they were less than 90 percent and calculated the geometric mean potential inhalation exposure as 0.006 mg/day.

This study met most of the OPPTS Harmonized Test Guidelines Group A, 875.1100 (dermal exposure), 875.1300 (inhalation exposure), 875.1500 (biological monitoring) Guidelines. The major issues of concern are: (1) Cotton clothing worn by the study participants was used as the dosimeter rather than a whole-body dosimeter underneath the clothing. Because dermal exposure monitoring was done concurrently with biomonitoring, the appropriateness of measuring dermal exposure via dosimetry is questionable; (2) There was considerable variability in the daily excretion of creatinine, and according to the authors, in some cases the values suggest that the urine collections were incomplete; (3) No information on the limit of quantitation (LOQ) was provided for diquat in clothing, handwash, or inhalation samples. Only a range of LOQ were provided for diquat in urine samples (between 0.50 and 3.77 ng/L); (4) Information on storage stability was not provided for dosimetry, handwash, or inhalation filter samples; and (5) The guidelines specifically state that data should be corrected if any appropriate field fortified, laboratory fortified or storage stability recovery is less than 90 percent. The registrant only corrected data if field recovery was less than 70% or more than 120%.

COMPLIANCE: The study sponsor and author stated that the study was conducted in compliance with the UK Principles of Good Laboratory Practice (The United Kingdom GLP Regulations 1997). These principles are in accordance with the OECD Principles of Good Laboratory Practice 1981 (OECD Environment Monograph No. 45). The sponsor further stated that the OECD international standards are acceptable to the United States Environmental Protection Agency and applicable to this study and, therefore, satisfy the requirements of 40 CFR Part 160 and 40 CFR Part 792. No GLP deviations were noted.

GUIDELINE OR PROTOCOL FOLLOWED: OPPTS Harmonized Test Guidelines Series 875, Occupational and Residential Exposure Test Guidelines, Group A: 875.1100 (dermal exposure), 875.1300 (inhalation exposure), and 875.1500 (biological monitoring).

There was no study protocol other than the CEM Analytical Services Ltd. Project Protocol included in the study.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Formulation: Reglone® - a wettable powder containing 36.4 percent active ingredient (ai).
Lot/Batch # formulation: No. FM 16
Purity: The purity of the diquat dibromide monohydrate reference standards was verified at 96.9% with an expiration date of May 1, 1997.
CAS #(s): CAS 2764-72-9
Other Relevant Information: EPA Reg. No. 10182-353, CTL test substance reference number Y00895/053

2. Relevance of Test Material to Proposed Formulation(s):

The wettable powder formulation sent to the field sites was labelled as Reglone® which is the same product name that appears on the label provided.

3. Packaging:

The product was supplied in 5 L containers.

B. STUDY DESIGN

The field portion of this study was conducted from September 29 to October 3, 1996 on a banana plantation close to the Atlantic coast of Guatemala. The plantation was divided into sections of approximately 0.5 to 0.75 hectare blocks divided by a series of canals. Twenty workers were monitored while spraying approximately one section each. The treated areas were similar with flat areas of dense plantation and deep sided gullies every 100 meters.

Weather conditions, such as wind speed and direction, shade temperature, and relative humidity were monitored at approximately hourly intervals during the loading and application. Temperature and humidity were measured using a Fisher Nist certified hygrometer/thermometer while wind speed was measured using a Dwyer Wind Meter. Air temperatures at the time of application ranged from 74.8EF and 97.6EF, relative humidity readings ranged from 57.1 to 109.4 percent for both applications, and there were no winds.

1. Number and type of workers and sites:

Twenty workers were monitored at one site. Four different workers were monitored on each of five days (total of 20 workers). All test subjects were male. Their experience ranged from 2 to 25 years. The workers' heights ranged from 1.50 to 1.70 meters and their weights ranged from 48 to 81 kg.

2. Replicates:

Each worker mixed, loaded, and applied Diquat for approximately 6 hours (275 - 323 minutes). During this time there was a short break for breakfast. Each worker was allocated a different section to spray to ensure there was no overlap. Each worker handled approximately 0.34 kg of Diquat (0.29 - 0.38 kg).

Appendix 3 (pages 26 and 27) of the Study Report contain observations on work practices, hygiene standards, clothing worn, eating, drinking, and smoking habits, and amounts loaded. Any activities or events that could potentially have led to increased exposure was also noted and included in Appendix 3. The authors stated that the ground was very wet which resulted in the workers' clothes getting very muddy. The climate was hot and humid on each day of application resulting in a lot of hand to face contact because workers had to wipe away their sweat.

3. Protective clothing:

All workers wore 100% cotton clothing. The clothing (also used as dosimeter) was comprised of:

- B. Long sleeved shirt (Marks and Spencer 100% cotton weave);
- C. Long trousers Meiklejohn workwear 100% cotton 3110 drill 280 g/m³);
- D. Protective Nitrile gloves;
- E. Faceshield;
- F. Rubber boots; and
- G. Long socks.

The clothing was supplied to the workers on the morning of the application day and dressing was supervised by a member of the study team. The trouser legs were worn outside of the boots.

Versar obtained a label for Diquat (EPA Reg. No. 10182-353) that outlined the requirements for the following personal protective equipments during mixing/loading and application:

- A. Long-sleeved cotton shirt and long pants;
- B. Waterproof gloves;
- C. Chemical-resistant footwear plus socks;
- D. Protective eyewear;
- E. Chemical-resistant headgear for overhead exposure, and
- F. A chemical-resistant apron when cleaning equipment, mixing or loading.

4. Mixing/loading/application method:

The spray was mixed by filling the “Guarany Plus” 16 L spray tank with water. The product was measured by pouring it into a graduated plastic measuring cylinder which was then tipped into the spray tank. Finally, water and Agral® was added to the spray tank.

5. Application Rate:

- Application rate(s): The target application concentration was 2 g diquat cation/L.
- Application Equipment: The product was applied using Knapsack Sprayers with blue flood-jet nozzles.
- Spray Volume: The target spray volume was 3L/ha (600 g ai).
- Equipment Calibration: The authors reported that the sprayers were calibrated prior to application.

The label provided with the study report was Reglone ® diquat desiccant (Zeneca Ag Products) Registration for Special Local Need for Distribution and Use only within the State of Florida (EPA Reg. No 10182-353). This label only provided application rate information for the burndown of tomato vines (1.5 pts of Reglone per acre in 60 to 120 gals of water per acre). Based on this information it is uncertain whether the maximum application rate was used.

6. Exposure monitoring methodology:

Urine: The absorbed dose of diquat was determined by measuring diquat in workers’ urine. Complete 24-hour urine samples were collected from all test subjects over a seven day period (i.e, one day prior to exposure through the morning of the sixth day after exposure). Two separate collections were made on the day of exposure. The first collection was made during the application and up until the time the workers first washed their hands. The second collection was for the remainder of the application day. Urine voids were made directly into clean polypropylene containers which were available to the test subjects at all times to ensure that all urine was collected. The test subjects were advised to use caution so that contamination of the urine with extraneous diquat did not occur. The workers were also instructed to exercise normal hygiene practices in terms of changing clothes and washing. The test subjects were asked to put their samples in as cool of a place as possible and out of direct sunlight. Test subjects were requested not to handle or have contact with diquat for a period of five days before and 5 days after exposure. After the exposure period, the total volume of each 24-hour set of samples were measured and two 50 mL aliquots were taken (primary and reserve) and stored in polypropylene containers. The samples were kept in a deep freezer until they were shipped frozen in dry ice to Zeneca Central Toxicology Laboratory in the U.K.

Control urine, used to prepare extracted standards and to dilute samples, was collected from employees at Zeneca Central Toxicology Laboratory who had no previous exposure to diquat.

Creatinine levels were measured in the urine samples as a qualitative measure of the completeness of the urine samples. Creatinine is excreted as a waste product by the kidney and synthesized in the body at a fairly constant rate.

Dermal: Dermal exposure was monitored using 100% cotton clothing as the whole body dosimeter. On completion of the application day, the clothing was removed and hung to dry on a line. The length of time that the clothing was drying is not specified in the Study Report. The clothing was sectioned by cutting it with scissors into arms, torso (which included the shirt and upper part of the trousers), and legs. Any dried dirt was brushed off. The individual parts were wrapped in aluminum foil, labelled, and placed in a labelled plastic bag. The plastic bags were placed in frozen storage until they were shipped frozen in dry ice to CEM Analytical Services, Ltd. in the U.K.

Hand: Potential hand exposure was monitored using a handwash procedure. Two handwash samples were taken at times when the workers would normally wash their hands, when they stopped for their breakfast break and upon completion of the day's work. The handwash was carried out by the workers washing their hands with two squirts of liquid "Simple" soap in one litre of water. On completion of the handwash samples, an aliquot of approximately 50 mL of the handwash solution was taken in a 60 mL labelled polypropylene container. The samples were wrapped in aluminum foil and placed into a labelled polythene bags and placed into frozen storage prior to shipment in dry ice to CEM Analytical Services, Ltd. in the U.K.

Inhalation: Potential inhalation exposure was monitored using a personal air sampling pump (SAC model 2244-PCX R4) with an SAC 25 mm glass fibre filter (Cat. No. 225-58F) which attached to the workers' belts. The fibre filter was attached to the pump by rubber tubing and positioned in the breathing zone of the worker by attaching it to the collar. The pumps were calibrated to operate at 2.0 ± 0.1 litre per minute. After the day's work the performance of the personal air sampling pumps was checked with the glass fibre filter attached. The glass fibre filter samples were wrapped in aluminium foil, placed in a labelled plastic bag and placed in frozen storage until shipment in dry ice to CEM Analytical Services, Ltd. in the U.K. The air sampling pumps were operated during mixing/loading and application, but were switched off during the breakfast break.

7. Analytical Methodology:

Urine:

Extraction method: Diquat was extracted from urine using solid phase extraction (SPE) and converted to the bipyridone derivative.

Detection methods: See Table 1

GC Column	KR100 501 25 cm x 4.6 mm id
Mobile Phase	92.5% ion pair buffer x 7.5% Acetonitrile
Injection Volume	50 FL
Flow Rate	1.25 mL/min
Fluorescence	λ excitation 360 nm λ emission 440 nm
Typical Retention time	9.3 min

Instrument performance and calibration: Calibration curves were prepared for each calibration run. The limit of quantitation for diquat in urine varied from 0.50 to 3.77 ng/mL.

Quantification: The concentration of diquat was quantified by liquid chromatography with fluorescent detection. The chromatography data system was used to measure peak areas for diquat. The concentrations of diquat in unknown samples were

calculated from a calibration curve based on the areas for the diquat peaks in the standard extracts. Results obtained were corrected where necessary for any dilutions made.

Dermal and inhalation

Extraction methods:

Cloth Material Samples: The torso clothing sections was extracted in 4000 mL 0.5 M sulphuric acid, the leg clothing sections in 2000 mL, and the arm clothing sections in 1000 mL. After soaking for 10 minutes with occasional gentle agitation, aliquots of the supernatants (500 mL for the torso, 250 mL for the legs and arms) were made up to 500 mL sulphuric acid.

Handwash Solution Samples: Diquat was extracted from handwash solution samples by diluting the sample to 500 ml with 0.5 ml sulphuric acid.

Air-Filters: Air filters were extracted in batches by soaking for 10 minutes in saturated ammonium chloride with occasional gentle agitation.

Detection methods: See Table 2.

Table 2. Summary of Chromatographic Conditions for Inhalation and Dermal	
Instrumentation	Varian 9000 series high performance chromatography system consisting of an autosampler, ternary gradient pump, a column oven, a variable wavelength UV/VIS detector and a computing integrator.
Column	Hichrom Spherisorb S5P phenyl, 25 cm x 4.6 mm i.d..
Column Temperature	40EC
UV Wavelength	310 nm
Mobile Phase	Water:Methanol (90:5 v/v) + 0.14% (w/v) sodium-1-octanesulfonate + 1.0% (v/v) diethylamine + 0.8% (v/v) orthophosphoric acid
Flow Rate	1.5 mL/min.
Injection Volume	200 FL
Retention Time	~ 4 minutes

Instrument performance and calibration: Repeated injections of a standard solution of diquat in saturated ammonium chloride at 0.1 Fg/mL were made into the HPLC operated under the conditions described above.

Quantification: The concentration of diquat in all three sampling media were determined by HPLC. Residues were quantified using a standard linear regression technique.

8. Quality Control:

Lab Recovery: There was no description of the concurrent laboratory recovery procedures, however, results for dermal and inhalation lab recovery were provided in Appendix B (pages 69-70) of the study report (see Table 3).

Table 3– Concurrent Laboratory Recovery - Diquat

Sampling Matrices	Concurrently Analyzed Laboratory Fortified Samples		
	Fortification Level	Range of Recovery (%)	Mean ± SD of Recovery (%)
Cotton Material	25 Fg/sample	90 - 110 (n=5)	99.4 ± 8.0
	250 Fg/sample	70 - 99 (n=9)	89.8 ± 9.8
Handwash Solution	10 Fg/L	84 - 95 (n=3)	90.7 ± 5.9
	100 Fg/L	82 - 105 (n=7)	96.0 ± 9.4
Air Filters	1.25 Fg/sample	98 - 98 (n=3)	98.0 ± 0
	12.5 Fg/sample	90 - 104 (n=3)	98.0 ± 7.2

Field recovery:

Urine: Duplicate 50 mL urine samples were prepared to assess recovery of diquat from urine on each exposure day that urine was collected. Urine samples from non-exposed subject were fortified at 2, 10, and 50 ng of diquat per mL and stored under the same ambient and frozen conditions as the 24-hour test samples. Untreated control samples were also stored under the same conditions. Recovery and test samples were analyzed concurrently.

All of the values for the control urine samples were below the limit of quantitation for the assay. The overall field fortification recovery was 90.1 percent. Mean recoveries for the samples fortified at 2, 10, and 50 ng/mL were 87.0% (range 69.0% - 114%), 85.1% (range 65.9% - 111%), and 98.1% (range 67.8% - 130%), respectively. According to the study authors, the low recovery values of R30, R33, R36, R42, R45, and R48 were probably due to an error in the fortification of the samples and those values were excluded when calculating the mean recovery values.

Potential Dermal Exposure: Five replicates of each of two fortification rates of 0.1 and 0.01 mL/sample (0.2 and 0.02 mg ai/sample, respectively) were applied to 100 cm² swatches of clothing. The same rates and replication were applied to 50 mL of a water/soap handwash solution. Untreated control clothing were also used to measure any background contamination. The samples were exposed for the duration of the working day and processed in exactly the same manner as the test samples.

On day 1, the field fortification site was located on the plantation in a position away from the treated area. However, on day 2, the authors reported that it was necessary to move the field fortification sites to another area on the plantation halfway through the day and in doing so some of the samples remained in direct sunlight for about 3 hours. On days 3, 4, and 5, the field fortifications were done at the hotel laboratory since it was impossible to carry out the field fortification on the plantation. The environmental conditions were reported to be the same at the hotel site.

The mean recovery of diquat from the clothing and handwash was 69% and 68%, respectively. On day one, the recovery of diquat from the clothing and handwash was 90% and 89%, and on day 2 the recovery was 80% and 125%, respectively. On days 3, 4, and 5, the recoveries were low at 59%, 56%, and 61%, respectively for the clothing, and 54%, 45%, and 29%, respectively, for the handwash.

Potential Inhalation Exposure: Field fortification solution was applied to glass fibre filters at 0.1 and 0.01 mL/filter (0.2 and 0.02 mg/ai, respectively). Three replicates were used for each rate. Untreated control samples were also exposed to measure any background exposure. All samples were placed at a site away from the loading area and away from any likely contamination. The field fortification samples were attached to personal air sampling pumps in the same way as those of the test subjects and they were run for the duration of the loading and application time and processed in the same manner as the test samples.

The mean recovery of diquat from glass fibre filters prepared under field conditions was 77% and ranged from 70 - 81%.

Formulation: A sample was sent to CEMAS for analysis and it measured 186.4 g diquat/L.

Tank mix: No information on tank mix analysis was provided in the Study Report.

Travel Recovery: Travel Recovery was not assessed in the Study.

Storage Stability: All samples were stored frozen prior to analysis. The field study was conducted between September 29 and October 3, 1996. Urine samples were analyzed between December 1996 and February 1997 (up to 153 days of storage). Dermal and inhalation samples were analyzed between November 1, 1996 and February 1, 1997 (up to 153 days of storage).

Control urine was spiked with diquat on the day of the study and treated in an identical manner to urine samples from the exposed workers to determine the stability under field conditions. Mean overall recovery was 90.1 percent. No information on storage stability for clothing or handwash samples was provided in the Study Report.

II. RESULTS AND CALCULATIONS:

A. EXPOSURE CALCULATIONS:

Systemic Absorption

The absorbed dose of diquat was determined by measuring diquat in worker's urine. Measurement of the urinary excretion of diquat has been confirmed as a suitable means of assessing absorption by the work of Feldman and Maibach (1974) who showed that 61% of an intravenous dose in humans was excreted in the urine as unchanged diquat over 6 days.

The absorbed dose of diquat was measured by collecting and analyzing workers' urine for a seven day period from the day prior to exposure until 5 days after the exposure day. The total amounts of diquat di-cation excreted over the six day period (excluding the pre-exposure day), expressed as F g/kg bodyweight, were calculated from the concentrations of diquat di-cation in urine, the urine volumes, and the bodyweights of the workers. The results for the absorbed dose of diquat based on urinary excretion are presented in Table 4. The total amounts excreted varied from 0.009 to 0.359 F g diquat di-cation/kg body weight. The total geometric mean absorbed dose of diquat was estimated to be 0.075 F g/kg bodyweight/day. In 14 of the 20 test subjects, diquat residues were only detected in the samples from 0-24 hours after the initial of exposure. For the remaining 6 test subjects, diquat residues were only found up to the day after exposure (24-48 hours). One test subject had detectable levels of diquat in the baseline sample (pre-exposure day sample). The registrant did not correct for field recovery; however, the data were adjusted for 61 percent of the dose eliminated in urine. It is not clear how values <LOQ were treated in the calculations. The authors stated that in a small number of individuals, there was considerable variability in the daily excretion of creatinine and in some cases the values suggest that the urine collections were incomplete.

Versar also presents the registrant's total amounts of residues excreted (see Table 5). This table presents the total amount of diquat handled and body weights from the registrant (see Table 1, page 190-20 of the Study Report) and also presents the excretion amounts calculated by the registrant (see Table 2, page 52).

Table 4. Absorbed Dose of Diquat from Urinary Excretion

Parameters			
	#Rep	Arith. Mean	Geo. Mean
Sampling Interval = 0-12 hours			
Diquat di-cation (F g/kg bodyweight)	20	0.046	0.084
Sampling Interval = 12-24 hours			
Diquat di-cation (F g/kg bodyweight)	20	0.019	0.059
Sampling Interval = 24-48 hours			
Diquat di-cation (F g/kg bodyweight)	20	0.023	0.021
Creatinine (g/24 hr)	20	0.990	0.936
Sampling Interval = 48-72 hours			
Diquat di-cation (F g/kg bodyweight)	20	<DOQ	--
Creatinine (g/24 hr)	20	1.015	0.963
Sampling Interval = 72-96 hours			
Diquat di-cation (F g/kg bodyweight)	20	<DOQ	--
Creatinine (g/24 hr)	20	1.0615	0.956
Sampling Interval = 96-120 hours			
Diquat di-cation (F g/kg bodyweight)	20	<DOQ	--
Creatinine (g/24 hr)	20	1.020	0.945
Sampling Interval = 120-144 hours			
Diquat di-cation (F g/kg bodyweight)	20	<DOQ	--
Creatinine (g/24 hr)	20	1.080	1.030
Total Absorbed Dose	20	0.125	0.075

Arith. Mean = Arithmetic Mean (average)
Std. Dev. = Standard Deviation

Geo. Mean = Geometric Mean

Note: The geometric mean is shown as (--) if a zero was a data point in the calculation. Calculations by the registrant using EXCEL 97 spreadsheet software. The absorbed dose of Diquat from Urinary Excretion is adjusted for 61% urinary excretion.

Potential dermal Exposure

The potential dermal exposure was calculated as the total amount measured on the clothes and in the handwash. The registrant calculated the geometric mean total dermal exposure as 78.2 mg/day. The total amount of diquat on the clothing was estimated as 74.5 mg/day, while the total amount of diquat in the handwash was estimated as 3.03 mg/day. The registrant corrected the values for field recovery where they were less than 70 percent and more than 120 percent.

Versar corrected the values for field fortification recovery where they were less than 90 percent (Table 6). Versar did not correct the values for field recovery where they were more than 120 percent. Versar calculated the geometric mean total dermal exposure as 81.01 mg/day.

Potential inhalation Exposure

The geometric mean potential inhalation exposure was calculated by the registrant as 0.004 mg/day. The registrant did not correct for field recoveries. Values were calculated using a 29 L/min breathing rate. The volume of air samples was calculated by multiplying the nominal flow of 2 L/min by the recorded time that the pumps were running.

Versar corrected the values for field fortification recovery where they were less than 90 percent (Table 6). Versar calculated the geometric

mean potential inhalation exposure as 0.006 mg/day.

Table 5. Total Amount of Diquat Di-cation Excreted Post Exposure

Worker Replicate	Amount Handled (kg)	Bodyweight (kg)	Total Diquat Excreted 0 to 144 hours ($\mu\text{g}/\text{kg}$ bodyweight) ¹
1	0.32	53	0.056
2	0.32	59	0.039
3	0.29	59	0.016
4	0.29	66	0.033
5	0.38	81	0.047
6	0.35	64	0.068
7	0.38	81	0.077
8	0.38	56	0.119
9	0.35	52	0.009
10	0.35	63	0.032
11	0.35	59	0.359
12	0.35	48	0.127
13	0.29	64	0.020
14	0.29	58	0.019
15	0.29	54	0.018
16	0.29	54	0.115
17	0.35	52	0.283
18	0.35	59	0.029
19	0.38	66	0.014
20	0.38	66	0.036

Table 6. Versar's Summary of Dermal and Inhalation Exposure by Worker

Replicate	Amount Handled (kg)	Exposure Time (min)	Pump Flow Rate (L/min)	Dermal Exposure ^a (mg/day)			Inhalation Exposure ^b (mg/day)		
				Total Potential Dermal Exposure	Arithmetic Mean ^c	Geometric Mean ^d	Total Potential Inhalation Exposure	Arithmetic Mean ^c	Geometric Mean ^d
1	0.32	310	2	32.9	91.847	81.012	0.00528	0.006	0.006
2	0.32	310	1.5	37			0.0132		
3	0.29	310	1.5	36.3			0.00396		
4	0.29	310	1.5	70			0.00528		
5	0.38	310	1.5	155			0.00417		
6	0.35	323	1.5	142			0.00417		
7	0.38	305	1.5	205			0.0139		
8	0.38	300	1.5	87.1			0.0139		
9	0.35	307	1.5	65.2			0.00812		
10	0.35	307	1.5	117.1			0.00464		
11	0.35	303	1.5	145.1			0.00696		
12	0.35	303	1.5	77			0.00348		
13	0.29	280	1.5	119			0.00492		
14	0.29	275	1.5	72			0.00615		
15	0.29	277	1.5	53.7			0.00861		
16	0.29	277	1.5	93.6			0.00738		
17	0.35	284	1.5	137			0.00429		
18	0.35	300	1.5	45.1			0.00286		
19	0.38	310	1.5	73.8			0.00429		
20	0.38	310	1.5	72.5			0.00286		

L = liters; min = minute

Footnotes

- a Dermal exposure = sum of the residues (mg/sample day) found in all whole body dosimeter sections; arms, torso, legs (upper), legs (lower), and handwash samples for each replicate. Corrected for field recovery < 90%. Not corrected for field recovery > 120%.
- b Inhalation exposure (mg/ day) = reported as total potential exposure in mg/day by the registrant. Corrected for field recovery <90%. Not corrected for field recovery > 120%.
- c Arithmetic mean calculated by averaging the residues for Replicates 1-20
- d Geometric mean calculated by the equation:

$$\frac{GM}{y} = \sqrt[n]{y_1 y_2 y_3 \dots y_n}$$

III DISCUSSION

A. LIMITATIONS OF THE STUDY:

This study met most of the requirements of OPPTS Harmonized Test Guidelines Series 875.1100, 875.1300, and 875.1500 Guidelines. The major issues of concern are: (1) Cotton clothing worn by the study participants was used as the dosimeter rather than a whole-body dosimeter underneath the clothing. Because done concurrently with biomonitoring, the appropriateness of measuring dermal exposure via dosimetry is questionable; (2) There was considerable variability in the daily excretion of creatinine and according to the authors, in some cases the values suggest that the urine collections were incomplete; (3) No information on the limit of quantitation (LOQ) was provided for diquat in clothing, handwash, or inhalation samples. Only a range of LOQ were provided for diquat in urine samples (between 0.50 and 3.77 ng/L); (4) Information on storage stability was not provided for dosimetry, handwash, or inhalation filter samples; and (5) The guidelines specifically state that data should be corrected if any appropriate field fortified, laboratory fortified or storage stability recovery is less than 90 percent. The registrant only corrected data if field recovery was less than 70% or more than 120%.

APPENDIX A

Compliance Checklist for “Diquat: Worker Exposure During Mixing, Loading, and Application of Reglone with Knapsack Sprayers”

Compliance Checklist

Compliance with OPPTS Series 875, Occupational and Residential Exposure Test Guidelines, Group A: Guidelines, 875.1500 (biomonitoring), 875.1300 (inhalation), and 875.1100 (dermal) is critical. The itemized checklist below describes compliance with the major technical aspects of OPPTS 875.1500, 875.1300, and 875.1100.

875.1500:

- S *The Agency requires investigators to submit protocols for review purposes prior to the inception of the study. Adequate pharmacokinetic data must exist to effectively interpret the data.* This criterion was probably met. Measurement of the urinary excretion of diquat has been confirmed as a suitable means of assessing absorption by the work of Feldman and Maibach (1974) who showed that 61% of an intravenous dose in humans was excreted in the urine as unchanged diquat over 6 days.
- S *Expected deviations from GLPs should be presented concurrently with any protocol deviations and their potential study impacts.* This criterion was probably met. There was no mention of a GLP deviations nor any mention of protocol deviations.
- S *The test substance should be a typical end use product of the active ingredient.* This criterion was met.
- S *The application rate used in the study should be provided and should be the maximum rate specified on the label. However, monitoring following application at a typical application rate may be more appropriate in certain cases.* The target application rate was 3L/ha (600 g ai). The label provided with the study report was Reglone ® diquat desiccant (Zeneca Ag Products) Registration for Special Local Need for Distribution and Use only within the State of Florida (EPA Reg. No 10182-353). This label only provided application rate information for the burndown of tomato vines (1.5 pts of Reglone per acre in 60 to 120 gals of water per acre). Based on this information it is uncertain whether the maximum application rate was used.
- S *Selected sites and seasonal timing of monitoring should be appropriate to the activity.* This criterion was probably met. According to the Study Report, Reglone ® is a broad spectrum herbicide for total vegetation control and one of it's main uses is application by knapsack sprayer along rights of way, fences, and railroad tracks. This study only took place at one test site in Guatemala.
- S *A sufficient number of replicates should be generated to address the exposure issues associated with the population of interest. Specifically, each study should include a minimum of 15 individuals (replicates) per activity.* This criterion was met. This study included 20 replicates.
- S *Test subjects should be regular workers, volunteers trained in the work activities required, or typical homeowners.* This criterion was met. The test subjects were regular workers with an average of 8 years of experience.
- S *The monitored activity should be representative of a typical working day for the specific task in order to capture all related exposure activities.* This criterion was mostly met. Clean-up activities were not monitored.
- S *The exposure monitoring period should be of sufficient length to ensure reasonable detectability of residues in biological media (e.g., blood and urine) consistent with pharmacokinetic data such as excretion profile, duration time, etc.* This criterion was met. The absorbed dose of diquat was measured by collecting and analyzing workers' urine for a seven day period from the day prior to exposure until 5 days after the exposure day.
- S *Biomonitoring should be conducted using methodologies based on the pharmacokinetic properties of the pesticide (parent compound and its metabolites) of concern (e.g., need validated pharmacokinetic models from humans or appropriate animal surrogate and appropriate route of exposure).* This criterion was met.
- S *Any protective clothing worn by study participants should be identified and should be consistent with the product label.* This criterion was mostly met. All workers wore long-sleeved shirt, long trousers, protective nitrile

gloves, faceshield, rubber boots, and long socks. Versar obtained a label for Diquat (EPA Reg. No. 10182-353) that specified that chemical-resistant headgear for overhead exposure, and a chemical-resistant apron be used when cleaning equipment, mixing or loading.

- S *If urine monitoring is being conducted, urine samples should be collected one or two days before participating in the applicator exposure monitoring activities and should continue on the day of exposure and for an appropriate time period after these activities have been completed, depending on the excretion kinetics of the compound. The 24-hour sample collection cycle should begin with the first void after beginning work activities and end with the first void on the following morning, continuing this 24-hour cycle on subsequent days.* This criterion was met. Complete 24-hour urine samples were collected from all test subjects over a seven day period (i.e one day prior to exposure through the morning of the sixth day after exposure).
- S *If blood monitoring is being conducted, baseline blood samples should be collected from each individual prior to exposure. Based on pharmacokinetics, postapplication exposure samples should be collected at the appropriate times before, during, and after exposure.* This criterion was not applicable. Blood monitoring was not conducted.
- S *Materials used for sample collection should not interfere with (e.g., absorb) the analytes of interest.* This criterion was met. Urine voids were made directly into clean polypropylene containers.
- S *Creatinine levels should be determined as a way of qualitatively monitoring completeness of urine collection samples. Specific gravity, as another measure of 24-hour sample completeness, should be performed as soon after collection as possible (and before sample storage).* This criterion was mostly met. Creatinine levels were determined. There was no mention of specific gravity measurements in the Study Report.
- S *Prior exposures to the test pesticide or structurally related compounds may interfere with study results. A brief history should be taken from each participant relating to known prior exposures to pesticides for at least the last 2 weeks, including reentry into potentially treated fields. For urine monitoring, there should also be a sufficient time period between such exposures and participation in the study to ensure adequate urinary clearance of the compound and its metabolites, based on pharmacokinetic data.* This criterion was mostly met. Workers were requested not to handle or have contact with diquat for a period of 5 days before and 5 days after the exposure day. One worker had detectible levels in his baseline sample.
- S *Validated analytical methods for the biological analyte (parent compound and its metabolites) of sufficient sensitivity are needed. Information on method efficiency and limit of quantitation (LOQ) should be provided.* This criterion was mostly met. Only a range of LOQ were provided (between 0.50 and 3.77 ng/L).
- S *Samples should be stored in a manner that will minimize deterioration and loss of analytes between collection and analysis. Biological monitoring samples (e.g., serum, plasma and urine) should be refrigerated or stored frozen prior to analysis. Whole blood should not be frozen. Information on storage stability should be provided.* This criterion was mostly met. Samples were stored frozen prior to analysis. Control urine was spiked with diquat and treated in an identical manner to urine samples from the exposed workers to determine the stability under field conditions. Mean overall recovery was 90.1 percent.
- S *Data should be corrected if any appropriate field fortified, laboratory fortified or storage stability recovery is less than 90 percent.* This criterion was met. Corrections were not made because field recovery was above 90 percent.
- S *Unless stability of the analyte has been established prior to initiation of the study, three samples of control (nonparticipant) should be fortified with two levels of the biological analyte (parent or metabolite(s), whichever is appropriate) for each experimental site.* This criterion was met.
- S *Each subject's absorbed dose should be expressed in terms of body weight using his/her own measured value, and as a cumulative total for each exposure period.. The arithmetic mean, range, standard deviation, and coefficient of variation should be calculated from the results of all individuals. Geometric mean, range and*

standard deviation may be calculated if the results are shown to be log-normally distributed. Other distributional data should be reported, to the extent possible (e.g., percentiles). This criterion was met.

875.1300

- *When both dermal and inhalation monitoring are required, field studies designed to measure exposure by both routes on the same subjects may be used. This criterion was met.*
- *The analytical procedure must be capable of measuring exposure to 1 ug/hr (or less, if the toxicity of the material under study warrants greater sensitivity). It is unsure whether this criterion was met.*
- *A trapping efficiency test for the monitoring media chosen must be documented. It is uncertain whether this criterion was met.*
- *Air samples should also be tested for breakthrough to ensure that collected material is not lost from the medium during sampling. It is recommended that at least one test be carried out where the initial trap contains 10X the highest amount of residue expected in the field. It is uncertain whether this criterion was met.*
- *The extraction efficiency of laboratory fortified controls is considered acceptable if the lower limit of the 95% confidence interval is greater than 75%, unless otherwise specified by the Agency. At a minimum, seven determinations should be made at each fortification level to calculate the mean and standard deviation for recovery. Total recovery from field-fortified samples must be greater than 50% for the study. These criteria were probably met. The mean recovery of diquat from glass fibres was 77 percent.*
- *If trapping media or extracts from field samples are to be stored after exposure, a stability test of the compound of interest must be documented. Media must be stored under the same conditions as field samples. Storage stability samples should be extracted and analyzed immediately before and at appropriate periods during storage. The time periods for storage should be chosen so that the longest corresponds to the longest projected storage period for field samples. This criterion was not met. There was no information in storage stability included in the study report.*
- *A personal monitoring pump capable of producing an airflow of at least 2 L/min. should be used and its batteries should be capable of sustaining maximum airflow for at least 4 hours without recharging. Airflow should be measured at the beginning and end of the exposure period. This criterion was met.*
- *Appropriate air sampling media should be selected. The medium should entrap a high percentage of the chemical passing through it, and it should allow the elution of a high percentage of the entrapped chemical for analysis. This criterion was met.*
- *If exposed media are to be stored prior to extraction, storage envelopes made from heavy filter paper may be used. The envelope must be checked for material that will interfere with analysis. Unwaxed sandwich bags should be used to contain the filter paper envelopes to help protect against contamination. It is uncertain whether this criterion was met.*
- *Personal monitors should be arranged with the intake tube positioned downward, as near as possible to the nose level of the subject. This criterion was met.*
- *Field calibration of personal monitors should be performed at the beginning and end of the exposure period. This criterion was met.*
- *Field fortification samples and blanks should be analyzed for correction of residue losses occurring during the exposure period. Fortified samples and blanks should be fortified at the expected residue level of the actual field samples. Fortified blanks should be exposed to the same weather conditions. This criterion was met.*

- *Data should be corrected if any appropriate field fortified, laboratory fortified or storage stability recovery is less than 90 percent.* This criterion was not met. Residue data was corrected where recovery values were less than 70 percent or more than 120 percent.
- *Respirator pads should be removed using clean tweezers and placed in protective white crepe filter paper envelopes inside sandwich bags. The pads should be stored in a chest containing ice until they are returned to the laboratory, where they should be stored in a freezer prior to extraction.* This criterion was met.
- *Field data should be documented, including chemical information, area description, weather conditions, application data, equipment information, information on work activity monitored, sample numbers, exposure time, and any other observations.* This criterion was met.

875.1100

- S** *The monitoring period should be of sufficient duration to result in reasonable detectability on dosimeters. Monitoring should be conducted before residues have dissipated beyond the limit of quantification. Baseline samples should be collected before the exposure activity commences.* This criterion was probably met. Baseline samples were collected and monitoring was conducted for seven days. There is no information on the limit of quantification in the Study Report.
- S** *The sampling techniques (e.g., patches, whole-body dosimeters, hand rinse, gloves, fluorescent tracer) should be appropriate to the activities being monitored. The construction materials and location (i.e., inside or outside clothing) of monitoring devices and numbers (e.g., patches) should be appropriate to the use scenario. Hand rinse solutions must be appropriate to the pesticide being evaluated (i.e., selection of aqueous surfactants vs. isopropanol or other solutions, based on the physical chemical properties of the pesticide being evaluated).* These criteria were not met. Because done concurrently with biomonitoring, the appropriateness of measuring dermal exposure via dosimetry is questionable.
- S** *Sufficient control samples should be collected.* This criterion was probably met.
- S** *Samples should be stored in a manner that will minimize deterioration and loss of analytes between collection and analyses. Information of storage stability should be provided.* This criterion was mostly met. Samples were stored frozen prior to analysis. However, there was no information on storage stability in the Study Report.
- S** *Validated analytical methods of sufficient sensitivity are needed. Information on method efficiency (residue recovery) and limit of quantification (LOQ) should be provided.* These criteria were probably met. A validated analytical method was used, however, there was no information of limit on quantitation in the Study Report.
- S** *Information on recovery samples must be included in the study report. A complete set of field recoveries should consist of at least one blank control sample and three or more each of a low-level and high-level fortification. These fortifications should be in the range of anticipated residue levels in the field study.* This criterion was probably met.
- S** *Raw residue data must be corrected if appropriate recovery values are less than 90 percent.* This criterion was not met. Corrections were made if field recovery was less than 70% or more than 120%.