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

OFFICE OF PREVENTION,
PESTICIDES AND
TOXIC SUBSTANCES

October 22, 2001

Memorandum

SUBJECT: Review of *Biomonitoring Assessment of Worker Exposure to Methyl Parathion during Cotton Scouting Following Applications of Penncap-M Microencapsulated Insecticide* (MRID No. 452047-01)

FROM: Renee Sandvig, Environmental Protection Specialist Renee Sandvig 10/23/01
Reregistration Branch II
Health Effects Division (7509C)

THROUGH: Al Nielsen, Branch Senior Scientist Al Nielsen 10/23/01
Reregistration Branch II
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TO: Laura Parsons, Chemical Review Manager
Reregistration Branch I
Special Review and Reregistration Division (7508C)

DP Barcode: D270370

Pesticide Chemical Codes: 053501

EPA MRID Numbers: 452047-01

Attached is a review of the post-application biomonitoring data submitted by Cerexagri, Inc. (formerly Elf Atochem North America, Inc.). This review was completed by Versar, Inc. on February 12, 2001, under supervision of HED. It has undergone secondary review in the HED and has been revised to reflect Agency policies.

Executive Summary

The data collected reflecting the workers exposure to methyl parathion from leaf surfaces of treated cotton meet most of the criteria specified in the U.S. Environmental Protection Agency's (US EPA) OPPTS Series 875, Occupational and Residential Exposure Test Guidelines, Group B: Postapplication Exposure Monitoring Test Guidelines, 875.2600, Biomonitoring Data. The data will be considered in future methyl parathion REDs.

Summary

The insecticide methyl parathion was applied to cotton plants in three geographical locations: California, Louisiana, and Texas. Cotton plants were treated with PENNCAP-M® Microencapsulated Insecticide, containing approximately 20.9 percent methyl parathion as the active ingredient (a.i.). The product is a flowable formulation consisting of a water suspension of polymeric-type microcapsules. The study was conducted to quantify potential worker exposure due to scouting cotton treated with methyl parathion. The cotton was treated with four ground spray applications of methyl parathion, each at an application rate of 1.0 pounds active ingredient (ai) per acre.

Volunteer study subjects performed a single day of cotton scouting either four days (Texas and Louisiana) or five days (California) after the last PENNCAP-M® application, when the cotton plants were 8 to 14 inches tall. There was approximately 4.5 hours of in-field exposure time, interrupted by 5 break-times, during which study subjects washed their hands. [Study subjects spent about 8 hours in their work clothing. The study subjects wore identical, new clothing provided by the study coordinator. Work clothing consisted of: long-sleeved shirts, undershirt, long pants, underwear, socks, and hat. All subjects wore closed shoes. Gloves were not worn by the study subjects.] In this study, methyl parathion exposure was quantified by measuring total 4-nitrophenol (4-NP) and its sulfate and glucuronide conjugates in urine samples (the analytical method hydrolyzes these conjugates to 4-nitrophenol equivalents). Twenty-four hour urine samples were collected for two days prior through 3 days after exposure, or 6 days total.

In general, 4-nitrophenol concentrations in urine measured on the day of exposure to treated cotton rose to average 6 fold higher than pre-screening baseline values in Louisiana and California. Urinary 4-nitrophenol levels doubled in Texas. Urinary 4-Nitrophenol levels declined quickly within 24 hours, returning to levels at or below pre-screening baseline values by Day 3 after exposure.

Conclusions

The study was in compliance with the major technical aspects of OPPTS Series 875 guidelines. There were issues and limitations of the data identified below.

- Two California workers, subjects #1 and #7, 4-NP values that were lower after exposure than before the exposure event.

- Creatinine levels seem to have been unusually low, relative to the other workers, on the days after exposure in two California workers and one Texas worker. The two California workers, subjects #1 and #10, had creatinine levels of 0.548 g/24hrs (levels ranged from 1.24 to 3.05 g/24hrs on the other monitoring days) and 0.300 g/24hrs (levels ranged from 1.01 to 2.49 g/24hrs on the other monitoring days), respectively. The Texas worker, subject # 5, had a creatinine level of 0.910 g/24hrs (levels ranged from 2.61 to 3.96 g/24hrs on the other monitoring days).

MEMORANDUM

TO: Renee Sandvig cc: 1000.001.01
Margarita Collantes

FROM: Diane Forrest/Pat Wood/ Susan Anderson

DATE: February 12, 2001

SUBJECT: Review of *Biomonitoring Assessment of Worker Exposure to Methyl Parathion During Cotton Scouting Following Applications of PENNCAP-M® Microencapsulated Insecticide* (MRID No. 452047-01)

This report reviews *Biomonitoring Assessment of Worker Exposure to Methyl Parathion During Cotton Scouting Following Applications of PENNCAP-M® Microencapsulated Insecticide*, submitted in support of re-registration requirements for the insecticide active ingredient methyl parathion. The requirements for this study are specified by the U.S. Environmental Protection Agency (US-EPA) in OPPTS Series 875 Occupational and Residential Exposure Test Guidelines, Group B: Postapplication Exposure Monitoring Test Guidelines 875.2600, and Parts C & D. The following information may be used to identify the Study:

Title:	*Biomonitoring Assessment of Worker Exposure to Methyl Parathion During Cotton Scouting Following Applications of PENNCAP-M® Microencapsulated Insecticide,* 330 pages
Sponsor:	Cerexagri, Inc. (formerly Elf Atochem North America, Inc.) Agrichemicals Division 2000 Market Street, 21st Floor Philadelphia, PA 19103-3222
Field Phase Investigators::	1) David Ennes, Research for Hire, Inc., 1696 S. Leggett St., Porterville, TX 93257 2) Nelson Prochaska, R&D Research, Inc., 7033 Hwy. 103, Washington, LA 70589 3) Mike Phillips, South Texas Ag. Center, Uvalde Div., Benson Loop Rd., Uvalde, TX 78802
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Report Date:	August 31, 2000 (Corrected Final)
Identifying Codes:	MRID No. 452047-01; AASI Study No. KP-2000-02; Morse Project No. ML00-0837-ATO; Draft Protocol MRID No. 449842-04

EXECUTIVE SUMMARY

The purpose of this study was to quantify potential worker exposure due to scouting cotton treated with the restricted use, organophosphate insecticide methyl parathion, formulated as a 20.9 percent product in PENNCAP-M® Microencapsulated Insecticide. Four ground spray applications were made 5 days apart at three geographic locations. Volunteer study subjects performed a single day of cotton scouting either four days (Texas and Louisiana) or five days (California) after the last PENNCAP-M® application, when the cotton plants were 8 to 14 inches tall. There was approximately 4.5 hours of in-field exposure time, interrupted by 5 break-times, during which study subjects washed their hands. [Study subjects spent about 8 hours in their work clothing.] In this study, methyl parathion exposure was quantified by measuring total 4-nitrophenol and its sulfate and glucuronide conjugates in urine samples (the analytical method hydrolyzes these conjugates to 4-nitrophenol equivalents). Twenty-four hour urine samples were collected for two days prior through 3 days after exposure, or 6 days total.

The target application rate (per application) was 1.0 lbs. ai/A, which was the maximum application rate specified on the label. The application volume ranged between 10 and 18 gallons/acre. The label only recommended minimum dilution volume for aerial application (2 gallons/acre) not ground application, which was the application method used in this study. [The Study Protocol specified an application volume of no more than 20 gallons/acre (±5 percent).]

While scouting cotton, study subjects wore identical, new clothing provided by the study coordinator. This consisted of: long-sleeved shirts, undershirt, long pants, underwear, socks, and hat. All subjects wore closed shoes. Gloves were not worn by the study subjects.

In general, 4-nitrophenol concentrations in urine measured on the day of exposure to treated cotton rose to average 6 fold higher than pre-screening baseline values in Louisiana and California. Urinary 4-nitrophenol levels doubled in Texas. Urinary 4-Nitrophenol levels declined quickly within 24 hours, returning to levels at or below pre-screening baseline values by the 3rd day after exposure. The study author calculated residue biological half-life with Microsoft Excel® 2000, plotting the natural logarithms of arithmetic means of five field data points (µg/kg body weight) against time (Day 0 - day of exposure, and Days 1, 2, and 3 post-exposure). First order kinetics were assumed. Predicted half-lives were: (1) *California*: 0.7 days ($R^2 = 0.86$); (2) *Louisiana*: 1.1 days ($R^2 = 0.71$); and (3) *Texas*: 0.8 days ($R^2 = 0.82$).

The study followed the OPPTS Series 875 Occupational and Residential Exposure Test Guidelines, Group B: Postapplication Exposure Monitoring Test Guidelines 875.2600, and Parts C & D in most respects. The following issues of potential concern were identified:

- Overall average fortified field control recovery and overall average concurrent laboratory control recovery values for 4-nitrophenol and its' glucuronide and sulfate conjugates were both less than 90 percent. The data were corrected for field recovery values.

- Two California workers, subjects #1 and #7, 4-NP values that were lower after exposure than before the exposure event.

- Creatinine levels seem to have been unusually low, relative to the other workers, on the days after exposure in two California workers and one Texas worker. The two California workers, subjects #1 and #10, had creatinine levels of 0.548 g/24hrs (levels ranged from 1.24 to 3.05 g/24hrs on the other monitoring days) and 0.300 g/24hrs (levels ranged from 1.01 to 2.49 g/24hrs on the other monitoring days), respectively. The Texas worker, subject # 5, had a creatinine level of 0.910 g/24hrs (levels ranged from 2.61 to 3.96 g/24hrs on the other monitoring days).

- At the California site, one application of an herbicide was made just before the study period and one application of an insecticide was made during the study period. At the Louisiana site, two applications of fungicides, three applications of a plant growth regulator, one application of an insecticide (an organophosphate), and two applications of herbicides were applied just before the study period. At the Texas site, one application of an insecticide (an organophosphate) and two applications of herbicides were made just before the study period. One application of an herbicide was applied during the study period.

STUDY REVIEW

Study Background

The purpose of this study was to quantify potential worker exposure due to scouting cotton treated with the restricted use, organophosphate insecticide methyl parathion (i.e., O,O-dimethyl O-p-nitrophenylphosphorodithioate, CAS No. 298-00-0). Methyl parathion was formulated as a 20.9 percent product in PENNCAP-M® Microencapsulated Insecticide. Four ground spray applications were made 5 days apart at three geographic locations. Volunteer study subjects performed a single day of cotton scouting either four days (Texas and Louisiana) or five days (California) after the last PENNCAP-M® application, when the cotton plants were 8 to 14 inches tall.

In this study, methyl parathion exposure was quantified by measuring 4-nitrophenol and its sulfate and glucuronide conjugates in urine samples (the analytical method hydrolyzes these conjugates to 4-nitrophenol equivalents). Twenty-four hour urine samples were collected for a total of 6 days (i.e., the 2 days prior to exposure, the day of exposure, and for 3 days following).

Study Subjects

The study subjects were "healthy Caucasian or Hispanic, males or females, ranging 19 to 53 years old, 62.5 to 76 inches in height, 116 to 285 pounds (53 to 129 kg) in weight, and had

experience as scouts/agricultural laborers." Worker experience varied in duration from 1 month to 10 years. "All workers read and signed Informed Consent forms. The workers were sequestered in a hotel beginning on the evening prior to the beginning of the minus-2 days urine collection period. [They] remained in the hotel (except to eat meals) during the entire monitoring period except on the day of exposure (cotton scouting) until the morning of the fourth day after the exposure event."

The study subjects were warned to avoid exposing themselves to a list of substances, e.g. polishes, paint solvents, any product containing almond essence, and certain drugs (see page 304 of the Study Report for a complete list).

Protective Clothing & Work Practices

While scouting cotton, study subjects wore identical, new clothing provided by the study coordinator. This consisted of: long-sleeved shirts, undershirt, long pants, underwear, socks, and hat. All subjects wore closed shoes. Gloves were not worn by the study subjects.

Test Site

The study was conducted at three test sites: 1) near Porterville, CA; 2) near Washington, LA; and 3) near Uvalde, TX. Test site diagrams may be found on pages 92-95 of the Study Report. One large treated plot was established at each test site. Concurrent dislodgeable foliar residue studies were conducted on separate treated and control plot areas.

Porterville, CA, Test Site

The treated plot (171 feet wide and 1,360 feet long) consisted of 54 rows of cotton, spaced 38 inches apart, and planted on a 0.5 percent slope. The soil was loamy fine sand. The prevailing wind was from the Northwest. The cotton crop (var. *Delta Pine 6102*) had been planted on May 4, 2000 The cotton plants were 14 inches tall by the time of the last application.

Washington, LA, Test Site

The treated plot (198 feed wide and 976 feet long) was planted on a 0.25 percent slope, and the soil was silty clay loam. The cotton crop (var. *Suregrow 821*) had been planted on March 23, 2000 The cotton plants were 12 inches tall by the time of the last application.

Uvalde, TX, Test Site

The treated plot (267 feet wide and 800 feet long) consisted of 5 blocks of growing cotton plants, each approximately 50 feet wide, and each one assigned to a designated cotton scout. The test site was planted on a 1.0 percent slope. The soil was clay loam. The prevailing wind was

from the East. The cotton crop (var. *SureGrow 125*) had been planted on March 30, 2000. The cotton plants were 8 inches tall by the time of the last application.

Crop Maintenance & Pesticide Use History

"Crop and pesticide use history for the three years prior to test substance application were documented in the field trial notebook." During the trial period, "the plots were maintained as per normal agronomic practice for cotton production. The test plots were not treated with any organophosphate pesticides during the trial period." The pesticides used at each test site between March and mid-June 2000 are listed below (application rates are listed on page 62 of the Study Report).

- *California* - Treflan® was applied before the study period and Kelthane® was applied during the study period
- *Louisiana* - Temik®, Ridomil®, PCNB®, Prowl®, Cotoran® and the plant growth regulator PGR IV® were all applied before the study period.
- *Texas* - Temik® 15G, Caparol®4L, and Assure® II were applied before the study period and Fusilade® DX was applied during the study peiod.

Materials and Application Methodology

A copy of the product label for PENNCAP-M® Microencapsulated Insecticide [EPA Reg. No. 4581-393] was provided for review. The product contained 20.9 percent methyl parathion at 2 lbs. ai/gallon. It is a flowable formulation consisting of a water suspension of polymeric-type microcapsules containing the active ingredient. In this study, PENNCAP-M® was applied at the maximum labeled application rate for cotton plants. The application volume ranged between 16.06 and 18.07 gallons/acre in California, between 10.01 and 11.26 gallons/acre in Louisiana, and between 10.43 and 10.71 in Texas.

Four applications, at 5-day intervals, were made before exposure monitoring of the cotton scouting operation began.[1] Applications were made using tractor drawn groundspray equipment. In California, 12 hollow cone nozzles were used, and the total swath width was 19 feet. In Louisiana, 12 flat fan nozzles were used, and the total swath width was 18 feet. In Texas, 16 flat fan nozzles were used, and the total swath width was 26.7 feet. No overhead irrigation was used. The spray equipment was calibrated before each application using the volume/time or time over plot method. The author states that: "The spray equipment was calibrated based on the distance and speed traveled and the total spray output at a given operating speed and pressure over a measured amount of time."

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[1] **California**: June 6, 11, 16, and 21, 2000
Louisiana: May 12, 17, 22, 27, 2000
Texas: May 7, 12, 17, and 23, 2000.

Work Performed

Five study subjects scouted cotton at each of the three test sites. "Work began at approximately 0600 hours. A full-day work cycle consisted of six scouting cycles. A scouting cycle was 60 to 75 minutes in duration of which 45 minutes was spent in the field actively moving through the field and touching plants, followed by a 15 to 30 minutes out-of-the-field break period.... At the end of the sixth scouting cycle workers remained in their work clothing for another two hours before bathing and changing into clean clothing.... Work finished at approximately noon (4.5 hours of in-field exposure). The only exception to this scouting exposure scenario was for Worker #1 at the LA site who... scouted 15 minutes less (4.25 hours of in-field exposure)." The author states that the total time spent in work clothing was "at least 8 hours" after the initiation of the exposure. Clothing worn by each worker was bagged and held after use.

It is noted that during most of the break periods, workers would consume liquids/food or take bathroom breaks, and each time, they washed their hands first with soap and water. Urine was collected during the breaks. Worker observations are provided on pages 70-72 of the Study Report, summarizing environmental conditions and work observations.

Environmental Conditions

On each application day, the following parameters were reported (see page 66 of the Study Report): application time, wind speed and direction, percent cloud cover, percent relative humidity, air temperature, soil temperature and general soil moisture conditions at the surface and subsurface. [On page 71 of the Study Report, it was noted that dew was present for a short time on the Louisiana test site.] The author states that data were either collected with onsite instruments or off-site weather station data were used. Summarizing conditions reported on the final application day at each test site:

- *California*: PENNCAP-M® was applied between 0825 and 0925 hrs. Wind speed was 1.6 to 2.7 mph from the SW/NE. There was no cloud cover, and ambient temperature was 88° F. Percent relative humidity was 40 percent. Soil temperatures at 2 and 4 inches were 92° F. and 85°F, and soil was dry. Cotton foliage was 14 inches high.

- *Louisiana*: PENNCAP-M® was applied between 0645 and 0713 hrs. Winds were calm. There was 20 percent cloud cover, and ambient temperature was 76° F. Percent relative humidity was 64 percent. Soil temperatures at 2 and 4 inches were 70° F. and 78° F, and soil was dry. Cotton foliage was 12 inches high.

- *Texas*: PENNCAP-M® was applied between 0645 and 0715 hrs. Winds were calm. There was no cloud cover, and ambient temperature was 71°F. Percent relative humidity was 40 percent. Soil temperatures at 2 and 4 inches were 72°F and 74°F, and soil was dry. Cotton foliage was 8 inches high.

Historical weather data were provided (see page 68 of the Study Report), but it is not known what period these data cover. Monthly maximum and minimum air temperatures were normal. The author states that in California and Texas, there were "no significant departure from the normal air temperature and historical rainfall amounts during the trial period... The Louisiana climatological data indicate that there was significantly less rainfall than normal..." No overhead irrigation was applied after the PENNCAP-M® applications and sampling began.

Urine Sample Collection

Study subjects were provided every morning with pre-weighed and coded 3-Liter UriSafe™ urine collection containers and a cooler with blue ice packs. New blue ice packs were provided each evening. Filled UriSafe™ containers were collected the following morning. New coolers, fresh ice packs, and UriSafe™ containers were then provided for the next 24-hour period. Samples (24-hour) were collected at minus-2 days, minus-1 day, day of exposure (Day 0), and Day 1, 2, and 3 after exposure. Each day's samples were transported to the field facility for processing and storage.

QA/QC

Sample Storage and Handling

Each day's urine sample was allowed to come to ambient temperature, acidified with hydrochloric acid (to free any conjugated residue), weighed, and the approximate volume recorded. The specific gravity was determined by weighing an aliquot of urine. Next, 100 mL aliquots of each acidified sample were placed into labeled amber HPDE bottles. Urine samples were stored frozen at the field facility until shipment to Morse Laboratories, Inc. via Federal Express courier service on dry ice. The balance of all the urine samples was shipped to Elf Atochem N.A. via ACDS freezer truck for storage.

Sample History

Sample history information was provided from date/time of sample collection through dates of sample analyses (see pages 79 to 90 and 139 to 146 of the Study Report). From sampling to analysis, field urine samples (N=115 samples) were stored as follows: (1) *Texas*: from 7 to 78 days prior to analysis; (2) *Louisiana*: from 4 to 16 days prior to analysis; and (3) *California*: from 9 to 43 days prior to analysis. Field-fortified samples (N= 108 samples) were stored as follows: (1) *Texas*: from 24 to 67 days prior to analysis; (2) *Louisiana*: from 54 to 69 days prior to analysis; and (3) *California*: from 31 to 40 days prior to analysis. Field control samples (N= 54 samples) were stored similarly.

Tank Mix Samples

Tank mix samples were collected. However, according to a Protocol Amendment 13 (see page 322 of the Study Report), they were not analyzed because "analysis of the samples was not deemed necessary to meet the study objectives."

Analytical Methodology

1. Analysis of 4-Nitrophenol

A proprietary method was used, Morse Laboratories Inc.'s SOP# Meth-120, entitled "*Determination of 4-Nitrophenol in Urine*," Revision #3, dated April 28, 2000. Briefly summarizing the principle of the method, sodium bisulfate and concentrated HCl were added to 8 mL of urine. Acid hydrolysis was performed at 100°C for 1 hour. A 4.5 mL aliquot of the hydrolyzate was extracted into toluene. The volume of the extract was concentrated, and derivatized with N-(tert-Butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA). The derivatized sample was further concentrated, and analyzed for the 4-nitrophenol tert-butyldimethylsilyl derivative using gas chromatography with mass selective detection. The retention time for this analyte was approximately 15 minutes. The target limit of quantitation (LOQ) was 1.0 µg/L and the target limit of detection (LOD) was 0.3 µg/L 4-nitrophenol or equivalent. Peaks were reasonably sharp.

2. Analysis of Creatinine

A proprietary method was used, Morse Laboratories Inc.'s Analytical Method No. Meth-111, entitled "*Quantitative Determination of Creatinine in Urine*," Original Revision, dated June, 1998. Briefly summarizing the principle of this colorimetric method, an aliquot of urine is reacted with an alkaline picric acid reagent in the presence of sodium borate to form an amber-colored creatinine-picrate complex. The concentration of creatinine in the sample is calculated against a known creatinine standard concentration based on absorbance of the resulting complex at 520 nm. The sensitivity of the method is 0.6 mg/dL based on instrument resolution of 0.01 absorbance units. Creatinine was reportedly stable in urine for 3 days at room temperature and for at least 5 days in the refrigerator.

Limits of Detection (LOD) and Limits of Quantitation (LOQ)

For 4-nitrophenol and its conjugates, the target limit of quantitation (LOQ) was 1.0 µg/L and the target limit of detection (LOD) was 0.3 µg/L 4-nitrophenol or, in the case of conjugates, 4-nitrophenol equivalents. For creatinine, the method sensitivity was 0.6 mg/dL based on instrument resolution of 0.01 absorbance units.

Compositing of Control Urine

Elf Atochem N.A. provided a specific procedure for compositing control urine whenever more than one container was used for collection (see page 210, Appendix III of the Study Report). Field samples were never collected in more than two containers. Most samples selected to be controls required compositing.

Concurrent Laboratory Recovery

Urine used for the preparation of concurrent laboratory (or procedural) controls came from two different sources: (1) workers prior to exposure from this study; and (2) workers prior to exposure from another study (Study No. KP-99-15). Urine for laboratory control use was "considered acceptable ... if its endogenous content of 4-NP (corrected for reagent blank) was less than approximately 1 µg/L.... all control urine samples were found to contain less than 1.2 µg/L 4-NP, except for three samples which contained 1.56, 1.66, and 1.90 µg/L, respectively."

Four fortification levels for 4-nitrophenol were analyzed: 1 µg/L (N=31); 50 µg/L (N=2); 100 µg/L (N=31); and 156 µg/L (N=2). Two fortification levels for the glucuronide conjugate were analyzed: 22.7 µg/L (N=31) and 114 µg/L (N=4). One fortification level for the sulfate conjugate was analyzed: 92 µg/L (N=4).

The overall concurrent (or procedural) laboratory recovery for 4-nitrophenol averaged 84 ± 15 percent (N=65), ranging from 61 to 125 percent. The overall concurrent laboratory recovery for 4-nitrophenol glucuronide conjugate averaged 80 ± 8.8 percent (N=35), ranging from 61 to 101 percent. The overall concurrent laboratory recovery for 4-nitrophenol sulfate conjugate averaged 85 ± 2.2 percent (N=4), ranging from 82 to 87 percent.

Fortified Field Recovery

Field-fortified samples were prepared three times at each test site, that is, on the day of exposure, Day +1 and Day +2 after exposure. The fortification levels were: 0, 2, 10, and 100 µg/L 4-nitrophenol, and six samples were prepared at each level on each of the three days.[2] No field-fortified recovery samples were prepared using either the glucuronide or the sulfate conjugates of 4-nitrophenol, or creatinine. The overall field fortified recovery averages were as follows: (1) *Texas*: 87 ± 11 percent (N=36), ranging from 63 to 111 percent; (2) *Louisiana*: 82 ± 18 percent (N=36), ranging from 49 to 117 percent; (3) *California*: 78 ± 13 percent (N=36), ranging from 49 to 113 percent. Fortification levels chosen corresponded well to the range of 4-nitrophenol levels found in field data. Only the field fortified samples prepared at the 10 µg/L 4-nitrophenol level were used to correct the data, since all of the field samples were closest to the

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[2] Originally, a 2 µg/L fortification level was to have been included, however the fortification solutions used to prepare these samples were prepared incorrectly at two test sites, and results at a third test site were invalidated because the blanks contained higher levels.

10 µg/L 4-nitrophenol fortification level. According to the 875 series guidelines, data should be corrected for all field recoveries less than 90 percent. Therefore, the biomonitoring data from the Lousiana and California sites were corrected. See Table 1 for a summary of the individual and overall field fortification recoveries.

Table 1. Summary of Field Fortification Recoveries*

Test Site	Fortification Level (µg/L)	Recovery* (percent)
Texas	10 100	90 ± 9.3 (N=18) 84 ± 11.8 (N=18)
	Overall Average	87 ± 10.8 (N=36)
Louisiana	10 100	82 ± 23.9 (N=18) 82 ± 10.9 (N=18)
	Overall Average	82 ± 18.3(N=36)
California	10 100	75 ± 15.9 (N=18) 82 ± 9.7 (N=18)
	Overall Average	78 ± 13.5 (N=36)

* Corrected for average procedural (laboratory) recovery within the analytical set.

Storage Stability Testing

Two storage stability tests were performed. First, the effect of storing urine samples in coolers was examined using the field fortified controls reviewed in the preceding section. Of the six field-fortified samples, three were "immediately placed in frozen storage" (referred to as "travelers" in the study), and "the other three were placed in a cooler with blue ice to mimic the storage conditions experienced by the worker-generated urine samples." These latter were referred to as "exposed spikes." The latter were kept on blue ice for between 24 and 30 hours, then placed in freezer storage. [The longest interval urine field samples were stored on blue ice was 30 hours.]

Results from this test with "travel" field-fortified samples yielded mean recoveries of: (1) *Texas*: 90 ± 12 percent (N=18), range = 67 to 111 percent); (2) *Louisiana*: 78 ± 21 percent (N=18), range = 49 to 115 percent); (3) *California*: 84 ± 13 percent (N=18), range = 62 to 113 percent).

Mean recoveries for "exposed" field-fortified controls were: (1) *Texas*: 84 ± 9.5 percent (N=18), range = 63 to 98 percent); (2) *Louisiana*: 87 ± 15 percent (N=18), ranging between 65 and 117 percent; (3) *California*: 73 ± 12 percent (N=18), ranging between 49 and 87 percent. [These values were corrected for procedural recovery.]

Secondly, the analytical report presents results of a test in which control urine samples were fortified with 50 µg/L 4-nitrophenol, and its glucuronide and sulfate conjugates, each in duplicate. These samples were placed in frozen storage at -20° ± 5° C., and then analyzed at 0, 30 and 31 days. [Note: Protocol Amendment #11 stated that previously planned 60 day storage interval samples were not to be conducted, because 30 day storage samples covered the "longest storage interval for field generated samples."] Average storage stability recoveries (corrected for procedural recovery) were: (1) *4-Nitrophenol*: 108 percent at 31 days; (2) *4-NP Glucuronide*: 92 percent at 30 days; and (3) *4-NP Sulfate*: 90 percent at 30 days.

Otherwise, the study author relied on the field-fortified controls for storage stability information. From the day of sampling to the day of analysis, field urine samples (N=115 samples) were stored as follows: (1) *Texas*: from 7 to 78 days prior to analysis; (2) *Louisiana*: from 4 to 16 days prior to analysis; and (3) *California*: from 9 to 43 days prior to analysis. Field-fortified samples (N=108 samples) were stored as follows: (1) *Texas*: from 24 to 67 days prior to analysis; (2) *Louisiana*: from 54 to 69 days prior to analysis; and (3) *California*: from 31 to 40 days prior to analysis. Field control samples (N= 54 samples) were stored similarly. The analytes appear to have been stable in urine through cooler/freezer storage.

CALCULATIONS

General statistical calculations were limited to calculation of the mean, range, and standard deviation using SYSTAT®, version 8.0. Field data were typically corrected using the field blank. The field data was also corrected for field recovery when the recovery value was less than 90 percent for the corresponding fortification level.

4-Nitrophenol Concentrations

Samples were analyzed in groups, each of which contained a reagent blank, a control sample, three fortified control samples (two containing 4-nitrophenol: 1 at the LOQ, and 1 at a higher level, plus 1 sample containing 4-nitrophenol glucuronide conjugate), and up to 12 field samples.

Peak response was converted to concentration units (µg/mL) using a four point standard curve, generating a power curve equation (i.e., $Y = Ax^b$), where y = peak response, x = concentration, and A, b are dependent variables. Next, the concentration in a specific urine sample was calculated using the equation which appears on page 123 of the Study Report, adjusting for dilutions. All sample results were corrected using the appropriate reagent blank.

The study author noted that: "Calculation of [glucuronide] conjugate concentrations applies to the determination of their recoveries only. Both the fortified control samples and their respective control samples were calculated in the same manner..." This involved using, a molecular weight conversion factor for the p-nitrophenol beta-d-glucuronide conjugate of 2.27 and 1.85 for p-nitrophenyl sulfate, relative to the 4-nitrophenol value of 1.00.

Creatinine Calculations

These samples were also analyzed in groups, each of which contained one fortified control sample, plus up to 36 field samples. The authors used the GraphPad Prism® software program to generate a standard curve for creatinine concentration (mg/dL) versus absorbance at 520 nm. Raw data were entered into a Microsoft Excel® 97 spreadsheet for analysis, according to the equation appearing on page 128 of the Study Report. The amount (grams) of creatinine produced by a study subject in a 24 hour period was reported.

RESULTS

In general, 4-nitrophenol concentrations in urine measured on the day of exposure to treated cotton rose to an average value of more than 6 time baseline values in Louisiana and California. Urinary 4-nitrophenol levels doubled in Texas. Two California workers (Subjects #1 and #7) seemed to have received very little exposure during cotton scouting, when compared to pre-exposure levels. Urinary 4-Nitrophenol levels declined quickly within 24 hours, returning to at or below pre-screening baseline values by Day 3 after exposure. See Tables 2-4 for a summary of these data for California, Louisiana, and Texas, respectively.

The study author calculated residue biological half-lives with Microsoft Excel® 2000, plotting the natural logarithms of arithmetic means of five field data points (µg/kg body weight) against time (Day 0 - day of exposure, and Days 1, 2, and 3 post-exposure). First order kinetics were assumed. Predicted half-lives were: (1) *California*: 0.7 days ($R^2 = 0.86$); (2) *Louisiana*: 1.1 days ($R^2 = 0.71$); and (3) *Texas*: 0.8 days ($R^2 = 0.82$).

Creatinine measured in each day's urine sample from the study subjects during the six day monitoring periods ranged from 0.910 to 4.38 g/24-hr (Texas), 1.03 to 5.93 g/24-hr (Louisiana), and 0.548 to 3.47 g/24-hrs (California). Creatinine levels seem to have been unusually low, relative to the other workers, on the days after exposure in two California workers and one Texas worker. The two California workers, subjects #1 and #10, had creatinine levels of 0.548 g/24hrs (levels ranged from 1.24 to 3.05 g/24hrs on the other monitoring days) and 0.300 g/24hrs (levels ranged from 1.01 to 2.49 g/24hrs on the other monitoring days), respectively. The Texas worker, subject # 5, had a creatinine level of 0.910 g/24hrs (levels ranged from 2.61 to 3.96 g/24hrs on the other monitoring days). [Specific gravity and urine weight and volume measurements were made at the field test facility. These data may be found on pages 73 to 78 of the Study Report.]

Table 2. Corrected 4-NP Residues in Cotton Scout Urine Samples - CA Site

Parameters	Sampling Interval = 0 Day			
	#Rep	Arith. Mean	Std. Dev.	Geo. Mean
Gross 4-NP (μg/L)	5	24.4661	17.3595	14.4123
Net 4-NP (μg/L)	5	19.8075	17.5247	8.6142
Gross 4-NP (total μg)	5	39.4630	22.4416	30.6150
Net 4-NP (total μg)	5	30.0686	25.0312	21.9820
Gross 4-NP (μg/kg weight)	5	0.5536	0.2830	0.4552
Net 4-NP (μg/kg weight)	5	0.4100	0.3295	0.3253
Net 4-NP (μg/70 kg weight)	5	28.6983	23.0649	22.7701
Creatinine (g/24 hr)	5	0.9776	0.5293	0.8302
	Sampling Interval = +1 Day			
Gross 4-NP (μg/L)	5	9.01844	4.9596	6.9760
Net 4-NP (μg/L)	5	4.2892	3.8054	6.0098
Gross 4-NP (total μg)	5	11.7304	3.5630	10.9997
Net 4-NP (total μg)	5	3.8132	3.4848	5.0556
Gross 4-NP (μg/kg weight)	5	0.1712	0.0467	0.1636
Net 4-NP (μg/kg weight)	5	0.05253	0.0489	0.06893
Net 4-NP (μg/70 kg weight)	5	3.6758	3.4239	4.8295
Creatinine (g/24 hr)	5	1.8660	0.3983	1.8332
	Sampling Interval = +2 Day			
Gross 4-NP (μg/L)	5	7.6985	2.7122	7.0389
Net 4-NP (μg/L)	5	3.0159	2.0435	2.9877
Gross 4-NP (total μg)	5	12.1357	3.0596	11.5246
Net 4-NP (total μg)	5	3.8559	2.2563	4.4051
Gross 4-NP (μg/kg weight)	5	0.1855	0.0576	0.1713
Net 4-NP (μg/kg weight)	5	0.05867	0.0355	0.06520
Net 4-NP (μg/70 kg weight)	5	4.1102	2.4834	4.5631
Creatinine (g/24 hr)	5	2.5880	0.5424	2.5458
	Sampling Interval = +3 Day			
Gross 4-NP (μg/L)	5	4.2799	1.5594	3.8938
Net 4-NP (μg/L)	5	0.5987	0.6731	1.4046
Gross 4-NP (total μg)	5	7.8985	2.3527	7.4168
Net 4-NP (total μg)	5	0.9680	1.0121	1.2436
Gross 4-NP (μg/kg weight)	5	0.1213	0.0436	0.1103
Net 4-NP (μg/kg weight)	5	0.01413	0.0144	0.0189
Net 4-NP (μg/70 kg weight)	5	0.9898	1.0055	1.3218
Creatinine (g/24 hr)	5	2.3040	0.5323	2.2552

Footnotes:

Arith. Mean = arithmetic mean (average) Geo. Mean = Geometric mean

Std. Dev. = Standard Deviation Gross 4-NP = Total 4-NP found in sample

Net 4-NP = Gross 4-NP - (Average 4-NP found in samples collected at -2 and -1 Day)

4-NP data corrected for field recovery of 75% at the 10 μg/L fortification level.

16

Table 3. Corrected 4-NP Residues in Cotton Scout Urine Samples - LA Site

Parameters	Sampling Interval = 0 Day			
	#Rep	Arith. Mean	Std. Dev.	Geo. Mean
Gross 4-NP (μg/L)	5	29.4487	17.1617	23.6119
Net 4-NP (μg/L)	5	24.5085	17.1060	17.6983
Gross 4-NP (total μg)	5	35.1951	9.8571	33.6673
Net 4-NP (total μg)	5	27.8341	10.9197	25.3621
Gross 4-NP (μg/kg weight)	5	0.4196	0.1615	0.3773
Net 4-NP (μg/kg weight)	5	0.3413	0.1620	0.2843
Net 4-NP (μg/70 kg weight)	5	23.8931	11.3401	19.8953
Creatinine (g/24 hr)	5	3.3380	0.6043	3.2958
	Sampling Interval = +1 Day			
Gross 4-NP (μg/L)	5	8.6463	4.4291	7.1738
Net 4-NP (μg/L)	5	3.7061	4.4963	1.0861
Gross 4-NP (total μg)	5	11.4146	4.9275	9.9638
Net 4-NP (total μg)	5	4.3061	4.5896	4.2006
Gross 4-NP (μg/kg weight)	5	0.1311	0.0697	0.1117
Net 4-NP (μg/kg weight)	5	0.05488	0.0574	0.0584
Net 4-NP (μg/70 kg weight)	5	3.8380	4.0173	4.0896
Creatinine (g/24 hr)	5	2.8420	1.0643	2.6012
	Sampling Interval = +2 Day			
Gross 4-NP (μg/L)	5	7.2854	3.0148	6.4832
Net 4-NP (μg/L)	5	2.6817	2.5740	2.1530
Gross 4-NP (total μg)	5	11.2927	3.6268	10.5567
Net 4-NP (total μg)	5	4.2780	4.1174	3.8973
Gross 4-NP (μg/kg weight)	5	0.1274	0.0459	0.1183
Net 4-NP (μg/kg weight)	5	0.0518	0.0488	0.0501
Net 4-NP (μg/70 kg weight)	5	3.6243	3.4130	3.5113
Creatinine (g/24 hr)	5	2.7340	0.8336	2.6346
	Sampling Interval = +3 Day			
Gross 4-NP (μg/L)	5	7.3634	2.4709	6.8678
Net 4-NP (μg/L)	5	2.5427	1.8412	2.7023
Gross 4-NP (total μg)	5	10.1683	3.7504	9.2588
Net 4-NP (total μg)	5	3.2549	3.8650	3.8488
Gross 4-NP (μg/kg weight)	5	0.1157	0.0490	0.1038
Net 4-NP (μg/kg weight)	5	0.0412	0.0456	0.0535
Net 4-NP (μg/70 kg weight)	5	2.8837	3.1923	3.7471
Creatinine (g/24 hr)	5	2.6380	0.9650	2.4382

Footnotes:

Arith. Mean = arithmetic mean (average) Geo. Mean = Geometric mean

Std. Dev. = Standard Deviation Gross 4-NP = Total 4-NP found in sample

Net 4-NP = Gross 4-NP - (Average 4-NP found in samples collected at -2 and -1 Day)

4-NP data corrected for field recovery of 82% at the 10 μg/L fortification level.

Table 4. 4-NP Residues in Cotton Scout Urine Samples - TX Site

Parameters	Sampling Interval = 0 Day			
	#Rep	Arith. Mean	Std. Dev.	Geo. Mean
Gross 4-NP (μg/L)	5	5.1700	3.1849	4.5017
Net 4-NP (μg/L)	5	2.9135	2.4828	3.2306
Gross 4-NP (total μg)	5	10.2600	3.5769	9.7132
Net 4-NP (total μg)	5	6.7908	4.4422	5.0542
Gross 4-NP (μg/kg weight)	5	0.1031	0.0241	0.1007
Net 4-NP (μg/kg weight)	5	0.0667	0.0383	0.0524
Net 4-NP (μg/70 kg weight)	5	4.6671	2.6788	3.6675
Creatinine (g/24 hr)	5	3.1000	1.0283	2.9049
	Sampling Interval = +1 Day			
Gross 4-NP (μg/L)	5	3.6840	0.7310	3.6247
Net 4-NP (μg/L)	5	1.1624	0.9637	0.7974
Gross 4-NP (total μg)	5	4.1340	2.6295	3.3057
Net 4-NP (total μg)	5	1.4360	2.1402	3.3853
Gross 4-NP (μg/kg weight)	5	0.0425	0.0274	0.0343
Net 4-NP (μg/kg weight)	5	0.0143	0.0225	0.0320
Net 4-NP (μg/70 kg weight)	5	0.9997	1.5753	2.2432
Creatinine (g/24 hr)	5	2.5760	1.3367	2.2245
	Sampling Interval = +2 Day			
Gross 4-NP (μg/L)	5	1.8382	0.9211	1.6254
Net 4-NP (μg/L)	5	0.0000	0.0000	---
Gross 4-NP (total μg)	5	3.2980	0.8883	3.1983
Net 4-NP (total μg)	5	0.4370	0.6100	1.0976
Gross 4-NP (μg/kg weight)	5	0.0337	0.0070	0.0332
Net 4-NP (μg/kg weight)	5	0.0040	0.0057	0.0097
Net 4-NP (μg/70 kg weight)	5	0.2792	0.3983	0.6797
Creatinine (g/24 hr)	5	2.9440	0.8474	2.8316
	Sampling Interval = +3 Day			
Gross 4-NP (μg/L)	5	2.1890	1.0311	1.9525
Net 4-NP (μg/L)	5	0.3255	0.4572	0.8010
Gross 4-NP (total μg)	5	3.3500	0.6739	3.2962
Net 4-NP (total μg)	5	0.5540	0.6455	0.8228
Gross 4-NP (μg/kg weight)	5	0.0342	0.0019	0.0342
Net 4-NP (μg/kg weight)	5	0.0051	0.0055	0.0079
Net 4-NP (μg/70 kg weight)	5	0.3558	0.3837	0.5498
Creatinine (g/24 hr)	5	3.6280	0.5979	3.5871

Footnotes:

Arith. Mean = arithmetic mean (average)
Geo. Mean = Geometric mean
Std. Dev. = Standard Deviation
Gross 4-NP = Total 4-NP found in sample
Net 4-NP = Gross 4-NP - (Average 4-NP found in samples collected at -2 and -1 Day)

Compliance Analysis

The itemized checklist below describes compliance with the major technical aspects of the relevant sections of the OPPTS Series 875 Postapplication Exposure Monitoring Test Guidelines Part B.

- *Typical end use product of the active ingredient used. Where metabolites, breakdown components, or contaminants of pesticide end-use products pose a potential toxicological concern, investigators may need to consider sampling for them on a case-by-case basis.* This criterion was met. This study monitored 4-nitrophenol in urine as a biomarker for methyl parathion exposure. A separate white paper [MRID 449744-01, dated November 18, 1999] documents the rationale for this choice.

- *Selected sites and seasonal timing of monitoring must be appropriate to the activity. The need for studies under different geographical/climatological sites should be considered.* This criterion was met. The study was conducted in three cotton-growing geographic locations (i.e., California, Texas, and Louisiana).

- *End use product applied by application method and equipment recommended for the crop. Application rate given should be at the least dilution and highest, label permitted, application rate. It is suggested that the product also be applied at a lower application rate. Where multiple applications are recommended, the minimum time interval between applications should be used.* This criterion was partially met. Methyl parathion was applied four times at a single rate, which was the maximum label rate (i.e., 1.0 lbs. ai/A). The application volume ranged between 10 and 18 gallons/acre, and the label only recommended minimum dilution volume for aerial application (2 gallons/acre) not ground application. [The Study Protocol specified an application volume of no more than 20 gallons/acre (±5 percent).] The product label does not limit the number of applications, or specify a minimum interval between applications.

- *Applications occurred at time of season that the end-use product is normally used to achieve intended pest control.* This criterion was met. The pesticide was applied to growing cotton, a time of the season when methyl parathion is normally applied to cotton. The product label does not specify an application interval for cotton. Methyl parathion was applied at 5 day intervals in the study.

- *Meteorological conditions including temperature, wind speed, daily rainfall, and humidity provided for the duration of the study.* This criterion was met. On each application day, the following parameters were reported (see page 66 of the Study Report): application time, wind speed and direction, percent cloud cover, percent relative humidity, air temperature, soil temperature and general soil moisture conditions at the surface and subsurface. Daily rainfall values were given in a later submission.

- *Storage stability, method efficiency (residue recovery), and limit of quantitation should be provided.* These criteria were met.

- *The Agency requires investigators to submit protocols for review purposes prior to the inception of the study.* This criterion was met.

- *Biomonitoring studies must be carried out concurrently with transferable residue studies.* This criterion was met. Dislodgeable foliar data were collected concurrently at these test sites. A separate study, identified as #KP-2000-01, was submitted to EPA separately.

- *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 replicates per activity and preferably 5 replicates (i.e., individuals) for each of three monitoring periods.* This criterion was met.

- *The exposure monitoring period must be of sufficient length to have reasonable detectability of residues in urine, and be representative of a normal activity.* This criterion was met. There were six scouting sessions, although interrupted by break-times during which subjects washed their hands with soap and water. Total in-field exposure time was approximately 4.25 hours, however study subjects stayed in their work clothes for two hours after that. Study subjects entered the treated test site either 4 or 5 days after the final pesticide treatment. The product label specifies a reentry time of 4 or 5 days (depending on the reentry time mandated due to local rainfall conditions).

- *Monitoring should be conducted before residues have dissipated beyond the limit of quantitation.* This criterion was met. The residues were above the LOQ on the day of the activity for all three sites. Although, the residues at the Louisiana site on the day of activity (day 4) were very low at only 0.0164 $\mu g/cm^2$, which is just above the LOQ of 0.01 $\mu g/cm^2$.

- *Baseline urine samples should be collected at least one day before participating in the post-application exposure monitoring activities and continue on the day of postapplication monitoring and for an appropriate time period after these activities have been completed, depending on the excretion kinetics of the compound.* These criteria were met. Pre-screen 24-hour urine samples were collected about 2 weeks before the exposure event. Baseline 24-hour urine samples were collected each day beginning 2 days before the exposure event, through the day of the exposure event, and for 3 days after the exposure event. Kinetics observed in the field data indicated a rapid drop off of 4-nitrophenol concentrations within this time period.

- *The 24-hour 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.

- *All urine samples should be logged in at the time of collection. Material used to construct containers used for urine collection should not interfere with (e.g., absorb) the analytes of interest. Light sensitive analytes should be protected from degradation.* It is not known whether these criteria were met. All urine samples were logged in, however, the other issues were not discussed in the Study Report.
- *Field data should be corrected if any appropriate recovery is less than 90 percent.* This criterion was met. Only the field fortified samples prepared at the 10 µg/L 4-nitrophenol level was used to correct the data, since all of the field samples were closest to the 10 µg/L 4-nitrophenol fortification level. The field recovery values at the Louisiana and California sites were 82 and 75 percent respectively at the 10 µg/L 4-nitrophenol level, therefore the biomonitoring data was corrected for field recovery at these sites.

- *All urine samples should be frozen after the specific gravity is measured.* This criterion was met.

- *A brief history should be taken relating to known prior exposures to pesticides for at least the last 2 weeks, including reentry into potentially treated fields.* This criterion was partially met. No formal discussion of activities performed by subjects within the last two weeks prior to biomonitoring was provided. However, the study protocol required that " each subject will have had no contact with the prohibited materials listed in [Study Protocol] Appendix 2 [see page 274 of the Study Review] during the 7 days prior to his first day of monitoring. In addition, each subject will be sequestered at a hotel convenient to the test site(s) from 3 days prior to the exposure through the third day after exposure. Finally, each subject will avoid using or exposure to the materials listed in Appendix 2 from 7 days prior to the beginning of monitoring until the end of monitoring. Compliance with or deviation from these requirements will be documented for each worker."

- *Creatinine levels should be determined as a way of qualitatively monitoring completeness of urine collection samples.* This criterion was met.

- *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 met.