

**AGRICULTURAL HANDLERS EXPOSURE TASK FORCE
(AHETF)**

**Airblast Applications to Trellis Crops in the West
Using Open or Closed Cab Equipment**

STUDY No. AHE36

Study Title: Determination of Dermal and Inhalation Exposure to Workers
in the West During Airblast Applications to Trellis Crops
Using Open or Closed Cab Equipment

PROTOCOL AUTHORIZATION

Read and Approved by:

AHETF Sponsor
Representative: David Johnson, Ph.D.

Signature _____

Date _____

Study Director: Eric Bruce, B.S.

Signature _____

Date _____

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1.0 GENERAL INFORMATION

Study Title: Determination of Dermal and Inhalation Exposure to Workers in the West During Airblast Applications to Trellis Crops Using Open or Closed Cab Equipment

Study No.: AHE36

Objective: The primary objective of this study is to generate data to determine the potential exposure for workers who make airblast applications of spray mixtures to trellis crops (e.g., grapes) using open or closed cab equipment. The secondary objective is to determine the potential exposure to workers who mix and load a liquid pesticide product using open-pouring or closed system techniques.

Proposed Experimental Start Date: July 25, 2006

Proposed Experimental Termination (Field Phase) Date: July 28, 2006

Proposed Experimental Termination (Analytical Phase) Date: January 30, 2007

Proposed Final Report Issue Date: May 30, 2007

Good Laboratory Practice:

This study will be conducted in compliance with the US EPA FIFRA Good Laboratory Practice (GLP) Standards (40 CFR 160) and will adhere to applicable AHETF and/or field facility standard operating procedures (SOPs) and field work practices.

Pesticide Assessment Guideline:

This study is based upon EPA's guidance documents for dermal and inhalation exposure measurement under Series 875: Occupational and Residential Exposure Test Guidelines. Data reporting will follow the requirements defined in these guidelines.

Quality Assurance:

Field and analytical phases of this study will be monitored by the AHETF Quality Assurance Unit (QAU) and/or QAU personnel of the relevant test laboratories while this study is in progress to ensure compliance with the FIFRA GLP regulations and adherence to this protocol and relevant SOPs. In addition, raw data and final report audits will be conducted by the AHETF Quality Assurance Unit:

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Field Laboratories: Worker exposure monitoring will be conducted by:

Access Research and Consulting, Inc.
4720 W. Jennifer Ave., Suite 106
Fresno, CA 93722

Principal Investigator: Brian Lange
Phone: 559-277-5272
Mobile: 559-647-9800
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Field logistics will be coordinated by one or both of the following:

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1696 S. Leggett St.
Porterville, CA 93257

Local Site Coordinator: John Corkins
Phone: 559-784-5787
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Coastal Research Services
1661 Mulberry Lane
Paso Robles, CA 93446

Local Site Coordinator: Michelle Mitchell
Phone: 805-239-5875
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Analytical Lab: The analytical phase will be conducted by:

To Be Determined.

2.0 INTRODUCTION

The Agricultural Handlers Exposure Task Force (AHETF) was formed to develop a generic exposure database for use by its member companies in assessing potential exposure to workers mixing, loading and/or applying pesticides. The database will be representative of worker activities and methods used in the handling of pesticides.

The degree to which individual workers are potentially exposed while handling pesticides depends on his/her specific activities and the mixing/loading and/or application equipment being used. Typically, worker activities are restricted to

mixing and loading equipment for application or to applying the pesticide with equipment that has already been loaded. However, in some cases it involves mixing, loading, and applying the pesticide. Individual studies conducted by AHETF may include monitoring some or all of these phases: mixing and loading (M/L) activities, application (A) activities, or mixing, loading and application (M/L/A) activities associated with the application of agricultural pesticides.

This study is an integral part of a multi-phase, multi-year series of field studies, which will form a generic database designed to provide estimates of exposure for workers who handle and apply pesticides. The study is based upon the guidelines contained in Series 875, Part A of the U.S. EPA Pesticide Assessment Guidelines: Occupational and Residential Exposure Test Guidelines, and will be conducted in accordance with EPA FIFRA Good Laboratory Practice Standards (GLPs), 40 CFR, Part 160.

3.0 OUTLINE OF STUDY

The primary purpose of this study is to monitor exposure to workers who make airblast applications of liquid pesticide spray solutions to trellis crops (e.g., grapes) using open or closed cab equipment. Each worker will apply at least three loads of diluted product during a period of time representative of a full day's work, with a target of at least 4 hours.

The secondary purpose of this study is to monitor exposure to other workers who mix and load the spray solutions by handling a liquid product. Mixing/loading will be done by open-pouring the liquid into a suitable tank or an eductor system or using closed systems. Each monitored mixer/loader may prepare solution for more than one application rig and monitoring time will involve work periods with a target of at least 4 hours.

All monitored workers will be professional agricultural workers who will be required to give their informed consent to participate in the study. The number of workers monitored will be determined based on available resources in the field and will be detailed in the raw data. The anticipated number of total replicates is thirteen (13).

Potential dermal exposure to the test substance will be measured using whole body dosimeters, hand washes, and face/neck wipes. All monitored workers will be asked to wear one layer of their own clothing (normal outer work garments, plus shoes and socks) over inner whole body dosimeters. The outer work garments will be WPS (Worker Protection Standard) compliant and consist of long pants and long-sleeved shirts. All WPS compliant garments worn by the workers will either be freshly laundered or new and free from pesticide residues. The worker will be asked to confirm that the garments were laundered prior to the field monitoring study.

Workers will wear appropriate Personal Protection Equipment (PPE) specified by the product label which includes chemical-resistant gloves and protective eyewear (except when inside enclosed cabs). Potential inhalation exposure for each worker will be measured by means of a personal air sampling pump and an OSHA Versatile Sampler (OVS) tube with a glass fiber filter and XAD-2 sorbent.

4.0 LOCATION OF THE FIELD PHASE OF THE STUDY

The field location will be one or more sites in the state of California (or in another state in the western U.S.). Full details of the locations, including field maps, will be recorded in the study file.

5.0 FIELD MATERIALS AND METHODS

5.1 TEST SYSTEM

The test system for this study is the workers handling pesticides according to label directions.

5.2 JUSTIFICATION OF THE TEST SYSTEM

Experienced agricultural workers handling pesticide products using commercial product packaging and typical mixing/loading or application techniques will be monitored since this provides the best estimate of potential dermal and inhalation exposure for the activities being studied.

5.3 TEST SYSTEM IDENTIFICATION – WORKERS

Adult workers will be selected who are experienced in the work activities under study and who consider themselves to be in good health. They should also have a willingness to cooperate in a study of this type and have no conflict of interest in the conduct or outcome of the study. The reproductive status of all potential female participants will be ascertained through the use of a supervised urine pregnancy test conducted within 24 hours prior to the initiation of monitoring. Any pregnant subjects will be excluded from the study. The volunteers will be at least 18 years of age.

A signed informed consent form will be obtained from each worker prior to their participation in the study. This protocol, as well as the informed consent form and worker selection process, will be reviewed and approved by an Institutional Review Board (IRB) prior to worker exposure monitoring.

Each worker will be provided with a full explanation of the study, its

requirements, and any potential risks. Workers will be advised of their right to withdraw from the study at any time and for any reason without jeopardizing their normal position with their employers. In order to maintain confidentiality in the final report, only the unique worker identity number will identify each worker.

Personal details including name, age, gender, previous experience in the work activity, and a general health statement will be provided by each worker. In addition, the body weight and height of each worker will be measured by researchers. All personal information will be maintained in the study file. Workers will be asked to bathe or shower prior to each day of monitoring to minimize any possible contamination from one day to the next and from any outside pesticide sources.

5.4 MIXING/LOADING STATIONS AND APPLICATION AREAS

Field maps and/or sketches will be provided in the raw data showing the exact locations where mixing/loading and application occur. Relative distances between mix/load areas and application areas will be recorded. These will include area and local maps or sketches for all sites involved in the study.

5.5 STUDY PERSONNEL - FIELD

The study team will be comprised of a sufficient number of people to conduct the following activities:

1. Assisting with the donning and collection of all dosimeters in a time-efficient manner to minimize the time from completion of the work cycle to sampling
2. Fortifying field recovery samples
3. Calibrating air sampling pumps and recording beginning and ending flow rates
4. Observing and recording all work practices, recording site details and treatment details
5. Taking a photographic record of representative study-related activities
6. Monitoring the accuracy of mixing/loading and application equipment

5.6 TEST SUBSTANCE

The test substance used in this study is a commercial pesticide product which is a liquid formulation. This formulation is categorized as a Liquid, emulsifiable concentrate.

5.6.a Test Substance Identification

Test substance:	Malathion 8EC or Gowan Malathion 8 (two names for the same product)
EPA registration	51036-214 or 10163-21
Lot number(s):	Will be recorded in the study raw data.
Container description(s):	Will be recorded in the study raw data.
Common name of active ingredient (ai):	Malathion
CAS No. of ai:	121-75-5
Nominal concentration of ai:	8 pounds ai per gallon
Source of product:	Micro Flo Company LLC or Gowan Company
Appearance:	Will be recorded in the study raw data.

Stability: The stability of the active ingredient(s) in the test substance under recommended storage conditions will be documented before the start of the study. Generally, AHETF will rely on data supplied by the product registrant that were submitted to support the EPA registration of the test substance. An expiration date and recommended storage conditions will be based on the stability data to ensure the test substance strength does not change appreciably prior to use in the study.

GLP purity analysis (content of active ingredient in the test substance) will be performed for each lot of test substance used in the study prior to the start of the study or concurrently with study conduct. Documentation of such analyses will be retained in the study raw data file.

Retained samples from each lot of the test substance used in the study will be archived with AHETF. At the discretion of the Study Director, these retain samples may be analyzed to determine whether there was any change in test substance strength during the study period.

5.6.b Justification for Use of Test Substance

Malathion is registered and approved for use in a wide variety of agricultural settings, including application to grapes. The justification for its use in this study is that it has been deemed suitable by the Sponsor as a surrogate compound for generating exposure data. The analytical methods have been validated and this product has the requisite degree of stability under field, storage, and transit conditions.

5.6.c Safety Precautions

A copy of the Material Safety Data Sheet and the product label will be made available to the study team and included in the study file. Label safety requirements will be explained to the workers involved in the study. All label-specified PPE and use directions will be followed by the workers.

5.7 MIXING/LOADING, APPLICATION PARAMETERS

Mixing/loading equipment:	All mixing/loading activities will involve transferring the liquid product from commercial product packaging into suitable application equipment and dilution with carrier. Transfer may be by open-pouring directly into a spray tank or eductor system or using a small-volume suction and extraction closed system such as a Chemprobe® or similar system to transfer directly to the spray tank.
Carrier:	Water
Target application rate:	Depends on target crop and field needs, but application rates will generally range from 0.5 to 2.0 lb. active ingredient (ai)/acre (A). Actual application rates will be documented in study raw data.
Target application volume:	Depends on target crop and field needs, but application volumes will generally range from 50 to 200 gal/A.

Application Method: Applications will be made using airblast equipment and either open or closed cab equipment.

Stage of crop growth at application: Generally about six feet high, with full foliage.

5.7.a Equipment Accuracy Verification

Mixing/Loading equipment accuracy will be verified according to field testing facility SOPs prior to use in this study. This will include equipment used to weigh, pump, or meter the test substance and carrier during the handling of these materials.

Copies of relevant facility maintenance records (if available) for all application equipment used will be obtained and retained with the field raw data. The Study Director will assure application equipment operation is acceptable according to SOP AHETF-10.D.

5.7.b Application

The volume of spray mixture applied will be determined and recorded in the field raw data, along with other critical measurements including application area and duration. The capacity of the equipment tank may be measured or verified by the use of mass flow meters or other suitable equipment prior to the study. Upon completion of spraying each load of diluted product, the amount of spray volume remaining in the tank(s) will be determined. Disposal of excess spray mixture will occur in accordance with any applicable regulations.

5.7.c Rationale for the Route of Administration

The above procedures for mixing/loading and application should represent typical agricultural practices for each particular site, test substance, and geographic location.

5.8 ENVIRONMENTAL CONSIDERATIONS

Exposure monitoring will not be conducted under meteorological conditions inappropriate for the conduct of the activity (e.g., excessive precipitation or excessive wind). Adequate protection from the elements should be provided for handling worker exposure samples in case of inclement weather.

5.9 DESCRIPTION OF WORK ACTIVITY

Workers who are monitored during mixing/loading activities will prepare at least three loads of spray mixture by transferring the test substance from representative product packaging into designated mixing or application equipment and diluting with water as appropriate for the intended application.

Mixing/loading may involve open-pouring the product directly into a suitable tank or eductor system, or using a small-volume suction and extraction closed system such as a Chemprobe® or similar system to transfer directly into a spray tank.

The amount of product to be handled by each applicator and major application parameters are suggested in the following section. The exact configuration and size of equipment will be dictated by what is available in the field; however the Study Director will make an attempt to utilize a variety of standard equipment throughout the study. When necessary, the worker will measure the required amount of product from the commercial packaging according to their normal procedures. Mixer/loaders may prepare spray solution for more than one application rig. Mixer/loaders may perform other activities between loads with the exceptions of handling other forms of the same active ingredient and cleaning any mixing/loading or application equipment (other than what was used by that worker during his mixing/loading task). Routine preparation, clean-up, and container disposal activities may be performed according to the workers' typical procedures for the equipment used during his mixing/loading task.

Workers who are monitored during application of spray mixtures (prepared by the mixer/loaders) will make airblast applications using open or closed cab equipment (see the table below for a breakdown). Fan and nozzle set-ups will be determined by the worker according to his/her usual practice.

For all workers, the mixing/loading and/or application parameters will be adjusted so the target of at least four hours of exposure monitoring are met. For example, the workers who mix/load smaller amounts of product might use smaller tank sizes and prepare less concentrated spray mixtures so that multiple loads are necessary and the work time is extended. Similarly, the spray volume and/or application rate may be adjusted so that applicators reach the target of at least four hours of monitoring time.

All workers (mixer/loaders and applicators) will wear the clothing and PPE required by the product label. For mixer/loaders, this includes long pants, long-sleeved shirts, shoes, socks, chemical-resistant gloves, and protective eyewear. For closed cab applicators, the chemical-resistant gloves and protective eyewear are not required to be worn except when exiting the cab

and contacting contaminated surfaces. The clothing and PPE can be provided by each worker as long as the Study Director agrees they are compliant with the Worker Protection Standard (WPS). However, AHETF will provide new chemical-resistant gloves for all workers. All items worn must be compliant with the Worker Protection Standard (WPS), and the clothing must have been laundered prior to use or be new. Any clothing items deemed unacceptable by the Study Director will be replaced by alternate clothing. Upon approval by the Study Director, workers may wear a hat.

5.10 NUMBER OF SUBJECTS AND ACTIVITIES

Nine different applicator workers (or replicates) will be monitored for exposure. In addition, the mixer/loaders needed to provide the spray solution may be monitored. The following scheme is suggested:

Application Parameters						
Rep. No.	Day	Crop, Cab Type	Gallons per acre	Rate (lb ai/Acre)	Treated Acres	lb a.i. Handled
A1	1	Trellis, Open	150	0.75	20	15
A2	1	Trellis, Open	150	0.75	33	25
A3	1	Trellis, Open	100	1.0	40	40
A4	2	Trellis, Open	100	1.5	40	60
A5	2	Trellis, Open	50	2.0	37	74
A6	3	Trellis, Closed	150	1.0	20	20
A7	3	Trellis, Closed	100	1.0	35	35
A8	4	Trellis, Closed	100	1.5	30	45
A9	4	Trellis, Closed	50	2.0	35	70
Totals:					300	374

Generally, one mixer/loader will be monitored on each day; however the number of mixer/loader replicates will be determined by the Study Director and will be based primarily on the availability of appropriate equipment and amounts of product handled. Mixer/loader replicates will be numbered sequentially during the study as M1, M2, etc.

Each applicator replicate should be performed by a separate worker, however it is acceptable to occasionally “re-use” workers (e.g., one person might be monitored during an application one day and during mixing/loading the next day). However, workers may not be monitored more than once per day of the study.

All workers will have prior experience in the work activity under study and will conduct their work activity according to their normal practice.

5.11 DERMAL EXPOSURE SAMPLING

Full details of procedures for dermal exposure sampling, and sample removal, are specified in the most recent versions of SOPs AHETF-8.A, 8.B, 8.C, 8.D, and 8.H. At the completion of the monitoring period, exposure samples will be taken in the following order to minimize cross contamination: inhalation samples, then hand washes, then face/neck wipes, and finally inner dosimeters as described below and in SOP AHETF-10.E.

Workers will wear one layer of work clothing over the inner dosimeters. The inner dosimeter will consist of 100% white cotton long underwear provided by the AHETF. The inner dosimeter is designed to represent the worker’s skin and will act as a collection medium that will be analyzed. It will be worn throughout the period of monitoring and removed at the end of the work period, with the assistance of a member of the monitoring team.

At the end of the monitoring period (and after the inhalation exposure equipment are removed as described below), the worker will first remove his/her gloves and shoes, and then enter a clean, private area for collecting the remaining samples. Once inside the private area, the worker will remove his/her outer clothing and socks. The outer layer of clothing will not be collected or analyzed.

Hand exposure will then be assessed (after chemical-resistant gloves are removed) by having the worker wash their hands in a 0.01% Aerosol OT solution according to a standardized washing procedure described in the most recent version of SOP AHETF-8.B.

Hands will be washed just prior to the exposure monitoring period. If washing facilities are not available, a hand wash sample procedure will be conducted and the rinsate discarded. A hand wash sample will be collected prior to eating, in addition, a hand wash sample will be collected whenever a worker would normally wash his/her hands (e.g., before using the toilet, before using tobacco, etc.). These interim hand wash samples will be numbered sequentially, as illustrated in Appendix 1. After a monitoring

replicate is completed, one final hand wash will be collected from each worker. The post-activity hand wash sample for each replicate will be the final hand wash sample for the monitoring period and receive the final sequence number for the replicate. This sample will be clearly marked as the post-activity hand wash. All hand washes collected during and at the end of the work period will be treated as separate samples. All hand wash samples will be placed in pre-labeled containers and placed in temporary frozen storage as soon as possible for transport to the analytical facility. Samples will be maintained in frozen storage until analysis.

Face/neck exposure will then be measured by wiping the entire face and neck areas (front and back of neck) with two gauze sponges, sequentially, that have been wetted with 0.01% Aerosol OT as described in the most recent version of SOP AHETF-8.C.

The face and neck area will be washed just prior to the exposure monitoring period. If washing facilities are not available, one dermal face/neck wipe sample (consisting of two gauze wipes or a wipe from a moistened paper towel) will be collected from each worker and the wipe(s) discarded. A dermal face/neck wipe sample (consisting of two gauze sponges) will be collected prior to eating. After each exposure replicate is completed, a dermal face/neck wipe sample will be collected from each worker after the hand wash sample is collected and before removal of the whole body dosimeters. Face/neck wipe samples will be wrapped in aluminum foil prior to placement in pre-labeled resealable plastic bags or placed directly into a pre-labeled glass jar. All wipes collected during the study will be combined in the same container, resulting in a single sample for analysis. If more than two samples (4 sponges) are in a sample container, the laboratory must be notified as to the number in the container. All face/neck wipe samples will be placed in pre-labeled containers and placed in temporary frozen storage as soon as possible for transport to the analytical facility. Samples will be maintained in frozen storage until analysis.

Finally, the inner layer of clothing (inner dosimeter) will be removed with the assistance of a member of the study team. For the open cab applicators and any mixer/loaders, each inner dosimeter will be sectioned into upper and lower arms, front and back torso, and upper and lower legs (6 sections). For the closed cab applicators, each inner dosimeter will be sectioned into two sections: upper body and lower body. Each section of inner dosimeter will be individually wrapped in aluminum foil, placed in pre-labeled containers and placed into temporary frozen storage as soon as possible for transport to the analytical facility. Samples will be maintained in frozen storage until analysis.

5.12 INHALATION EXPOSURE SAMPLING

Full details of the personal air-sampling method, attachment of pumps, monitoring of workers, and pump calibration are given in the most recent versions of SOP AHETF-8.D and 10.A. Suitable low-volume personal air-sampling pumps and OVS tubes with a glass fiber filter and XAD-2 sorbent are required. Valid calibration equipment, specified in SOP AHETF-10.A, and Tygon[®] (or equivalent) tubing are also required. The pumps and calibration equipment will be uniquely labeled and this information recorded in the raw data records.

Before the work commences, the sampling pump will be attached to a belt around the waist of the worker to be monitored. Tygon[®] tubing (or equivalent) attached to the inlet valve of the pump will be placed over the shoulder of the worker and attached to the air-sampling tube. A clip will be used to attach the tube to the collar of the worker, thus positioning it in the breathing zone of the worker. The inlet of the air-sampling tube will be facing downward, similar to the nasal passage of a worker.

Each pump will be calibrated, as specified in SOP AHETF-10.A, to a nominal sample flow rate of approximately 2 L/min and will operate for the duration of the exposure monitoring period. Flow rates will be measured before and after each exposure monitoring period and detailed records of flow rates and sampling durations will be maintained in the raw data records.

The pumps will be turned on immediately prior to the start of the monitoring period and will operate continuously until the end of the period. Detailed time logs will be maintained to allow the exposure period to be calculated as either the total time or the time actually spent in the work area (e.g., excluding time in buildings for bathroom breaks or lunch breaks).

Periodically throughout the monitoring period, the pumps will be checked to ensure they are still running and the tubing checked to ensure that there are no kinks in it. Workers will be instructed to inform a study team member if the pump fails to operate or the tubing becomes kinked.

If a pump stops operating during the work cycle, it will be replaced with a pre-calibrated replacement pump or given fresh batteries as soon as possible. Only the pump or batteries will be changed, the same sampling tube and tubing will continue to be used. At the conclusion of each exposure monitoring period, after the final flow rate has been recorded, the OVS tube will be disconnected from the tubing leading to the pump. The OVS tube will be sealed at both ends, placed in a pre-labeled container, and placed in temporary frozen storage as soon as possible for transport to the analytical

facility. Samples will be maintained in frozen storage until analysis.

5.13 CONTROL OF BIAS

Sampling bias will be controlled by sampling multiple workers over a period representative of a typical work day, and by sampling over the entire body of each worker. Quality control samples in the field and in the laboratory also act as methods for controlling bias.

5.14 FIELD RECOVERY EVALUATION

Full details regarding field recovery evaluation procedures for all sampling media are given in the most recent version of SOP AHETF-8.E. The SOP instructions for “spiking using vial spikes” will be followed.

Sample matrix fortifications designed to assess the stability of the active ingredient under field, storage and transit conditions in or on the sampling materials (inner dosimeters, hand wash solutions, face/neck wipes, head patches, and air sampling matrices) will take place on at least two days of exposure monitoring.

Field fortification suspensions of the test substance diluted in water, or solutions of active ingredient in an appropriate solvent, will be prepared and pre-measured into vials by the analytical laboratory and shipped to the study team for field recovery evaluation on all matrices except OVS tubes. Vials with suspensions of diluted test substance will be shipped and stored under chilled conditions until used in the field. Vials with solutions of active ingredient in solvent will be shipped and stored under frozen conditions until used in the field. The OVS tubes will be pre-spiked with the active ingredient (generally in an organic solvent) at the analytical laboratory and kept frozen until their use in the field.

Storage conditions of the individual vials used for fortifications, and of the fortified OVS tubes, will be specified by the analytical laboratory and the actual storage details will be recorded in the study file. Note that suspensions of test substance diluted in water should never be frozen. Any unused vials or unused fortified OVS tubes will be returned to the analytical laboratory.

With the exception of OVS tubes, the entire contents of the fortification vials will be applied to the sampling media. Field fortifications will be conducted at the following levels during the study.

Matrix:	Fortification Level
----------------	----------------------------

	($\mu\text{g}/\text{sample}$):
Inner Dosimeters	5, 100, and 5,000
Face/neck Wipes	5, 100, and 2,500
Hand Wash	5, 100, and 5,000
OVS Tubes	0.05, 1.0, and 50

On each study day where field fortifications are conducted, samples of each matrix will be fortified in triplicate at each of the levels shown above. Field fortifications will be conducted on at least two study days, and at the Study Director's discretion, on additional days.

After fortification, the inner dosimeters and OVS tubes will be exposed to ambient conditions (i.e., weathered) for the longest expected exposure monitoring period in a location away from possible contamination (e.g., upwind of mixing/loading and application operations). Inner dosimeter samples will be covered with a single layer of shirt material during weathering. Segments representing any body area may be used for inner dosimeter fortification samples. An air sampling system will be set up in a manner similar to that of the workers, in which a pump will continuously draw air through the pre-spiked filter and OVS tube for the entire duration of the work period.

Hand wash and face/neck wipe samples will be fortified and immediately placed in frozen storage without exposure to ambient conditions.

In addition, on each study day where field fortifications are conducted, duplicate samples of the inner dosimeters fortified in the field at the highest level, and duplicate OVS tubes fortified in the laboratory at the highest fortification level, will be processed for immediate frozen storage and used as travel spikes. These travel spikes will be analyzed only if deemed necessary by the Study Director, for example to help determine the cause of unusually low field fortification recovery results.

Finally, on each study day where field fortifications are conducted, two untreated control samples of each matrix will be processed similar to the field fortification samples (i.e., some are weathered).

Packaging, storage and shipment of the field fortification samples will be the same as for the experimental exposure samples.

5.15 OBSERVATIONS

Observations will be recorded throughout the monitoring period while the workers perform their tasks according to SOP AHETF-10.C. Any specific occurrences that could affect exposure will be noted on the observation forms. Measurements will be made of the amount of test substance mixed/loaded or applied. A detailed time log will be maintained for all activities. A photographic record will be taken of representative study related activities during exposure monitoring.

The amount of test substance handled by mixer/loaders will be determined by summing the volume of test substance added to the spray mixture(s). Full containers of the product may be assumed to contain the net contents printed on the label. Researchers may measure the amount of product remaining in partial containers to determine the volume of product used (by subtracting from the full container weight). The total volume of spray mixture prepared will be determined based on readings from a flow meter, volume marks on mixing tanks, or other appropriate means.

The volume of spray mixture handled by applicators will be determined by measuring the amounts loaded into the spray tank(s), measuring or estimating the amounts remaining after each application, and calculating and summing the differences. The amount of test substance handled will be calculated from the amount of spray mixture applied by taking into consideration the nominal concentration of each load sprayed (e.g., in terms of pounds of product per gallon of spray mixture).

5.16 ENVIRONMENTAL MONITORING

Environmental conditions, including air temperature, relative humidity, wind speed, and wind direction will be recorded by means of an on-site, portable weather station at least hourly during exposure monitoring. If an on-site weather station is not available or practical, measurements from the nearest NOAA (National Oceanic and Atmospheric Administration) weather station may be referenced. Measuring equipment will be calibrated as per the field contractor's SOP. Additionally, observations concerning pertinent weather conditions, such as amount of cloud cover, degree of sunshine, rainfall, relative humidity, etc. for each day of monitoring will be recorded in the field raw data.

6.0 SAMPLE IDENTIFICATION, SHIPPING AND STORAGE

6.1 SAMPLE IDENTIFICATION

The sample identification process is described in the most recent version of SOP AHETF-8.F. Samples will be identified and tracked by unique sample numbers assigned by AHETF. Sets of example sample identification numbers are appended to this protocol (Appendix 1). During the analytical phase of the study, the laboratory may assign its own sample numbers as long as the AHETF number is cross-referenced and included in the documentation of the sample.

6.2 SHIPPING

Samples will be shipped frozen to the analytical laboratory for storage prior to analysis. A full chain of custody record will be available for each sample.

6.3 STORAGE

All samples will be placed into frozen storage as soon as possible after collection; the analytical laboratory will store samples under frozen conditions pending analysis. Freezers will be monitored and the temperatures documented.

7.0 ANALYTICAL PROCEDURES

Experimental exposure and field recovery samples will be analyzed according to the analytical methods specified in the analytical section of this protocol. The methodology will have been validated for use in the relevant matrices prior to the initiation of the sample analyses.

7.1 REFERENCE SUBSTANCE(S)

The reference substance for this study is the analytical standard used by the analytical laboratory to prepare analytical standard solutions.

Common name:	Malathion
CAS No.:	121-75-5
Purity:	Will be recorded in the raw data
Source:	Will be recorded in the raw data
Lot no.:	Will be recorded in the raw data

The Study Director or an authorized representative will obtain analytical standard from the AHETF. Receipt of the standard will be documented, including label identification, date of receipt, person receiving the standard, and the amount received. Preparation of all stock and serially diluted solutions will be documented.

The stability of the analytical standard (reference substance) will be documented before the start of the study. Generally, AHETF will rely on data supplied by the product registrant that were submitted to support the EPA registration of the technical grade active ingredient. An expiration date and recommended storage conditions will be based on the stability data to ensure the analytical standard strength does not change appreciably during conduct of the study. Analytical standards are to be stored under the recommended conditions.

GLP purity analysis (content of active ingredient in the reference substance) will be performed for each lot of reference substance used in the study prior to the start of sample analyses. Documentation of such analyses will be retained in the study raw data file.

7.2 ANALYTICAL METHODS

The latest revisions of the following validated analytical methods will be used:

Analytical Method No. ARTF-AM-005 entitled, "Determination of Diazinon and Malathion in Inner Dermal Dosimeters."

Analytical Method No. ARTF-AM-006 entitled, "Determination of Diazinon and Malathion in Hand Wash Solutions."

Analytical Method No. ARTF-AM-009 entitled, "Determination of Diazinon and Malathion in OVS Air Sampling Tubes."

Analytical Method No. ARTF-AM-010 entitled, "Determination of Diazinon and Malathion in Facial/Neck Wipes."

Equivalent instrumentation, apparatus, and reagents may be substituted for those specified in the method. All substitutions must be clearly documented in the raw data.

7.3 ANALYTICAL DESIGN

All analytical procedures, techniques and matrices will be provided by the AHETF. Procedures and techniques will be followed as rigidly as possible. No changes are permitted without the prior approval of the AHETF Analytical Monitor and the Study Director.

All data will be measured against a standard curve (five-point minimum) that brackets the levels of the matrix spikes. If necessary, a solvent blank for the standard solutions will be injected prior to the standard solutions for each run.

Analytical data sets for the study will be considered acceptable if the following criteria are met. If these criteria cannot be met, the analytical monitor must be contacted immediately.

- 1) The limit of determination, r^2 , or the regression coefficient, r , must be reported for all curves to demonstrate sufficient linearity of detector response in the range of residues quantified. All r^2 values must be 0.90 or greater or all r values must be 0.94 or greater.
- 2) Back calculations of the standard to the calculated curve which is based on the standards run in a set of samples will be performed for all analytical sets. The back calculations of the standards to the curve will be around $\pm 15\%$ for all standards but the lowest concentration standard which may back calculate to around $\pm 20\%$. No standard will be discarded from a set unless there is a good reason for its being discarded and not without consultation with the analytical monitor.

A minimum of two laboratory spikes must be included in each analytical set. For large analytical sets, include approximately one spike for every ten field samples. The spiking concentrations will bracket the expected levels in the field samples. The LOQ is defined in each analytical method.

For all samples wrapped in aluminum foil, the inner surface of the foil wrapping will be rinsed with at least 50 mL of extraction solvent, which will be added to the total extract volume. The final volume of solvent used must be documented.

The filter, plus front and rear sorbent sections of the OVS tubes, (along with the retainer ring and sorbent section separators) will be analyzed together as one unit.

7.4 STATISTICAL METHODS

Chromatographic quantification (either GC or HPLC depending on the method) will be achieved using a standard curve obtained from peak heights or areas of injections of several concentrations of standards. The standard curve will be a least squares fit unless otherwise approved by the AHETF Analytical Subcommittee. Means and standard deviations (arithmetic and/or geometric), and coefficients of variation may be calculated on the data generated.

8.0 STUDY RECORDS

8.1 FIELD RECORDS

Raw data will be obtained to cover all aspects of the study, including but not limited to the following:

1. Test and reference substance lot numbers, receipt and storage location(s) use records
2. Crop description and growth stage
3. Mixing/loading equipment details
4. Application equipment details
5. Application equipment maintenance records (retained in the study file)
6. Environmental conditions for the entire monitoring period
7. Personal details of workers, including consent forms
8. Trial location maps, including description, dimensions, and exact locations of plots and mixing/loading station(s)
9. Pounds active ingredient handled, acres treated, time exposed and/or volume of liquid applied
10. Dermal exposure sampling information
11. Inhalation exposure sampling information, including pump identification, calibration, flow rates and times of sampling
12. Field recovery information for all sampling media
13. Test and reference substance, and sample storage temperature records
14. Observations on work practices; including photographs
15. Sample information (including inventory, chain of custody)

Field raw data will be recorded directly into the study notebook provided by AHETF. All data generated during this study will be kept in files bearing the study number.

8.2 ANALYTICAL RECORDS

All study-specific original documents and data generated in the course of this study, including but not limited to the following, will be maintained and turned over to the AHETF when requested, or at the completion of the study.

1. Analytical worksheets, chromatograms, methods, residue calculation sheets and other pertinent analytical data
2. Laboratory notebooks or bench sheets used to record details of the analyses
3. Chromatograms and/or machine-generated analysis reports and data
4. Spreadsheets and other calculated data
5. Chain of custody records

In addition to the above study-specific raw data, the following records must also be kept, and true copies submitted with the raw data:

- a. Storage conditions for reference substances and samples
- b. Reference substance use log
- c. Balance and instrument log book pages
- d. Communications logs or records

Following completion of the field or analytical portion of the study, copies of the relevant records will be indexed and sent to the Study Director for preparation of the final report. All original raw data will be transferred directly to the AHETF GLP study archive at Quality Associates, Inc., 9017 Red Branch Road, Suite 102, Columbia, MD 21045 and stored for an indefinite period.

9.0 DATA HANDLING

9.1 COMMUNICATION OF RESULTS

Results will be communicated from Principal Investigators to the Study Director and the designated AHETF Study Monitors on a regular and timely schedule.

9.2 STATISTICAL METHODS

Proposed calculations are limited to the calculations specified in Section 7.4.

10.0 QUALITY ASSURANCE

AHETF intends that all regulatory studies are conducted in accordance with the FIFRA GLP Standards (40 CFR 160). Field and analytical aspects of this study will be monitored by the relevant quality assurance unit(s) (QAU) personnel of the test laboratories and/or AHETF while this study is in progress to ensure compliance with the FIFRA GLP regulation and adherence to this protocol and relevant SOPs. The QAU(s) will submit copies of its/their inspection reports to the Study Director and AHETF Sponsor Representative (40 CFR part 160.35 [4]). The final report will be audited by the AHETF QAU to ensure that the contents of the report accurately describe the conduct and findings of the study.

The QAU(s) of the individual contractors will also submit copies of their QA reports to the AHETF QAU. The final report will contain a Quality Assurance Statement from the QAU of each contributing laboratory conducting QA audits, and from the AHETF QAU.

11.0 SAMPLE RETENTION

All sample extracts, extracted sample matrices, unanalyzed fortification matrices, and analytical standards will be retained until the Study Director and Analytical Monitor determine they are no longer useful. These materials are the property of the AHETF and will be stored or disposed of in a safe and lawful manner by the appropriate authorized personnel with the approval of AHETF and with QA verification at the performing facility.

12.0 PHASE REPORTS

Separate final reports will be prepared for the field and analytical phases of each monitoring study.

12.1 FIELD REPORT

Upon completion of the field phase, the field laboratory will submit a report to the AHETF and the Study Director in a format specified by the AHETF. The field report will be appended to the final study summary report and must contain, but is not limited to containing the following:

1. Identification of the location of the study, and the general environmental conditions during the exposure monitoring period(s)
2. A record of the mixing, loading, and/or application, including a description of the workers and their activities
3. A summary of worker observations identifying any specific occurrences

- that may contribute to unusual worker exposure
4. A detailed summary of the amount of test substance handled or applied for each worker
 5. A detailed summary of the length of time each worker was monitored
 6. A complete description of the field recovery evaluation with a summary of specific handling and weathering of all field samples
 7. A complete description of collection, handling, storage, and shipping of field samples

12.2 ANALYTICAL REPORT

At the completion of the analytical phase, the analytical laboratory will submit a report to the AHETF and the Study Director in a format specified by the AHETF. The analytical report will be appended to the study report and must contain, but is not limited to containing the following:

1. Results of analysis
2. An analytical laboratory QAU statement giving dates of inspections and dates that findings were reported to the Study Director and AHETF Management
3. A detailed description of the methods
4. Example calculations
5. A summary of the recovery data
6. Representative chromatograms of control, treated, fortified samples and calibration standards
7. A typical standard curve

13.0 FINAL STUDY REPORT

A final summary report will be prepared by the Study Director according to a standardized format provided by AHETF. The original signed copy of the final study summary report will be archived at the AHETF GLP study archive.

14.0 PROTOCOL CHANGES

14.1 AMENDMENTS

Amendments to this protocol during the course of the study are permissible. Amendments to this protocol must be approved by the Study Director and be acknowledged by the Sponsor. Protocol amendments are to be documented in accordance with SOP AHETF-2.C.

14.2 DEVIATIONS

GLP deviations are to be documented on the "Statement of GLP Compliance" in the report. A description of any and all deviations to the protocol must appear in the final report. Any deviations to the protocol, lab SOPs or GLPs, or situations that may affect the integrity of the study must be communicated to the Study Director in a timely manner. Protocol deviations are to be documented in accordance with SOP AHETF-2.C.

APPENDIX 1: Example Sample Identification Numbers

A. Exposure Samples

Example sample numbers for the first open cab applicator replicate, designated as Rep A1:

Sample ID Number	Description
36-WS-A1-ID-BL	Worker Sample, Rep A1, Inner Dosimeter, Lower Body
36-WS-A1-ID-BU	Worker Sample, Rep A1, Inner Dosimeter, Upper Body
36-WS-A1-AR-01	Worker Sample, Rep A1, Air Sampling Tube
36-WS-A1-FW-01	Worker Sample, Rep A1, Face/Neck Wipes
36-WS-A1-HW-01	Worker Sample, Rep A1, 1st interim Hand Wash
36-WS-A1-HW-02	Worker Sample, Rep A1, 2nd interim Hand Wash
36-WS-A1-HW-xx	Worker Sample, Rep A1, post Hand Wash

Notes:

36: refers to the study number AHE36.

WS: indicates the sample is a “Worker Sample”.

A1: refers to the replicate number and indicates the first applicator replicate in the study.

ID, AR, FW, and HW: refers to the exposure dosimeter type as indicated in the descriptions.

BL and BU: refers to various body area sections of inner dosimeters. Note that the closed cab applicator replicates will have six inner dosimeter sections (as shown for the mixer/loaders).

xx in the post hand wash sample refers to the final sequential number for that replicate. For example, if only two hand wash samples are collected for a given monitoring replicate the first (with number ending 01) would be described as “... 1st interim Hand Wash” and the second (with number ending 02) would be described as “... post Hand Wash”.

Each subsequent applicator replicate would be assigned a sequential replicate number: A2, A3, A4, etc.

A. Exposure Samples (cont.)

Example sample numbers for the first mixer/loader replicate, designated as Rep M1:

Sample ID Number	Description
36-WS-M1-ID-LA	Worker Sample, Rep M1, Inner Dosimeter, Lower Arms
36-WS-M1-ID-UA	Worker Sample, Rep M1, Inner Dosimeter, Upper Arms
36-WS-M1-ID-FT	Worker Sample, Rep M1, Inner Dosimeter, Front Torso
36-WS-M1-ID-RT	Worker Sample, Rep M1, Inner Dosimeter, Rear Torso
36-WS-M1-ID-LL	Worker Sample, Rep M1, Inner Dosimeter, Lower Legs
36-WS-M1-ID-UL	Worker Sample, Rep M1, Inner Dosimeter, Upper Legs
36-WS-M1-AR-01	Worker Sample, Rep M1, Air Sampling Tube
36-WS-M1-FW-01	Worker Sample, Rep M1, Face/Neck Wipes
36-WS-M1-HW-01	Worker Sample, Rep M1, 1st interim Hand Wash
36-WS-M1-HW-02	Worker Sample, Rep M1, 2nd interim Hand Wash
36-WS-M1-HW-xx	Worker Sample, Rep M1, post Hand Wash

Notes:

36: refers to the study number AHE36.

WS: indicates the sample is a “Worker Sample”.

M1: refers to the replicate number and indicates the first mixer/loader replicate in the study.

ID, AR, FW, and HW: refer to the exposure dosimeter type as indicated in the descriptions.

LA, UA, FT, RT, LL, and UL: refer to various body area sections of inner dosimeters.

xx in the post hand wash sample refers to the final sequential number for that replicate. For example, if only two hand wash samples are collected for a given monitoring replicate the first (with number ending 01) would be described as “... 1st interim Hand Wash” and the second (with number ending 02) would be described as “... post Hand Wash”.

Each subsequent mixer/loader replicate would be assigned a sequential replicate number: M2, M3, etc.

B. Field Fortification Samples

Example sample numbers for field fortification samples on Study Day 1:

Sample ID Number	Description
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36-FF-01-ID-L1	Fortification sample, Day 01, Inner Dosimeters, 1st Low level
36-FF-01-ID-L2	Fortification sample, Day 01, Inner Dosimeters, 2nd Low level
36-FF-01-ID-L3	Fortification sample, Day 01, Inner Dosimeters, 3rd Low level
36-FF-01-ID-M1	Fortification sample, Day 01, Inner Dosimeters, 1st Mid level
36-FF-01-ID-M2	Fortification sample, Day 01, Inner Dosimeters, 2nd Mid level
36-FF-01-ID-M3	Fortification sample, Day 01, Inner Dosimeters, 3rd Mid level
36-FF-01-ID-H1	Fortification sample, Day 01, Inner Dosimeters, 1st High level
36-FF-01-ID-H2	Fortification sample, Day 01, Inner Dosimeters, 2nd High level
36-FF-01-ID-H3	Fortification sample, Day 01, Inner Dosimeters, 3rd High level
36-FF-01-AR-L1	Fortification sample, Day 01, Air sampling tube, 1st Low level
36-FF-01-AR-L2	Fortification sample, Day 01, Air sampling tube, 2nd Low level
36-FF-01-AR-L3	Fortification sample, Day 01, Air sampling tube, 3rd Low level
36-FF-01-AR-M1	Fortification sample, Day 01, Air sampling tube, 1st Mid level
36-FF-01-AR-M2	Fortification sample, Day 01, Air sampling tube, 2nd Mid level
36-FF-01-AR-M3	Fortification sample, Day 01, Air sampling tube, 3rd Mid level
36-FF-01-AR-H1	Fortification sample, Day 01, Air sampling tube, 1st High level
36-FF-01-AR-H2	Fortification sample, Day 01, Air sampling tube, 2nd High level
36-FF-01-AR-H3	Fortification sample, Day 01, Air sampling tube, 3rd High level
36-FF-01-FW-L1	Fortification sample, Day 01, Face/Neck wipes, 1st Low level
36-FF-01-FW-L2	Fortification sample, Day 01, Face/Neck wipes, 2nd Low level
36-FF-01-FW-L3	Fortification sample, Day 01, Face/Neck wipes, 3rd Low level
36-FF-01-FW-M1	Fortification sample, Day 01, Face/Neck wipes, 1st Mid level
36-FF-01-FW-M2	Fortification sample, Day 01, Face/Neck wipes, 2nd Mid level
36-FF-01-FW-M3	Fortification sample, Day 01, Face/Neck wipes, 3rd Mid level
36-FF-01-FW-H1	Fortification sample, Day 01, Face/Neck wipes, 1st High level
36-FF-01-FW-H2	Fortification sample, Day 01, Face/Neck wipes, 2nd High level
36-FF-01-FW-H3	Fortification sample, Day 01, Face/Neck wipes, 3rd High level

B. Field Fortification Samples (cont.)

Sample ID Number Description

36-FF-01-HW-L1	Fortification sample, Day 01, Hand Wash, 1st Low level
36-FF-01-HW-L2	Fortification sample, Day 01, Hand Wash, 2nd Low level
36-FF-01-HW-L3	Fortification sample, Day 01, Hand Wash, 3rd Low level
36-FF-01-HW-M1	Fortification sample, Day 01, Hand Wash, 1st Mid level
36-FF-01-HW-M2	Fortification sample, Day 01, Hand Wash, 2nd Mid level
36-FF-01-HW-M3	Fortification sample, Day 01, Hand Wash, 3rd Mid level
36-FF-01-HW-H1	Fortification sample, Day 01, Hand Wash, 1st High level
36-FF-01-HW-H2	Fortification sample, Day 01, Hand Wash, 2nd High level
36-FF-01-HW-H3	Fortification sample, Day 01, Hand Wash, 3rd High level

Notes:

36: refers to the study number AHE36.

FF: indicates the sample is a “Field Fortification” sample.

01: refers to the Study Day on which the field fortification samples were obtained; a Study Day means a day on which field fortifications or exposure monitoring was performed and Days are numbered sequentially, e.g., Day 1, Day 2, Day 3, etc. (even though the Days might not be sequential days on the calendar).

ID, AR, FW, and HW: refer to the exposure dosimeter type as indicated in the descriptions above.

L1, L2, L3, M1, M2, M3, H1, H2, and H3: refer to three replicate samples for Low, Mid, or High fortification levels.

C. Field Fortification Samples – Travel Spikes

Example sample numbers for travel spikes on Study Day 1:

Sample ID Number	Description
-------------------------	--------------------

36-FF-01-ID-T1	Fortification sample, Day 01, Inner Dosimeters, 1st Travel spike
36-FF-01-ID-T2	Fortification sample, Day 01, Inner Dosimeters, 2nd Travel spike
36-FF-01-AR-T1	Fortification sample, Day 01, Air Sampling Tube, 1st Travel spike
36-FF-01-AR-T2	Fortification sample, Day 01, Air Sampling Tube, 2nd Travel spike

Notes:

36: refers to the study number AHE36.

FF: indicates the sample is a “Field Fortification” sample.

01: refers to the Study Day on which the field fortification samples were obtained; a Study Day means a day on which field fortifications or exposure monitoring was performed and Days are numbered sequentially, e.g., Day 1, Day 2, Day 3, etc. (even though the Days might not be sequential days on the calendar).

ID and AR: refer to the exposure dosimeter type as indicated in the descriptions above.

T1 and T2: refer to two replicates of Travel spikes.

D. Field Fortification Samples – Controls

Example sample numbers for field control samples on Study Day 1:

Sample ID Number	Description
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36-FF-01-ID-C1	Fortification sample, Day 01, Inner Dosimeters, 1st Control sample
36-FF-01-ID-C2	Fortification sample, Day 01, Inner Dosimeters, 2nd Control sample
36-FF-01-AR-C1	Fortification sample, Day 01, Air Sampling Tube, 1st Control sample
36-FF-01-AR-C2	Fortification sample, Day 01, Air Sampling Tube, 2nd Control sample
36-FF-01-FW-C1	Fortification sample, Day 01, Face/Neck wipes, 1st Control sample
36-FF-01-FW-C2	Fortification sample, Day 01, Face/Neck wipes, 2nd Control sample
36-FF-01-HW-C1	Fortification sample, Day 01, Hand Wash, 1st Control sample
36-FF-01-HW-C2	Fortification sample, Day 01, Hand Wash, 2nd Control sample

Notes:

36: refers to the study number AHE36.

FF: indicates the sample is a “Field Fortification” sample.

01: refers to the Study Day on which the field fortification samples were obtained; a Study Day means a day on which field fortifications or exposure monitoring was performed and Days are numbered sequentially, e.g., Day 1, Day 2, Day 3, etc. (even though the Days might not be sequential days on the calendar).

ID, AR, FW, and HW: refer to the exposure dosimeter type as indicated in the descriptions above.

C1 and C2: refer to two replicates of Control samples.