Department of Pesticide Regulation
Environmental Monitoring and Pest Management Branch
1020 N Street, Room 161
Sacramento, California 958143624

Study Protocol for Residues of Forestry Herbicides in Plants of Interest to Native Americans, Phase Two: Dissipation and Off-Site Movement Study 159

Revised
December 12, 1997

I. INTRODUCTION

The use of native plant materials is a tradition among Native American tribes in California. Native Americans use many different plants for food, basketry, and other cultural activities. The California Indian Basketweaver Association has alerted the Department of Pesticide Regulation (DPR), the U.S. Environmental Protection Agency - Office of Pesticide Programs, and the U.S. Forest Service of potential exposures to forestry herbicides that may be occurring to Native Americans through the use of plant materials. These unique exposure scenarios are not characterized in the risk assessment of these herbicides. While the U.S. Forest Service has established programs within their national forests to work with tribal representatives to identify collecting areas and protect the areas from herbicide spraying, not all Native Americans participate in these programs; they may collect plant materials in unidentified locations. Additionally, Native Americans are concerned that the protective measures may not be sufficient.

There are three herbicides used for site preparation and release operations in conifer tree plantations in California's national forests. Glyphosate is a nonselective, postemergent contact herbicide. Triclopyr is a systemic herbicide used extensively to control woody weeds and many broad-leaf weeds. Hexazinone is a contact and residual herbicide. All three herbicides are applied

from the spring through fall months. These herbicides are applied by commercial applicators under contract with the U.S. Forest Service. While most applications are made by ground application (backpack sprayers), some large acreage projects may utilize aerial application of granular products.

The U.S. Forest Service has requested the assistance of DPR in assessing exposure of basketmakers to forestry herbicides. In order to estimate the potential exposure, the residue concentrations of forestry herbicides must be determined. Therefore, this project is being conducted by the DPR's Environmental Hazards Assessment Program (EHAP), under contract to the U.S. Forest Service.

The residues of forestry herbicides in plant materials will be determined in two phases. Phase one involved the development of sampling and analytical methodology, and pilot sampling. The results from phase one were used in the planning for phase two. The two primary objectives for phase two are described below.

II. OBJECTIVES

A. In areas that have been treated, determine the dissipation rate of glyphosate, hexazinone, and triclopyr in selected plants of interest to Native Americans. The dissipation rate will be determined by measuring herbicide concentrations in plants over time.

B. In areas adjacent to applications, determine the frequency of occurrence of glyphosate, hexazinone, and triclopyr in plants of interest to Native Americans. How far herbicides move away from treated areas (off-site movement) will be determined by counting the number of positive and number of negative plant samples at various distances.

III. SPONSOR

U.S. Department of Agriculture
U.S. Forest Service
Pacific Southwest Region
630 Sansome Street
San Francisco, California 94 111

IV. TESTING FACILITIES AND PERSONNEL

The testing facilities are located at:

Department of Pesticide Regulation
Environmental Hazards Assessment Program
1020 N Street, Room 161
Sacramento, California 958 14-5624

Department of Pesticide Regulation
Environmental Hazards Assessment Program
3971 Commerce Drive, Suite D
West Sacramento, California 9569 1

California Department of Food and Agriculture
Center for Analytical Chemistry
3292 Meadowview Road
Sacramento, California 95832

This study will be conducted by EHAP, under the general direction of Kean Goh, Program Supervisor. Key personnel are listed below:

Study Director (Project Leader):	Randy Segawa
Senior Staff Scientist:	Heinz Biermann
Field Coordinators:	Adrian Bradley and Clarice Ando
Statistician:	Terri Barry
Chief Chemist:	Cathy Cooper
Quality Assurance Officer:	Cindy Garretson
Contact Person:	Madeline Brattesani

Responsibilities of the key personnel are described in EHAP Standard Operating Procedure ADMN002.00 (Supplement 1). Authorship of the final report should include, but not be limited to Kean Goh, Randy Segawa, Adrian Bradley, and Terri Barry.

Questions concerning this monitoring should be directed to Madeline Brattesani at (916) 324-4100; fax, (916) 324-4088; e-mail, mbrattesani@cdpr.ca.gov.

V. TEST SUBSTANCES

Accord@, active ingredient glyphosate [isopropylamine salt of N-(phosphonomethyl) glycine], CAS 1071-83-6

Pronone® 10G, active ingredient hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione], CAS 51235-04-2

Velpar® L, active ingredient hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione], CAS 51235-04-2

Garlon® 4, active ingredient triclopyr [(3,5,6-trichloro-2-pyridinyloxy) acetic acid, butoxyethyl ester], CAS 55335-06-3

Most of the applications DPR will monitor will be directed treatments (i.e. individual plants will be treated). The application rate will depend on the number and size of target plants. **The typical application rates for these**

herbicides are 1.5 pounds glyphosate per acre, 3.5 pounds hexazinone per acre, and 1 .0 pounds triclopyr per acre.

For each treatment unit monitored, the following information will be recorded:

total amount of product used (pounds or gallons)
total area of the treatment unit
date and time of start of treatment
date and time of end of treatment
location of treatment unit

VI. SELECTION OF TEST SYSTEM

The test system will consist of the areas treated with herbicides in the Eldorado, Sierra, and Stanislaus National Forests. These are the only three national forests in California that will be conducting herbicide applications during the next year.

For the purposes of this study, herbicide applications have been classified by active ingredient and method of application. Currently, the U.S. Forest Service uses four different products each containing one of three active ingredients. These herbicides can be applied from the ground or air. The following types of applications are currently used:

hexazinone/air:	Pronone® 10G/air
hexazinone/ground:	Pronone® 10G/ground
	Velpar® L/ground
glyphosate:	Accord®/ground
triclopyr:	Garlon® 4/ground

Four of the five application methods will be studied. Pronone® applications by ground will not be monitored. This is primarily due to funding limits. In addition, dissipation of Pronone® should be the same whether it is applied by air or ground. Aerial applications were chosen because they probably have greater off-site movement than ground applications.

VII. EXPERIMENTAL DESIGN/STUDY PLAN

A. Dissipation

Dissipation of glyphosate, hexazinone and triclopyr will be measured in four plant species:

Arctostaphylos spp. (manzanita-berries)
Ceanothus cuneatus (buck brush-shoots)
Ericameria arborescens(golden fleece-foliage)
Pteridium aquilinum var. pubescens (bracken fern-roots)

These plants were selected based on the results of the pilot sampling in phase one, plant part category, plant availability, and discussions with Native Americans. Specifically, the 14 plants studied in phase one were categorizied according to the part sampled (and used by Native Americans) and one plant from each category was selected for study. Food plants included elderberry, manzanita berry, and oak-acorn. Brush plants included bitter cherry, buck brush, deerbrush, dogwood, and willow. Foliage plants included deergrass, golden fleece, pearly everlasting, and watercress. Root plants included bracken fern rhizome and soaproot. The following plants were not selected for dissipation monitoring because they occur with low frequency within proposed treatment areas: bitter cherry, deergrass, dogwood, oak-acorn, willow, and watercress. Of the remaining plants, manzanita was selected over elderberry because of Native American preference, much greater abundance of manzanita, and higher frequency of herbicide detection. Buck brush was selected over deerbrush because of Native American preference (although not unanimous) and higher frequency of herbicide detection. Golden fleece was selected over pearly everlasting because of its availability throughout the dissipation study and higher frequency of herbicide detection. There was no strong Native American preference in this case. Bracken fern was selected over soaproot because of Native American preference.

For each of the 16 treatment-plant combinations (4 treatments X 4 plants), four treatment areas or units will be sampled. The actual number of units is unknown since an individual treatment unit may be sampled for more than one plant type or herbicide. An individual unit may also be sampled for both dissipation and **off-site movement. Each treatment unit will be sampled up to seven times after**

application: l-3 days after application, 3-5 weeks, 7-9 weeks, 11-13 weeks, 19-21 weeks, 27-29 weeks, and 35-37 weeks. Sampling may be discontinued if two consecutive samples show no detectable residue. Additional sampling dates may be added and additional samples may be collected if residues are detected throughout the study period. The sampling schedule may be modified if circumstances (e.g., inclement weather or fire) prevent access to the sampling areas. The appropriate plant parts will be sampled only if available; for example, manzanita berries will be unavailable during the first several sampling intervals.

Number of Samples = 4 treatments X 4 plants X 4 units X 7 periods = 448

B. Off-Site Movement

Off-site movement of glyphosate, hexazinone, and triclopyr will be measured in up to 13 plant species, the same ones studied in phase one:

Anaphalis margaritacea (pearly everlasting-foliage)
Arctostaphylos spp. (manzanita-berries)
Ceanothus cuneatus (buckbrush-shoots)
Ceanothus integerrimus (deer brush-shoots)
Chlorogalum pomeridianum (soaproot-bulb)
Comus spp. (dogwood-shoots)
Ericameria arborescence (golden fleece-foliage)
Muhlenbergia rigens (deergrass-stalks)
Prunus emarginata (bitter cherry-shoots)
Pteridium aquilinum var. pubescens (bracken fern-roots)
Rorippa nasturtium-aquaticum (watercress-foliage)
Salix spp. (willow-shoots)
Sambucus mexicana (elderberry-berries)

For each of the four treatments, six treatment areas or units will be sampled. The actual number of units is unknown since an individual treatment unit may be sampled for more than one herbicide. An individual unit may also be sampled for both off-site movement and dissipation. Each treatment unit will be sampled up to three times after application: l-3 days after application, 3-5 weeks, and 11-13 weeks. The sampling schedule may be modified if

circumstances (e.g., inclement weather, fire) prevent access to the sampling areas. Each time a unit is sampled, a single sample will be collected at each of four distances. The distances will range from 5 to 100 feet from the edge of the treated area. The specific distance for each sample will be recorded. Any of the 13 plants may be sampled, but priority will be given to the four plants sampled for dissipation. All samples will be collected from the drainage or seepage areas that are buffered (untreated) within the treatment units, or adjacent to waterways downstream of the treatment units. These areas have the highest probability of plants containing herbicides due to water runoff.

Number of Samples = 4 treatments X 6 units X 3 periods X 4 samples = 288

Additional miscellaneous samples will be collected and analyzed as described in Appendix 1.

VIII. SAMPLING AND ANALYTICAL METHODS

A. Sampling Methods

If a sufficent number of plants are available, each sample will be a composite collected from 3 to 20 plants. Since most of the plants to be sampled do not occur throughout the sampling area, plant selection cannot be randomized. Representative plants will be selected from designated sampling areas within the treatment units. The method for collecting plant samples is described in EHAP Standard Operating Procedure FSOTOO1.OO (Supplement 2). All samples will be refrigerated or frozen from the time of collection until laboratory analysis. All sampling information and analytical results will be recorded on the chain of custody form (Supplement 3) as described in EHAP Standard Operating Procedure ADMN006.00 (Supplement 4).

B. Analytical Methods

Preliminary analytical methods were developed in phase one (Supplement 5). These analytical methods are currently being revised and validated. The analytical methods will be validated, and the standard operating procedures **written and approved prior to the analysis of any samples.**

C. Quality Assurance/Quality Control

This study will comply with U.S. EPA requirements for Good Laboratory Practices (40 CFR Part 160).

Method Detection Limits were determined according to EHAP Standard Operating Procedure QAQC001.00 (Supplement 6) and U.S. EPA procedure (40 CFR, Part 136, Appendix B) as part of phase one. The Method Detection Limit for each chemical and plant will be given in the analytical Standard Operating Procedures.

Method validation has been completed or is near completion for each of the 13 methods to be used for the dissipation monitoring. Validation follows EHAP Standard Operating Procedure QAQC001.OO, including the following: For each method a series of three replicate spiked samples are analyzed at three different spike levels, 0.3 ppm, 3.0 ppm, and 30 ppm. The spike levels were chosen based on the range of concentrations detected in phase one. The mean recovery and standard deviation will be calculated for each method. Assuming there is no difference with spike level, warning limits will be established at the mean recovery plus two times the standard deviation and the mean recovery minus two times the standard deviation. Control limits will be established at the mean recovery plus three times the standard deviation and the mean recovery minus three times the standard deviation.

Number of Samples = 12 methods X 9 samples = 108

Storage stability tests are in progress. Two replicate spiked samples will be analyzed at the following storage times: 0, 3, 6, and 9 weeks after spiking.

Number of Samples = 12 methods X 8 samples = 96

Laboratory continuing quality control will follow EHAP Standard Operating Procedure QAQCOOl .OO and include the following:

matrix blank: 1 sample per extraction set
matrix spike: 1 sample per extraction set

Any spike samples falling outside the warning or control limits will have the appropriate steps taken as described in EHAP Standard Operating Procedure QAQCOO 1 .OO.

D. Sample Storage, Transport and Tracking

Sample storage and transport are described in EHAP Standard Operating Procedure QAQC004.00 (Supplement 7). Sample tracking is described in EHAP Standard Operating Procedure QAQC003.00 (Supplement 8)

IX. DATA ANALYSIS

For methods that have been fully validated, sample results will be reported as parts per million, fresh weight basis (equivalent to milligrams of herbicide per kilogram of plant material). For methods that have been partially validated, sample results will be reported as concentration estimates: low concentration = 1 - 10 times the reporting limit, medium concentration = 10 - 100 times the reporting limit, and high concentration = more than 100 times the reporting limit.

Data collected for determining the dissipation of glyphosate, hexazinone and triclopyr will be analyzed by linear regression analysis. Concentration data will be transformed by natural logarithm before analysis. The regression analysis will describe the natural logarithm of concentration as a function of days elapsed since application. Each regression equation will be checked for lack of fit before it is used to estimate the rate of dissipation. Provided the observed decay is first order, the slope of the linear regression function quantifies the rate of dissipation. Unbiased back-transformed concentrations will be obtained from the predicted natural logarithm concentrations by using the back-transform routine developed by Powell (1991).

Data collected to investigate the potential for off-site movement of glyphosate, hexazinone, and triclopyr will be analyzed first as frequency of detections of each compound in off-site samples. If sufficient detections are obtained, further analysis may be conducted to investigate the frequency of detections at various distances from the field. Regression analysis of concentration (or natural

logarithm concentration) as function of distance from the site may also be performed depending upon the results. In the case of commonly found plant species, it may be possible to compare detections by plant species.

X. ESTIMATED TIMETABLE AND NUMBER OF SAMPLES

Experimental Start Date:	March 1997
Experimental Termination Date:	March 1999
Laboratory Analysis Completed:	April 1999
Data Analysis Completed:	August 1999
Study Completion:	December 1999

Progress reports will be issued every six months beginning in June 1997.

Because of funding limitations, field sampling will be divided between two years. The exact division will depend on the number and types of plants in the selected treatment units. It is anticipated that most of the dissipation treatment units will be initiated in the first year so that the sampling can be carried over into the second year if necessary. Conversely, it is anticipated that most of the off-site treatment units will be sampled in the second year.

Number of Samples = 448 dissipation + 288 off-site + 204 QC
= 940

XI. RECORDS TO BE MAINTAINED

The following documents will be maintained at the testing facility as described in SOP ADMNO05.00 (Supplement 9).

- All raw data other than those records maintained by the laboratory

- The study protocol bearing original signatures of the study director, sponsor, and quality assurance officers, including amendments and documentation of deviations

- All correspondence necessary to reconstruct the study

- All progress reports and audits

- Documentation of the training and experience of personnel involved in the study

- A copy of the final report

XII. REFERENCES

Powell, S. 1991. Implementation in the SAS® System of the Bradu and Mundlak minimum variance unbiased estimator of the mean of a lognormal distribution, ***in Proceedings of the Sixteenth Annual SAS Users Group International Conference.*** SAS Institute Inc., Cary, NC.

APPENDIX 1 - MISCELLANEOUS SAMPLES

The following additional monitoring will be conducted. However, the sampling and analytical methods may not be fully validated and this part of the study will not follow Good Laboratory Practices. DPR will fully fund this part of the study.

A. Redbud Monitoring

Cercis occidentalis (redbud-shoots) is frequently used in the preparation of Native American baskets. However, it has not occurred in any of the treatment units sampled to date. A test application of hexazinone (Velpar® L)will be made to an area that contains redbud in the Sierra National Forest. This test site will be used to determine the dissipation of hexazinone in redbud. The same study design as described above will be used for this site.

B. Mushroom and Acorn Monitoring

Quercus spp. (oak-acorns) and mushrooms are frequently consumed by native Americans. However, they did not occur in any of the treatment units sampled to date. Samples of these species will be collected from inside treatment units, if they occur. As in phase one, only qualitative analytical methods will be used. Sampling and analysis will only detect the occurrence of herbicides, not the exact concentration.

APPROVALS

Randall L Yeddeloh — 12/22/97

Sponsor (U.S. Forest Service) — Date

John D. Sanders — 1/15/98

Management (Dept. Pesticide Regulation) — Date

Randy Segawa — 12-16-97

Study Director (Dept. Pesticide Regulation) — Date

Cindy Garrett — 1/8/98

Quality Assurance Officer
(Dept. Pesticide Regulation) — Date