Appendix H. California Department of Fish and Games Procedure for Fish Tissue Sample Collection, Laboratory Methods and Fish Tissue Quality Control Results.

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1.0 Sample Collection

1.1 Sample Identification

Each sample will be uniquely identified by a number previously designated. This number will also be used on the sampling forms. Labels with adhesive backing and with the sample number will be used. Extra labels will be available. Should more labels be required, they will be prepared with a permanent marking pen in the field. Ziplock bags used to carry samples will be labeled by writing appropriate identification directly on the bag or label using a permanent making pen.

1.2 Field Storage of Samples

The fish collected by the Yurok Tribe will be double wrapped in aluminum foil, the wrapped fish placed in a ziplock bag, and the bags kept in chilled storage in the field. Insulated ice chests and frozen plastic-encased coolants (i.e., blue ice) will be used. For long-term field storage of samples, dry ice will be used. The sampling team will have sufficient number of ice chests and frozen coolants to assure that samples remain chilled throughout the day. The samples must always be kept in the possession of the sampling team until they are transferred to the custody of the laboratory. Since the ice chests will have to be kept in a locked car or truck, the vehicle should be parked in the shade to the extent possible. Ice chests will be cleaned with water and stored uncovered after each day. Sealed refrigerants will be washed with water and put into a freezer for reuse.

1.3 Storing and Shipping Samples

1.3.1 Storage at the Laboratory

The samples received at the Department of Fish and Game laboratory will be kept in secured refrigerators or freezers. Refrigerators will be kept at 4 degrees C, and freezers will be kept at -15 degrees C or cooler. Storage will be in an environment where the sample identification numbers will remain attached. Mechanical refrigerant units shall be used. The of ice as a refrigerant for sample storage is not allowed.

1.3.2 Shipping

All samples will be refrigerated or frozen during shipment through the use of cold packs or dry ice. Ice is not acceptable as a refrigerant. Samples will be shipped in insulated containers.

2.0 Sample Custody

The Department of Fish and Game chain-of-custody procedures for sample tracking are initiated during the time of actual sample collection by field collection Yurok Tribe personnel and maintained throughout the time the samples are in the possession of Yurok Tribe field collection personnel. Since the data generated will be used for scientific purposes, a Record of Custody will

be followed for non-regulatory purposes.

2.1 Record of Custody

The person responsible for sample collection must originate the chain-of-custody record. The sampler will clearly label the sample with the project name, sample location, field identification number, the date and time of sampling, and name and initials. This information is entered on the chain-of-custody record along with information concerning the sample type, the analyses to be performed and the sample container. The individual collecting the samples will be responsible for the custody of the samples until they are transferred or properly dispatched. If samples are hand-carried to the Fish and Game laboratory personnel, custody of samples will be transferred to the laboratory sample custodian. Samples transported by commercial carrier will have chain-of-custody record accompanied the samples when transported to the laboratory. The chain-of-custody form will be placed inside a ziplock bag and transmitted along with the samples. Each subsequent custodian of the sample(s) must complete a new line on the chain-of-custody and enter the date of receipt.

The sample custodian at the Department of Fish and Game Pesticide Investigations Unit (PIU) laboratory will carefully inspect each sample for chain-of-custody documentation, sample labeling, packing lists, and for the condition of the sample. Any discrepancies or problems associated with sample shipment will be documented on the chain-of-custody form.

After inspection, the samples will be entered into the laboratory sample tracking logbook, and will be assigned a unique sample identification number. The following information shall be included when samples are logged-in:

- date received and by whom
- date sampled and by whom
- required analysis
- sample location
- sample matrix

The sample custodian will ensure that the samples are either retained in secure storage or are in the possession of the authorized analyst during the time in which the samples are in the laboratory. The chain-of-custody form will accompany the samples through analysis. The completed chain-of-custody form will retained as a permanent part of the project record.

2.2 Sample Handling, Storage, and Holding Times

All samples will be handled, prepared, transported, and stored in a manner designed to minimize bulk loss, analyte loss, contamination, or biological degradation. The tissue samples must be frozen to prevent degradation or volatilization. Samples will be stored frozen for the maximum holding time (six months) for analyses.

3.0 Study Design

3.1 Procedure for Processing Fish Samples

Samples of fish collected by the Yurok Tribe will be analyzed for 2,4-D, triclopyr, and atrazine residues by the California Department of Fish and Game Water Pollution Control Laboratory (WPCL). The California Deaprtment of Fish and Game PIU will process the fish for analyses using Standard Operating Procedure B-5 (see attached). A sample (100 grams) of entire fish will be submitted for analyses; similar (same species and similar size) fish from the same collection area will be composited for analyses. The number, size (standard length), and species of each sample will be noted on the COC. The sample will be processed using a Brienkman Bottle 400 Mixer/Homogenizer that will be chemically cleaned after each use. The sample will be placed in a chemically-clean glass jar.

3.2 Quality Assurance/Quality Control Procedures

A set of ten samples will constitute a batch. For each batch of samples, percision (relative percent difference on duplicate samples) and accuracy (relative percent error on spiked samples) estimtes will be provided by WPCL.

3.3 Detection Limits

The detection limits are:

Analyte	ng/g
2,4-D (& dichlorphenol?)	50
Triclopyr (& trichloropyridinol?)	50
Atrazine (& hydroxyatrazaine?)	50 10

B-5 SOP for Tissue Sample Extraction for Pesticide Residue Analysis

1. The person preforming necropsies must wear plastic or rubber gloves, a lab coat or apron and when necessary, a mask if the possibility of disease is present. Person preforming necropsies must be able to identify fish and wildlife anatomy to ensure proper tissue extraction.

2. Keep all samples refrigerated until time of analysis.

3. Put down a clean sheet of aluminum foil, dull side up, on the stainless steel necropsy table to prevent contamination from previous dissections.

4. Wash glassware and any dissecting utensils as described in the SOP B-7. Rewash utensils between specimens and between tissues (if necessary).

5. Following extraction of tissue, place the tissue sample in a chemically clean (see SOP B-7) glass jar, and place a clean, square of aluminum foil over the opening of jar (or use a Teflon® lined lid), and seal lid tightly.

6. Label each bottle with your name, date, "P" number of sample, sample location, and contents of sample.

7. Make sure labels are on jars in plain view and written in indelible ink. Complete a Chain of Custody form (FG-1000) for each sample or sample set. The samples should be refrigerated in a secure location until transported to the WPCL (or other laboratory) for analysis. The COC should be kept with any other maps or paperwork associated with the sample or investigation (i.e., FG-406) in a secure location. If samples cannot be transported to the laboratory within one to two hours, these should be frozen at a temperature of 0 °C.

8. Wash down the necropsy table with soap and water.

9. Place the remaining specimens in a large garbage bag and store in freezer in lock up in the warehouse until it can be disposed of properly. Make sure the specimens are correctly labeled with actual contents, date of storage, "P" number, and name of investigator.

B-7 SOP for Cleaning Glassware and Utensils for Pesticide Residue Analysis

Laboratory glassware must be clean and free of any substance that may influence the results of chemical tests. We will adopt the procedures in accordance with the USEPA (1991) recommendations.

NEW PLASTIC ITEMS:

New plasticware does not generally require thorough cleaning before use. Plastic containers used to hold water samples for trace-metal analysis should be soaked in 10% (v/v) nitric acid solution and rinsed with deionized water. New glassware must be soaked overnight in 10% nitric acid solution. NOTE: If unopened "certified trace-clean" sample bottles and jars are used for water and tissue samples, this is not necessary.

CONTAINERS AND UTENSILS:

1. Soak 15 minutes in tap water, and scrub with detergent..
2. Rinse twice with tap water.
3. Carefully rinse once with fresh, dilute (10% v/v) nitric acid to remove scale, metals, and bases. Use protective safety clothing and equipment when acid rinsing. Clean up immediately any spilled acid solution.
4. Rinse twice with deionized water.
5. Rinse once with full-strength, pesticide-grade acetone to remove organic compounds (inside of the fume hood).
6. Rinse three times with deionized water.

OTHER COMMENTS:

This procedure is used for all glassware including beakers, glass funnels, watch glasses, and bottles. Dissecting utensils or any piece of laboratory equipment used to extract tissue samples for analysis are washed in warm, soapy water, and rinsed in tap water, petroleum ether, deionized water, and then air dried. The items are then placed on clean, aluminum foil until used.

STATE OF CALIFORNIA
DEPARTMENT OF FISH AND GAME

PESTICIDE LABORATORY REPORT

1701 Nimbus Road, Suite F
Rancho Cordova, California 95670

Lab No: P-2250 Date Received: 06/05/01

E.P. No. L- 263-01 Sample: fish tissue

Index: K112 PCA: E2794

To: Mr. Kean Goh
California Department of Pesticide Regulation
Environmental Monitoring and Pest Management
1001 I Street
Sacramento CA 95812-4015

Report Date: 09/07/01

Background

Fish samples were collected by the Yurok tribe from McGarvey Creek (MCG) and the West Fork Blue Creek (WFB) and analyzed as part of a larger study related to the environmental fate of forest herbicides. Samples were processed by DFG and analyzed for residues of the herbicide triclopyr and its metabolite 3,5,6-trichloro-2-pyridinol and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and its metabolite 2,4-dichorophenol.

RESULTS OF EXAMINATION

Table 1. List of fish species, collection dates, weights (g) and lengths (mm).

SAMPLE NO.	Species	Collection Date	Weight (g)	length (mm)
MCG413	sculpin	04/13/01	19.7	104.7
WFB413	trout	04/13/01	11.7	104
WFB501	trout	05/01/01	10.1	95.2
MCG501	sculpin	05/01/01	19.5	104.7
WFB508	trout	05/08/01	23.3	115
MCG508	trout	05/08/01	20.8	112

Table 2. Residues (ng/g, fresh weight basis) of triclopyr, 3,5,6-trichloro-2-pyridinol, 2,4-D and 2,4-dichlorophenol[1].

SAMPLE NO.	Triclopyr	3,5,6-trichloro-2-pyridinol	2,4-D	2,4-dichorophenol
MCG413	ND[2]	ND	ND	ND
WFB413	ND	ND	ND	ND
WFB501	ND	ND	ND	ND
MCG501	ND	ND	ND	ND
WFB508	ND	ND	ND	ND
MCG508	ND	ND	ND	ND

[1]Minimum detection limits: triclopyr, 1 ng/g; 3,5,6-trichloro-2-pyridinol, 5 ng/g; 2,4-D, 2 ng/g; and 2,4-dichlorophenol, 5 ng/g.

[2]ND = no detectable residues ≥the minimum detection limit.

Table 3. Accuracy and precision values for laboratory analyses.

Analyte	Sample Date	Accuracy[1]	Precision[2]
2,4-D	04/13/01	95.5 % (MS/MSD) 101 % (LCS/LCSD)	1.05 % (MS/MSD) 3.96 % (LCS/LCSD)
Triclopyr	04/13/01	107 % (MS/MSD) 95.5 % (LCS/LCSD)	3.74 % (MS/MSD) 5.2 % (LCS/LCSD)
2,4-dichorophenol	05/08/01	56 % (MS/MSD) 91% (LCS only)	3.57 % (MS/MSD)
3,5,6-trichloro-2-pyridinol	05/08/01	75.5 % (MS/MSD) 67 % (LCS only)	6.60 % (MS/MSD)

[1] Mean recovery values for matrix spike and matrix spike duplicate samples (MS/MSD) and laboratory control spikes and laboratory control spike duplicates (LCS/LCSD).

[2] Calculated as relative percent difference between matrix spike and matrix spike duplicate samples (MS/MSD) and laboratory control spikes and laboratory control spike duplicates (LCS/LCSD), when available.

Conclusions

Laboratory analyses did not reveal detectable residues of the herbicides or their metabolites.

PESTICIDE INVESTIGATIONS UNIT
OFFICE OF SPILL PREVENTION AND RESPONSE

By _____
Joel Trumbo
Principal Investigator

Approved _____
Brian Finlayson, Chief
Pesticide Investigations Unit

cc:

Total Cost of investigation:	0
Chemical analysis:	$8,250.00
Assessment and report:	0