SAINT REGIS MOHAWK TRIBE, ENVIRONMENT DIVISION

Screening of Organophosphates and Carbamates in Tobacco Waste

Quality Assurance Management Plan

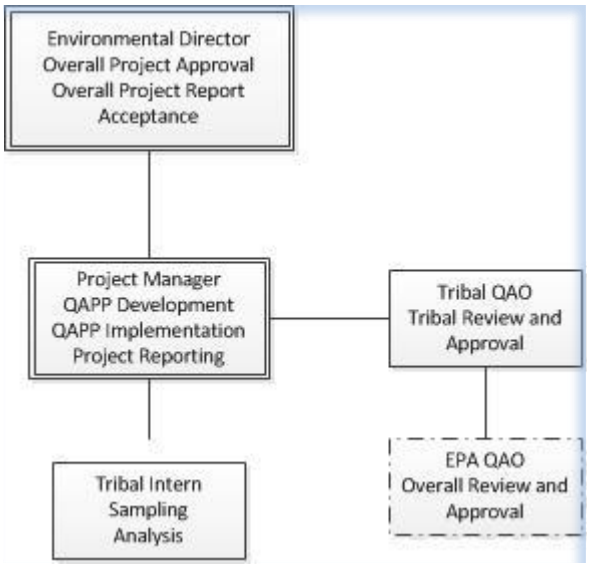
1/14/2013

Approvals

Les Benedict, Project Manager	Date
Aarti Reddy, USEPA, Pesticides Chemical/Environmental Engineer USEPA, Region 2, Pesticides Team	Date
Angela Benedict-Dunn, Tribal Quality Assurance Officer	Date
Kai Tang, USEPA, Regional Quality Assurance Officer	Date
Ken Jock, Director	
Denean Cook, Administrative Assistant	
Dan Bero, Procurement	

This document communicates the required measures for meeting the stated goals and objectives of the Tribe's Quality Assurance Management Plan (QAMP) requirements in characterizing tobacco waste for pesticide residues. It defines the roles and responsibilities of those involved with sampling and analysis and steps that will be taken to maintain and assess data quality throughout the stated project.

Organizational Chart



Distribution List

Note that paper copies need not be provided to individuals if equivalent electronic information systems can be used.

Les Benedict, Project Manager – Revisions, Implementation

Staff Intern - Sample Collection, Implementation

Angela Benedict-Dunn, TQAO, File

Kai Tang, USEPA QAO - File

Project/Task Organization

Name, Title	Organization	Role	Responsibility
Les Benedict, Assistant Director	Saint Regis Mohawk Tribe (SRMT)	Project Manager	QAPP development, project oversight, revisions
Angela Benedict-Dunn	SRMT	TQAO	Tribal level QAPP review and approval
Kai Tang	USEPA, Region 2	RQAO	EPA regional level QAPP review, review coordination and approval
Aarti Reddy	USEPA, Pesticides Team	Program Manager	Technical review, project approval
Ken Jock, Director	SRMT	General oversight	Project approval
SRMT, Project Intern	SRMT	Fieldworker	Sample collection, sample

			analysis
Denean Cook, Administrative Assistant	SRMT	Resource allocation	Budgetary approval
Dan Bero, Procurement Officer	SRMT	Resource procurement	Implement tribal policies in obtain services/supplies
Russell Phillips, Solid Waste Transfer Station Operations Manager	SRMT, Transfer Station	Waste handling decision maker	Utilize data analysis and interpretation for operational decisions
Lawrence Thompson, Recycling Coordinator	SRMT, Transfer Station	Waste stream decision maker	Utilize data analysis and interpretation to waste stream handling

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Problem Definition/Background

The Saint Regis Mohawk Tribe, Solid Waste Program, Transfer Station accepts solid waste materials on a daily basis from the Mohawk community of Akwesasne. Materials are brought in by contract haulers, individuals and commercial enterprises. These materials are held at the transfer station and regularly transported to permitted sanitary landfills in New York State.

The State of New York, Solid Waste Management Act of 1988 established policy that includes the disposal of solid that can't be reused, recycled or in the production of energy by other methods including composting. Overall, the policy provides benefits such as energy savings and pollution reduction by eliminating the need to transport it to a landfill. The Transfer Station is examining its operations to decrease operational costs including diverting compostable materials from the landfill thereby avoiding tipping fees. One of the compostable materials is tobacco waste disposed of by cigarette producers operating in Akwesasne.

Before the Tribe can integrate tobacco waste into a composting program it needs to obtain assurances that the final compost product doesn't contain residual pesticides and place consumers at unnecessary risk. The tobacco industry relies heavily on pesticides for crop management. There is a lot of controversy concerning the disclosure and accuracy of information about pesticide residues in tobacco because of alleged industry secrecy and accuracy of reporting of tests they conduct. The most up to date information available for this project is the Government Accounting Office (GAO) report, Pesticides on Tobacco, Federal Activities to Assess Risks and Monitor Residues published Ennisko:wa/March 2003.

The report cites domestic commonly using 37 pesticides for use on tobacco by EPA, many that are also used on food crops. Residues in varying ranges remain on tobacco from application in the field to the time a consumer smokes it. Table 1 lists commonly used pesticides listed by the GAO. This project is interested in all the chemical insecticides but will be limited to those most easily measured for (**bolded**), at least for the initial screening. Identification of this selected list will be used as an indicator for further investigation if detected and detected at elevated levels.

Table 1 Pesticides Commonly Used on Domestic Tobacco, 1990-98. USEPA, GAO.

Pesticides Commonly Used on Domestic Tobacco, 1990-98	
Primary use(s)	Pesticide
Insecticide	Acephate, **aldicarb**, Bacillus thuringiensis, **carbaryl**, **carbofuran**, **chlorpyrifos**, **diazinon, disulfoton**, endosulfan, ethoprop, fenamiphos, fonofos, imidacloprid, **malathion**, methidathion, methomyl, spinosad, trichlorfon
Herbicide	Benefin, clomazone, diphenamid, isopropalin, napropamide, pebulate, pendimethalin, sethoxydim, sulfentrazone

Fungicide	Dimethomorph, mancozeb, mefenoxam, metalaxyl
Plant growth regulator	Ethephon, flumetralin
Plant growth regulator, herbicide	Maleic hydrazide
Fumigant, insecticide	Chloropicrin
Fumigant, insecticide, herbicide	Methyl bromide
Fungicide, insecticide, herbicide	1,3-dichloropropene (1,3-D)

Table 2 is a list of organophosphates and Carbamates and associate detection limits that the screening is capable of detecting.

Table 2

Organophosphate	**PPB**
Azinphos methyl	**0.8**
Chlorpyrifosmethyl	**1.0**
Chlorpyrifos ethyl	**1.3**
Diazinon	**1.0**
Dichlorvos	**0.5**
Dicrotophos	**20**
Disulfoton	**25**
Ethion	**3.9**
Malathion	**1.4**
Parathion	**1.0**
Phorate	**4.0**
Phosmet	**0.7**
Carbamates	**PPB**
Aldicarb	**10**
Carbaryl	**160**
Carbofuran	**1.2**

There is some literature concerning composting of tobacco waste but it is primarily concerned with nicotine and carbon levels[1]. The primary concern in this study was the fate of nicotine, considered toxic, which decreased by over 80% in concentration.

In end this project will seek to determine whether or not the tobacco waste is safe to introduce as a compostable material knowing that it may end up in uses for home gardening and landscaping.

[1] Nur Okur, Ege Üniversitesi, Ziraat Fakültesi, Toprak Bilimi ve Bitki Besleme Bölümü, A Blok 35100 Bornova, İzmir, Turkey. Evolution of enzyme activities during composting of tobacco waste.

Project/Task Description

The project has two tiers: Tier 1 is to screen tobacco waste for organophosphates and carbamates OP/C utilizing a modified Ellman assay; Tier 2 is to analyze tobacco waste and composted tobacco waste through Gas Chromatograph-Mass Spectrometry (GC-MS) or High Performance Liquid Chromatography (HPLC) analysis for OP/C that may have been identified in Tier 1. This particular QAPP will address Tier 1 sampling and analysis. Tier 2 will be developed based on the results of Tier 1 results.

Tobacco waste entering the Transfer Station will be separated from other solid waste. It is typically dropped off in bagged containers. The tobacco will be sorted by facility. The waste from each facility will be homogenized (mixed) in a bin large enough to contain and mix the sample. Temporally spaced samples will be collected to represent changes in blends or source changes that might be experienced at the cigarette manufacturing facility. The bin and mixing tool will be constructed of a material that is hard surfaced and easy to clean.

Note that these samples do not represent "official samples" as defined by USEPA, Federal Insecticide, and Rodenticide Act (FIFRA) Inspection Manual, February 2002 since the purpose for sampling is non-regulatory in nature. The samples are considered "residue samples" as defined in the manual:

> *"These samples are collected in accordance with federal sampling procedures and the sample containers are identified with the sample number and the inspector's initials. Photographs should be taken of the sampling area in such a way to provide the reviewer with perspective of what the inspector did and observed in the sampling area. Photographs should be identified in the same manner and with the same number as the sampling container."*

The samples will be collected in accordance with Sample Procedures as defined by the FIFRA Inspection Manual. The samples that are collected will be placed in 500 ml glass amber sample jars, each uniquely identified. Sample size for Tier 1 is defined by the ELISA kit which requires 20 gm of sample, the FIFRA Inspection Manual indicates enough sample be collected to accommodate analysis, splits and duplicates. The samples will be documented using sample data sheets and chain of custody forms. Samples will be stored and batched for analysis.

Sample collection and processing will occur at the Transfer Station located at 179 County Route 43, Ft. Covington, NY (Map 1). Processing will take place at the facility. Samples will be transported and stored and then analyzed at the Environment Division lab located at 449 Frogtown Road, Akwesasne, NY Map 1.

Map 1

Sample preparation and analysis consists of methanol extraction of material for colorimetric and spectrophotometer assays (qualitative and quantitative respectively) based on detection of OP/C inhibiting Acetyl Cholinesterase (Ach-E) enzyme. The assay procedure provides for negative and positive controls as well as duplicates, blanks and standards for calibrations.

Quality Objectives and Criteria for Measurement Data

Objectives and criteria for sampling are to collect tobacco samples which represent waste streams entering the solid waste transfer station facility from several sources for pesticide residue analysis. The criteria for representative sampling include:

- The waste must be identified by source or the facility that it originates from
- The waste must be homogenized thoroughly before samples are drawn
- The waste must be collected over a minimum three month time period from each facility

Problem Statement

The decision unit for the Tier 1 and 2 objective is annual tobacco waste volume/tonnage collected at the Transfer Station. The estimated annual volume/tonnage is approximately 770 cu-yds/208 tons. There is a question of whether or not the tobacco waste contains pesticide residues that would pose a risk to humans if used in compost.

Diverting compostable waste from the landfill will reduce the Tribe's disposal costs and translate into fuel savings and vehicle emissions reductions.

The objective for the Tier 1 sampling is to determine the presence of OP/C in tobacco and to quantify select OP/C to develop a refined Tier 2 analytical plan, if necessary.

Planning Members are:

Les Benedict, Assistant Director
Russell Phillips, Operations Supervisor
Lawrence Thompson, Recycling Coordinator

Decision Makers are:

Ken Jock, Director
Les Benedict, Assistant Director
Aarti Reddy, USEPA, Pesticides Chemical/Environmental Engineer

Resources

Staff:

- Project Manager
- Student Intern

Supplies:

- Abraxis OP/C titer kit
- Abraxis Microphotomer, PN 475101 with 490 nm filter
- Reagents: methanol (pesticide grade)
- Equipment: 125 mL pre-cleaned glass bottles with screw caps, 13 x 125 mm glass test tubes, serological pipets, micropipet (100 mL), vortex mixer, scale or balance.
- Sample jars, required
- Sample labels, required
- Data Sample sheets
- Chain of Custody sheets

Deadlines

Order Abraxis OP/C kits – July 2013
QAPP Approval required – April 2013
Sample Collection – April-December 2013
Train in use of kits – August 2013
Sample Analyses – January 2014
Data Review – January - March 2014
Project Report and Recommendations - June 2014

The Decision

The Tier 1 decision to be made is to identify specific OP/C in sampled tobacco waste for refined analysis.

The Tier 2 decision to be made is to divert tobacco waste from the landfill and use it as a composting feedstock if it doesn't represent a pesticide exposure risk to end users of compost made from it.

Alternative Actions

Tier 1 -

- Conduct a full analytical scan for OP/C
- Accept the Tier 1 screening as sufficient for Tier 2 decision making

Tier 2 -

- Do not use tobacco waste as a compost feedstock and continue to dispose of it at the landfill.
- Use tobacco waste in compost feedstock and place warnings/restrictions on its use.

Inputs to the Decision

Tier 1 –

- Confirmed Positives for OP/C
- Mean concentration (PPB) for individual OP/C

Tier 2 –

- Mean Concentration for OP/C
- Concentration Range for OP/C
- Risk posed by OP/C residual

Study Boundaries

Tier 1 and 2 domain(s)

- Temporally spaced samples for tobacco waste entering the transfer station within a 3 month time period
- Organizationally defined sources of tobacco waste (strata)

Decision Making Scale

Tier 1 – The scale is multi-scale requiring involvement between the Tribe and USEPA.

Tier 2 - The scale is multiscale requiring involvement of various tribal departments including operations, finance and administration and with USEPA.

Practical Constraints on Data Collection

- Supply chain
- Tobacco waste stream flow changes

Decision Rule

Tier 1 –

- See Table 2, OP/C Limit of Detection
- If OP/C are positive, then:
    - Analyze in tier 2 for OP/C that are detected

Tier 2 –

While the following table is specific to pesticide and commodity it is worth noting that the FDA has a zero tolerance level for these pesticides. The table is included as a reference and doesn't imply that it should have applicability for tobacco compost. It may be prudent to know that

ENFORCEMENT LEVELS FOR PESTICIDES HAVING A ZERO TOLERANCE

The following table lists the level at which an enforcement action may be considered when residues of these pesticides are found in a commodity that the cited regulation specifies as having a zero tolerance. *The analytical levels are based on the level at which residues of these pesticides can be detected, quantified, and confirmed in the particular commodity.*The enforcement levels specified for raw agricultural commodities also apply to their corresponding processed commodity.

Table 3

40 CFR Section	Pesticide	Commodity (see note "a") Level (ppm)	
180.131	Endrin	Vegetables 0.05	Cottonseed 0.05
180.139	Perthane	Milk 0.2 (fat basis)	
180.169	Carbaryl	Grains 0.2	
180.174	Tetradifon	Milk 0.4 (fat basis)	
180.190	Diphenylamine	Milk (see note "b")	

Note "a": Commodities cited as broad food classes of vegetables and grains are limited to only those specific commodities listed in the regulation as having a zero tolerance.
Note "b": No level can be prescribed at this time due to lack of analytical experience with this pesticide.

Limits on Decision Errors

Tier 1 –

The use of screening kits has been evaluated by several sources. EPA evaluated an OP/C kit to detect OP in drinking water. They evaluated the kits for accuracy, false positive and negative rates precision, matrix and interference rate as well as operational considerations.

In their evaluation of the kit for qualitative results they found accuracy of 100% (9/9), precision 90% (19/21), 2 false positives (2/6) and no false negatives[2].

The OP/C screening kit was evaluated using a range of leafy vegetables with the results displayed in Table 5. The table shows the degree of agreement between % Inhibition and Corrected Inhibition for many of the vegetable except for Kang Kong, Kai Lan and Long Beans, there was agreement within 10%. There is a fair degree of linearity (Table 6).

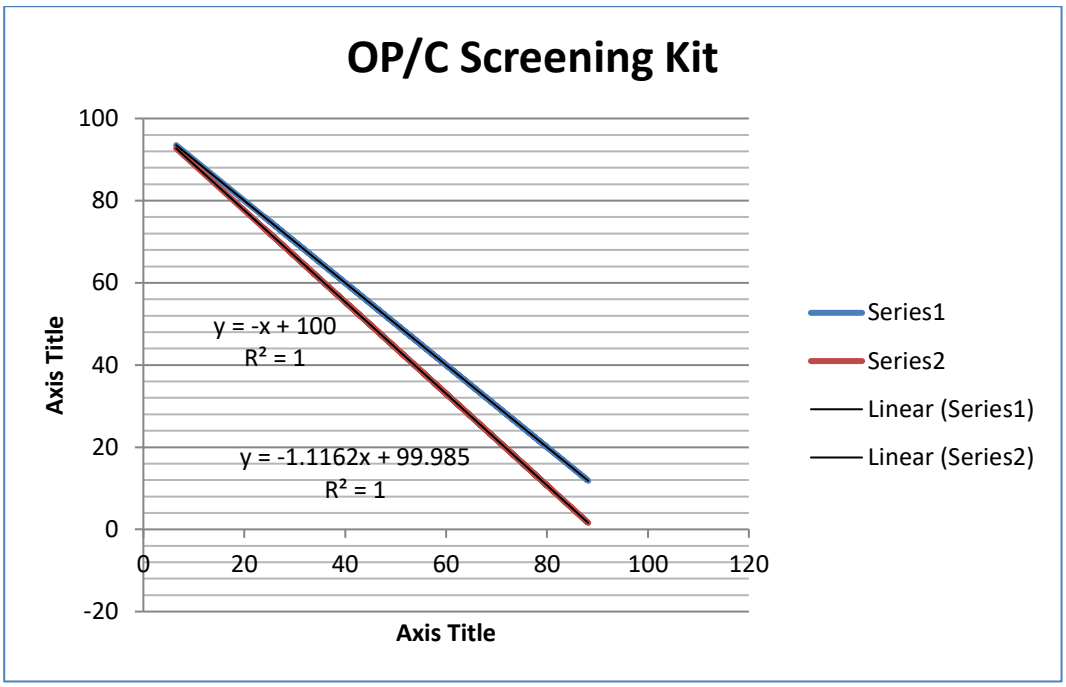
Table 4

Sample	Designation	OD 405 nm	B/Bo	**% Inhibition (100 – B/Bo)**	Corrected Met Ext (ref)	**Correct % Inhibition**
A	Local Sawi	1.139	41.24	**58.76**	46.04	**53.96**
B	Siew Pak Choy	1.171	42.40	**57.60**	47.33	**52.67**
C	Kang Kong	2.212	80.09	**19.91**	89.41	**10.59**
D	Bayam/Spinach	.0663	24.00	**76.00**	26.80	**73.20**
E	Kai Lan	2.336	84.58	**15.42**	94.42	**5.58**
F	Long Beans	2.435	88.16	**11.84**	98.42	**1.58**
G	Sawi (China)	0.18	6.50	**93.50**	98.42	**92.72**
Ext. Meth	50% extr methanol	2.474	89.57	10.43	7.28	0.00
Neg.	50% methanol	2.762	100	0.00	100.00	
Pos.	5 ppb Diazinon	0.229	8.29	91.71		

Information provided by ABRAXIS, LLC.

[2] The Environmental Technology Verification Program. ETV Join Verification Statement. USEPA. Batelle. 2006.

Table 5 OP/C Screening Kit



Tier 1 is screening and as such the analytical results will be considered to make the conclusion that OP/C's are *present* or *not present*.

Tier 1 Null Hypothesis: There are no OP/C present in tobacco waste.

The decision is to conduct a refined laboratory analysis if there are OP/C present in tobacco waste.

Alpha error rate – 95% confidence is desired, alpha = 0.05, Probability of < 0.05 of rejecting Null hypothesis
Beta error rate – 0.20
Standard Deviation – 2
Number of sub-samples per incremental sample - 2
Number of Increments per Sample – 10

Tier 2 – To be determined

Optimize the Design

The project will utilize multi increment sampling, an approach developed that pools several individual increments within a decision unit. The approach minimizes sampling and subsampling errors, normalize data distribution and optimizes analytical costs. Samples are systematic-random (there is an equal chance of selecting each unit from within the population when creating the sample) and include replicates.

Figure 1 is a comparison of "discrete sampling" with "incremental sampling". The model explains a "path" for an area. In the case of this effort the sampling points are tobacco loads, the decision unit is the time period during which the tobacco is collected.

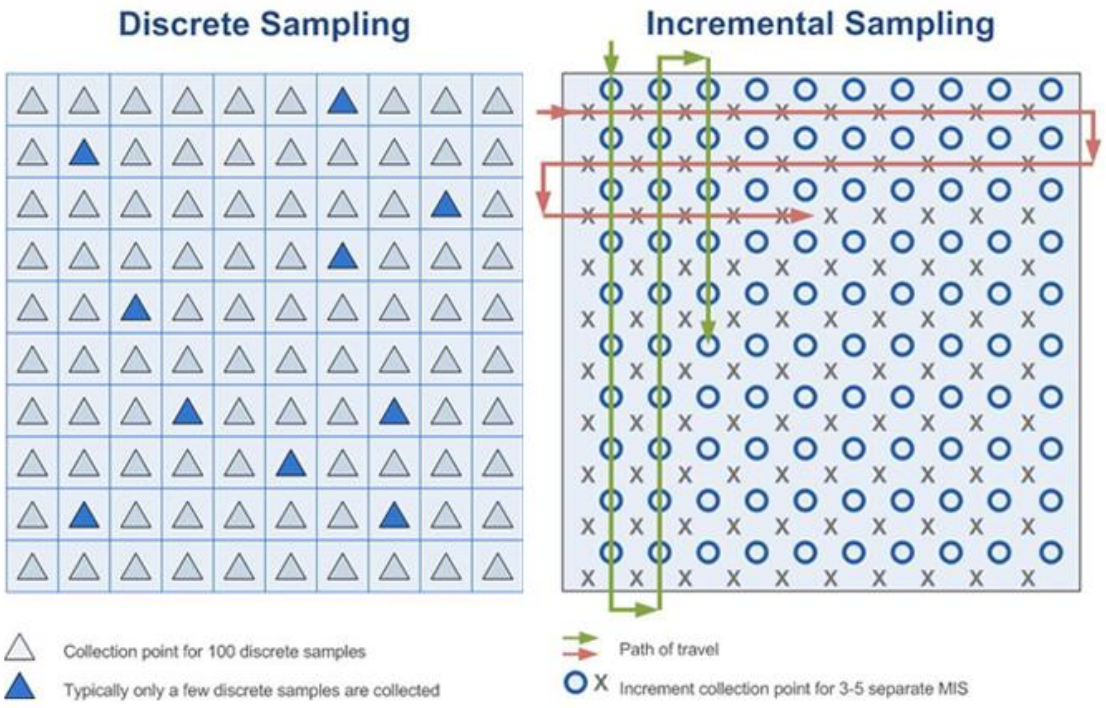


Figure 1 Discrete Sampling and Incremental Sampling Models

There are approximately 6 manufacturers that deliver waste tobacco to the transfer station.

Table 6 Sample Scheme

	Manufacturer/# Samples					
	A	B	C	D	E	F
Quarter 1	4	4	4	4	4	4
Quarter 2	4	4	4	4	4	4
Quarter 3	4	4	4	4	4	4
Incremental Samples	10	10	10	10	10	10
Total Subsamples	2	2	2	2	2	2
Total Samples	12	12	12	12	12	12

Special Training/Certification

The intern will require orientation with the QAPP and training in the use of the Abraxis kit. Training is provided by Abraxis at a nominal cost with the sale of their kits. The certificate of training for any staff will be kept with project file.

Orientation with the QAPP will consist of the intern being required to read the kit followed by a face-to-face meeting with the project manager. The meeting will consist of a briefing and overview of the project, tasks, sampling design and timeline. The intern will have an opportunity to ask questions about the project before starting.

Training in the use of the Abraxis kit will consist of on-site training by a representative of the company. The training will provide hands on learning of equipment and reagent use and handling, micropipette use, spectrophotometer set up and calibration, use of standards, controls and sampling interpretation.

The intern will be provided with basic health and safety training and use of personal protective equipment (PPE) that will be used while sample collection.

Documents and Records

The project manager will ensure that everyone involved with the project has a signed and dated QAPP prior to initiating work. If any changes are made personnel likewise will be provided copies of the revised QAPP. The QAPP will be distributed electronically with “received receipt” and “read receipt” function of the Outlook e-mail messaging system. Signed and dated QAPP copies and project reports and documents will be retained by the project manager in hardcopy in a file cabinet. Electronic copies of the project QAPP, reports and documents will be maintained in the Division file server which is regularly backed up.

The intern will be provided a copy of the Abraxis screen kit instructions before handling samples.

The final project report will consist of a summary of field collection activities, appendices of field data sheets, chain of custody sheets, equipment calibrations and data points. The report will identify the number of positives and negatives, results of positive and negative controls, duplicates, total number of samples collected, total number of samples collected by manufacturer, etc.

DATA GENERATION AND ACQUISITION

All samples will collected using a sample data sheet (Appendix E). Each sample jar will be uniquely identified with a sample number. Corresponding to each sample number will be person's name collecting sample, sampling date, corresponding manufacturer name and sample type (sample of subsample). A sample custody sheet will be used to track samples from field collection to storage until batch sampling and handling in the laboratory.

The project manager will oversee sample collection and make corrections on site if they deviate from sampling procedure. The project manager will inspect and review data sheets and chain of custody sheets as they are created and make corrections as required. Incorrect entries will be struck out and a correct entry made where it is legible. All entries will be made in permanent ink.

Data generated from the screening, positive or negative results of each sample will be recorded directly in MS Excel workbook. The workbook will be saved on the department file server that is backed up regularly.

Sampling Process Design (Experimental Design)

Samples to be analyzed = 72
Field Blank = 4
Negative Control = 1
Positive Control = 1
Wipe Samples, mix container = 6 total for project
Rinsate sampling tools = 6 total for project

Total = 90

As each manufacturers waste enters the transfer station the tobacco will be homogenized using a clean and rinsed container. Wipe samples will be collected from the mixing container and analyzed. Rinsate from cleaning of sampling tools will be collected and analyzed. Subsamples will serve as duplicates.

The anticipated precision for duplicates is 90% precision, 100% accuracy. Duplicates and other controls not meeting these criteria will be rerun.

The rate of sampling will be 4 random samples for each manufacturer per quarter. If a manufacturer doesn't generate enough in three quarters to create the required number of samples, sampling will continue into the next quarter.

Schedule

Figure 2 Sampling Schedule

The sampling scheme will demonstrate any seasonal variations in OP/C if there are any. The combined total and average number of positive and negative detections of OP/C for manufacturer will also be determined.

The incremental sampling scheme will consist of tobacco waste being emptied into a container and mixed with a stainless steel tool.

Since the sample mixing container and tool will be used for each set of samples between manufacturers the container and tool will be cleaned and rinsed and dried. A wipe sample will be collected between collections to identify any bias from cross contamination. Likewise, rinsate from the mixing tool will be collected.

The Abraxis screening kit contains enough materials for 96 analyses.

Sampling Methods

The FIFRA manual instructs the collection of dry samples:

> *Insert a clean unused plastic tube diagonally through the bag, when obtaining the sample, not just from one location in the bag.*

The FIFRA manual is similar in approach to ASTM D5451 applicable to sampling soils and similar fine-grained cohesive materials. In this method a "trier" or tubular metal device is used to obtain a sample. Triers vary in length but typically up to an inch in diameter. Readily available is the LaMotte soil sampling tube or XL Multipro Sector Probe, 30 ml capacity.

Figure 3 Sampling probe in use

For materials delivered in bags, the trier will be inserted diagonally through bags or piles for sample collection. Each sample (500 ml jar) will consist of increments collected from each bag of tobacco delivered. These increments will be deposited into a mixing pan and mixed. The resultant mix will be used to fill a 500 ml jar. The jar is to be lightly compressed to fill to ensure enough material in case reanalysis is required.

If the material is delivered loose it will be transferred to a large clean mixing container. EPA waste sampling guidance[3] recommends the use of scoops, spoons, push tubes, augers and other devices for sampling of piles. Most appropriate for tobacco waste is a spoon or push tube as they work well for materials <1" in size. The material will be mixed and piled and sampled using the trier. The trier will be inserted diagonally through the pile for sample collection to obtain an increment. The increment will be placed into a clean pan and mixed. The resultant mix will be used to fill a 500 ml jar. The jar is to be lightly compressed to fill to ensure enough material in case reanalysis is required.

The number of increments for each sample will be equivalent to the number of bags representing each manufacturer on the day it arrives at the transfer station. Where the bag exceeds the size capacity of the trier, the bag will be broken down into smaller piles and sampled. It will not be necessary to clean the mixing pan or tools for materials sampled on the same day from the same manufacturer when creating the incremental samples.

[3] USEPA. SESD Operating Procedure. Waste Sampling. November 1, 2007.

Only one incremental sample is to be collected per delivery. A total of 4 samples will be collected within a quarter. If less than 4 samples are attainable, sampling will continue until 4 samples are collected.

2 subsamples will be collected for each sampling event, this will be equivalent to a duplicate. Subsamples will be based on random selection.

Field blanks will consist of empty sample jars transported into the field that are handled in the same manner as regular sample jars. They will be opened, closed and labeled as all other samples. They will be analyzed with methanol rinsate in the lab.

Wipe samples will be collected from sample mixing containers using a clean wipe that is placed in a 500 ml jar. The wipe sample will identify cross contamination between samples.

The trier will be field cleaned and rinsed. Rinsate from the field cleaning will be used to identify cross contamination between sampling.

Sample Handling and Custody

All samples will be clearly labeled with sample ID number, collectors name, sample date, time of collection and the words "tobacco waste" indicating the matrix. Sample collection documentation will consist of a sample data sheet that is completed in the field with all relevant information (Sample Data Sheet, Appendix E)

All samples collected will be placed into an insulated cooler that contains ice packs (no ice). The cooler will contain materials to prevent breakage of sample jars from rough handling. The samples will be held in a refrigerator between 35 and 38 degrees F (1.7 to 3.3 degrees C) for short-term, up to 3-months. The long-term holding time for the samples is 1-year in a freezer around 0 degrees Fahrenheit or -13 degrees Celsius.

Sample custody will be maintained in the possession of the intern at all times. A chain of custody form (Appendix F) will be used to track the samples from field collection, storage in the lab to final lab analysis.

Samples will be transported directly to the Environment Division and placed in the lab refrigerator until analyzed.

Sample data sheets and chain of custody sheets will be handed in to the project manager upon storage in refrigerator/freezer. The project manager will store the originals in a file cabinet (unbound) with the project files and make them available as required.

Analytical Methods

Before each sample can be analyzed 20 gm (one cigarette weighs about a gm) of sample must be extracted with 40 ml of analytical grade methanol. The extracts then can be used for the OP/C tests.

The extract will be allowed to stand for several minutes to allow particulates to settle. Additionally, Abraxis recommends the preparation of a negative control for pigmented samples that may cause interferences. A control using "certified organic tobacco" will be used to account for turbidity effects if any.

20 gm of tobacco waste will be removed from the sample jar and placed in a clean 125ml bottle. A volume of methanol equal to twice the weight of the sample, about 40 ml, in bottle with tobacco. The jar will be gently shaken for 30 seconds and allowed to settle for 3 minutes. Extract is removed from the 125 ml jar by gently pipetting extract and storing in 100 ml, pre-cleaned sample vials. Each jar and vial will be labeled with an ID corresponding to each field sample collected. The 100 ml sample vials will stored until a batch can be analyzed with the Abraxis kit. Analytical duplicates will be obtrained from the vial at a 10% rate. Vials will be archived in a freezer for future reference.

Analysis will be performed using the Abraxis, colorimetric OP/Screen test. The test kit consists of plates for sample analysis, negative control and positive controls as well as matrix controls. A photometer is required to analyze the plates at 490 nm. The results are recorded.

Interpretation

Calculate the OP/C concentration on the sample by multiplying the assay result by the appropriate factors introduced by the procedure:

> assay result (ppb) x (vol. methanol (mL)/weight of sample (g)) x vol. extract (mL)/ volume of extract (mL) weight of sample (g) vol. extract (mL)

High readings or positive (+) readings are indicative of the presence of pesticides and will warrant further investigation, Tier 2 analysis.

The complete step-by-step OP/C screen test is located in Appendix B.

Quality Control

Field Sampling –

Subsamples, equivalent to duplicates - 12
Field blanks - 4
Mixing tool rinsate - 6
Mixing container rinsate - 6

Analysis –

The Abraxis kit contains a high positive control with the assay kit. It is a 5 ppb Diazinon in distilled water. It is to be included with every run and will be treated in the same manner as the unknowns.

Blanks and duplicates will be used for quality control checks in addition the kit positive control.

Checks will be performed for each assay run and in this case only once unless problems arise and additional runs are required.

After the methanol extract has been prepared, removal of the extract from contact with the sample and dilution of the extract to 50% methanol/water should proceed without delay. ***The batch size should not exceed the number of samples which can be extracted and diluted in one hour or less.***

Instrument/Equipment Testing, Inspection, and Maintenance

The only equipment that may require testing, inspection and maintenance is the photometer. It is factory certified before shipped.

Instrument/Equipment Calibration and Frequency

The instrument at the begin of a run is zeroed by insertion of a blank. It is calibrated using the blank and a single standard. Additionally, an interference standard, consisting of a blank extract of "organic tobacco" will be introduced to compensate for turbidity created by tobacco extract (discussed earlier as negative control).

In the event the instrument doesn't operate properly analysis will be suspended and samples stored while the unit is sent to the supplier for repair/replacement.

Inspection/Acceptance of Supplies and Consumables

The Abraxis kit will be inspected for completeness of shipment when it is received at the department. A complete kit contains:

- 20 assay tubes with white caps and 500 ul buffer
- 1 negative control test tube
- 1 positive control test tube
- 1, 1ml oxidizer vial
- 1, 2ml neutralizer vial
- 1 Ach-E reagent dropper bottle
- 1 Substrate dropper bottle
- 1, 2ml Chromogen dropper bottle
- 1, 2ml Stopping solution dropper bottle
- 22, 100 ul pipettes
- 2, 3 ml transfer pipettes
- 1 work station box
- Reagents stored in refrigerator

Supplies are ordering using uniquely numbered purchase orders, receipt of materials is tracked by invoices. Equipment is tracked by Tribal inventory. Records of purchase request, ordering, receipt and inventory will be tracked and stored by maintaining this information with project files.

Reagents will be checked to ensure expiration dates haven't been exceeded for the purpose of this project. The photometer will be inspected and checked for proper operation. Any deficiencies will be brought to the attention of the supplier and corrected before start of analysis. Any deficiencies or corrections will be noted.

The OP/C Screen Kit needs to be refrigerated until use, and then all of the reagents must come to room temperature before they can be used.

Non-direct Measurements

Not applicable.

Data Management

Data will be generated in the field from field sampling. This data will be in the form of data sheets and chain of custody forms. All records will be securely stored with the program manager at the end of each work day.

Data stored in electronic format, e.g. excel spreadsheet, will be stored on the departments file server. Access is restricted to authorized users. Data is protected through weekly backup of server files.

ASSESSMENT AND OVERSIGHT

Assessments and Response Actions

Tier 1 sampling and analysis is non-complex and as such assessments will be minimal. The assessments will primarily consist of direct oversight of the intern performing the work by the project manager. The manager will supervise work to ensure completion and conformance to step-by-step instructions allowing progression to subsequent steps only after approval by the manager.

Supervision will be on a daily basis. Any corrections will be noted.

Tobacco samples and extracts will be archived in the lab freezer in the event samples need to run again.

Reports to Management

The project will generate a data summary and recommendations report after all samples have been collected and analyzed.

The intern will be required to generate an Excel spreadsheet with the data and provide it to the project manager.

DATA VALIDATION AND USABILITY

Positive and negative controls will be reviewed to ensure they were run correctly and responded correctly. If they are incorrect, they will be run again.

Data Review, Verification, and Validation

Positive controls must react showing the presence of pesticides. If it shows otherwise the entire batch is suspect and must be run again. The negative control must not react and not indicate the presence of pesticides. If a negative control reacts positively the batch must be run again.

Verification and Validation Methods

The project manager will review documents and data to verify that project objectives for sampling and analysis have been conducted according to established criteria. Any deviations will be noted including results of field audits or inspections. This information will be incorporated into the final project report.

Reconciliation with User Requirements

Field Sampling

Field data will be analyzed statistically and graphed to demonstrate temporal characteristics of tobacco deliveries for each of the manufacturers. Deliveries should indicate an even distribution of deliveries over a time period and not be biased toward any one month or date to be representative of material flow over a 3 month time period unless deliveries were lacking and had to extend into another quarter. Any bias will be reported in the project report.

Field duplicates should both be reported to be the same result, positive or negative. If any duplicate is questionable the sample will be run again.

Analysis

Calibrations should be within manufacturers specifications.

Positives control, negative control and lab duplicates will be reported on. Controls should react as expected, if not the batch is suspect and should be rerun. The same requirements will hold true for blanks. Analytical duplicates should react the same way, either positive or negative and if different they will be rerun.

Any results and reruns will be reported on in the summary report.

Data Analysis

Descriptive statistical analysis to be performed for the data:

- Mean % Inhibition
- Maximum % Inhibition
- Minimum % Inhibition

Precision - Duplicates reported as RPD (relative percent difference)

Percent difference=100 percent x |Value1-Value2|/(Value1+Value2)/2

Accuracy - The linearity of the calibrations performed for each analytical run is the determination of accuracy. The specifications of the instrument for linearity is 0.000-1.500 Optical Density (OD) (+/-2%). The default coefficient value for the instrument is 1.000. The instrument receives a 2 point calibration using a standard and a blank.

Completeness - (sampling and analytical)

%C = 100% X [V/T]

%C = percent completeness
V = number of measurements judged valid
T = total number of measurements

Waste Handling

Tobacco materials generated will be disposed of in regular waste. Minimal quantities of liquid waste, e.g. methanol rinsate, sample residue will be allowed to evaporate under a lab fume hood before disposal of sample containers.

Appendices

A – Evaluation of Abraxis OPC Kit
B – AOP C TB User's Guide
C – ETV Joint Verification Statement
D – Combo Miniphotometer
E - Sample Data Sheet
F – Chain of Custody Sheet