NORTH COAST LABORATORIES LTD

SOP CODE	VERSION	COPY	EFFECTIVE DATE	LOCATION OF ORIGINAL	WP FILE
ME 0011	02	J	08 / 30 / 89		VITA

	NAME	TITLE	DATE
PREPARED BY:		PRINCIPAL SCIENTIST	8/7/89
APPROVED BY:		STUDY DIRECTOR	8/30/89
APPROVED BY:		QA OFFICER	8/30/89
APPROVED BY:		LAB DIRECTOR	8/7/89
VALIDATED BY:			/ /

1.0 TITLE

Procedures For Extraction of VITAVAX, VITAVAX SULFOXIDE, and VITAVAX SULFONE From Soil; Conversion of the Extracted Analyte to Aniline, and Subsequent Determination of Aniline by TSD-Gas Chromatography.

2.0 SCOPE

To set forth detailed procedures for the analysis of VITAVAX, VITAVAX SULFOXIDE, and VITAVAX SULFONE as ANILINE.

3.0 PURPOSE

To assure that all analyses of soils for VITAVAX as Aniline are carried out in a consistent manner at NCL.

4.0 DEFINITIONS

NA

5.0 MATERIALS

5.1 Materials required for extraction of VITAVAX from soils

5.1.1 # 10 sieve.
5.1.2 Top loading balance capable of weighing accurately to 0.01 g.
5.1.3 Centrifuge tubes capable of holding 10 g soil and 30 ml acetone.
5.1.4 Wrist arm shaker.
5.1.5 Centrifuge capable of spinning tubes (from above) to produce a clear soil extract.
5.1.6 25 ml graduated cylinders.
5.1.7 100 ml round bottom boiling flasks.
5.1.8 Rotary evaporator capable of evaporating acetone.
5.1.9 Spider attachment for rotary evaporator.
5.1.10 Waterbath capable of holding water temperature @ 28-32 degrees C.
5.1.11 Glass 60 degree funnels for 9 cm. paper.
5.1.12 9 cm. "Shark skin" filter paper (S&S).
5.1.13 Repipet capable of delivering 25 ml acetone.

5.2 Materials required for conversion of VITAVAX to Aniline.

5.2.1 Pasteur pipets.

5.2.2 5 ml reaction (RX) vials; Supelco # 3-3299 or equivalent.
5.2.2.1 Reaction vial caps; Supelco # 2-3264 or equivalent.
5.2.2.2 Reaction vial cap septa; Supelco # 2-3269 or equivalent.
5.2.3 Electric heater block capable of holding reaction vials from section 5.2.2 at 110-115 degrees C for 90 minutes.
5.2.4 10ul, 25ul, 100ul, 1ml, 2.5ml, & 5ml syringes.
5.2.5 1.5 ml or other suitable storage vials for final extract.

5.3 Materials required for gas chromatographic analysis of aniline.
5.3.1 10 ul syringe.
5.3.2 Gas chromatograph equipped with nitrogen-phosphorous specific detector, 0.53 mm DB-5 capillary column, and data system capable of peak identification and quantification.

5.4 Required chemicals
5.4.1 Required chemicals for extraction of Vitavax from soil.
5.4.1.1 Pesticide grade acetone.
5.4.1.2 Furnaced sodium sulfate.
5.4.2 Required chemicals for conversion of VITAVAX to aniline.
5.4.2.1 1:3 (v/v) sulfuric acid. 333ml sulfuric acid up to 1 L with deionized water.
5.4.2.2 10 N. NaOH. 400 g reagent grade NaOH up to 1 L with deionized water.
5.4.2.3 Pesticide grade benzene.
5.4.3 Required chemicals for manufacture of standards.
5.4.3.1 Aniline, neat, 99+% purity. Available from Chem Service. Note that this standard is subject to destruction by exposure to oxygen and light. Store in the dark in freezer.
5.4.3.2 VITAVAX neat standard, 99+% pure. Available from Uniroyal Corp. Naugatuk, Conn.
5.4.3.3 VITAVAX SULFOXIDE, 99+% pure. Available Uniroyal Corp. Naugatuk, Conn.
5.4.3.4 VITAVAX SULFONE (PLANTVAX), 97+ % purity. Available from Uniroyal Corp. Naugatuk, Conn.
5.4.3.5 Pesticide grade methanol or equivalent.

6.0 PROCEDURE
6.1 Flow chart of method. See exhibit 1 this SOP.

6.2 Procedure for extraction of VITAVAX from soil.

6.2.1 Sieve soil through 10 mm sieve.

6.2.2 Weigh out a ten g portion of soil to the nearest 0.1 g into a suitable centrifuge tube, see sec. 5.1 this SOP.

6.2.3 Weigh out a twenty gram portion of sieved soil into a suitable cup for percent moisture determination. See SOP ME 0007 01.

6.2.4 Spike soil sample in centrifuge tube at this time if required. See sec. 6.4.2.5 this SOP.

6.2.5 Using a Repipet, deliver 25 ml. of pesticide grade acetone into each centrifuge tube containing a soil sample.

6.2.6 Attach centrifuge tubes to wrist arm clamps in a horizontal position.

6.2.7 Set shaker on "5" (medium) shaking action.

6.2.8 Set shaker timer for 30 minutes.

6.2.9 After end of shaking period, place the centrifuge tubes in the centrifuge and spin at a suitable speed/time combination to yield a clear supernatant. For the Sorval centrifuge, set on "16" and take up to 10K RPM then turn off. This is sufficient to settle the soil suspension. Place 4 tubes at a time in the centrifuge and spin them while decanting the previous 4 tubes. CAUTION: DO NOT leave centrifuge unattended when set above "12". DO NOT allow RPM to exceed 12K at any time.

6.2.10 Decant 20 ml of the supernatent into a 25 ml. graduated cylinder.

6.2.11 Pour 20 ml of the supernatent into a 100 ml boiling flask thru about 2 spoonulas of sodium sulfate contained in a 60 degree funnel lined with "shark skin" filter paper.

6.2.11.1 Rinse the graduated cylinder onto the sodium sulfate with 2 ml of acetone.

6.2.11.2 Rinse the sodium sulfate with a second 2 ml portion of acetone.

6.2.12 Repeat steps 6.2.5 thru 6.2.11.2. Forty ml. of the original 50 ml of extractant is now in the 100 ml boiling flask. Because of this dilution factor results obtained by this method must be multiplied by 1.2. Set the G.C. data system to do this automatically.

6.2.13 Mount the flasks either directly onto the rotary evaporator or onto the "spider" attachment for evaporation of multiple samples.

6.2.14 Rotary evaporate the acetone to approximately 2 to 3 ml at 28-30 degrees C and 22-24" Hg. Use caution when bringing vacuum up to prevent boiling of acetone.

6.2.15 After removing the boiling flask from the evaporator, roll flask a few revolutions in the ultrasonic cleaner to redissolve any residue which may have precipitated onto the glass.

6.2.16 Using a pasteur pipet, transfer the concentrated extract to a 5 ml reaction vial. Using the technique described in section 6.2.15 rinse the flask twice with 1 ml portions of acetone. Transfer each portion to the reaction vial.

6.3 Procedure for conversion of VITAVAX to Aniline.

6.3.1 Ebulate acetone in the reaction (RX) vial to 0.1-.3 ml under nitrogen using the 6 position ebulators, under a fume hood.

6.3.1.1 Ebulate to less than 0.1 ml w/ nitrogen using a pasteur pipet as a nozzle. This is necssary to eliminate the last traces of acetone. Each vial should take 2-3 min.

6.3.2 Add 0.5 ml of 1:3 sulfuric acid to each RX vial.

6.3.3 Securely cap each vial with septum top. This cap must hold quite a bit of pressure during the hydrolysis step so use only septa and caps which are in good condition. Use septa a maximum of 2 times. A septum leak during hydrolysis will ruin the sample. Always carefully inspect the level of liquid in the RX vial after hydrolysis to make certain none has been lost. Also inspect the cap for signs of damage or leakage. If there is any doubt about the integrity of the sample at this stage it must be redone from the beginning of the analysis.

6.3.4 Place vials in heater block which has been preheated to 110 degrees C. After about 5-10 min. remove vials from block one at a time & retighten caps. Wear thick rubber gloves for this operation.

6.3.5 After 90 min, remove the vials, place in an appropriate rack and set rack in a pan of cold tap water to cool tubes to room temperature or below.

6.3.6 Very carefully add one ml of 10 N. NaOH to each vial. Tilt the vial to about a 20 degree angle from the vertical and allow the NaOH to run down the side of the vial and layer onto the acid in the vial. DO NOT mix at this stage. Aniline in basic solutions is very volatile. If you observe any bubbling at this stage the analysis must be restarted. Quickly cap the vial and agitate to mix contents. Cool the vial to or below room temperature under cold tap

water.

6.3.7 Add exactly 2 mls of pesticide grade benzene to the vial from a 2.5 ml syringe. Cap the vial and place on Vortex mixer set on "high" for 30 sec. Place vial in a wide mouth 50 ml. plastic centrifuge tube with a half paper towel plug in the bottom. Bring the Sorval centrifuge up to 4 K RPM quickly and shut off. This will separate the phases. Pipet at least 1 ml. of the benzene phase into a 1.5 ml. storage vial.

6.4 Manufacture of standards and spikes.

6.4.1 Standards

6.4.1.1. Aniline standards are made up to be equivalent to certain levels of VITAVAX standards since VITAVAX is the analyte of interest, and it is converted to aniline during the course of this analysis.

6.4.1.2 Aniline 1,000 ppm stock standard

6.4.1.2.1 10 ul of 99+ % Aniline neat standard into a 10 ml volumetric flask. Bring the flask up to volume with pesticide grade methanol.

6.4.1.3 Aniline 395 ppm primary standard, (1,000 ppm VITAVAX equivalent).

6.4.1.3.1 Using a 5 ml syringe, add 3.95 ml of the Aniline 1,000 ppm stock standard to a 10 ml volumetric flask and invert at least 5 times to mix.

6.4.1.4 Aniline 39.5 ppm secondary standard, (100 ppm VITAVAX equivalent).

6.4.1.4.1 Using a volumetric pipet, transfer 1 ml of 395 ppm primary std. to a 10 ml vol. flask. Cap flask and invert at least 5 times to mix.

6.4.1.5 Aniline 3.95 ppm tertiary standard, (10 ppm VITAVAX equivalent).

6.4.1.5.1 Using a volumetric pipet, transfer 1 ml of 39.5 ppm secondary standard to a 10 ml volumetric flask. Cap flask and invert at least 5 times to mix.

6.4.1.6 Manufacture of Aniline working standards.

6.4.1.6.1 0.05 ppm VITAVAX equivalent (1X DL) standard.

6.4.1.6.1.1 Using a 50 ul syringe, add 50 ul of the tertiary Aniline std to 2,000 ul pesticide grade bezene in a glass vial. Add one drop of 0.1 N NaOH and gently invert 5 times. Transfer about 1.5 ml of the std.

to a 1.5 ml storage vial. DO NOT transfer any of the aqueous phase (bottom layer) to the storage vial. Store in refrigerator in dark. Note: benzene freezes at 5 degrees C. Do not store standards or samples in the freezer.

6.4.1.6.2. 0.25 ppm VITAVAX equivalent std.

6.4.1.6.2.1 Exactly as per section 6.4.1.6.1.1 except use 25 ul of the secondary Aniline std.

6.4.1.6.3. 1.50 ppm VITAVAX equivalent std.

6.4.1.6.3.1 Exactly as per section 6.4.1.6.1.1 except use 15 ul of the primary Aniline std.

6.4.1.6.4. Note that all standard values are given relative to a 10 gram soil sample.

6.4.2 Manufacture of spikes for method proof.

6.4.2.1 Spikes are produced separately from VITAVAX, VITAVAX SULFOXIDE and VITAVAX SULFONE.

6.4.2.2. 1,000 ppm primary standard for each component.

6.4.2.2.1 Weigh out 0.010 g of VITAVAX and VITAVAX SULFOXIDE neat std. into separate 10 ml volumetric flasks.

6.4.2.2.2 Weigh out 0.0103g of VITAVAX SULFONE into a third flask. (The reason for weighing out 0.0103g is to compensate for the lower purity of the neat std.)

6.4.2.2.3 Bring flasks up to volume with pesticide grade methanol. Cap flask and invert at least 5 times to mix.

6.4.2.3 100 ppm secondary standards for each component.

6.4.2.3.1 Using a 1 ml syringe, measure out 1.0 ml primary standard into a 10 ml volumetric flask. Bring up to volume with pesticide grade methanol. Cap and invert at least 5 times to mix.

6.4.2.4 10 ppm tertiary standard for each component

6.4.2.4.1 Exactly follow procedure in section 6.4.2.3.1 except use 1 ml of secondary std rather than 1 ml of primary std.

6.4.2.5 Spiking soil for method proof. Note: separate spikes are made for VITAVAX, VITAVAX SULFOXIDE and VITAVAX SULFONE.

6.4.2.5.1 0.05 ppm spike (detection limit). Spike into separate 10 g soil samples, 50 ul of VITAVAX-tertiary std., 54 ul of VITAVAX SULFOXIDE tertiary std., and 57 ul of VITAVAX SULFONE tertiary std. The different spiking volumes account for the diferent molecular weights of VITAVAX and its 2 oxidation products.

6.4.2.5.2. 0.25 ppm spike. Exactly follow procedure section 6.4.2.5.1 this SOP except spike seperate 10 g soil samples with 25, 27 and 29 ul of VITAVAX, VITAVAX SULFOXIDE and VITAVAX SULFONE secondary standards.

6.4.2.5.3. 1.50 ppm spike. Exactly follow procedure in section 6.4.2.5.1 this SOP, except spike seperate 10 g soil samples with 15, 16 and 17 ul of VITAVAX, VITAVAX SULFOXIDE and VITAVAX SULFONE primary standards.

6.4.3 Manufacture of spikes for soil field samples.

6.4.3.1 Spikes are produced from a mixture of VITAVAX, VITAVAX SULFOXIDE AND VITAVAX SULFONE.

6.4.3.2. 1,000 ppm primary standard for each component.

6.4.3.2.1 Weigh out 0.100 g of VITAVAX and VITAVAX SULFOXIDE neat std. into a 100 ml volumetric flask. Weigh out 0.103 g VITAVAX SULFONE into the same flask. The reason for weighing out 0.103 g is to compensate for the lower purity of the neat standard.

6.4.3.2.2 Bring flask up to volume with pesticide grade. Methanol. Cap flask and invert at least 5 times to mix.

6.4.3.3 100 ppm secondary standard for each component.

6.4.3.3.1 Using a 1 ml syringe, measure out 1.0 ml of the primary standard into a 10 ml volumetric flask. Bring up to volume with methanol. Cap and invert flask at least 5 times to mix.

6.4.3.4. 10 ppm tertiary standard for each component

6.4.3.4.1 Exactly follow procedure in section 6.4.3.3.1 except use 1 ml of secondary rather than 1 ml of primary std.

6.4.4 Spiking soil samples for field sample QC.
6.4.4.1 0.05 ppm (detection level) spike.

6.4.4.1.1 Using a 25 ul syringe, spike with 17.7 ul of the 3 component tertiary standard into a centrifuge tube containing 10 g of soil. Note that this volume of spike corrects for the added molecular weight present in VITAVAX SULFOXIDE and VITAVAX SULFONE as compared to VITAVAX. The volume of the spike also accounts for the conversion of the VITAVAX forms to Aniline.

6.4.4.2 0.25 ppm spike.
6.4.4.2.1 Exactly follow procedure in section 6.4.4.1.1, except spike with 8.84 ul of the 3 component secondary standard.

6.4.4.3 1.5 ppm spike.
6.4.4.3.1 Exactly follow procedure in section 6.4.4.1.1 this SOP, except spike with 53.0 ul of 3 component secondary std.

6.5 Instrumental analysis conditions.

6.5.1 Instrument: OR-GC-6.
6.5.2 Method Name: Aniline.
6.5.3 Method File: Ana.
6.5.4 Channel: 0.
6.5.5 Run Time: 6 min.
6.5.6 Flow Rate: Set Flow controller on "085".
6.5.7 Make Up Gas: 25 ml/ min.
6.5.8 Oven temp: 90 C, hold 0, final temp 105, program @ 3 C/min, hold 0, 2nd prog. final temp 250, @50 C/min, hold 2 min.
6.5.9 Injection temp: 220 C.
6.5.10 Detector temp: 300 C.
6.5.11 Column Type: 0.53 mm X 30 m capillary, DB-5.
6.5.12 Column ID:
6.5.13 Amount inj: 1 ul.
6.5.14 Det. type: TSD.
6.5.15 G.C. meth.: 1.
6.5.16 D.S. channel: 0.
6.5.17 Detector: A.
6.5.18 TSD mV: 4.25-5.00.

6.6 Notes on G.C. operation: TSD response for 0.25 ppm aniline working std. should be 22 K to 32 K area counts. TSD mV is normally 4.5 to 5.

7.0 CALCULATIONS.

7.1 Calculating value of aniline as Vitavax equivalent in soil samples.

7.1.2 Values on chromatograms are in ppm (ug/g) Vitavax equivalent of aniline. Values were calculated as follows:

Soil samples size: 10 g
Detection limit: 0.05 ppm (ug/g)
therefore total amount of Vitavax to be detected in 10 g soil = (0.05 ug/g)(10 g) = 0.5 ug.
If final value of sample extract and standard equals 2 mL (2000 uL) then standards at detection limit must be made up so that 0.5 ug are present in 2 mL. This means the actual concentration of aniline (Vitavax equivalent) in the 2 mL of solvent for a detection level standard = $\frac{0.5 \text{ ug}}{2 \text{ mL}}$ = 0.25 ug/mL. If the tertiary standard is 10 ppm (ug/mL) aniline (Vitavax equivalent) then 50 uL of tertiary standard into 2 mL solvent must be used. For example:

$$(50 \text{ uL})(10 \text{ ng/uL})\left(\frac{1}{2 \text{ mL}}\right) = 250 \text{ ng/mL} = 0.25 \text{ ug/mL}$$

7.1.3 For a 5X DL standard (0.25 PPM in soil) there must be 1250 ng/mL in the standard solution (5) (250 ng/mL). Therefore the most convenient way to make this standard is to use 25 uL of 100 ppm Vitavax equivalent secondary standard.

$$(25 \text{ uL})(100 \text{ ng/uL})\left(\frac{1}{2 \text{ mL}}\right) = 1250 \text{ ng/mL} = 1.25 \text{ ug/mL}$$

7.1.4 For a 30X DL standard (1.50 ppm in soil) there must be 7500 ug/uL in the standard solution (30) (250 ng/uL). Therefore the most convenient way to make this standard is to use 15 uL of 1000 ppm Vitavax.

8.0 REPORTING AND DOCUMENTATION.

8.1 Sample preparation and extraction.

8.1.1 See SOP _____.

8.2 Analysis

8.2.1 Automatic data transfer (ADT) is a computer driven method of reporting the final results of an analysis to the client file and of producing reports which summarize QC data for a chromatographic run. See SOP _____ for an explanation of the procedure.

NORTH COAST LABORATORIES LTD

SOP CODE	VERSION	COPY	EFFECTIVE DATE	LOCATION OF ORIGINAL	WP FILE
ME	0011	02	08 / 30 / 89		VITA

EXHIBIT 1
FLOW CHART OF VITAVAX AS ANILINE METHOD

START

Weigh out 20 g portion of 10 mm sieved soil

Determine moisture content on the portion

Weigh out 10 g portion of 10 mm sieved soil

Place 10 g portion of soil in centrifuge tube

spike if required

Add 25 ml acetone to tube

Shake on wrist arm shaker set on "5" for 30 minutes

Bring up to 10,000 rpm on centrifuge

Decant 20 ml acetone from tube into 25 ml grad.cyl.

Pour acetone thru sodium sulfate into a 100 ml.boiling flask.

Repeat extraction, centrifugation, and decantation steps as above. Add the second 20 ml extract to the 1 st in the 100 ml boiling flask.

Evaporate to approx. 3 ml @ 28-30 C and 22-24 mm Hg

Transfer acetone to 5 ml RX vial with 2 X 1 ml acetone rinses

Ebulate extract to near dryness @ 28-30 C under Nitrogen

Add 0.5 ml 33% sulfuric acid

Carefully seal vial and heat @ 110 C for 90 min.

Cool vial to or below RT in cold tap water

Very carefully layer 1 ml 10 N. NaOH onto sulfuric acid in RX vial

Cap vial and agitate to mix

Cool in cold tap water

Add 2.0 ml benzene to RX vial

245