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# U.S. Army Corps of Engineers

New England District
Concord, Massachusetts

# QUALITY ASSURANCE PROJECT PLAN

## Volume IV
## Appendices E and F

DCN: GE-021601-AAHM

March 2001

## Environmental Remediation Contract
## General Electric (GE)/Housatonic River Project
## Pittsfield, Massachusetts

Contract No. DACW33-00-D-0006

Task Order No. 0002



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**FINAL**

# QUALITY ASSURANCE PROJECT PLAN

# ENVIRONMENTAL REMEDIATION CONTRACT
# GENERAL ELECTRIC (GE) HOUSATONIC RIVER PROJECT
# PITTSFIELD, MASSACHUSETTS

## Volume IV—Appendices E and F

Contract No. DACW33-00-D-0006
Task Order No. 0002
DCN: GE-021601-AAHM

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# APPENDIX E

# STANDARD OPERATING PROCEDURES – INVESTIGATORS

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# APPENDIX E-1

# EXTRACTION OF ANIMAL TISSUES FOR RESIDUE ANALYSIS AND PERCENT LIPID DETERMINATION [USGS-TILLITT]

ECRC SOP P.461

Date Prepared: 6/17/98

Date Revised: 8/21/98



# EXTRACTION OF ANIMAL TISSUES FOR RESIDUE ANALYSIS AND PERCENT LIPID DETERMINATION

## I. Introduction

This SOP describes a procedure for extraction of animal tissue samples for a variety of residue analyses. Additionally, it includes the procedure for percent lipid determination for tissue samples. The extracts generated are suitable for subsequent cleanup using procedures outlined in other SOPs.

## II. Blending Tissue with Sodium Sulfate

This SOP assumes that you will be starting with ground tissue samples. The day before you plan on extracting a group of tissue samples, thaw the samples and weigh appropriate aliquants of tissue samples into half-pint, pint, or quart canning jars. Add four times the sample wet weights of $Na_2SO_4$ to the jars. Immediately and thoroughly mix the tissue and desiccant together, using stainless steel rods or spatulas. Allow the tissue-sodium sulfate mixtures to interact for about an hour before stirring them again. Occasionally stir the samples again throughout the day so as to prevent hardening of the tissue-$Na_2SO_4$ mixtures. [NOTE: On rare occasions, the sample may still appear wet after the required period of interaction with the recommended amount of sodium sulfate. More can be added at the analyst's discretion without significantly affecting the analysis.] The following day, thoroughly stir the samples one more time. Samples are now ready for homogenization with a blender.

Blending is performed with a standard commercial blender. The blade assembly fits the mouth/threads of regular (not wide-mouth) canning jars so the sample is blended in the jar it is stored in. The blades are washed with soap and water plus acetone, hexane, and methylene chloride between samples. The rubber ring-washer that is supplied with commercial blenders for use between the blades and the jar is not used. It is replaced with ring cut from Teflon sheet material. The sample/sodium sulfate mixture is stirred with a steel rod or spatula to break up clumps and the sides of the jar are scraped to free adhering particles. The blade assembly and ring are affixed and the sample is homogenized by inverting and/or shaking the blender while it is running until the sample has been reduced to powder. Lipid-rich sample material will accumulate on the blade

shaft just under the point of attachment of the rotating blades. After the blade assembly is removed, this accumulation is scraped back into the sample with a spatula. The sample is now ready for extraction.

## III. Extraction

Use the table at the end of this section to determine the appropriate extraction column and solvent volume for the mass of sample you are extracting.

A. Record all relevant information on the Sample Extraction Record sheet(data base number, submitter's description, grams extracted, etc.).

B. Set up clean glass extraction columns fitted with removable Teflon stopcocks and glass tips. Place a wad of glass wool over the stopcocks and rinse the column assembly with acetone, hexane, and $CH_2Cl_2$. Add 1-2 cm of anhydrous $Na_2SO_4$ to the columns and rinse with hexane and $CH_2Cl_2$.

C. Transfer the dry samples into the columns. Spike QC samples, surrogates, radiolabelled recovery standards, or any other procedural internal standards at this point. Cover samples with 1-2 cm of anhydrous $Na_2SO_4$. Place collection flasks under the columns and rinse sample mixing containers with $CH_2Cl_2$ (3 x 10 - 20 mL) and pour rinsings on columns. Allow the rinses to drain into the column. Apply the volume of $CH_2Cl_2$ designated "Soak" in the table below. Allow this solvent to drain to within 2 cm of the column bed and close the stopcock. Let the columns stand for aproximately 30 min. Apply the volume designated "Extraction" from the table and allow it to drain through the columns at a flow rate of 3-5 mL/minute. After the columns have stopped dripping, fully open the stopcocks and apply the "Wash" solvent.

| Sample Size (g) | Extraction Column (i.d x l; cm) | Extraction Solvent Volumes (mL $CH_2Cl_2$) | | | |
|---|---|---|---|---|---|
| | | Soak | Extraction | Wash | Total |
| 5 | 1 x 30 | 15 | 50 | 15 | 80 |
| 10 | 2 x 30 | 20 | 100 | 20 | 140 |
| 15 | 2 x 30 | 30 | 150 | 30 | 210 |
| 25 | 4 x 30 | 50 | 225 | 50 | 325 |
| 50 | 4 x 30 | 100 | 450 | 100 | 650 |

## IV. Determination of Percent Lipid

**Water baths for all $N_2$- and rotary evaporations must be at 35° or cooler.** Rotary evaporate the tissue extracts to small volumes and use $CH_2Cl_2$ rinses to transfer the lipid solutions to appropriately-sized volumetric containers. [For example, 5-g extracts may be transferred to calibrated 10-mL culture tubes. 100-mL volumetric flasks are more appropriate for 50-g extracts]. Dilute samples to the correct volume. **Imperative:** Before proceeding further, cap the volumetric flasks, and shake them thoroughly to homogenize the solutions. Do duplicate percent lipid determinations on one of the samples in each extraction grouping.

A. Record relevant information on Percent Lipid Content Data Sheet.

B. Label a series of shell vials for the group of samples you are processing and precondition them at 100 C for 30 min. From this point on, it is advisable to handle the vials with forceps to avoid fingerprints. Weigh the vials to the nearest 0.001 g. (The preconditioning removes moisture, fingerprints, etc., that can significantly affect the analysis. This effect is most notable in the procedural blanks.)

C. Using a volumetric pipette or syringe, transfer measured aliquots of the lipid extract to weighed shell vials.

D. Evaporate solvent at 70° C using heating block inside exhaust hood. Check the vials every 30 min - 1 hr. until the solvent has been removed. Increase the temperature to 100 C for 1 hour. (More than 1 hour at 100 C causes an increase in lipid weights, possibly due to oxidation of lipid components.)

E. Reweigh shell vials and use the following equation to determine percent lipid for each sample:

$$\%\ Lipid \quad \frac{Lipid\ Weight}{Sample\ Weight} X \frac{Sample\ Extract\ Volume}{Volume\ for\ Lipid\ Determination} X\ 100$$

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# APPENDIX E-2

# SAMPLE TRANSMITTAL, RECEIPT, AND INVENTORY [USGS-TILLITT]

USGS
science for a changing world


Date Prepared: August 29, 1991
Date Revised: February 12, 1999



# SAMPLE TRANSMITTAL, RECEIPT, AND INVENTORY

The Environmental Chemistry Branch of the Columbia Environmental Research Center (CERC) has certain procedures which must be followed in the transmittal and receipt of samples that are to be processed by the Branch. Adherence to these instructions will facilitate incorporation of samples into the Branch's sample management system.

## Sample Transmittal

1. The individual responsible for transmission of samples **(from outside CERC or internally)** must contact Jesse Arms of CERC via phone (573-876-1856) PRIOR TO any sample transmission[1]. He will send you the necessary forms to fill out and provide additional instructions. Information on methods of preservation used during transmission can be provided at this time. **NOTE: PARTICULARLY SPECIFY ANY SAMPLES REQUIRING -80°C STORAGE REQUIREMENTS; CONTACT PERSONALLY - DO NOT LEAVE VOICE MAIL.**

2. The person responsible for the samples, or designated individuals, must fill out the attached "Batch History" form in detail. Failure to complete this form in its entirety will delay and may jeopardize sample processing.

3. The form entitled "Chain of Custody Record" must be completed. Each sample is to be listed on an individual line along with other requested information. The header and appropriate footer information must be filled out for each sheet that is used.

4. For samples being transmitted **to CERC from outside:** place all completed forms in protective covering and put in packaging with samples. Seal all boxes, coolers, or other packaging so that it can be indicated if tampering has occurred. Once transmission (shipment) has taken place, immediately contact Jesse Arms (573 876-1856) and provide shipment and expected date of arrival information. **Do not leave a voice mail; a direct contact with specified CERC personnel prior to shipment must be established.** Following this phone call, specified CERC personnel must complete the "Pre-Shipment" section of the form entitled "Sample Shipment Record."

5. For samples being transmitted **from one group to another within CERC:** steps 1-4 above must be completed. Following notification of J. Arms or designate, manual transmission (hand-carrying) of samples to the Analytical Building is appropriate. However, contact J. Arms beforehand to assure completion of the transmission process. Completion of "Pre-Shipment" section of "Sample Shipment Record" is still required by specified CERC personnel (J. Arms).

[1]If Jesse Arms is unavailable, contact Tom May at 573-876-1858.

6. For samples being transmitted **from CERC Chemistry to a second party outside CERC:** A yellow Batch History form (samples out) must be completed along with "Chain of Custody Record" for all samples, following instructions in steps 2 and 3. This documentation is packaged and shipped with samples to the outside party. A copy of documentation is placed in a green pocket file and filed in specified Analytical Building Rm. 5 file drawer. At time of shipment, CERC personnel (J.Arms or alternate) will call the outside party and inform them of shipment details. Once the shipping details have been verified, fill out the "Pre-Shipment" section of the "Sample Shipment Record." When the outside party notifies CERC of sample receipt, J. Arms or alternate will obtain information to fill out "Receipt" portion of "Sample Shipment Record." This completed "Sample Shipment Record" is filed with other documentation in the appropriate green file pocket.

**Sample Receipt (receipt at CERC)**

1. J.Arms or alternate CERC personnel must immediately notify responsible party at CERC when samples are received. Also J. Arms or alternate CERC personnel must complete a sample check-in procedure following guidelines depicted in "Receipt" section of form entitled "Sample Shipment Record."

**Sample Inventory (at CERC)**

1. Samples are logged-in or inventoried into the Chemistry Division sample management system by J.Arms or alternate CERC personnel following guidelines depicted on a form entitled "Sample Log-in."

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# APPENDIX E-3

# MICROSOMAL PREPARATION OF LIVER TISSUE [USGS-TILLITT]

ECRC SOP P.123

Date Prepared: 2/23/97

Date Revised: 2/8/99

**FOR USERS OTHER THAN ECRC STAFF, THIS IS FOR REFERENCE ONLY. THIS IS NOT A CITABLE DOCUMENT.**

## Microsomal Preparation of Liver Tissue

I. General

Biotransformation reactions involving xenobiotic compounds often are catalyzed by enzymes that occur in the liver, intestine, lung, blood, and kidney of many species. Enzymes are proteins whose presence is usually measured by their rate of activity. Many factors, i.e., high temperature, different pH, proteinases, heavy metals that bind to the protein, absence of thiol reducing agents, etc. can change the enzymatic activity. The following procedure outlines a technique used to prepare tissue to be used in xenobiotic metabolism assays.

Note: It is best to run the EROD assay (see SOP P.124) on the same day as the microsomal preparation. To complete the microsomal preparation and the EROD assay within 8 hours, no more than 44 samples should be attempted. Prepare all reagents (stored at –20-0 °C) and label all tubes prior to assay date.

II. Equipment

Insulated container with crushed ice
Kimwipes
Balance that weighs to 0.0001g
Omni handheld homogenizer with stirrer and tubes (Model TH S/N -1797 or equivalent)
Fisher centrifuge tubes, 12 x 75 mm (for Savant high speed centrifuge)
Beckman ultracentrifuge tubes (polyallomer 13 x 64 mm, Cat. # 355644)
Frozen ice pack test tube holder (kryorack)
Vortex
Spatulas
Pasteur pipets and bulbs
Gilson P5000 pipettor or equivalent
4 plastic beakers
Cryovials (1.5 – 2.0 ml)
Beckman Ultracentrifuge (L8-55, located in B3 lab or equivalent)

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ECRC, USGS-BRD
February 18, 1999 (12:56PM), C:\SHELLY\EROD\FILES\MICROSOME-SOP.DOC

Savant Centrifuge (HSC10K, located in Physiology Laboratory or equivalent)
Rotor for Beckman (Type 50.4 Ti, located in the ultracentrifuge)
Rotor for Savant (HSR-30, located in Phys. Lab refrigerator)

III. Chemicals/Reagents

Phosphate Buffer, pH 7.4 at 4 °C (approx. 220 ml per day assuming 44 samples)
Ultrapure water in 4 beakers, approx. 400 ml per beaker (to rinse homogenizer)

IV. Quality Assurance/Quality Control

Each day microsomes will be prepared from at least one positive control sample.

V. Procedure

Note: Keep all samples and solutions cold, but NOT frozen, on crushed ice or in frozen ice pack test tube holder. Do not place frozen ice pack in freezer with sample tissue; the samples will freeze.

A. First thing in the morning:

1. Turn on the cooler to the Savant centrifuge by sliding two white buttons forward on right top side of cooling unit.

2. Turn on the Beckman Ultracentrifuge and set parameters (i.e. 4 °C, 30,000 rpm, and 50 minutes).

B. To homogenize fresh or frozen tissue:

1. Label 12 x 75 mm centrifuge tubes with sample ID.

2. Place first 12 x 75 mm labeled tube in a small beaker, place on balance, and tare.

3. Using a spatula, dig out ~0.5 g of liver tissue (± 0.05 g) and place in tube. Place tube in kryorack or on ice and record weight of tissue (to nearest 0.0001 g) in notebook. Note: this is easiest when sample is still firmly frozen. It may be useful to store liver tissue samples in dry ice prior to removing the 0.5 g subsamples.

4. Repeat steps 1-3 for remaining samples.

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5. Put 1.5 ml cold (4 °C) phosphate buffer (pH 7.4) in each tube, rinsing the sides of the tube as the buffer is added.

6. Using the Omni hand-held homogenizer, homogenize the sample at medium speed for 20 seconds. Wait for 10 seconds (leave stirrer in tube), then homogenize another 20 seconds.

7. Raise stirrer probe above liquid while motor is still on. Once stirrer is out of liquid, turn homogenizer off. **Do not remove the stirrer from the tube until homogenizer is off!**

8. Place sample in kryorack or on ice. Rinse homogenizer probe in 4 different beakers of UP water. The same order should be followed each time, with the fourth beaker being the cleanest rinse. Do not use solvent or soap to rinse the Omni hand-held homogenizer. The rinse water should be changed every 5-6 samples, or when the last rinse appears dirty.

9. Repeat steps 5-8 for remaining samples. Samples must remain COLD to ensure no loss of enzymatic activity. Cold temperatures dramatically slow enzyme reaction rates. Therefore, proteolytic destruction of the cytochrome P450 IA enzymes is minimized.

VI. Supernate - S9 fraction using Savant Centrifuge

A. Place an empty 50 ml glass beaker on balance and tare.

B. Place first 12 x 75 sample tube into beaker and bring to a total weight of 5.0 g (± 0.1 g) with cold phosphate buffer. The weight of the tube plus its contents now equals 5 g. Repeat until all samples are done.

C. If there are less than 22 samples, align tubes in cold rotor with opposite partners. If odd number of tubes, add one more tube filled with buffer to a weight of 5.0 g.

D. Place rotor in cold (4°C) Savant centrifuge and tighten screw top completely.

E. Close and lock centrifuge lid.

F. Spin samples at 8500 rpm (9000 rcf) for 25 minutes. Bring the speed up SLOWLY! During this spin, start homogenizing next 22 samples, if applicable.

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G. After Savant spin, remove 12 x 75 mm tubes from centrifuge and place in kryorack or on ice. KEEP SAMPLES COLD!

H. Repeat steps A-G on next set of 22 samples, if applicable.

VII. Microsomes

A. Pour off supernates from above S9 (Savant) spin into respective Ultracentrifuge tubes and place in kryorack or on ice. These supernates are the S9 fractions.

B. Place an empty beaker on balance and tare. Bring all ultracentrifuge tubes containing S9 supernates up to 6.0 g (± 0.1 g) with cold phosphate buffer. The weight of the tube plus its contents now equals 6.0 g.

C. Repeat steps A and B on second set of 22 samples, if applicable.

D. Remove the rotor (Type 50.4 Ti) from the Beckman Ultracentrifuge. Be careful, it's very heavy! If the rotor is placed outside the ultracentrifuge, then place it inside its protective, blue cloth bag.

E. Place Ultracentrifuge tubes in the Beckman rotor; if less than 44 samples, make sure all tubes have an opposite partner. If there is an odd number of tubes, add one tube calibrated to 6.0 g with buffer.

F. Using forceps or gloved fingertip, push all vials to the bottom of each rotor opening.

G. Wipe any moisture from rotor and cap, place cap on rotor, and tighten.

H. Place rotor in Beckman Ultracentrifuge.

I. Close Ultracentrifuge lid, making sure it locks.

J. Press "display" on Ultracentrifuge and check settings. They should read 4 °C, 30,000 rpm, and 50 minutes. Correct if necessary.

K. Press "time" then "autorun" to start Ultracentrifuge. Make sure Ultracentrifuge reaches 30,000 rpm. If not, turn it off and let it come to a complete stop. Then release the vacuum, check your setup and setting, and try again.

Note: Once the centrifuge is operating properly, you have approx. 50 minutes to label microsome storage tubes, make EROD solutions, and/or put unused liver

samples back in -80 °C freezer.

L. When Ultracentrifuge run is complete, release the vacuum by pressing the "off" button under "vacuum" on the left. Make sure to fill in data for Beckman logbook.

M. When vacuum is completely released, open lid and remove rotor.
N. Take out one sample at a time and place in kryorack or on ice.

O. Wipe moisture from rotor and return to the ultracentrifuge. Turn off Ultracentrifuge and Savant microfuge.

P. Pour off the supernate and discard. KEEP THE PELLET!

Q. Place 1.0 ml cold phosphate buffer into each tube on top of pellet.

R. Using a pasteur pipet (9-inch), resuspend the pellet by scraping sides and pipetting up and down. Try to keep bubbles to a minimum. IMPORTANT: Change pipets between each sample!

S. Pipet each suspension into a labeled cryovial and place in kryorack or on ice. KEEP SAMPLES COLD!

T. The samples are ready to be used for EROD assay (see SOP P.124).

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# APPENDIX E-4

# CALIBRATION CHECK OF 96 WELL MICROPLATE ABSORBANCE AND FLUORESCENCE READERS [USGS-TILLITT]

NFCRC SOP: P.475

Date Prepared: 5/16/96

Date Revised: 6/22/98

# Calibration Check of 96 Well Microplate Absorbance and Fluorescence Readers

## I. INTRODUCTION

This SOP describes a preparative scheme for statistically checking the accuracy of pipetting techniques, microplates, pipets, and microplate readers. This SOP also describes the materials needed, the preparation of appropriate solutions, the pipetting and reading methods, and the statistical analysis of the data. These procedures are based on the prior work of Harrison and Hammock 1988.

## II. LISTING OF REAGENTS AND APPARATUS FOR ABSORBANCE READER

A. Ultra Pure Water
B. Tween 20 (to provide a meniscus)
C. McCormick yellow food coloring dye
D. 100 mL graduated cylinder
E. Two 100 mL Erlenmeyer flask with stoppers
F. Two Plastic Solution Trays
G. Micropipettor(s) capable of delivering 200ul
H. 96-well microplate(s)

## III. PREPARATION OF DYE SOLUTION FOR ABSORBANCE READER

Measure out 100 mL of water (all water should be ultra pure) into the graduated cylinder. Pour about 50 mL of this water into the Erlenmeyer flask. Using a positive displacement pipettor, pipet 50 $\mu$L of Tween 20 into the flask. Draw up water into the pipet repeatedly to rinse out all of the Tween 20. Pour the remaining 50 mL left in the graduated cylinder into the flask. Stopper the flask, or close with parafilm, and swirl to thoroughly mix the solution. Foam will appear on top of the water. Measure out 40 mL of the solution into the graduated cylinder being careful to minimize the amount of foam transferred. Pour the 40 mL into the second flask. Next, squeeze out one (1) drop of yellow dye into the flask. Stopper the flask and swirl until a consistent yellow color is achieved. Pour the dye solution and the remaining 60 mL of aqueous Tween 20 solution (0.05%) into separate plastic trays. This dilution of the yellow food coloring dye approximates a typical enzyme immunoassay endpoint, giving 0.700-1.200 absorbance at 405nm in a flat-bottom polystyrene plate.

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Note: Sixty milliliters are left for rinsing and or prewetting the plate and 40 mL are used for absorbance readings. If larger volumes are needed, remember to make any necessary dilutions.

## IV. PIPETTING and ABSORBANCE READING METHODS

To ensure the most accurate results, these techniques should be followed: touch the sides of the wells with the pipet tip and use volumes around 200 $\mu$l to avoid variability due to the meniscus from the Tween 20.

Wash a new plate with aqueous 0.05% Tween 20, tap plate on a paper towel to remove excess liquid, and load the plate with 200ul/well of the dye solution in 0.05% Tween 20.

Perform the desired experiments, varying the pipets, plates, volumes, and other settings and techniques.

In each experiment, after loading the wells, read the plate(s) at a wavelength of 405 nm with a reference wavelength of 650 nm a total of five times. Next, turn the plate around 180 degrees and read again a total of five times using the dual wavelengths. (If desired, repeat the ten readings above without using the reference wavelength.) If the reader is equipped with an automatic shaker, let the plate shake for 10 seconds before each reading.

## V. LISTING OF REAGENTS AND APPARATUS FOR FLUORESCENCE READER (CYOTOFLUOR 2300)

A. PBS Buffer (approx. 15 ml)
B. 58 $\mu$M resorufin solution
C. Single-tip pipettor capable of 120 $\mu$l volumes
D. 96-well microplate(s)

## VI. PREPARATION OF RESORUFIN SOLUTION FOR FLUORESCENCE READER

The resorufin solution used should ideally be a concentration that falls within the linear range of the resorufin curve. For example, 40 pmol/20$\mu$l works wells. The 58 $\mu$M resorufin stock solution should be tested spectrophotometrically to determine its true concentration, then that concentration used to make a dilution to 15 ml of 40 pmol/20$\mu$l solution. For example, if the tested stock concentration was 44 $\mu$M, 113 $\mu$l should be

added to 14.887 ml PBS buffer. Mix thoroughly. The resulting solution should be kept in a foil-wrapped centrifuge tube until use.

## VII. PIPETTING AND FLUORESCENCE READING METHODS

To ensure accurate results, good pipetting technique should be used. Each well will receive 120 $\mu$l of solution, so a pipettor that has been proven to accurately dispense this amount should be used. Pipet the solution up and down a few times to prewet the tip, then touch the tip to the side of the well to ensure a complete dispense.

Read the plate using an excitation filter of 530 nm and an emission filter of 590 nm. Set the number of scans to 5 and the scan cycle to 60. This reading will take approximately five minutes. Export the resulting data file to EXCEL. Then turn the plate 180 degrees and repeat the reading process.

## VIII. STATISTICAL ANALYSIS

For data analysis using EXCEL, use the second and third readings in the normal orientation (N1 and N2, respectively), and the first reading in the reversed orientation (R1). Rearrange the R1 values to correct for the reversed orientation (ie-well A1 becomes H12, etc.).

Calculate the plate mean for each reading. Then calculate the mean and standard deviation for each column and each row (CM and RM). Finally, determine the columnwise standard deviation as a percentage of the plate mean (RSD%). To do this, divide the standard deviation for each column or row by the plate mean, and multiply by 100.

Calculate N2-N1 and N2-R1 for each well; this will yield two sets of individual well differences for repeated normal and repeated reverse readings. For each set, determine the absorbance grand mean (average of the two plate means). For the N2-N1 value, add the N2 and N1 plate means, then divide by 2. For the N2-R1 value, add the N2 and R1 plate means, then divide by 2.

Next, calculate the mean of the differences of each column and row (CM and RM). Then determine the columnwise and rowwise means as a percent of the absorbance grand mean (%CM and %RM). To do this, divide the column or row mean by the grand absorbance mean, and multiply by 100. Finally, determine the columnwise and rowwise percent coefficient of variation (CW%CV and RW%CV). To do this, divide the standard deviation of the entire row or column by the absorbance grand mean, then multiply by

100. A plot of CM and RM versus column and row number will reveal any error patterns.
Also, calculate the range of columnwise and rowwise difference by subtracting the lowest %CM or %RM value from the highest value. Determine the coefficient of variation of the plate by dividing the standard deviation of the plate by the absorbance grand mean and multiplying by 100. Finally, calculate the maximum absolute individual well difference as a percent of the absorbance grand mean.

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References:

Harrison, R.O. and Hammock, B.D. 1988. "Location dependent biases in automatic 96-well microplate readers". *J.A.O.A.C.* 71(5) 981-987.

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# APPENDIX E-5

# PROCEDURE FOR THE DETERMINATION OF 7-ETHOXYRESORUFIN-O-DEETHYLASE (EROD) ACTIVITY IN MICROSOMES FROM LIVER TISSUE USING 96-WELL MICROTER PLATES [USGS-TILLITT]

ECRC SOP: P.124

Date Prepared: 3/5/97

Date Revised: 9/28/98

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# Procedure for the Determination of 7-Ethoxyresorufin-O-Deethylase (EROD) Activity in Microsomes from Liver Tissue Using 96-well Microtiter Plates

## I. General:

Microsomes are small membrane-enclosed vesicles derived from the endoplasmic reticulum and contain enzymes involved in xenobiotic metabolism. One such enzyme system found in microsomes is that of cytochrome P4501A. The cytochrome P450 monooxygenase (MO) system is probably one of the most extensively studied biochemical indicator systems of contaminant exposure or their effects in fish and wildlife (Stegeman and Hahn 1994). Cytochrome P450 MOs are inducible enzymes, in other words they increase in response to a variety of environmental contaminants (e.g. PCBs, PAHs, PCDDs and PCDFs). This inductive response is the key to the potential for using MOs as biomonitoring tools. The following procedure outlines the measurement of enzymatic activity of cytochrome P4501A in fish liver samples as assessed by Ethoxyresorufin-O-deethylation (EROD).

## II. Equipment:

Fluorometric multiple well plate reader (Cytofluor 2300, Millipore Corp. or equivalent)
8-channel repeating pipettor (Matrix Impact, 1250 $\mu$l capacity or equivalent)
96-well flat bottom microtiter plates (CytoPlate, minimal fluorescence plate from (PE Biosystems)
Temperature controlled waterbath (calibrated to 25°C)
Timer
50 ml screw-cap centrifuge tubes
Bench-top plate shaker (TiterPlate Shaker, Lab-line Instruments, Inc or equivalent)
Vortex
Crushed ice in an insulated container
Tissue paper (i.e. Kimwipes)
Frozen ice pack test tube holder (e.g. Kryorack)
Incubator

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III. Chemicals/Reagents:

Note: See SOP Attachment for reagent preparation and storage.

~5 mls per plate of Phosphate buffer, pH 7.4 at 25°C
~5 mls per plate of 10 mM Ethoxyresorufin (ER) at 25°C
~5 mls per plate of 4.3 mM NADPH at 25°C
Resorufin standards 0, 5, 10, 20, 40, 80, 160, and 320 pmol/20 $\mu$l
BSA Standards 0, 10, 20, 30, 40, 50, and 60 $\mu$g/20 $\mu$l
~30 ml of 1.08 $\mu$M Fluorescamine/20 $\mu$l solution (in acetone)
~15 ml per plate of Sodium Phosphate buffer (pH 8)

IV. Procedure:

Note: Prior to Cytofluor scanning, keep all samples and solutions cold, but not frozen, on crushed ice or in frozen ice pack test tube holder. Keep reagent solutions needed at 25°C in waterbath.

A. First thing in the morning:

1. Remove 10 µM Ethoxyresorufin (ER), 4.3 mM NADPH, and 58 $\mu$M resorufin solutions from freezer and phosphate buffer from refrigerator. Place in 25°C waterbath.

2. Turn on computer, open Cytofluor software, do systems maintenance. Once monthly, calibrate Cytofluor using a resorufin solution according to SOP P.475, "Calibration Check of 96 Well Microplate Absorbance and Fluorescence Readers."

3. Check ER, NADPH, and resorufin solutions for correct absorbance using a spectrophotometer (see section VI.B)

4. Obtain liver samples and prepare microsomal fractions according to SOP P.123.

5. After microsomal prep is done, prepare resorufin standards and BSA standards to be run with that day's samples. (This can be done while the microsomes are in the Ultracentrifuge.)

B. Resorufin standard plate preparation:

1. Remove 1 sterile 96-well plate (CytoPlate) from its package and label it "r" in the bottom right corner.

2. Add 50 $\mu$l of 10 $\mu$M ER (at 25°C) to wells A1-A6 through H1-H6.

3. Add 50 $\mu$l of 4.3 mM NADPH (at 25°C) to wells A1-A6 through H1-H6.

4. An average of six replicates of each resorufin standard is taken.
- Place 20 $\mu$l of 0 pmol/20 $\mu$l resorufin standard into wells A1-A6.
- Place 20 $\mu$l of 5 pmol/20 $\mu$l resorufin standard into wells B1-B6.
- Place 20 $\mu$l of 10 pmol/20 $\mu$l resorufin standard into wells C1-C6.
- Place 20 $\mu$l of 20 pmol/20 $\mu$l resorufin standard into wells D1-D6.
- Place 20 $\mu$l of 40 pmol/20 $\mu$l resorufin standard into wells E1-E6.
- Place 20 $\mu$l of 80 pmol/20 $\mu$l resorufin standard into wells F1-F6.
- Place 20 $\mu$l of 160 pmol/20 $\mu$l resorufin standard into wells G1-G6.
- Place 20 $\mu$l of 320 pmol/20 $\mu$l resorufin standard into wells H1-H6.

5. Shake in plate shaker for 1 minute then wipe any fingerprints or dust from bottom of plate with tissue paper (kimwipe) and place plate in Cytofluor 2300.

6. Set the number of scans to 10 and the scan cycle to 60. Set the excitation filter to 530 nm (C), the emission filter to 590 nm (C), and the sensitivity to 3 The Cytofluor will then scan the resorufin plate 10 times, taking sixty seconds per scan. (This will take approx. 10 minutes. The protein standard plate can be prepared during this time.)

7. Save the file with appropriate name as a .csv file and export to EXCEL. The file name should include your initials, the date, and a reference to the plate type. For example, m62498r, where 62498 is June 24, 1998, and "r" stands for "resorufin."

C. EROD Assay on sample plate:

Note: Each plate has its own baseline or background fluorescence that has to be subtracted from sample fluorescence. Wells A1, A2, and A3 serve as the background subtract wells on each plate and contain positive control microsomes,

ethoxyresorufin, and phosphate buffer, but **NO NADPH**. Replace the volume of NADP with phosphate buffer, pH 7.4.

1. Remove a sterile 96-well microtiter plate and label it "a" in the bottom right corner.

2. Vigorously vortex microsome samples until any large particles are broken up and sample is homogenous (~30 s).

3. Add 5.0 $\mu$l of positive control (PC) microsomes to wells A1, A2, and A3. (This serves as the background subtract for the EROD assay).

4. Wells A4, A5, and A6 receive **NO MICROSOMES**. (This serves as the background subtract for the protein assay, which will be run later on this same plate.)

5. Add 5.0 $\mu$l of sample microsomes, each in triplicate, to wells starting with A7, A8, and A9. Move across the plate, continuing until entire plate is full. Make sure each sample gets three wells and that plate setup is recorded in the lab notebook. The positive control microsomes should be run in three locations on the plate, i.e. 9 wells.

6. Using the Matrix 1250 $\mu$l multichannel pipettor, add 50 $\mu$l of 10 $\mu$M ER (at 25°C) to every well.

7. Using the Matrix 1250 $\mu$l multichannel pipettor, add 50 $\mu$l of 4.3 $\mu$M NADPH (at 25°C) to every well **EXCEPT** A1, A2, and A3.

8. Add 50 $\mu$l of phosphate buffer (pH 7.4 at 25°C) to each well.

9. Shake the sample plate for ~30 seconds and then pre-incubate the plate at 25°C for 10 minutes. This can be done in the waterbath or in an incubator.

10. Wipe any fingerprints from the bottom of the plate and place the plate in the Cytofluor.

11. Scan plate same as resorufin plate. Set the number of scans to 10 and the scan cycle to 60. Set the excitation filter to 530 nm (C), the emission filter to 590 nm (C), and the sensitivity to 3.

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12. Save file with appropriate name as a .csv file and export to EXCEL. or example, m62498a denotes that plate "a" was run on June 24, 1998.

13. Repeat steps D1-11 until all samples are finished. (The next plate can be started while the previous plate is being scanned.)

D. Protein standard plate preparation:

1. Remove 1 sterile 96-well plate (Cytoplate) from its package and label it "p" in the bottom right corner.

2. Add 50 $\mu$l of 10 $\mu$M ER (at 25°C) to wells A1-A6 through H1-H6.

3. Add 50 $\mu$l of 4.3 mM NADPH (at 25°C) to wells A1-A6 through H1-H6.

4. An average of six replicates of each BSA standard is taken.
- Place 20 $\mu$l of 0 $\mu$g/20 $\mu$l BSA standard into wells A1-A6.
- Place 20 $\mu$l of 10$\mu$g/20 $\mu$L BSA standard into wells B1-B6.
- Place 20 $\mu$l of 20 $\mu$g/20 $\mu$l BSA standard into wells C1-C6.
- Place 20 $\mu$l of 30 $\mu$g/20 $\mu$l BSA standard into wells D1-D6.
- Place 20 $\mu$l of 40 $\mu$g/20 $\mu$l BSA standard into wells E1-E6.
- Place 20 $\mu$l of 50 $\mu$g/20 $\mu$l BSA standard into wells F1-F6.
- Place 20 $\mu$l of 60$\mu$g/20 $\mu$l (6.0mg/ml) BSA standard into wells G1-G6.

5. Add 150 $\mu$l of $NaPO_4$ buffer (pH 8) to each well.

6. Shake plate in plate shaker for 1 minute at speed 6.

7. Remove plate from plate shaker and add 20 $\mu$l of 1.08 $\mu$M fluorescamine solution to each well. (Original SOP calls for an extra 20 $\mu$l to be added to row G, the highest BSA level, but experimental results show that this is not necessary.)

8. Shake plate in plate shaker for an additional minute at speed 6.
NOTE: Allow the reaction to proceed for 10-30 min before scanning. Keep this time interval the same for standards and sample plates. If standard plate is incubated for 15 min prior to reading fluorescence, then sample plates should each be incubated 15 min prior to reading their fluorescence.

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9. Wipe any fingerprints from the bottom of the plate with tissue paper (Kimwipe) and place plate in the Cytofluor.

10. Set the number of scans to 1 and the number of cycles to 0. Set the excitation filter to 400 nm (A), the emission filter to 460 nm (A), and the sensitivity to 3. The Cytofluor will then scan the plate 1 time taking 1 minute.

11. Save file with appropriate name as a .csv file and export to EXCEL. For example, m62498p denotes a protein standard plate run on June 24, 1998.

E. Fluorescamine protein assay on sample plates.

Note: Protein determination assay is done directly **after** EROD assay on the **same** plate.

1. Add 150 $\mu$l of $NaPO_4$ buffer (pH 8) to each well of every sample plate.

2. Shake sample plate(s) in plate shaker for 1 minute at speed 5.

3. Remove plate(s) from plate shaker and add 20 $\mu$l of 1.08 $\mu$M fluorescamine solution to each well.

4. Shake plate(s) in plate shaker for an additional minute at speed 5. Allow reaction to proceed for 10 to 30 minutes before scanning. See note in IV.D.8 above.

5. Wipe any fingerprints from the bottom of the plate and place the plate in the Cytofluor.

6. Scan plate same as protein standard plate. Set the number of scans to 1 and the number of cycles to 0. Set the excitation filter to 400 nm (A), the emission filter to 460 nm (A), and the sensitivity to 3.

7. Save file with appropriate name as a .csv file and export to EXCEL. For example, m62498pa denotes that the protein assay for plate "a" was run on June 24, 1998.

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V. Calculation of EROD Activity in EXCEL Software:

Note: The macros used will run in EXCEL 4 or EXCEL 5.

A. Determination of Protein Standard Curve (mprtstd.xlm macro)

1. Open mprtstd.xlm macro file. File name to be run will be inserted at cell A52. (This is done automatically when the program is run on the file.)

2. Open .csv file containing protein standard data.

3. Run mprtstd.xlm macro by choosing MACROS/RUN. Click on desired macro to run when dialog box appears, then click OK.

4. Use "File" "Save As" to save the resulting transformed file as an .xls file in the appropriate subdirectory.

5. Close all files except protein standard .xls file.

B. Determination of Resorufin Standard Curve (resmicro.xlm macro)

1. Open resmicro.xlm macro.

2. Open .csv file containing resorufin standard data.

3. Run resmicro.xlm macro by choosing MACRO/RUN. Click on desired macro when dialog box appears followed by OK.

4. Save the resulting transformed file as an .xls file in an appropriate subdirectory.

5. Close all files except protein standard .xls file and resorufin standard .xls file.

C. Determination of Protein Content Loaded into Each Sample Well (prtmicro.xlm macro)

1. Open prtmicro.xlm macro and .csv file containing protein assay data for plate "a".

2. Copy slope and intercept values from protein standard .xls file and paste to cell

G17 of protein assay .csv file.

3. Run prtmicro.xlm macro by choosing MACRO/RUN. Click on desired macro when dialog box appears.

4. Save as .xls file in appropriate subdirectory.

5. Repeat steps 12-14 for other protein sample plates (i.e., "b", "c", etc.).

6. Close all files except protein standard .xls, resorufin standard .xls, and protein assay .xls.

D. Determination of EROD Activity in Each Sample (argmicro.xlm)

1. Open first EROD sample plate (i.e., "a").

2. Copy the slope and intercept values from the resorufin standard .xls file to cells AO1 and AO2 of the EROD assay .csv file.

3. Copy and transpose the protein values from the protein assay .xls file to the EROD assay .csv file. Paste to cell AQ1 by choosing paste special and clicking the transpose box. The protein values will now be running horizontally across the spreadsheet. (Be sure that the sample plate protein file, pa.xls, remains open during transposition.)

4. Open agrmicro.xlm macro

5. Run record 4 of the macro on the EROD assay .csv file.

6. Run record 5 of the macro without moving the cursor.

7. Save as .xls file in appropriate subdirectory.

8. Repeat steps 1-7 on other EROD sample plates (i.e. "b", "c", etc.).

E. Description of Macros

1. Protein Standard Curve (mprtstd.xlm)
   a. Takes average of relative intensity unit (RIU) values from background subtracts wells (i.e. wells A1, A2, A3, A4, A5, and

A6.)
b. Takes average of RIU values from BSA standard wells and subtracts background subtract value.
c. Plots average RIU values (y-axis) against nominal BSA standard concentrations (x-axis).
d. Calculates slope and y-intercept values. This is used in determining protein concentrations in sample plate wells.

2. Resorufin Standard Curve (resmicro.xlm)
   a. Takes average of RIU values from background subtract wells (i.e. wells A1, A2, A3, A4, A5, and A6.)
   b. Takes average of RIU values from resorufin standard wells and subtracts background subtract value.
   c. Calculates slope and y-intercept values. This is used in determining resorufin concentrations in sample plate wells.

3. Protein Assay (prtmicro.xlm)
   a. Takes average of RIU values from background subtract wells (i.e. wells A4, A5, and A6).
   b. Calculates protein concentrations (mg) of sample wells by using linear regression analysis (y=mx+b) where y=RIU, x=protein (mg), m=slope from protein standard plate, and b=y-intercept from protein standard plate.

4. EROD Assay (argmicro.xlm, record 4)
   a. Arranges data from background subtract wells (A1, A2, and A3) together and inserts 8 rows between each well.

5. EROD Assay (argmicro.xlm, record 5)
   a. Takes average of RIU values from background subtract wells (i.e. wells A1, A2, and A3) over 10 scans and subtracts them from sample RIU values.
   b. Calculates pmol resorufin for each well for each scan by using linear regression analysis (y=mx+b), where y=RIU value, m=slope from resorufin standard plate, x=pmol resorufin, and b=y-intercept from resorufin standard plate.
   c. Uses least squares method to calculate a straight line that best fits the data (i.e. pmol resorufin produced after 10 scans or 10 minutes) and returns an array (i.e. slope, y-intercept, $r^2$, etc.) that describes that line.
   d. Normalizes EROD rate to protein by dividing rate (pmol/min)

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by protein concentration in well. EROD now expressed in pmol resorufin/min/mg protein.
e. Copies well designation and respective EROD rate to the right for reference.

VI. Quality Assurance/Quality Control:

General
All experimental information will be recorded in bound notebooks and copies maintained in a separate secured area. Instrument printouts and computerized data tables will be uniquely labeled and cross-referenced to the project notebook. The accuracy of all such measurements will be independently checked. Copies of the computerized data files will be maintained in the way of hard copies in a project notebook and file, and on floppy disk in the project file. All equipment used in these studies will be routinely inspected and calibrated, and preventative maintenance performed. A logbook will be kept for each instrument to document its use, performance, maintenance, and calibration.

A. Equipment Calibration Checks
1. Pipettors - All pipettors will be checked prior to beginning a new project and monthly when in regular use. Pipettors are checked by gravimetrically determining their accuracy and precision.
2. pH meter - The pH meter should be completely calibrated, including temperature calibration, prior to beginning a new project. In addition, the pH meter is recalibrated with fresh buffer solution each day it is used.
3. Cytofluor - The Cytofluor will be calibrated prior to beginning a new project and monthly when in regular use. Refer to SOP P.475 for instructions.

B. Reagent and standard checks:
1. Resorufin: absorbance is measured at 571 nm with a micromolar extinction coefficient of $5.4 \times 10^{-2}$.
2. Ethoxyresorufin: absorbance is measured at 464 nm with a micromolar extinction coefficient of $2.3 \times 10^{-2}$.
3. NADPH: absorbance is measured at 340 nm with a micromolar extinction coefficient of 6.3.
4. For all three spectrophotometric checks, the formula below applies, with the path length being 1 cm:

Concentration ($\mu$M) = Absorbance/(Ext. Coeff. x Path Length)

C. Sample Analysis

1. Each microsomal fraction will be analyzed in three wells. One sample per plate will be prepared in triplicate (i.e. 3 separate microsomal preps.). Also, the positive control microsomes will be run in three locations on each plate (i.e. 9 wells, or 3 groups of 3 wells).

2. OPTIONAL: At least 20% of the sample will be done in triplicate by initially splitting the liver tissue into 3 portions when preparing the microsomes. Microsomes prepared from each portion will then be analyzed in triplicate. The combined mean of all portion replicates will be referred to as the sample mean.

D. Data Analysis

1. The $r^2$ value of the resorufin and protein standard curves will be $\geq 0.99$ and $\geq 0.97$, respectively.

2. The $r^2$ value of the sample slope created by the production of resorufin over time in each well will be $\geq 0.99$.

3. The coefficient of variation (C.V.) between replicates of the same sample will be $\leq 20\%$.

4. The C.V. between positive control replicates will be $\leq 20\%$.

5. The C.V. of the sample mean will be $\leq 25\%$.

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Approved by:

Paul R. Heine 2/22/99

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**Attachments to SOP P.124 - Recipes**

A. Phosphate Buffered Saline (PBS)

| Component | Quantity |
|---|---|
| Sodium Chloride (NaCl) Sigma S9625 | 8.0 g |
| Potassium Chloride (KCl) Sigma P4504 | 0.2 g |
| Sodium Phosphate Dibasic ($Na_2HPO_4$) Sigma S0876 | 1.15 g |
| Sodium Phosphate Monobasic ($NaH_2PO_4$) Sigma S0751 | 0.2 g |
| Ultrapure water | 1000 ml |

1. Put approximately 800 ml U.P. water in 1 liter graduated container and place on stir plate with stir bar in place.
2. Add all components while stirring, dissolving first in weigh boat before adding to container.
3. Bring up to volume with U.P. water.
4. Titrate pH to 7.8 with 5N NaOH and or 6N HCl.
5. Decant in clean, 500 ml glass bottles.
6. Store tightly covered in refrigerator.

B. Phosphate Buffer (pH 7.4)

1. Mass 14.2 g Sodium Phosphate Dibasic (see above for info) into 1 liter ultrapure water.

2. Mass 3.4 g Potassium Phosphate ($KH_2PO_4$, Sigma P5379) into 250 ml ultrapure

water.

3. Combine 800 ml of solution #1 and 200 ml of solution #2.

4. Adjust pH to 7.4 with 5N NaOH and or 6N HCl.

5. Decant into clean, 500 ml glass bottles.

6. Store tightly covered in refrigerator.

C. Sodium Phosphate Buffer

1. Put approximately 800 mls ultrapure water in a 1 liter container and place on stir plate with stir bar in place.

2. Add 6.127 g Sodium Phosphate Dibasic and 0.8218 g Sodium Phosphate Monobasic, stirring until dissolved.

3. Bring up to volume with ultrapure water.

4. Adjust to pH 8 with 5N NaOH and or 6N HCl.

5. Decant into clean, 500 ml glass bottles.

6. Store tightly covered in refrigerator.

D. 1.08 $\mu$M Fluorescamine

1. Mass 0.039 g fluorescamine (Sigma F9015).

2. Add fluorescamine to 52 ml acetone.

3. Store at room temperature in a dark colored bottle, preferably in a dark place such as the reagent cabinet.

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E. Resorufin Standards - Due to deterioration of stored resorufin, fresh standards should be made each day the EROD assay is run. A stock solution of 58 $\mu$M stock is made weekly and stored in the freezer. The concentration of the stock solution is checked spectrophotometrically daily prior to making the standards.

1. 272 $\mu$l of 58 $\mu$M resorufin stock and 728 $\mu$l PBS = 320 pmol/20 $\mu$l
   Note: This first dilution should be adjusted depending on what the true concentration of the solution is that day (determined by spectrophotometer). The rest of the dilutions can then be made as stated.
2. 500 $\mu$l of A and 500 $\mu$l of PBS = 160 pmol/ 20 $\mu$l
3. 500 $\mu$l of B and 500 $\mu$l of PBS = 80 pmol/ 20 $\mu$l
4. 500 $\mu$l of C and 500 $\mu$l of PBS = 40 pmol/ 20 $\mu$l
5. 500 $\mu$l of D and 500 $\mu$l of PBS = 20 pmol/ 20 $\mu$l
6. 500 $\mu$l of E and 500 $\mu$l of PBS = 10 pmol/ 20 $\mu$l
7. 500 $\mu$l of F and 500 $\mu$l of PBS = 5 pmol/ 20 $\mu$l
8. 500 $\mu$l of PBS = 0 pmol/ 20 $\mu$l (for blank wells)

F. Bovine Serum Albumin Standards (BSA, Sigma A2153) - These can be frozen; the amount is enough for 2-3 protein standard plates.

1. 12 mg BSA and 2 ml PBS = 120 $\mu$g/20 $\mu$l
2. 750 $\mu$l A and 250 $\mu$l PBS = 90 $\mu$g/20 $\mu$l
3. 500 $\mu$l A and 500 $\mu$l PBS = 60 $\mu$g/20 $\mu$l
4. 500 $\mu$l B and 500 $\mu$l PBS = 45 $\mu$g/20 $\mu$l
5. 500 $\mu$l C and 500 $\mu$l PBS = 30 $\mu$g/20 $\mu$l
6. 500 $\mu$l E and 500 $\mu$l PBS = 15 $\mu$g/20 $\mu$l
7. 500 $\mu$l PBS = 0 $\mu$g/20 $\mu$l

G. 10 $\mu$M Ethoxyresorufin

1. Put 38 ml of PBS into a 50 ml centrifuge tube.

2. Add 2 ml of 200 $\mu$M Ethoxyresorufin stock solution and mix. This amount may need to be adjusted to obtain the correct concentration. The 200 $\mu$M ER stock solution is made by dissolving powdered ER in methanol. The resulting solution is stored in a foil-wrapped, dark colored bottle in the freezer. It is relatively stable as long as it is kept away from light.

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3. Should be used the same day it is made.

H. 4.3 $\mu$M NADPH

1. Put 40 ml PBS into a 50 ml centrifuge tube. Cover tube with aluminum foil.

2. Mass 0.144 g β-NADP, add to PBS, and mix.

3. Can be stored in freezer, but it is best to use it the day it is made.

I. 58 $\mu$M Resorufin Stock

1. Make superstock by measuring 5 mg resorufin into 25 ml methanol (Resorufin from Molecular Probes). Record actual weight and calculate molarity.

2. Make appropriate dilution with superstock in methanol to get 58 $\mu$M resorufin stock.

3. Store in foil-wrapped tube in freezer.

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# APPENDIX E-6

# PREPARATION OF SULFURIC ACID/SILICA GEL [USGS-TILLITT]

Date Prepared: 10/19/87

Date Revised: 8/21/98



# PREPARATION OF SULFURIC ACID/SILICA GEL (SA/SG)

## I. Introduction

Two different preparations of Sulfuric Acid/Silica Gel (SA/SG) are used by ECRC's Organic Chemistry Section in pH-reactive clean-up procedures. The characteristics that differ between the various SA/SG preparations include the gravimetric ratio of sulfuric acid to silica gel and the grade and mesh of silica gel. This SOP details the preparation of standard 40/60 (w/w) SA/SG and coarse 70/30 (w/w) SA/SG.

## II. Safety Precautions

Because silica gel can cause severe physical damage if inhaled, as can sulfuric acid if allowed to contact the skin, respiratory tract, or eyes, the necessary precautions as noted in the ECRC Safety Manual should be adhered to with both of the two components as well as with the finished product.

## III. Preparation of the Silica Gel

A. Standard 60/40 SA/SG is prepared from Grade 62, 60-200 mesh silica gel. Coarse 70/30 SA/SG is prepared from Davisil Grade 635, type 60A, 60-100 mesh silica gel. Place the appropriate silica gel in a large glass column containing a plug of glass wool at the bottom. Tap the column to settle the silica gel.

B. Pour dichloromethane (pesticide residue grade or equivalent) into the column and let the solvent pass through the adsorbent bed and begin dripping from the stopcock. Close the stopcock and allow the adsorbent to soak for ~ 30 min. Open the stopcock and drain the solvent to waste. Use 500 mL dichloromethane for every 300 mL of silica gel.

D. When dripping ceases, apply $N_2$ pressure (2-3 psi) until the column is no longer cold to the touch (the dichloromethane has evaporated).

E. Pour the dry powder in a shallow layer into a glass pan. Cover the pan tightly with acetone-rinsed aluminum foil. Place in 130°C oven overnight.

## V. Preparation of SA/SG

A. Tare a one-liter glass bottle. Because of the hygroscopic nature of the chemicals being used, the following steps in this procedure should be performed as quickly as possible. Also, because of the limited shelf-life of SA/SG, a quantity only in slight excess of the amount immediately needed should be prepared at one time.

B. Fill the bottle approximately 2/3 full with washed silica gel and note the mass of the silica gel.

C. **For 60/40 SA/SG:** Compute 0.66 of the mass of the silica gel in the bottle, place this mass of concentrated sulfuric acid in the bottle.*

**For 70/30 SA/SG:** Compute 0.43 of the mass of the silica gel in the bottle, place this mass of concentrated sulfuric acid in the bottle.*

*NOTE: Ignore the fact that acid isn't 100% pure.

D. Cap with a Teflon-lined screw cap, and shake vigorously until there are no lumps and the contents are a free-flowing powder. Tumble on a bottle roller for a minimum of two hours.

E. Label the bottle showing the contents, the identification number of this SOP, and the date prepared. Keep the bottle tightly capped at all times and store at room temperature. **The maximum shelf life of this product is six months**, so discard the unused portions after this length of time. Also, if before the six months have elapsed, if the SA/SG appears lumpy, clings to the mouth of the bottle, or is noted to contain dark specks, it should be discarded.

*Adapted from an original procedure (SOP C5.67) prepared by Jon A. Lebo, Research Chemist.*

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# APPENDIX E-7

# PREPARATION OF POTASSIUM HYDROXIDE-TREATED SILICA GEL [USGS-TILLITT]

Date Prepared: 6/24/98

Date Revised: 8/21/98



# PREPARATION OF POTASSIUM HYDROXIDE-TREATED SILICA GEL

## I. Introduction

Potassium hydroxide-treated silica gel is a component in a variety of ECRC's clean-up procedures. It is essentially silica particles carrying a thin, evenly-distributed coating of KOH, but is generally referred to as potassium silicate or KS.

It should be recognized that the term "KS" is very likely inaccurate. Any actual reaction of the KOH with the silica particles is probably confined to the surface of the silica gel particles, leaving the greater portion of the mass of the adsorbent unreacted. The adsorbent described by this SOP is not water-soluble but is very hygroscopic. Two of the three possible potassium silicates (*meta-* and *tetra-*) are are soluble in water. The third (*di-*) is not water-soluble but unlikely to be hygroscopic. But other than this (and the fact that "KS" represents an impossible coupling of a potassium atom and a sulfur atom), KS is a perfectly good designation, deeply ingrained in our "bench language", so it will be used in this SOP.

## II. Organization of the SOP

Sections VI-XI are divided into two paragraphs. The first paragraph of each section has been taken with minor editorial changes from ECRC SOP C5.69 by Jon A. Lebo, and describes the production of very large amounts of adsorbent using equipment custom-fabricated for that purpose. As long as that equipment is available, the procedure is applicable and the instructions will be retained. The second, italicized paragraph contains instructions for smaller quantities of sorbent

## III. Apparatus for Large-scale Procedure

A. Heated water bath (Chromalox, Series 400)
B. 12-L roundbottom flask with 55/50 female ground-glass opening
C. Flask support rack with Teflon rollers
D. Motor for revolving the flask (Dayton Permanent Split Capacitor Gear Motor)
E. Teflon stopper for 12-L flask
F. Neck collar around flask for attachment of motor drive shafts
G. Large capacity glass column with 60/40 female ground-glass opening
H. 60/40 male ground-glass elbow
I. Two-gallon wide-mouth bottle (for tumbling the KS)

J. Bottle roller (for tumbling the KS)
K. Cork ring (to support the 12-L flask on the benchtop)

## IV. Reagents

A. Silica gel (SG-60; 70-230 mesh, ASTM; E. Merck, Darmstadt, GFR)
B. Methanol (OmniSolv™, E. Merck, Darmstadt, GFR; or the equivalent)
C. KOH pellets (Reagent grade, 85% or higher purity)
D. Dichloromethane (Pesticide Residue Grade, or the equivalent)

## V. Safety

In addition to the standard safety considerations when dealing with organic solvents and silica dust, special attention is called to methanolic KOH. Potassium hydroxide is extremely caustic, and severe burns can result from contact with the skin. Methanolic solutions of KOH are especially dangerous in this regard because not only is the KOH more reactive in this dissolved form, but if splashed or spilled, the caustic material is spread over a wide area. Proper protective safety gear must be worn.

## VI. Preparation of Methanolic KOH Solution

Pour 4500 mL of methanol into the 12-L round bottom flask. Weigh out 1008 g of KOH pellets and combine them with the methanol. Plug the flask with the Teflon stopper. Submerge the flask support rack in the water bath. Cradle the flask containing the methanol and KOH in the rack, tilt the flask toward the gear motor, and attach the two parallel drive shafts to the collar around the neck of the flask. (The water bath should contain water to a depth of approx. 10 cm for this and all subsequent operations.) Turn on the gear motor and allow the flask to revolve until the KOH has dissolved. There are two advantages in dissolving the KOH by this method. Ordinarily the flask would have to be swirled by hand to dissolve the KOH, and there would be a risk of breakage. Also, the dissolution of the KOH in methanol generates heat which could cause boiling were this heat not dissipated in the water bath.

***Small scale:*** *Pour 750 mL of methanol into a 2-L round bottom flask. Weigh out 168 g of KOH pellets and combine them with the methanol. Plug the flask with the Teflon stopper. Hold the flask under a stream of cold tap water to dissipate heat. Swirl the flask, venting the stopper occasionally till the KOH is dissolved.*

## VII. Addition of the Silica Gel

Remove the flask containing the methanolic KOH from the water bath. After the flask has been removed, preheat the water bath to 55°C. Place the flask containing the KOH solution in a fume hood. With the aid of a large funnel, slowly add 1800 g of silica gel

into the solution. This must be done slowly to avoid the formation of lumps. Add the silica gel a little at a time, swirling the flask, and allowing each addition to sink to the bottom of the solution before adding more. The addition of the entire 1800 g of silica gel to the methanolic KOH should require approximately 30 min.

***Small scale:*** *Place the flask containing the KOH solution in a fume hood. Slowly add 300 g of silica gel to the solution. Add the silica gel a little at a time, swirling the flask, and allowing each addition to sink to the bottom of the solution before adding more.*

## VIII. Revolving the Flask at 55° C

Stopper the flask, and cradle it in the flask support rack. As before, tilt the flask toward the gear motor and attach the drive shafts to the collar around the neck of the flask. Verify that the temperature of the water bath is 55°C. Turn on the gear motor and allow the flask to revolve in the bath for 90 min.

***Small scale:*** *Use a rotoevaporator with a heated bath for this operation. Rotate the flask in the heated water without vacuum for 90 min.*

## IX. Transferring the KS to the Column for Washing and Drying

Set up the large column in its custom-made stand. Insert a large plug of glass wool in the column and tamp it down to the bottom. Remove the 12-L flask from the water bath, place it on the bench top, and dry the outside of the flask with paper towels. Insert the large polypropylene funnel into the mouth of the column, and put two or more wires underneath the funnel (into the ground glass joint) to allow air to escape and to prevent spattering of the methanolic KOH. Place a clean, wide-mouth 4-L jar underneath the column. Swirl the flask vigorously until all solid material is suspended and the contents are a slurry. Quickly pour as much of the slurry as possible into the funnel before the solid material in the flask sinks to the bottom of the methanolic KOH and is no longer pourable. There will no longer be enough methanolic KOH in the flask to re-suspend the remaining solid material. Substitute an empty, clean, wide-mouth jar for the one underneath the column containing the effluent methanolic KOH. Transfer the solution from the first jar back to the flask, and use it to re-suspend the solid material by swirling the flask. As before, transfer as much of the slurry as possible to the funnel. Repeat the above steps until the flask is empty, then similarly use effluent methanolic KOH to rinse all solid material from the funnel down into the column. Remove the funnel, and cover the mouth of the column with the aluminum foil.

***Small scale:*** *Affix a glass column (~4 cm dia.) to a ring stand, plug the bottom with glass wool, and place a clean 1-L collection vessel under the stopcock. Remove the 2-L flask from the water bath and dry the outside of the flask with paper towels. Insert the large funnel into the mouth of the column and put a wire or paper clip underneath the*

*funnel to allow air to escape. Swirl and suspend the material in the 2-L flask and pour the contents into the column. When most of the solvent has drained into the collection vessel, add that solvent back into the 2-L flask to suspend and transfer the sorbent that you didn't get the first time. Repeat until you have transferred all the sorbent to the column.*

## X. Washing the KS

When the liquid level in the column has descended to the level of the top of the settled KS, wash the adsorbent with 600 mL of fresh methanol. Repeat this step twice. When the liquid level in the column has descended to the top of the KS for the fourth time, wash the adsorbent with 1 L of dichloromethane. Repeat this step twice. After most of the dichloromethane has run through the KS, blow nitrogen gas through the KS, using the 60/40 ground glass elbow. Adjust the nitrogen pressure through the column to approx. 2 psi, and allow the nitrogen to blow overnight. Check the column the next day. If the outside of the column still feels cold, there is residual dichloromethane in the KS. Continue blowing nitrogen through the column until it is no longer cold.

***Small scale:*** *When the liquid level in the column has descended to the level of the top of the settled KS, wash the adsorbent with 100 mL of methanol. Repeat this step twice. When the last methanol wash has reached the top of the KS, wash the adsorbent with 170 mL of dichloromethane. Repeat this step twice. After most of the dichloromethane has run through the KS, blow nitrogen gas at ~ 2 psi through the KS, using a ground glass elbow (or a stopper wrapped in teflon tape) and appropriate tubing. Allow the nitrogen to blow several hours or overnight until the column no longer feels cold.*

## XI. Activating and Tumbling the KS

Pour the KS into shallow layers in glass pans. Cover the pans tightly with aluminum foil, and activate the KS overnight (at least 12 h) in an oven at 130°C. Remove the KS from the oven and recombine the KS from all of the pans in an 8-L wide-mouth bottle. Cover the bottle opening tightly with a Teflon, polyethylene, or polypropylene sheet held around the neck of the bottle by a rubber band. Tumble the KS on a bottle roller for at least 2 hr. Remove the large bottle from the bottle roller and place the KS in one or more 4-L solvent bottles. Cover the openings with aluminum foil and store the bottles in the oven at 130°C.

***Small scale:*** *Tumble and store the KS in a 1-L screw-capped bottle as described above.*

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# APPENDIX E-8

# ALUMINA CLEANUP OF PCDD/PCDF FRACTIONS FROM HPLC-CARBON [USGS-TILLITT]

Date Prepared: 10/21/91

Date Revised: 8/21/98

**Note: This is an ECRC in-house document. It is not citable for publication purposes.**

# ALUMINA CLEANUP OF PCDD/PCDF FRACTIONS FROM HPLC-CARBON

## I. INTRODUCTION

This SOP describes how the reverse-flow toluene fractions from dispersed carbon containing PCDDs and PCDFs will be purified on basic alumina prior to GC/MS analyses. Non-polar biogenic materials, chlorinated naphthalenes, and most PCB congeners will be removed in the first fraction and discarded. The collected (second) fractions from alumina will contain the PCDD and PCDF congeners.

## II. MATERIALS

A. Columns: Glass 1-cm i.d. column equipped with a reservoir and fitted with a Teflon stopcock.

B. Solvents:
   a. nonane and isooctane (keeper solvents)
   b. 5/95 $CH_2Cl_2$/hexane; V+V (made with UV grade or Fisher universal grade solvents)
   c. $CH_2Cl_2$

C. Alumina: Fisher basic alumina, 60-325 mesh, received from the manufacturer as Brockman activity grade I. The alumina is prewashed with ~ 1 liter $CH_2Cl_2$ per 500g, placed in a cake pan and air dried for ~ 1 hr., then dried for 8 hr. (and subsequently stored) in a 190°C oven.

## III. PREPARATION OF ALUMINA COLUMNS

A. Dry packing: Remove the supply of alumina from the oven just before setting up the column array. Place a small plug of glass wool in each column directly above the stopcock, and above this place a 1-cm layer of anhydrous sodium sulfate. Weigh 8.0 grams of alumina and pour it over the sodium sulfate. Tap the column to settle the adsorbent and top the column with another 1-cm layer of anhydrous sodium sulfate.

B. Presaturation of alumina: Pour ~30 mL of the 95/5 eluant onto each column. Allow it to pass through to waste, stopping its flow when its level is slightly above the top of the

column bed (The solvent level is lowered to the top of the column bed immediately prior to sample application).

## IV. ELUTION PROFILES

A. Azobenzene elution: On each day that alumina cleanups are to be performed, calibrate the fractionation procedure by chromatographing azobenzene on the alumina as follows. Tamp small wads of glass wool in the bottoms of three pasteur pipets (the long tips of the pasteur pipets may be broken off for convenience). In each pipet, place 0.5 - 1.0 cm. Florisil. Assemble and pack three alumina columns in the normal way except insert the three mini-Florisil columns in place of the glass tips in the stopcocks of the columns. After the column rinse has drained, arrange to collect column effluent in 100-mL graduated cylinders. Place ~4 mg of azobenzene (100µL of 40 mg/mL solution) in a culture tube, dilute it to ~0.5 mL with 5/95 $CH_2Cl_2$/hexane; V+V. Apply the material to the alumina columns, as described below. Add an excess of eluant and observe the yellow band as it traverses the alumina and is adsorbed on the Florisil mini-columns. Record at what volume of total eluate the mini-columns first begin to turn yellow. Multiply this volume by 0.4. The resultant volume will be the total amount of 5/95 $CH_2Cl_2$/hexane eluant that comprises the DUMP fraction for each sample that is fractionated on alumina that day (see below).

B. Other elution profiles: When deemed appropriate by the chemists in charge of the work units for which this SOP is applicable, other elution profiles may be required to further prove the reliability of the alumina fractionation procedure. The profiles may involve such radiolabeled analytes as PCB congener 77, PCB congener 52, 2,3,7,8-TCDD, or OCDD; or such cold analytes as Aroclor or Halowax mixtures, or PCDD and PCDF mixed standards.

## V. SAMPLE FRACTIONATION

A. Nitrogen evaporation: Samples are generally received from carbon fractionation in 15 mL graduated culture tubes in a mixture of toluene and $CH_2Cl_2$. **All of the toluene must be removed before Alumina fractionation or losses of some of the dioxin and furan congeners will result.** Using the N-EVAP™ (nitrogen evaporator/water bath) set at approximately 35°C, **evaporate the samples *just to* dryness**. Immediately add ~0.5 mL solvent (5/95 $CH_2Cl_2$/hexane, if the samples are to be immediately processed; nonane, if they aren't) to the dried extracts and **ensure dissolution by sonicating the extracts.**

B. Application of sample and elution of dump fraction: Several (8-10) samples can be cleaned up on alumina simultaneously. For each alumina column that is simultaneously used, there should be on hand a graduated cylinder containing the appropriate volume of 5/95 $CH_2Cl_2$/hexane. Each graduated cylinder containing the premeasured eluant should have its own disposable pipette and bulb, as should each culture tube containing a concentrated sample.

First, lower the solvent level in the alumina columns just to the sodium sulfate and transfer the extract solutions from the culture tubes to the corresponding alumina columns. Then transfer 0.5 mL of eluant from the graduated cylinders to the corresponding culture tubes. Use the solvent to rinse the insides of the tubes, then, after the original solution applications have descended below the levels of the $Na_2SO_4$, transfer the first tube rinse solutions to the corresponding alumina columns. Apply the solutions just above the $Na_2SO_4$ segments. Allow the initial applications and subsequent rinses to run into the alumina beds.

Repeat the above-described tube-rinse and application sequence with two more 0.5-mL portions of 5/95 $CH_2Cl_2$/hexane for each sample. When the fourth applications (third rinses) have descended below the $Na_2SO_4$, gently pour the eluant remaining in the graduated cylinders into the column reservoirs.

C. Recovery of PCDD/PCDF fraction from alumina: Allow the 5/95 $CH_2Cl_2$/hexane eluant to descend to the level of the tops of the $Na_2SO_4$ segments. This eluant is the DUMP fraction and is discarded. Place 125mL round-bottomed flasks beneath the alumina columns. Elute the PCDDs and PCDFs from each column with a 25-mL portion of $CH_2Cl_2$. After collection, add ~1mL isooctane to each flask, rotoevaporate the solvent down to ~1mL, and transfer (with 3 $CH_2Cl_2$ rinses) to the original tubes that held the samples before the alumina procedure.

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# APPENDIX E-9

# TISSUE ANALYSIS FOR PCBS AND LOW-LEVEL PLANAR HALOGENATED HYDROCARBONS [USGS-TILLITT]

ECRC SOP P.186
Date Prepared: 1/16/91

Date Revised: 8/21/98



# TISSUE ANALYSIS FOR PCBs AND LOW-LEVEL PLANAR HALOGENATED HYDROCARBONS

## I. Introduction

This SOP describes a preparative scheme for tissue samples in which PCBs, PCDDs, and PCDFs are targeted. The purified extracts generated at the end of the procedure are ready for high performance gel permeation chromatography (HPGPC) followed by GC/ECD analyses (or GC/ECD semiquantitative screening), or fractionation by high performance carbon chromatography into coplanar fractions and PCDD-PCDF fractions. The extracts may be analyzed at ppt levels and therefore must be of sufficient quality to be reduced to a final volume of as low as 10 µL. The sequence of steps described in this SOP includes blending of the tissue with sodium sulfate, extraction, percent lipid determination, and reactive cleanup. HPGPC is detailed in SOP P.464.

## II. Safety Considerations

In performing the operations delineated in this SOP, all aspects of the ECRC Safety Plan shall be adhered to. Volatile or flammable solvents shall be handled only in the fume hood. Personnel shall wear lab coats, gloves, safety glasses and other appropriate protective gear. Radiolabeled materials such as $^{14}$C-2,3,7,8-TCDD shall be worked with only above the specially designed absorbent paper. This is only a partial listing of relevant safety precautions. Analysts should refamiliarize themselves with the Safety Plan before embarking on this project.

## III. Listing of Reagents and Apparatus

A. Glassware - *before use, rinse <u>all</u> glassware with acetone, hexane, dichloromethane, and toluene.*

B. Anhydrous sodium sulfate ($Na_2SO_4$) prepared as per ECRC SOP C5.8

C. Silica gel SG-60 prepared as in ECRC SOP P.270.

D. Standard 40/60; W/W; sulfuric acid/silica gel (SA/SG) prepared as per ECRC SOP P.270.

E. Coarse 30/70; W/W; sulfuric acid/silica gel (COARSE SA/SG) prepared in accordance with ECRC SOP P.270.

F. Standard potassium silicate (KS) prepared as per ECRC SOP P.271.

G. Blender blades, stainless steel rods and spatulas. Preparation: Rinse this equipment with acetone, hexane, then dichloromethane.

## IV. Blending Tissue with Sodium Sulfate

This SOP assumes that you will be starting with ground tissue samples. The day before you plan on extracting a group of tissue samples, thaw the samples and proceed as follows. Weigh aliquants (probably 25-g wet weight portions) of the tissue samples into pint or quart canning jars. Add four times the sample wet weights of $Na_2SO_4$ to the jars. Immediately and thoroughly mix the tissue and desiccant together, using stainless steel rods or spatulas. Allow the tissue-sodium sulfate mixtures to interact for about an hour before stirring them again. Occasionally stir the samples again throughout the day so as to prevent hardening of the tissue-$Na_2SO_4$ mixtures. The next morning, thoroughly stir the samples one more time. Samples are now ready for homogenization with a blender and extraction.

## V. Extraction

*NOTE: Due to the tremendous concentration factors involved in this procedure (25g. sample to a few microliters), it is essential that special attention be given to cleanliness and proper technique. All sample contact surfaces (columns, flasks, test tubes, caps, etc.) should be cleaned immediately before use by generous rinsing with acetone, hexane, dichloromethane, and toluene.*

A. Prepare 4-cm i.d. extraction columns as follows. Insert stopcocks in the columns and rinse with acetone, hexane, dichloromethane, and toluene. When the columns have dried, place wads of glass wool at the bottoms of the columns and cover the wads completely with sodium sulfate. Pour in the freshly blended tissue-sodium sulfate mixture and tap the columns to settle the mixture, eliminate voids, and level the top surface of the mix.

B. Spike surrogates (procedural internal standards) and, when applicable, radiolabeled and cold analytes into the tissue-$Na_2SO_4$ mixtures at this point. Using an appropriately sized syringe, dispense the correct volume of spiking solution onto the sample mix.

Rinse any remaining spiking solution from the syringe needle tip onto the matrix with a small volume of methylene chloride dispensed from a pasteur pipet.

C. After spiking, deposit $Na_2SO_4$ to segment heights of about 2 cm over the packed material, pouring the sodium sulfate down the sides of the columns to dislodge the small particles of sample mix adhering to the sides. For all samples that received spikes, rinse the sides of the columns with 2-3 mL dichloromethane and allow them to stand for 30 minutes before proceeding further. Place 500-mL boiling flasks that have been rinsed with the same sequence of solvents as the columns beneath the columns. Open the stopcocks, and carefully pour 50 mL of dichloromethane into each solvent reservoir. When the levels of the solvent in the reservoirs have descended to approx. 1 cm. above the tops of the upper $Na_2SO_4$ segments, close the stopcocks and allow the tissue-desiccant mixtures to soak in the $CH_2Cl_2$ for at least an hour. Then, allow the extraction solvent level to decend to the top of the sodium sulfate layer and gently pour 225 mL of $CH_2Cl_2$ into the reservoirs and open the stopcocks to obtain $\leq 2$ drops/second flow. When this volume of solvent has drained from the column completely, add another 50 mL methylene chloride to the columns and collect it. Keep the reservoirs covered with aluminum foil except when adding solvent.

## VI. Determination of Percent Lipid

Rotary evaporate the tissue extracts to volumes of ~25 mL. Use $CH_2Cl_2$ rinses to transfer the lipid solutions to 100-mL volumetric flasks and to dilute them to the 100-mL marks. For details of lipid determination, the analyst is referred to ECRC SOP P.461.

## VII. Reactive Adsorbent Cleanup - First Stage

The following preliminary cleanup step can accommodate no more than **four grams** of total lipid per chromatography column. If you have verified (in Step VI.) that any of the volumetric flasks contain more than four grams of lipid, the extracts in them should be split in two or more approximately equal portions for the chromatographic/reactive cleanup described below. Each portion of a split sample will then be applied to its own chromatography column, and the eluates recombined afterward.

A. Prepare 4-cm i.d. chromatography columns by attaching stopcocks and then placing wads of glass wool in the bottoms of the columns. Deposit anhydrous sodium sulfate over the glass wool to segment heights of ~1 cm.

B. For the sake of convenience and consistency, the adsorbents will be dispensed by volume using a graduated cylinder. (When measuring the volumes, tap or shake the cylinder in a consistent manner to settle the material being measured, thereby acheiving an accurate measurement. It is not necessary to carry this "settling" operation to

extremes). First, add 25 mL of KS to each column. Tap the columns to settle the adsorbent and produce a level top surface. Next, add 25 mL SA/SG, again settling and leveling. Place a 1-cm layer of sodium sulfate over the SA/SG. Finally, add 50 mL COARSE SA/SG to the columns and settle. Presaturate the columns with 80-100 mL of dichloromethane, allowing the solvent to decend just to the top of the adsorbent.

C. Quantitatively apply the remaining (after % lipid determinations) lipid solutions to the columns described above. Open the stopcocks and allow the samples to sink into the adsorbent. Observe the discoloration resulting from the sample's reaction with the COARSE SA/SG and close the stopcock when the discoloration has reached the sodium sulfate layer that separates the two acidic adsorbents. Stir this COARSE SA/SG layer thoroughly with a metal spatula to remove the gas bubbles that will have evolved there. Disturb the sodium sulfate layer as little as possible. The stirring process will cause discoloration of the remaining sample solution that has not passed into the adsorbent. Rinse the residue on the spatula back into the sample solution thoroughly with dichloromethane. Open the stopcocks and, when the sample solutions have decended into the adsorbent, rinse each volumetric flask with three 5-mL portions of $CH_2Cl_2$, adding these rinsings to the column. When these rinses have decended into the adsorbent, wash the column walls with another three 3-mL portions of dichloromethane, allowing each one to sequentially sink into the column. Then close the stopcock and deposit approx. 1 cm. of sodium sulfate onto the top of the column. Gently pour 150 mL of $CH_2Cl_2$ into the column reservoirs. Open the stopcocks to obtain a flow rate of approximately 2-3 drops/second and collect all effluent in 500-mL boiling flasks.

**NOTE:** In some instances, the total lipid content of individual samples within a set may consistently fall below 1 gram. A good example of this is when a small portion of the raw extract is set aside to be processed separately for the H-4-II E Bioassay. In this case, spiking of AHH-active recovery standards is performed on the raw extract after this portion has been removed. The reserved portion often contains 10% or less of the total sample and, therefore, less than a gram of total lipids. For samples such as these, the reactive column configuration may be altered. A 2-cm i.d. is used in place of the 4-cm one. The volumes of the adsorbents are adjusted to 10 mL KS, 10 mL SA/SG, and 15 mL COARSE SA/SG. The column is presaturated with 25-30 mL dichloromethane. The column is eluted with 50 mL dichloromethane, collected at 1 drop/second (3 mL/min.) into a 125-mL flask.

## VIII. Reactive Adsorbent Cleanup - Second Stage

A. Rotary evaporate extracts to volumes of about 20 mL, add 6 mL of isooctane to each flask, and rotary evaporate to 3-mL volumes. Use identical flasks containing 20 and 3 mL of solvent to make volume comparisons if necessary.

B. Clean extracts up on silica gel and SA/SG as follows. In 1.0-cm i.d. columns, pack the following ingredients from bottom to top: a plug of glass wool; a 1-cm segment of anhydrous sodium sulfate; a 10-mL segment of silica gel SG-60: a 3-mL segment of KS; a 5-mL segment of SA/SG; and a 1-cm segment of anhydrous sodium sulfate. Presaturate the column with the eluant, 3% dichloromethane; 97% hexane (V+V). Place 125-mL flat bottom flasks underneath the columns. Transfer the extracts to the tops of the adsorbent columns and rinse the 500-mL flasks with three 3-mL portions of the eluant, sequentially applying the rinsings to the columns. Gently pour 45 ml of the eluant into the reservoirs and allow it to pass into the flasks at 1 drop/second (3 mL/min.) flow rate.

C. Add 1-2 mL of isooctane to each flask (as a "keeper" rather than for solvent-exchange purposes) and rotary evaporate extracts to about 3 mL. Transfer the extracts to graduated culture tubes through pasteur pipet-filters. These filters are prepared by tamping small wads of 3-micron GFD filter into the bottoms of pasteur pipets and passing a 2-3 ml methylene chloride rinse through the assemblies to remove any extraneous material or stray glass fibers. Place the filters in the receiving culture tubes and transfer the concentrated extracts through them. Rinse the sample flasks with three sequential methylene chloride rinses, passing them through the filters also. The sample extracts may now be reduced in volume and transferred to autosampler vials in preparation for HPGPC (ECRC SOP P.464). After HPGPC, the samples can proceed to fractionation on HPLC-Carbon or to GC/ECD analysis/screening after the appropriate solvent exchange.

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# APPENDIX E-10

# ORGANOCHLORINE PESTICIDE ANALYSIS: FRACTIONATION OF COMPLEX MIXTURES ON SILICA GEL/ODS [USGS-TILLITT]

Date Prepared: 06/15/98

Date Revised: 08/21/98



# ORGANOCHLORINE PESTICIDE ANALYSIS: FRACTIONATION OF COMPLEX MIXTURES ON SILICA GEL/ODS

## I. INTRODUCTION

Analysis of environmental sample extracts frequently requires chromatographic separation of the constituents into less complicated mixtures in order to obtain viable instrumental detection and quantitation. This SOP details a non-destructive fractionation scheme that utilizes size exclusion chromatography followed by adsorbent chromatography to purify sample extracts and separate contaminant residues into two fractions: 1) PCBs and non-polar pesticides and 2) slightly polar pesticides. Hexane and methylene chloride are the primary elution solvents. The method is applicable to extracts from a variety of matrices including plant and animal tissues, water, SPMDs, sediments, and soils. Specific extraction procedures are included in SOP's P.186, P.187, and P.461.

## II. Preliminary Clean-up

The adsorbent fractionation outlined here is designed to be performed on extracts that have undergone a lipid-removal step that is non-destructive to the OC pesticides. The extract should not contain more than a few miligrams of lipid. For high-lipid extracts (primarily animal tissues), the preliminary treatment of choice at ECRC is open-column GPC (SOP C5.155), followed by HPGPC (SOP C5.191). For low-lipid extracts, such as those obtained from plant tissue, sediments and soils, the open-column GPC treatment is not necessary. However, these extracts are often highly colored and application of the extracts on HPGPC can seriously compromise the expensive separation column due to the strong adsorption of the pigments on the polystyrene divinylbenzene resin. Extracts of these matrices should be cleaned on Florisil before the HPGPC step.

Glass chromatography columns, 30cm x 1cm I.D. with 75-mL reservoirs and Teflon stopcocks are used. Tamp a small wad of glass wool in the bottom of each column and cover it with ~1cm of anhydrous sodium sulfate. Add 5 grams of Florisil (60-100 mesh), tap the column a little to settle it, and cover the Florisil with another 1 cm sodium sulfate. Add approx. 25 mL of methylene chloride and allow it to pass through the column to a waste collection vessel. Stop the flow when the solvent has just reached the sodium sulfate. Replace the waste collection vessel with a 125 mL RB flask. Quantitatively apply the sample extracts (in 1-3 mL volumes) to the columns with three sequential 2-mL rinses of methylene

chloride. Elute the columns with 80 mL methylene chloride. Reduce the solvent by rotoevaporation and proceed with HPGPC. After these procedures have been implemented, the following silica-gel/ODS (SODS) fractionation is performed.

## III. SODS Column Characterization

It is necessary, when initiating this procedure, to characterize the performance of the adsorbent columns. This is necessary when there is a change in any major parameter such as the adsorbent lot, source of solvent, or *person performing the procedure*.

Silica gel 60, 70-230 mesh, E. Merck, is prepared by washing with methylene chloride and activating (and storing) at 130°C for at least 24 hours prior to its use. Refer to ECRC C5.67, Section 4. Silica gel is very hygroscopic and is prone to deactivation by humidity during the weighing and column packing operation. **IMPORTANT: UNACCEPTABLE VARIATION will be introduced into this procedure if the silica gel is deactivated by humidity.** To minimize this significant source of variability, the following procedures have been adopted. Prior to performing a set of samples, an adequate number of 5-g portions of the silica gel (still warm from the oven) are weighed into glass scintillation vials and replaced in the oven. Just prior to performing a set of columns, enough portions are removed from the oven and placed in a dessicator to cool. When the columns are set up, the portions are removed from the dessicator and packed in the columns as quickly as possible.

ODS (octadecyl silica; $C_{18}$), 40$\mu$m particle size, 60Å pore size, sorbent quality, is prepared by washing with methylene chloride. The washed sorbent is placed in a fritted glass column and dried by passing $N_2$ upward through the column frit and washed sorbent. After most of the solvent is removed, the sorbent is spread out in a glass cake pan and air-dried before storage in a tightly-capped glass bottle. NOTE: The ODS must not be oven dried. Even at temperatures as low as 130°C, ODS will decompose.

The elution solvents used in this fractionation are 100% hexane for fraction 1 and 55/45 hexane/methylene chloride for fraction 2. The fraction 2 solvent storage life should not exceed two weeks.

A. Prepare four 5-gram silica gel columns in the same manner as described earlier in this document for Florisil. Deposit one gram of ODS over the top layer of sodium sulfate, followed by another 1cm layer of sodium sulfate. Add approx. 20 mL of hexane and allow it to pass through the column to a waste collection vessel. Stop the flow when the solvent has just reached the sodium sulfate.

B. Place 1 mL methylene chloride in four clean test tubes. Spike each tube with 20-30K counts of $^{14}$C-labelled pentachloroanisole (PCA). Apply the solutions to the columns and allow them to sink into the column beds. Add 32 mL hexane to each of the columns

and collect it as one fraction. Collect five more 1-mL fractions from each of the columns in graduated tubes.

C. Rotoevaporate the 35-mL portions to approx. 3 mL and transfer them to scintillation vials. Transfer the 1 mL portions to scintillation vials as well and count the fractions on the scintillation counter. PCA is the first OC of fraction 2 (SG-2) to begin eluting, therefore, the presence of counts indicates the volume required to elute fraction 1 (SG-1). At the time of this writing, the volume established for SG-1 ranges between 62 and 65 mL.

## IV. SODS Fractionation

A. Prepare the required number of SODS columns as described in Section III.

B. The sample extracts for fractionation should be in ~3mL alkane. Measure, in a graduated cylinder, the volume of hexane that was established for SG-1 by the column characterization procedure. Place a labelled 125-mL round bottomed flask under the stopcock. Using a Pasteur pipet, quantitatively transfer the sample to the SODS column and allow it to sink just to the top of the column bed. Rinse the sample flask three times with 1-2 mL portions of the hexane, from the premeasured volume, allowing each rinse to sink to the bed before adding the next one. Then add the remaining solvent from the graduated cylinder, stopping collection when the solvent has reached the column bed.

C. Remove the collection flask which now contains SG-1 and replace it with another labelled 125-mL round-bottom. The volume of 55/45 hexane/methylene chloride that has been established for SG-2 is 80 mL. Add this volume and allow it to drain into the flask.

D. Rotoevaporate SG-1 fractions to approx. 3mL and quantitatively transfer them to graduated tubes with hexane rinses. Add a few mL of isooctane to the SG-1 fractions as a "keeper" solvent prior to rotoevaporation. Add 5 mL isooctane to the SG-2 fractions and reduce the volumes to ~3 mL by rotoevaporation. Quantitatively transfer these fractions to graduated tubes with hexane rinses. The fractions, with appropriate volume adjustments, are ready for instrumental analysis.

## V. Standard Compositions

The following is a list of the compounds normally quantified in an analysis using this SOP. PCBs are represented by a 1:1:1:1 mixture of Aroclors 1242, 1248, 1254, and 1260.

Silica Gel Fraction I:

PCBs
Hexachlorobenzene
Heptachlor
p,p'-DDE
Mirex

Silica Gel Fraction II:

α-BHC
Pentachloroanisole
γ-BHC (Lindane)
β-BHC
δ-BHC
Dacthal
Oxychlordane
Heptachlor epoxide
*trans*-Chlordane
o,p'-DDE
*cis*-Chlordane
*trans*-Nonachlor
Dieldrin
o,p'-DDD
Endrin
p,p'-DDD
*cis*-Nonachlor
o,p'-DDT
p,p'-DDT
Methoxychlor

Samples undergoing this analysis are spiked with any of several compounds in known amounts for the purpose of recovery correction and verification that the fractionation of the mixture is being performed correctly. The compounds usually used for this purpose, and the fraction in which they elute, are listed below.

| | |
|---|---|
| PCB congener #030 (I-030) | SG-1 |
| PCB congener #204 (I-204) | SG-1 |
| 2,4,5,6-tetrachloro-m-xylene | SG-1 |
| dibutyl chlorandate | SG-2 |
| *cis*-permethrin | SG-2 |

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# APPENDIX E-11

# ANALYSIS OF TETRA- THROUGH OCTA-SUBSTITUTED POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS BY GAS CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY [USGS-TILLITT]

ECRC SOP: P.482

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# Analysis of Tetra- through Octa-Substituted Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans by Gas Chromatography-High Resolution Mass Spectrometry

## Introduction

This standard operating procedure describes the instrumental analysis of tetra- through octa-2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) using GC/High Resolution MS (GC/HRMS). It outlines the general method of GC/HRMS analysis, and specifically the operation of the VG 70-250S GC/HRMS and PDP 11/73 data system.

## Materials

**Solvents:** The following pesticide grade distilled-in-glass solvents may be used: nonane, hexane, isooctane, dichloromethane, toluene, and isopropanol.

**Chromacol semi-conical vials:** (0.9 mL) crimp-top vials are stored in a sealed container until being used once for GC/HRMS.

## Spiking and Standard Solutions

**Quantitative Standards:** The compositions of the surrogate standard (procedural internal standard), native-only spiking standard, and calibration standard solutions for PCDFs and PCDDs are shown in Table 1. Before extraction, sample/sodium sulfate homogenates are spiked with 600pg of the PCDD/PCDF Surrogate Standard, using 15 $\mu$L of ECRC Standard 174W at 40 pg/$\mu$L. Before GC/MS analysis, each PCDD/PCDF fraction is spiked with 1000 pg of the PCDD/PCDF Internal Standard, using 10 $\mu$L of ECRC Standard 72W at 100 pg/$\mu$L. The final volumes are then adjusted to between 20 and 35 $\mu$L.

**Qualitative Standards:** A PCDD/PCDF window-defining solution, ECRC Standard mixture of 80W and 81W at approximately 20 pg/$\mu$L (Table 2), is used to determine the SIM windows for monitoring. Low levels of 2,3,7,8-substituted analytes may be added to this standard to check sensitivity. Identification of 2,3,7,8-substituted analytes is aided by the use of a positive control tissue sample (e.g. PC 6806 positive control Carp from Saginaw Bay, MI). To assure at least partial gas chromatographic resolution for 2,3,7,8-TCDD, a mixture (161W) of five close-eluting TCDD isomers (two elute as pairs) including 2,3,7,8-TCDD and 1,2,3,4-TCDD at about 50 pg/$\mu$L is analyzed.

**Accurate Mass Calibration Standard for the Mass Spectrometer:** Perfluorotetradecahydro-phenanthrene is used for determining resolving power (R.P.) for mass resolution and for providing consistent accurate mass calibration.

**Sample Sets with Accompanying Quality Control Samples:** All sample tracking forms and all labeling for physically tracking the samples are prepared in advance. Samples are submitted for analysis preferably in sets of 16 consisting of 10 samples, two replicates (triplicate sample), a procedural blank, a matrix blank, a matrix spike, and a positive control matrix sample. Whenever possible, archived matrix blank (e.g. ECRC 654C, formerly 355C-2, bluegill; ECRC 356C-1 pond sediment) and positive control samples (e.g. ECRC 6806 carp from Saginaw Bay, MI and ECRC 235C-1 sediment from Saginaw Bay) will be used for ongoing quality assurance.

**Sample Receipt for GC/HRMS Analysis:** Purified and concentrated sample extracts are ready for analysis after the final Basic Alumina Cleanup. Extracts are in dichloromethane and stored at ambient temperature in 15 mL centrifuge tubes with Teflon-lined caps, awaiting final sample preparation. Labels on the sample containers are checked and reconciled with the tracking sheets from the Sample Preparation group.

**Sample Preparation for GC/HRMS Analysis:** The dichloromethane alumina fraction of each sample is blown down to about 500 $\mu$L, 250 $\mu$L of toluene is added and the sample is vortexed. The remaining dichloromethane is evaporated under a gentle stream of nitrogen to a final volume of 50 to 100 $\mu$L, with vortexing to rinse the tube.

The small volume of each extract is then transferred to a 0.9 mL Chromacol semi-conical vial using a glass transfer pipet. **It is important to rinse the walls of the tube well with several rinses and by capping the tube (as a precaution) and vortexing it.** Two additional dichloromethane or hexane rinses of about 75 to 100 $\mu$L each are added to the insert and then a final rinse of nonane (as a keeper).

The transferred eluate in the Chromacol vial is carefully evaporated under a gentle stream of nitrogen (purified by a carbon filter) to about 25 $\mu$L. If the concentrated eluate is to be stored for weeks prior to GC/HRMS analysis, then 25 $\mu$L of toluene is added to each vial before storage. Just prior to analysis, the final extracts at about 15 to 25 $\mu$L are spiked with the internal standard solution (10 $\mu$L of ECRC Standard 72W, $^{13}$C-1,2,3,4-TCDD at 100 pg/$\mu$L) and vortexed. **It is very important to insure that the internal standard solution mixes well with the extract. Failure to do so will result in either apparently low or high recoveries.**

## Gas Chromatography/High Resolution Mass Spectrometry

**Instrumentation and Chromatographic Conditions:** GC/HRMS analysis is performed using a HP 5890A capillary gas chromatograph interfaced to a VG 70-250S high resolution mass spectrometer. An HP 7673 autosampler is used to introduce 2 $\mu$L of the enriched extract from a conical vial onto a 5 m x 320 $\mu$m deactivated fused silica retention gap via a heated direct injection technique using a Restek Uniliner Insert. The analytes of interest are separated on a 50 m x 200 $\mu$m x 0.11 $\mu$m Ultra-2 (DB-5 equivalent) capillary column with an initial hold of 1 min at 120°C followed by a ramp to 200°C at 20°C/min, another ramp to 310°C at 2.3°C/min, and a final hold of 7 mins. The carrier gas is He maintained at about 44 psig with an initial linear velocity 27 cm/s. The analytical column is connected directly to the MS. The retention gap is connected to the column using a fused silica press-tight connector. The instrumental conditions are summarized in Table 3, and general operation and maintenance are contained in the system's operation manuals.

**GC/HRMS Detection Procedure:** The VG GC/HRMS system is tuned to 10,000 R.P. using perfluorotetradecahydrophenanthrene, and retention time windows for five ion groups are established for the $Cl_{4-8}$ PCDFs/PCDDs using the window-defining standard mixture (80W/81W-2). These ion groups are monitored sequentially during the temperature program, with data acquisition times (min.) typically: 14:00-19:50, 19:55-25:15, 25:20-31:15, 31:20-37:25, and 37:30-50:00, respectively. Within each group, two most abundant ions are measured for positive identification and quantitation of each analyte. The ion responses are quantitated and averaged, unless interferences occur. Other ions are monitored for interferences and for PCB #169 carryover from the HPLC-PGC Carbon column.

All ions from the five ion groups monitored using the SYStem **DFDD798** are listed in Table 4. For each ion group, lock-mass and lock-mass-check ions are used to maintain and verify the accuracy of mass measurement. Also in each group, two ions monitor for each homolog of native PCDFs, PCDDs, PCDTs, PCPhs (phenanthrene/anthracenes), and $^{13}C$-labelled PCDFs and PCDDs, except for $^{13}C$-OCDF which is not present. Other ions monitor interferences, e.g. $Cl_{5-10}$-diphenyl ethers (DPEs) whose fragment ions are exactly the same mass as $Cl_{4-8}$-PCDFs, $Cl_{6-7}$-PCNs whose ions are 0.11 Da less than $^{13}C$-$Cl_{4-5}$-PCDDs, $Cl_{5-7}$-Terphenyls (PCTs) whose ions are 0.056 Da higher than $^{13}C$-$Cl_{6-8}$-PCDDs, and $Cl_5$-biphenylene whose ion is 0.026 Da less than TCDD. Except for PCDPE interferences, other potential interferences listed above are typically avoided by GC/HRMS at 10,000 R.P. (overlap of $<$ 10% peak valley) unless the interferences are much higher (e.g. hundreds of times) and at levels that may also distort GC chromatography and affect the lock mass ion intensity.

**Chromatographic and Mass Spectral Resolution:** Chromatographic columns (50 m x 200 $\mu$m x 0.11 $\mu$m film HP Ultra-2 (or equivalent 60 m DB-5) are selected and temperature programmed on the basis that they must resolve 2,3,7,8-TCDD from 1,2,3,7/1,2,3,8-TCDD (and from 1,2,3,4-TCDD) by a resolution factor of at least 0.5. Column performance is verified by analyzing the qualitative standard 161W described above, standards of individual components, and observing the chromatographic resolution of the TCDDs, HxCDDs, and HxCDFs. Relative retention times for all other targeted congeners are evaluated with respect to labeled analogs. It should be noted that isomer-specific confirmation of all analytes cannot be attained on the Ultra-2 or DB-5 column. The co-elution of one or more TCDF isomers with native 2,3,7,8-TCDF is the greatest concern followed by one or more PeCDFs with 2,3,4,7,8-PeCDF, and a lesser concern is the potential co-elution of 1,2,3,6,8,9-HxCDD with 2,3,4,6,7,8-HxCDD.

Mass resolution checks are made using some of the interferences listed above and also of $^{13}C$-labeled congeners. In Group 1, the monitored $M^{+\cdot}$ ion of TCDD (m/z 319.8965) is only 0.04 Da less than the $(M+4)^+$ ion of $^{13}C$-TCDF and 8000 R.P. is needed for complete resolution. In Group 2, monitored ions for PeCDD (m/z 353.8576) and $^{13}C$-PeCDF (m/z 353.8970) also differ by only 0.04 Da and 9000 R.P. is needed for complete resolution.

## SPECIFIC VG GC/HRMS STEPS

**Setup of the VG/Fisons GC/HRMS:** Ensure that the data system's hard-disk space is sufficient for acquiring the upcoming sample set by using **HOU**sekeeping in VG> mode or **FRE** in RSX mode. Tuning the GC/HRMS for high resolution should begin after preliminary instrument checks (vacuum and temperature readings) have been made. While the GC oven and transfer lines are cool ($<$ 75 °C) and GC column head pressures are low ($<$ 10 psi), residual levels of gas ions (He, $H_20$, CO, $N_2$, $O_2$, and Ar) should be checked for leaks and relative sensitivity at 230 V on the photomultiplier.

**Obtaining Ion beam/Leak-checking:** Prepare to obtain an ion beam by opening the isolation valve and calling up the software program **ICP.** Type **V** for Voltage and initially input **5000** before pushing the OPERATE console button to activate the filament and accelerating voltages. While in Voltage mode, increase voltages incrementally by 1000 to **8000** by typing the values in. If some arcing occurs and continues, reduce voltage until it stops or quickly hit STANDBY console button. Switch to Magnet mode by typing **M** and enter the mass m/z **28** for both CO and $N_2$. You may need to widen the SPAN on the console panel to about $10^4$ , increase the oscilloscope sensitivity to 5 mV/division, and slightly adjust the trackball (side-to-side) to locate the ions. You can quickly assess the initial mass resolution by observing m/z 28, because ions for these two gases will be resolved above 3000 (R.P.). If the ion responses are similar to those recorded in the VG log book previously, type **40** for argon and begin leak-checking the column connections (especially the glass press-tight ones). Allow enough time (elution time of argon, e.g. 4-7 min) to ensure not missing leaks near the injector. After fixing any leaks, the GC oven should be ramped at least once through its temperature range to elute any residual bleed on the column and then leak-checked again to help ensure that the leaks don't reoccur during operation. Set the INLET TEMPERATURE CONTROLLER Channel 7 (new transfer line heater) to 305 °C and D.reen to 310 °C. Also check calibrant inlet heaters for 210 °C on S.Reentr and 180 °C for S.Reservoir. Allow at least 45 minutes to fully heat up before starting the autosampler (see below).

**Source Tuning for High Resolution:** Tuning, especially for high resolution, requires optimum settings on the various slits and lenses to narrow the ion beam peak width as necessary as possible with as little loss of peak height as possible. Different combinations of tuning parameters may still give high resolution, but usually at a significant loss in sensitivity. It is assumed that an ion beam has been obtained for the leak-checks above. Open the collector slit. The ion beam should become more square top and may become higher. If the residual gas ions of CO and $N_2$ seem low during leak checking and/or if the resolution seems to be higher than desired (e.g. > 10,000 R.P.), open the source slit a little (e.g. half a turn) by turning the brass control on top of the source housing counterclockwise. The ion beam should both broaden and increase in height. Next adjust the ion source controls, COARSE and FINE FOCUS, DEFLECT. If the DEFLECT controls are not in a middle range (10 to 2 o'clock), double-check the variable slit opening (micrometer control just below the reservoir) for ion response. Also, check that the ion repeller is low and usually slightly negative (e.g. -2 V). An incorrect ion repeller setting may later significantly decrease the responses of higher mass ions at lower accelerating voltages (e.g 6000 V) during selected ion monitoring. Reduce electron energy below 40 eV and find an optimum setting between 30 and 40 eV. Using the panel meter, verify that the filament trap current is being maintained at set levels and note that the filament current required is < 4.8 A.

**ESA Tuning for High Resolution:** Further tuning using the Electrostatic Sector Analyzer (ESA) controls on the console is important near 5000 R.P. A 3300 R.P. resolution will be achieved if the ion beam (e.g. m/z 293 of perfluorotetradecahydrophenanthrene) peak width fits within one of the 10 screen divisions at full-scale and SPAN marked with the dot between $10^3$ and $10^4$. If the beam fits within two of the screen divisions at full-scale and SPAN marked $10^3$ a 5000 R.P. will be achieved, if within one division a 10,000 R.P. If not >3300 R.P., close the source slit (brass knob clockwise) slightly and then close the collector slit slightly to triangulate the beam. It is important that the ION ENERGY control, which adds slightly to the basic accelerating voltage, is set and maintained at its mid-point within its range of controlling the ion beam. For example, a mid-point of the range from 3.1-8.7 would be 5.9. Y-FOCUS is another important control that dramatically affects high resolution. There are 10 other ESA lenses that can be tuned in order of their distance from the ion source, although no absolute tuning order works all the time. The Y-DEFLECT lens is nearest the source and

# APPENDIX E-12

# ANALYSIS OF SELECTED NON-O-CHLORO-SUBSTITUTED POLYCHLORINATED BIPHENYLS BY GAS CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY [USGS-TILLITT]

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# Analysis of Selected Non-*o*-Chloro-Substituted Polychlorinated Biphenyls by Gas Chromatography-High Resolution Mass Spectrometry

## Introduction

This standard operating procedure describes the instrumental analysis of selected non-*ortho*-chloro-substituted polychlorinated biphenyls (non-*o*-PCBs) using GC/high resolution MS (GC/HRMS). It outlines the general method of GC/HRMS analysis, and specifically the operation of the VG 70-250S magnetic sector mass spectrometer and data system.

## Materials

**Solvents:** The following pesticide grade distilled-in-glass solvents may be used: nonane, hexane, isooctane, dichloromethane, toluene, and isopropanol.

**Chromacol semi-conical vials:** (0.9 mL) are stored in a sealed container until being used once.

## Spiking and Standard Solutions

**Quantitative Standards:** The compositions of the $^{13}$C-surrogate standard (procedural internal standard), native-only spiking standard, and calibration standard solutions for non-*o*-PCBs are shown in Table 1. Also listed in Table 1 is a PCB interfering-congeners standard for QC that has PCBs 87, 110, and 129 at 250 pg/$\mu$L (25 ng total in the vial) and $^{13}$C-PCBs at the same concentration as in the calibration standards. A better alternative to spiking samples with the native-only non-*o*-PCB spiking standard (Table 1) is to use a 1:1:1:1 mixture of Aroclors (1242:1248:1254:1260), ECRC 88W, which contains both AHH-active and potentially interfering PCB congeners in concentrations relatively similar to environmental samples.

Before extraction, sample/sodium sulfate homogenates are spiked with 5 ng of the $^{13}$C-labeled non-*o*-PCB Surrogate Standard (congeners 77, 126, and 169) using 50 $\mu$L of ECRC Standard 191W-4 at 100 pg/$\mu$L nonane. In the final extract just before GC/HRMS analysis, each non-*o*-PCB fraction is spiked with 5 ng of the $^{13}$C-labeled non-*o*-PCB Internal Standard (congener 101) using 50 $\mu$L of ECRC Standard 90W-2 at 100 pg/$\mu$L nonane. The final volumes are then adjusted to between 50 and 100 $\mu$L.

**Qualitative Standards:** To verify adequate MS resolution for the $^{13}$C-labeled PCBs ($\geq$ 4,000 R.P. with $<$ 10% overlap), a Halowax 1014 standard of polychlorinated naphthalenes (PCNs) can be analyzed.

**Accurate Mass Calibration Standard for the Mass Spectrometer:** Perfluorodecalin is used for determining high mass resolution (e.g. 10,000 R.P.) and for providing consistent accurate mass calibration.

**Sample Sets with Accompanying Quality Control Samples:** All sample tracking forms and all labeling for physically tracking the samples are prepared in advance. Samples are submitted for non-*o*-PCB analysis preferably in sets of 16 consisting of ten samples, two replicate samples, a procedural blank, a matrix blank, a matrix spike, and a positive control matrix sample. Whenever possible, archived matrix blank (ECRC 355C-1 bluegill) and positive control samples (ECRC 6806 carp from Saginaw Bay, MI and ECRC 235C-1 sediment from Saginaw Bay) will be used for ongoing quality assurance.

**Sample Receipt for GC/HRMS Analysis:** Purified and concentrated sample extracts are received from Sample Preparation after the final HPLC-carbon cleanup and concentration. Extracts are typically in a mixture of toluene/dichloromethane/hexane and are stored at ambient temperature in 15 mL centrifuge tubes with Teflon-lined caps, awaiting final sample preparation. Labels for vials are prepared and checked with those on the sample containers and reconciled with the tracking sheets from the Sample Preparation Section. Samples are then stored in the GC/MS preparation lab. In general, care must be taken to avoid long-term exposure to laboratory air and dust, which contain PCBs from former use in fluorescent light capacitors and ballasts.

**Final Sample Extract Preparation for GC/HRMS Analysis:** HPLC-carbon eluates of toluene/dichloromethane-hexane are evaporated under a gentle stream of nitrogen (purified by a carbon filter) to a final volume of about 200 $\mu$L. This volume is then transferred to a 0.9 mL Chromacol semi-conical vial using a glass transfer pipet. **It is important to rinse the walls of the tube well by several small rinses and vortexing it.** A total of three rinses (150-200 $\mu$L each) in the tube are added to the Chromacol vial. For one rinse, combine 100 $\mu$L of nonane. Use 200 $\mu$L hexane each for two additional rinses.

The transferred eluates in the Chromacol vial are carefully evaporated under a gentle stream of nitrogen (purified by a carbon filter) to about 35 $\mu$L. If the concentrated eluates are to be stored prior to GC/HRMS analysis, then 25 $\mu$L of toluene is added to each vial before storage. Just prior to analysis, the final extracts at about 50 $\mu$L are spiked with the internal standard solution (50 $\mu$L of ECRC Standard 90W-2, $^{13}$C-2,2',4,5,5'-PeCB (#101) at 100 pg/$\mu$L), and vortexed. **It is very important to insure that the internal standard solution mixes well with the extract. Failure to do so will result in either apparently low or high recoveries.** To avoid a potential cross-contamination of sample vials, the internal standard can be added first to the vials before transfering the eluates from the tubes. Care must then be taken not to evaporate the vial contents to dryness and risk also losing the more volatile internal standard.

## Gas Chromatography/High Resolution Mass Spectrometry

**Instrumentation and Chromatographic Conditions:** GC/HRMS analysis is performed using a HP 5890A capillary gas chromatograph interfaced to a VG 70-250S high resolution mass spectrometer. An HP 7673 autosampler is used to introduce 2 $\mu$L of the enriched extract from a conical vial onto a 2.5 m x 530 $\mu$m deactivated fused silica retention gap via a cool on-column injection technique. The retention gap-column connection is made using a fused silica press-tight connector. The analytes of interest are

separated on a 50 m x 200 $\mu$m x 0.11 $\mu$m Ultra-1 (Hewlett Packard, or DB-1 equivalent) capillary column with an initial hold of 1 min at 120°C followed by a ramp to 240°C at 2.2°C/min, another ramp to 305°C at 5°C/min, and a final hold of 9 mins. The carrier gas is He maintained at 45 psig with an initial linear velocity 27 cm/s. The analytical column end is positioned into the MS source through a transfer line heated at 310°C. The instrumental conditions are summarized in Table 2, and general operation and maintenance are contained in the system's operation manuals.

**GC/HRMS Detection Procedure:** The VG GC/HRMS system is tuned to 10,000 R.P. using perfluorodecalin, and two ion groups are established for non-*o*-PCBs: 1). $Cl_3$-PCBs (#37, if targeted), $Cl_4$-PCBs (77 and 81), and $Cl_5$-PCBs (126); 2). $Cl_6$-PCBs (169). These ion groups are monitored sequentially during the temperature program. Within each group, two most abundant ions are measured for positive identification and quantitation of each analyte. The ion responses are quantitated and averaged, unless interferences occur. Other ions are monitored for interferences and for breakthrough in the cleanup step.

All ions from both ion groups monitored using the **SYS**tem **NONPCB10K** are listed in Table 3. In Group 1, two ions each are monitored for PCB 37, 77 and 81, and 126, $^{13}$C-PCBs 77 and 126, $^{13}$C-PCB 101 (IS), and $Cl_{4-6}$-PCNs. Some PCN ions differ from those of $^{13}$C-PCBs by only a 0.1 Da, thereby assuring a continual check that mass resolution is at least 4,000 R.P. (with $<10\%$ overlap assuming similar concentrations) to avoid potential PCN interference. Other monitored ions are for residual $Cl_{5-7}$ PCBs that can interfere with PCBs 77, 81 and 126, $Cl_4$-PCDFs for possible breakthrough on HPLC-carbon, and $Cl_6$-diphenyl ethers (DPEs). In Group 2, two ions each are monitored for PCB 169, and $^{13}$C-PCB 169. Other monitored ions are for $Cl_{7-9}$-PCBs, $Cl_{7-8}$ PCNs, $Cl_{3-5}$ PCTs, and $Br_5$-diphenyl ethers.

**Setup of the VG/Fisons GC/HRMS:** The data system's hard-disk space should be checked to be sufficient for acquiring the upcoming sample set. Use either **HOU**sekeeping in VG> mode or **FRE** in RSX mode. Tuning the GC/HRMS for high resolution should begin after preliminary instrument checks (vacuum and temperature readings) have been made. While the GC oven and transfer lines are cool (< 75 °C) and GC column head pressures are low (< 1 psi), residual levels of gas ions (He, $H_20$, CO, $N_2$, $O_2$, and Ar) should be checked for leaks and relative sensitivity at 230 V on the photomultiplier.

**Obtaining Ion beam/Leak-checking:** Prepare to obtain an ion beam by opening the isolation valve and calling up the software program **ICP.** Type **V** for Voltage and initially input **5000** before pushing the OPERATE console button to activate the filament and accelerating voltages. While in Voltage mode, increase voltages incrementally by 1000 to **8000** by typing the values in. If some arcing occurs and continues, reduce voltage until it stops or quickly hit STANDBY console button. Switch to Magnet mode by typing **M** and enter the mass m/z **28** for both CO and $N_2$. You may need to widen the SPAN on the console panel to about $10^4$ , increase the oscilloscope sensitivity to 5 mV/division, and slightly adjust the trackball (side-to-side) to locate the ions. You can quickly assess the initial mass resolution by observing m/z 28, because ions for these two gases will be resolved above 3000 (R.P.). If the ion responses are similar to those recorded in the VG log book previously, type **40** for argon and begin leak-checking the column connections (especially the glass press-tight one). Allow enough time (elution time of argon, e.g. 5-7 min at head pressure < 10 psig) to ensure not missing leaks near the injector. After fixing any leaks, the GC oven should be ramped at least once through its temperature range to elute any residual bleed on the column and then leak-checked again to help ensure that the

leaks don't reoccur during operation. Set the INLET TEMPERATURE CONTROLLER **Channel 7** (newer transfer line heater) to 305 °C and **D.reen** to 310 °C. Allow at least 30 minutes to fully heat up before starting the autosampler (see below).

**Introducing Calibrant into MS Instrument:** Purge out any residue from the heated box reservoir near the ion source (close left valve while right valve is opened). Tune the GC/HRMS using the calibrant, perfluorodecalin, which is liquid and is injected (< 1 $\mu$L) into the reservoir with a designated syringe. In **ICP**, type **293** and locate the ion which should approach the maximum 10V response at a photomultiplier setting above 320V. If so, pump away some of the calibrant by quickly opening and closing the right valve on the reservoir.

**Source Tuning for High Resolution:** Tuning, especially for high resolution, requires optimum settings on the various slits and lenses to narrow the ion beam peak width as necessary as possible with as little loss of peak height as possible. Different combinations of tuning parameters may still give high resolution, but usually at a significant loss in sensitivity. It is assumed that an ion beam has been obtained for the leak-checks above. Open the collector slit by turning the servo-motor control slightly to the right. The ion beam should become more square top and may become higher. If the residual gas ions of CO and $N_2$ seemed low during leak checking and/or if the resolution seems to be higher than desired (e.g. > 10,000), open the source slit a little (e.g. half a turn) by turning the brass control on top of the source housing counterclockwise. The ion beam should both broaden and increase in height. Next adjust the ion source controls, COARSE and FINE FOCUS, DEFLECT. If the DEFLECT controls are not in a middle range (10 to 2 o'clock), double-check the variable slit opening (micrometer control just below the reservoir) for ion response. Check that the ion repeller is low and usually slightly negative (e.g. -2.5V). An incorrect ion repeller setting may later significantly decrease the responses of higher mass ions at lower accelerating voltages (e.g. 6000V) during selected ion monitoring. To optimize the ion repeller setting, type **V** then **6000**, and adjust the ion repeller for the maximum response of the ion, e.g. m/z 293 of perfluorodecalin. Type **8000** to return to 8000V then **M** to return to mass mode in **ICP**. Reduce electron energy below 40 eV and find an optimum setting between 30 and 40 eV. Using the panel meter, verify that the filament trap current is being maintained at set levels and note that the filament current required is < 4.8A.

**ESA Tuning for High Resolution:** Further tuning using the Electrostatic Sector Analyzer (ESA) controls on the console is important near 5000 R.P. A 3300 R.P. resolution will be achieved if the ion beam (e.g. m/z 293 of perfluorodecalin) peak width fits within one of the 10 screen divisions at full-scale and SPAN marked with the dot between $10^3$ and $10^4$. If the beam fits within two of the screen divisions at full-scale and SPAN marked $10^3$ a 5000 R.P. will be achieved, if within one division a 10,000 R.P. If not >3300 R.P., close the source slit (brass knob clockwise) slightly and then close the collector slit slightly to triangulate the beam. It is important that the ION ENERGY control, which adds slightly to the basic accelerating voltage, is set and maintained at its mid-point within its range of controlling the ion beam. For example, a mid-point of the range from 3.1-8.7 would be 5.9. Y-FOCUS is another important control that dramatically affects high resolution. There are 10 other ESA lenses that can be tuned in order of their distance from the ion source, although no absolute tuning order works all the time. The Y-DEFLECT lens is nearest the source and should be adjusted earlier than other ESA lenses. In increasing order of distance from the source are ROTATE 1, Z-DEFLECT, Y-FOCUS, Z-FOCUS 1, Z-DEFLECT 1, CURVE 1, CURVE 2, ROTATE 2, Z-FOCUS 2, and Z-DEFLECT 2.

After an initial pass through the ESA controls, the beam may have become more square-topped so the collector slit control can be turned counterclockwise very slightly to trangulate and narrow the beam without losing more than 25% of its height. If the result is close to 10,000 R.P. (one screen division at full-scale and 10% valley at SPAN $10^3$), one or two more stages of fine tuning of the ESA controls may suffice. If not, restrict the source slit (brass knob) some more to further narrow but also shorten the beam and follow with slight adjustment of the collector slit servo. Typically at 10,000 R.P. with the VG 70-250S GC/HRMS, the ion beam transmission is in the range of 4-8% that of an ion beam at about 1000 R.P.

If an analyst is unable to achieve near 10,000 R.P., then corrective action is required. Ion source cleaning might be needed if there is a relatively high emission value. A 5-day bake-out of the flight tube might be needed followed by the next day cool down and subsequent reposition of the magnet. The source slit might still be dirty, requiring a clean substitute.

**Acquiring GC/HRMS Selected Ion Monitoring Data:**

**Signal Zero Check:** Before acquisition begins, check the signal zero of the GC/HRMS. With the GC/HRMS on OPERATE mode, briefly switch off the High Voltage Power Supply (below the vacuum gauges). Set the photomultiplier to the expected acquisition value (e.g. **350**). From the VG> prompt, enter **{CTRL}** \ (simultaneously) then @VT to switch to the RSX operating system. Next, enter **RUN $MIKRO** [CR], enter **12** for the data system interface, @ and then **X** at least 10 times to read the 16-bit A/D output in octal. Octal values should include at least a few 000001 or 000002 values. If values are above 3 or consistently 0, enter **O** (letter O) and a 4 digit value, currently about 4500. Check the output again using **X**, and enter a different **O value** until the right output is given. Type **I** to finish, then **{CTRL} C** to exit, then **@ LOGIN** to return to the VG> prompt. Remember to switch on the High Voltage Power Supply.

**SYS (SIR) System Setup of Mass Spectrometric Acquisition:** Set up the GC/HRMS selected ion monitoring data acquisition by entering **SYS (SIR)/U**, choose the appropriate system definition (e.g. NONPCB10K) from the list by typing **'U**, to update the acquisition. On the menu's left half, type in the new acquisition file name by **DAT**, **RES**olution if different than 10,000, and the total number of injections expected for (plus several more as spares by **SMP**). If the GC column or conditions have changed since previous use, an initial test analysis of a calibration standard or Aroclor mixture must be done to update the **GTM** (beginning and end times) of each ion group. Quickly glance at the list of ions in each group to ensure that there is a designated lock mass ion (colored blue) and it is correctly designated. Also for each ion group, a lock-mass-check ion is used to verify the accuracy and consistency of mass measurement. Type **>** at almost any line to switch to the other ion group. To delete an ion from **SYS (SIR)**, make the ion's mass very high (e.g. **995**), type **'I** to reorder the ions, move the cursor to that ion, then type **0** (zero) to remove it (the last ion in the group). To change to the correct lock mass ion, if reordering the ions moved its designation, type **-***, then move to the correct ion and type ***. Below in the menu is a **TXT** (text) line for the description of each sample or standard injection with up to 64 characters. Typing **>** or **<** at the **TXT** line increments to higher or lower sample injection numbers. The **TXT** line used for **CHR**omatograms of the GC/HRMS data can be changed later in **CHR** to allow reordering of sample/standards if necessary during the acquisition of the entire sample set. Print out a hardcopy of the **SYS**tem pages for both ion groups for future reference using **'H, 'H,2,2** or **'H,3,2**.

**SYS (GCC) System Setup of Gas Chromatographic Conditions:** Typing **GCC** within any SYS (SIR) menu line will transfer you to setup of GC conditions. Type **'U** to choose **PCBONCOL.** On the right half of page 1 of the **GCC** system menu, check that **AQS** (MS acquisition system) is the same as from **SYS (SIR)** and input the new acquisition file name by **DAT**. Print out a hardcopy of the **SYS**tem pages for future reference using **'H, 'H,2,2** or **'H,3,2**. Type **'G** to proceed with the MS's automated calibration procedure just prior to injection of the first vial. Check that the FILTER on the console is set at 0.01 ms.

**SYS High Resolution Calibration Process:** After acquiring one or more slow calibration scans, the data system displays an SIR calibration if it is within tolerance specified in **SYS (SIR)** ($\pm$ 25 ppm or $\pm$ 0.0075 Da for m/z 300). If no peaks are found, the analyst may have forgotten to switch the high voltage supply back on. Another scan will proceed if more than one is specified in **SYS (SIR)**. Print a hardcopy if this calibration was successful by using **'H, 'H,2,2** or **'H,3,2,** because it is not stored anywhere as a QC check. Then type **'G,** to exam the lowest and highest mass calibrant ions for Group 1. Again print a hardcopy if the two peaks (first and last calibration masses) are centered and their peak widths are within tolerance (two block widths). If not, hit the **{F11}** key to prompt the data system to recenter them. Wait another few seconds to be sure the ions do not significantly drift, then print a hardcopy when it is satisfactory. Note also the relative response of the two ions, especially the higher one. If the higher ion is significantly low, adjust the ion repeller slightly to attempt to increase the ion's response. If everything is satisfactory, type **'G** to do the same process for Group 2. Similarly, print hardcopies of Group 2 calibration and peak examination graphs when they are satisfactory. A final **'G** will prompt the data system to exit the calibration process and do final checks. If the GC conditions are at READY state, the data system will prompt, "READY FOR GC INJECTION." Make sure the photomultiplier is set to the appropriate operating voltage (350-380V).

**Final Preparation of GC and GC-Autosampler:** Because the HP GC has two different types of injectors, it is necessary to check several items before starting the autosampler. Ensure that the autosampler tower is positioned over the correct injection port (cool-on-column). Check that the autosampler control cable is plugged into the correct injector (front) jack in the back of the autosampler master control box. Check the position of the toggle switch inside the top of the autosampler tower for the appropriate injector (cool-on-column). Open the clear plastic door, remove the 10 $\mu$L syringe, clean it, and reinstall it in the slot and tighten the screw and support. If any burrs on the needle snag a Kemwipe, inspect the needle with a magnifying glass and smooth it or replace the syringe with a new one. Ensure that the syringe is the correct type (tapered) for the cool-on-column injector used. Dump out any residual solvent from the first rinse vial and fill with a combination of isopropanol and nonane, recently removed from a 4-L bottle. Dump out any residual solvent from the second rinse vial and fill with nonane, recently removed from a 4-L bottle. Start the GC autosampler (which starts the process for GC/HRMS acquisition) either at the autosampler tower or at the START button on the GC.

**Monitoring Calibrant Level and Overall Operation During GC/HRMS Acqusition:** Several minutes after injection, note the approximate solvent elution time and whether the SOLVENT TRIP on the Source Vacuum Gauge properly responds to pressures higher than its setpoint ($\sim 4 \times 10^{-5}$ mbar) and temporarily shuts off the filament and high accelerating voltage. Just before the time designated in **SYS (SIR)** for data collection to begin, type **CHR** {file name} and then as soon as data collection begins, type the letter for the lock-mass-check ion (e.g. **P**) for Group 1. If the ion response of the lock-mass-check ion exceeds 30,000, carefully pump away some of the excess by quickly opening/closing the right (rear) black valve on the calibrant reservoir. Check other ion response levels for background,

stability, and baseline zero. Check analyte retention times of a calibration standard to ensure that GC chromatographic performance and ion group switching is appropriate. Periodically monitor the lock-mass ion and other ions on the oscilloscope during acquisition using a **TIME** setting of about 1. Periodically check the syringe filling and rinsing functions for any abnormal operation and replenish any low solvent rinse vials. In **CHR**, review the data for analyses just acquired. Check the ion response(s) for the $^{13}$C-PCB #101 IS for consistency and for comparable response to GC/HRMS analyses of previous sample sets. Ensure that samples with high analyte responses, near 65,000 data saturation, are diluted by a factor of at least 20 and reinjected in the sample set. Always check the calibrant (lock-mass-check ion) level before leaving work. On average, more calibrant is needed every 15-20 injections (18-24 hrs). It is best to add calibrant either at the beginning or near the end of a GC/HRMS analysis to avoid affecting the baselines of the other ions at any analyte retention times.

**Sample Dilution with Additional $^{13}$C-PIS:** Samples eluates are diluted whenever native PCBs exceed the ratio of the highest native standard to $^{13}$C-labelled standard. Dilutions should be at least a factor of 20. If the sample eluate solution in the vial is at a known volume (~100$\mu$L), transfer 5$\mu$L of it (5%) to a new, clean vial, add 50$\mu$L of $^{13}$C-surrogates, and add 45$\mu$L of nonane. If the original solution is not known, an alternative method is to add 19 times more of $^{13}$C-surrogates to the original vial (e.g. 95$\mu$L of 91W-1), if the native PCB ion responses are not near saturation.

**Analyst-assisted Computer Automated Data Processing using the VG/Fisons GC/HRMS:** Various software modules within the VG/Fisons GC/HRMS data system and in a PC are used for data processing and each will be detailed below. In summary, **PKD** detects and integrates peaks and **PKR** tabulates peak retention times and areas. **PKQ** identifies peaks of PCB congeners based on retention time windows in **.TRG** (target) files and quantitates using calibration standard curves computed in **.CAL** files and grams spiked/extracted entered in **.MTH** (method) files. **RPG** converts **.RES** (result) files of final concentrations into ASCII-readable format (**.RPT** files). Combinations of these modules can be linked together in comprehensive **RUN** files for unattended operations. On a PC as a second terminal to the GC/HRMS, the **.RPT** files are imported via **Kermit** mode within the program, **SMARTERM Office**. A basic program, **VGAPPEND**, joins each set of two ASCII lines into one for proper importing into **Lotus 1,2,3** as a summary quantitation report of one or more PCB congeners in the entire sample set. **Lotus** template files called **PARS-** have parse dimensions set and macros to import, parse, and perform calculations using the **.PRN** files made using **VGAPPEND**. Final excerpted tables are made using **Microsoft Excel** as part of an ECRC Final Laboratory Report.

**NPCBPLO - First RUN File: GC/HRMS Ion Chromatographic Print Out, Digital Filtering, Peak Detection and Initial Integration.** After GC/HRMS acquisition has finished (and a tape backup has been made), GC/HRMS ion chromatograms are printed out in groups and in order selected by the analyst. A direct interactive way is by typing the program **PLO CHR {file name}/U** and updating any parameters, e.g. number of ion chromatograms on a page and their order of printing. A more complete method is to use a **RUN** file, e.g. **NPCBPLO**, which prints out ion chromatograms from all of the samples, integrates digitally-filtered peaks with **PKD**, tallies peaks in a **.PKS** file using **PKR**, and prints showing the integrations using a **PLO QUA {file name}** command.

**Initial Testing With PKD:** Before starting the **RUN** file (**NPCBPLO**) to integrate peaks from the entire data set, **PKD** may be tested first using at least the lowest standard and/or a low-level sample from the data set. An ideal **PKD** will use an optimum **THR**eshold value to tag the smallest real peaks while ignoring baseline drift and will not impose many shoulders on all larger peaks. The **THR** value

will vary somewhat depending on the ions' baseline "zero" levels. **THR** values of about 0.10-0.25 have been used for ion baseline intensity levels near 1.0 and values of 0.25-0.40 have been worked well for baselines ranging from 1.5-3. In **PKD**, use -3 for digital filtering and typically, 5 for differential window, and 3 for baseline points.

**PKR** parameters are typically enclosed in a command line in a **RUN** file. They include sample, injection and SIM group numbers; ions specified in alphabetical TRACES; peak filter by size of area and width; and time range. Note that a sufficiently wide-enough time range needs to be specified around a peak, especially after a peak elutes, to ensure that the integration finds a next valley or baseline to complete a peak. If not, an ion peak, especially a large peak with some tailing, will not be included in a **.PKS** file using **PKR**.

After **PKD** and **PKR** (and the rest of the RUN file if set up) have been completed in the initial data processing, check the file size of **.PKD** files (in directory [70,20]) and of **.PKS** files (in directory [70,41]) to ensure that peak integrations can be moved with **DBS** or deleted with **DBD** in **CHR**. The **.PKD** file size should not exceed 2048 blocks (1024 kbytes). If the file just exceeds the maximum size, rerun **PKD** with either a slightly higher threshold or exclude some of the insignificant ions in the entire set. The **.PKS** file from **PKR** should not exceed about 750 blocks (384 kbytes), corresponding to a maximum number of about 2044 rows in the peaks table. If a **.PKS** file does exceed these limits, it can be used in quantitation, but cannot be edited using **DBE**.

**Inspection of GC/HRMS Ion Chromatographic Data:** The integrated GC/MS ion peaks, plotted using **PLO QUA {file name}** or as part of the **RUN** file, must be inspected. Usually, the only problem is to eliminate or move a peak shoulder on the right-hand side of a peak. Call up the **PKD**'d file {e.g. N36PCBS1} with **CHR** {file name} and display the integrations using **DBA**. Eliminate a peak shoulder by moving the trackball cursor "+" to the shoulder, typing **DBD**, hitting middle button of the trackball, and typing **[ENTER]**. Be careful, once you've eliminated a peak shoulder, you cannot undo your action other than rerunning **PKD** and **PKR**. Move or shift a split peak shoulder by moving the trackball cursor to the shoulder, typing **DBS**, hitting the middle button of the trackball, and typing **[ENTER]**. Although the **PKD** file is updated by these modifications to the peak integrations, the **PKR** file is not. Typically, **PKR** must be rerun to revise the peak areas in the **.PKS** file as part of a third **RUN** file with quantitations by **PKQ**.

The use of **DBE** (data base editor) is sometimes also required to edit the revised **.PKS** file before beginning **PKQ**. Occasionally, chromatographic peaks are sufficiently skewed that discrete peaks are tagged instead of shoulders. Type **DBE.,,PKS** (. indicates a space) to list the **.PKS** files and then **'E** to edit. Whenever an entry line is inserted or deleted in **DBE**, the counter in the far right column (column 24) must be increased or decreased accordingly. Move to that column in **DBE** by typing **'S{row},24.** After changing the counter entry, move back to the column 0 again using **'S{row},0.**

**DBEMTHCHR - 2nd RUN FILE -- Sample Text Headers Incorporated Into Method (.MTH) and Manual Input of Sample Mass (g) and Standard Amounts for Quantitation:**

**DBEMTHCHR** is a short-running **RUN** file to sequentially incorporate each sample textual description line from **CHR** into a database table called a method file (.MTH). Before running **DBEMTHCHR**, type **DBE ,,MTH** to list the **.MTH** templates and existing .MTH files. Move to or type **MTHWIDE7**

file, then type 'C {file name}, such as 'C **N36PCB** to create a full-width template for **N36PCB.MTH**. Exit **DBE** by either 'X or 'Q.

Type **RUN**, move to **DBEMTHCHR** in the listing, then type 'E to edit the names of both global variables **%19** and **%95**, **CHR TXT** line and **.MTH** name, respectively, with the name of the newly-created **.MTH** file. Quit editing by 'Q, then type 'G to begin running the highlighted **RUN** file, **DBEMTHCHR**.

After the **RUN** file has completed, edit the **.MTH** file just created by typing **DBE ,,MTH** and then 'E {file name}. Manually enter each sample mass (g) in Column 1. For **Sample Type** (Column 2), designate either **C** for a calibration standard or **U** for an unknown. For **Amount Injected** (a divisor, Column 4), typically enter 1 unless there is an unusual amount of $^{13}$C-surrogate standard added to a sample or standard. For **Concentration** (Column 5), enter the appropriate amount. For native analytes, enter total pg in a calibration standard of 100 $\mu$L; for $^{13}$C-analytes, enter **100** for 100%). Enter **Target Peak File Name** in Column 6 (See below for instructions about **.TRG** files).

**Building/Modifying a Target File (.TRG), A List of Analytes to be Quantitated Using PKQ:** A target file is a table which lists ions of analytes and of internal or surrogate standards. Type DBE ,,TRG to list the names of all existing target files. The file named TRG is the default empty template file; **DO NOT DELETE THIS FILE**. To build a .TRG file using the empty template file (**TRG**), type 'C {file name}, such as 'C **N36PCBCL5** to create a target file. Or preferably, move the cursor with the trackball to a target file from the previous set, e.g. **N35PCBCL5**, and type 'C {file name}, such as 'C **N36PCBCL5**. An internal standard ion is always listed on the first row of any target file and typically every subsequent odd row. In column 1, designate the internal standard as **RS** to make it also serve as a GC reference standard to adjust the corresponding analyte ion retention times. Ions for more than one **RS** can be used, as long as the corresponding analyte ions (just below) are matched up with them on alternate rows.

In any target file, target standard concentration (Column 3) should only have an entry in each row there is an ion for the **RS** or internal standard. Also in these rows of **RS** ions, it is very important to scroll right to **Multiple CAL Name** (Column 11) and enter an appropriate unique name for the next analyte ion which follows. For example, **N36126E** would designate **N36** sample set, analyte PCB **126**, and ion trace **E**.

Most of the columns in the target file must be filled in. Typically not used are **RRF%** (Column 4), **CYCLE/SCAN** (Column 8), **External Std File Name** (Column 12), **External Sam** (Column 13), and **External Inj** (Column 14).

*When the target file has been built or revised, exit using 'Q. From the list of .TRG files on the screen, type 'L to list the newly built or revised target file to the printer (set to Mode 5).*

*Type DBE ,,MTH and type 'L to list the appropriate method file. Ensure that the Target Peak File Name (Column 6) in the .MTH file has the correct .TRG file name. Whenever analytes in samples extend the entire calibration range (0.25 to 2500), two calibration curves (1 full curve, 1 lower-level* curve) must be made using two different **.TRG** file names with different ion names and two different **.MTH** files. For the lower-level calibration curve, the **.MTH** file should drop at least the highest standard or if necessary, the highest two standards.

**Quantitation of Analytes:** The concentration of each PCB congener detected is inherently self-corrected to account for losses throughout the entire method (extraction, isolation of analytes, and GC/HRMS analysis). For each native congener ion monitored, a software-computed calibration curve describes ion peak area responses from wide-ranging calibration standards to those of a $^{13}$C-labeled surrogate standard congener kept at the same amount in the calibration standards. Concentrations of the native PCB congeners in calibration standards typically range from 0.25 to 2,500 pg/$\mu$L. Each calibration curve is specifically matched to the range of analyte responses in the sample set.

The concentration of each analyte detected, **C** is self-corrected to account for losses through the whole analysis by the following equation:

$$\mathbf{C = A_a S / R_{ap} A_p M}$$ where $\mathbf{A_a}$ = Peak area of native PCB analyte
$\mathbf{A_p}$ = Peak area of $^{13}$C-PCB surrogate standard
$\mathbf{M}$ = Spiked-sample mass (g) with $^{13}$C-PCB surrogate
$\mathbf{R_{ap}}$ = Response factor depicted by a regression curve fit $A_a S / A_{ap}$ * (Varying amounts of analyte)
$\mathbf{S}$ = Amount of $^{13}$C-PCB surrogates spiked into sample and calibration standards

**Calculation of Percent Recovery of $^{13}$C-PCB Surrogates to Determine Method Efficiency:** To account for variations in GC/HRMS analysis, a known amount of internal standard ($^{13}$C-PCB #101) is spiked into each final extract and used to calculate the amounts of the $^{13}$C-PCB surrogates recovered in the final extract before any dilution is made. The efficiency of the extraction and cleanup procedures is measured by comparing the quantity of the surrogates detected in the *final* isolated extract (awaiting GC/HRMS analysis) with the quantity spiked into the sample at the beginning of the extraction step. Of course, the same amount of $^{13}$C-PCB #101 IS is also in each calibration standard. It should be noted that response factors between the instrumental internal standard and the various other labeled congeners may be affected by activity in the vials, GC retention gap, GC column, or GC/MS transfer line.

**Quantitative Ion Calibration Curves:** These curves provide response factors $R_{ap}$ for each native PCB analyte ion and $R_{pi}$ for each $^{13}$C-PCB PIS ion. The intercepts of these response factors are checked to insure they make a negligible contribution to the calculated amount of analyte. These response factors are determined experimentally for the system for each block of sample sets. Ion response data are subjected to regression analysis. The best curve fit is chosen within the calibration range needed to match the amount of each native PCB in the samples. The ratio of the amount of native PCB to the amount of the $^{13}$C-PCB surrogate (independent variable) is compared to the ratio of the peak area of native to the peak area of $^{13}$C-PCB surrogate. Because of the wide calibration range needed for native PCBs in various samples, **quadratic** curve fits for $R_{ap}$ are usually most appropriate and usually work best with a weighting factor of 1 and a maximum weighting factor of 999. For the calibration curves for the $^{13}$C-PCB surrogate, however, **linear** curve fits for $R_{pi}$ are necessary because of the constant amount of $^{13}$C-PCB surrogate in each sample and standard. The system is calibrated within each set of analyses using all available and appropriate calibration solutions (at least three) chosen to span the anticipated levels in the samples to be analyzed. Calibration standards are interposed after each four sample analyses.

**N**PCBPKQ - 3rd RUN FILE -- Quantitation of Non-*o*-PCB Analytes and ASCII Report:**
(* sample set number)

Initially within the **RUN** file, **PKR** must often be rerun to rewrite correct peak areas in the **.PKS** file following the removal of incorrect peak shoulders from initial integration using **PKD** (p.8, above). If **PKR** has already been rerun and possibly **DBE** has been used to edit the **.PKS** file, **REM** (remark) out the two initial lines of **N**PCBPKQ RUN** file using **.;** and do not rerun PKR.

The basic quantitation program, **PKQ**, can be accessed using the update mode, **PKQ /U** and is typically used several times to quantitate separate PCB analytes in a **RUN** file, e.g. **N36PCBCL5**. It is usually easiest to modify or adapt an existing **RUN** file using **PKQ** rather than completely rewriting another. Type **'O {file name}** to create another **RUN** file from one already existing..

There are two possibilities for editing, line-by-line editing in **RUN** and screen editing of the converted ASCII text version of the **RUN** file using the **RSX** editor **EDT**. To line-by-line edit in **RUN**, **'E** to edit that file, and **/C/abc/xyz** to change **abc** in the line to **xyz**.

To screen edit using **RSX EDT** first go into **RUN**, type **'CT** {temp file name} to convert the existing **PKQ** file to a text file. Exit **RUN** by typing **'X** and go into **RSX** by simultaneously hitting **{CTRL}** \. Type **EDT [70,40]**{temp file name**.TXT;1**} and start editing. The most useful feature of **EDT** for a long file is to search and replace a previous sample set number with the current number. To search and replace in **EDT** requires the following sequences:

Use **keypad .** to Select and move cursor to **Block** desired text.
(For a new sample set number, you may need to first type this number at the beginning.)
Cut blocked text using **keypad 6**
Hit **keypad PF1** then **keypad PF3** to **Search for** what to replace
Hit **keypad PF3** to **Find** the next time the text to replace appears in the file
Hit **keypad PF1** and **keypad 9** to **Replace**
Hit **keypad PF3** to **Find** the next time the text to replace appears in the file

When finished editing, type **{CTRL Z}** and then **EXIT [70,40]**{real file name**.TXT;1**} to give it a new meaningful name, e.g. **N37PCBCL5.TXT;1**. Type **@LOGIN** to return to **VG>**, type **RUN**, type **T** to access **.TXT** files, type **'CR** to convert to a **RUN** file which should be ready to run.

Typically, **PKQ** is requested to adjust retention times, to graph the first ion **CAL**ibration specified, not to force the curve through zero, and to use a quadratic curve fit with a weighting factor of 1. One exception is that for $^{13}$C-surrogate standards at the same amount in each calibration standard, a linear curve fit must be used. After **PKQ** is updated in the **RUN** file, it is called up by:

**PKQ {.PKS** file name**},{.RESults** file name desired**},{.MTH** file name**}/R**

where **/R** requires that **PKQ** search each entry in the **.PKS** file from the beginning to ensure it will be found even if entered in the **.PKS** file out of sequence. **PKQ** already presumes the types of files so do **not include file name extensions**. After **PKQ** completes calibrating all ions specified in the **.TRG** file, the **RUN** file selects and prints out each ion calibration graph, designated by **'C** {ion name in **.TRG** file}. After **PKQ** finishes, a table of each ion's response factors is printed out by **RPG CALRPT,{.CAL file name}(file name.CAL)**. Within the parenthesis example given, the extension **.CAL must be used with RPG**.

For non-*o*-PCBs, it is usually best to quantitate $Cl_4$-PCBs (77 and 81) together using **PKQ**, then PCB 126 and 169, each separately using **PKQ**. PCB 77 usually needs two separate calibration curves; the other PCBs only occasionally need separate curves.

ASCII-Reports of the quantitation results and also of calibrations are done using **RPG** (Report Generator). **RPG** command files can be created and edited within the VG software using **RPG** {file name}/**E**. When finished, type **{CTRL}Z** then **EXIT**. It will automatically save the latest revision along with the two previous versions if present and will exit to the VG prompt **VG>**. Arithmetic calculations can be performed using **RPG**, therefore analyte final concentrations can be determined to specified significant figures. Do not flippantly change the layout format of the various columns of the report! Any change in width of any column will likely disrupt the parsing of the report columns into **Lotus 1,2,3**. For **RPG** to save the ASCII report to a **.RPT** file requires a /- be added at the end of the **RPG** command line, otherwise the report will be printed out on the printer.

When reviewing new or modified **RUN** files, it is important to ensure that a wait statement in the form of **.WAIT PKQ** or **.WAIT RPG** follows a **PKQ** or **RPG** command line so that the computer's activities are in sync with its data processing. If automated data processing will be set up easier using multiple **RUN** files, another **RUN** file can be executed using **.XQT** {file name}, e.g. **N36PCBPK2.**

Each ASCII-converted quantitation report is transferred to a PC, appended, and is parsed and incorporated into a Lotus 1-2-3 spreadsheet. File transfer is done on the PC using **SmarTerm Office** to log on to the VG data system as a second terminal and then uses **KERMIT** (inside of **SmarTerm**) to retrieve a **.RPT** report. Manual retrieval uses the sequence in **SmarTerm** in VG's **RSX** mode: **CHD 3,54, KER** to enter **KERMIT, SET LINE TT15:, SET DEF 70,42, SEND N36CL5.RPT** or the appropriate report's file name. To Exit **Kermit**, type **{CTRL}Z,** then type **CHD 70,17**, and finally **@LOGIN** to return to the VG > prompt.

An automatic macro file will be developed to replace the older VGXFER macro that ran on the DOS version of SmarTerm. Because the **.RPT** file contents extends wider than 132 characters, it wraps to a second line. The QBASIC program **VGAPPEND** joins each pair of lines past line 17 and also renames the quantitation results report to {filename}**.PRN**.

**LOTUS 1-2-3 -- Parsing and Tabulation of PCB Congener Results:**
Empty template Lotus worksheet files have been made for each PCB, e.g. **PARS-126.123**, for a group of non-*o*-PCB congeners, or for all $^{13}C$-labeled PCBs. Each template file has two macros built-in, one (**PARSE**) which imports a **.PRN** file, parses the columnal data correctly, and saves the parsed file as a Lotus worksheet (**.123**). The second Lotus macro program (**CALCULATE**) averages concentrations determined from two ions, computes relative standard deviation of the concentrations and then calculates the variance in ion peak area ratios for quality assurance purposes.

**Final Laboratory Report Tables--QA/QC Report Criteria (Below) and Action Taken if Necessary:**
A Lotus final report spreadsheet table is compiled for native PCB congeners and another for recovery of $^{13}C$-PCB surrogates. Appropriate concentration values are selected based on the criteria below, ND, NQ, and less than values are tabulated, and footnotes added to identify out-of-spec QA/QC conditions, such as low recovery. The acceptable range of percent recoveries is 25 to 125%. Whenever percent recoveries are outside the acceptable range, samples in sample set should be reextracted and analyzed,

if possible. Concerning blank samples, corrective action should be taken if analyte amounts in procedural and method blank samples exceed by **three times** the mean values in the ongoing compilation tables of QA samples. Corrective action should also be taken whenever analytes in environmental samples are below levels than in QA blank samples. Molecular ions of interfering PCBs, mostly PCB #129 (see below), should be monitored to ensure their fragment ion responses do not contribute significantly to the responses of the respective non-*o*-PCBs. Whenever an interfering PCB, e.g. PCB #129, contributes ≥10% of the target non-*o*-PCB, e.g. #126, the interfering amount must be subtracted from the initially determined concentration of the non-*o*-PCB. If the interference contributes ≥50% of the target non-*o*-PCB, the sample extract should be rerun through HPLC-carbon and reanalyzed. If GC chromatographic resolution degrades significantly from that described below, GC/MS analyses should stop and appropriate corrective action should be taken, e.g. new retention gap and further cleanup of sample extract. After everything is completed and the final laboratory report has been signed and sent, make two sets of PC diskette backups, 1 set for the data folder and another set stored in a diskette storage box.

**Use of "Not Quantifiable" at Concentrations near the Detection Limit or from Interferences:** If peaks are detected for both principal ions but their ion ratio is not within the acceptable range, then "**NQ**" (not quantifiable) is reported, and the lesser value contributed by substituting the areas of each interfering "peak" for the analyte response is reported. If only one ion of the cluster has significant interference, the analyte concentration is determined by responses of the remaining cluster ion(s). If only two ions have been monitored in the cluster and one has interference, there is no second ion ratio to check and **NQ** must be reported. The analyte concentraton is reported as less than the smallest amount determined from the ions in the cluster.

**Use of "Not Detectable" at the Detection Limit of a PCB analyte:** If no peak is detected for either one or both of the principal ions, then "**ND**" (not detected) is reported. In each sample set, procedural and matrix blanks should have background levels of PCB congeners as low as possible, ideally "**ND.**" For optimum trace-level analysis of each sample set, it is also important to include a sufficiently low calibration standard to have ion responses with signal-to-noise ratios near the criterion of 3:1. Based on the lowest standard, a detection limit for each analyte in each sample can be determined based on residual noise manually integrated compared with the ion response of the appropriate $^{13}$C-PCB surrogate.

**Resolution of Analytes Gas Chromatographic and Mass Spectral Resolution:** HPLC-carbon separations of non-*o*-PCBs from other PCB congeners typically results in a fraction with >99% enrichment and preferably >99.9%. Residual carryover of major PCB congeners may still interfere with subsequent chromatographic (and mass spectral) analysis. Molecular ion responses of these major congeners are measured to ensure their fragment ion responses do not contribute significantly to the responses of the respective non-*o*-PCBs. Whenever an interfering PCB, e.g. PCB #129, contributes ≥10% of the target non-*o*-PCB, in this case PCB #126, the interfering amount must be subtracted from the initially determined concentration of the non-*o*-PCB. If the interference contributes ≥50% of the target non-*o*-PCB, the sample extract should be rerun through HPLC-carbon and reanalyzed.

GC/HRMS ion chromatograms of non-*o*-PCBs and of other PCB congeners in the non-*o*-PCB fraction from HPLC-carbon are shown in Figures 1-2. Figure 1 shows the PCBs in a chicken egg spiked with 40 μg of Aroclors 1242:1248:1254:1260; Figure 2 shows the PCBs in a large-scale cormorant egg dialysis sample.

Gas chromatographic resolution of the selected non-*o*-PCBs is better with a 50m HP Ultra-1 (DB-1 equivalent) capillary column than with a DB-5 column. Column performance is verified by analyzing standards of individual components and Aroclor mixtures. Similarly, relative retention times for all other congeners are evaluated with respect to labeled analogs. The following congener multiplets are at least partially resolved on an Ultra-1 (DB-1) column, as shown in Figure 1, to complement their resolution by mass except for small interfering fragment ions:

3,4,4'-TriCB (#37) (Optional) : 2,2',3,5'-TeCB (**#44**), elutes ~7 sec earlier than #37
: 2,2',3,4'-TeCB (**#42**), elutes ~8 sec later than #37
: and 2,3,3',6-TeCB (**#59**)

3,4,4',5-TCB (**#81**) : 2,2',3,4,5'-PeCB (**#87**), elutes ~9 sec later than #81; fragment ion 3%

3,3',4,4'-TCB (**#77**) : 2,3,3',4',6-PeCB (**#110**), elutes ~13 sec later than #77; fragment ion 0.7%
: 2,2',3,3',6,6'-HxCB (**#136**), elutes ~7 sec earlier than #77

The Ultra-1 column barely resolves the following multiplet, which requires optimal removal of PCB #129 by the HPLC-carbon column to minimize interference from the fragment ion even at 10,000 R.P.:

3,3',4,4',5-PeCB (**#126**): 2,2',3,3',4,5-HxCB (**#129**), elutes ~1-2 sec later than #126; (fragment ion 3.5%)

**Criteria for Confirmation:** For the positive identification and quantitation of a particular congener, the following additional criteria must be met:

(1) The peak areas for the selected ion responses must be greater than three times the background noise (S/N > 3);

(2) For congeners with isotopically-labeled analogs, the ion peaks for the native must occur at retention times from -1 to +3 sec that for the corresponding $^{13}C$-labeled ion peaks, which elute about 1 sec earlier than the native ion peaks;

(3) For the two principal ion responses, the ion ratio must be within the acceptable range (generally ±15%). These ion ratios are determined experimentally for the system during calibrations, compared with the theoretical values, and are tracked for quality assurance.

If additional ions are monitored during analysis, they will be included for quantitation and confirmation unless interfering compounds are detected. For ion responses very near the noise levels, or analytes with interferences, the final confirmation is left to the judgment of the analyst.

**Reminders for Optimum VG data processing:**

1. Check for and remove incorrect peak shoulders from integration.

2. Rerun **PKR** (using overwrite) to amend the pk shoulder removals and/or in more extreme cases, manually edit the **.PKS** integration table.

3. In **RHI** (Range of Highest Retention Time in Ion Group Window) of **PKR**, use * in group 1 (meaning go to the end of the group acquisition time) to ensure including a very large, slightly tailing PCB 77 peak.

4. Preview the detected peak chromatograms to best choose the range of standards to use in calibration. If PCB #169 is very low in all samples, restrict the range of calibration standards to match the corresponding levels in the samples.

**VG GC/MS Files, Related to Each Sample Data Set, To Be Archived (Currently on TK-50 Tape):**
Various VG files are pertinent to quantitative analyses of a set of samples and standards. They consist of the following in the specified subdirectories [ ]:

[70,20] **.DAT** (original/smoothed chromatographic data files) and **.PKD** (integrations)
[70,21] **.SYS** (system acquisition files), **.GCC** and **.SIR**
[70,40] **.RUN** and **.TXT**
[70,41] **.MTH, .TRG, .PKS, .CAL, .RES**
[70,42] **.RPT** and **.RPG**

To locate just the files of interest pertinent to the sample data set, go into RSX by typing {CTRL} \ then type **PIP**. If the pertinent data files have been acquired and data processed when other processing has not been done, use **/DD:01-JUN-98:15-JUN-98** (example dates) within **PIP** to specify only those files. If necessary, you can also use a * wildcard with (e.g. **36**) to help isolate only those files associated with the **N36PCB** set. Then list files in **PIP** by **[70,20]/FU** (full listing) or by **[70,20]/LI** (abbreviated listing). Print out this listing in **PIP** by **LP:=[70,20]/FU** (use print mode 5 or condensed mode).

Because the program **BRU** (Backup and Restore Utility) for Disk to Tape apparently does not recognize certain limited dates, it is best to move the desired files to equivalent unused subdirectories beginning with either **71, 72, or 73**. Depending on the size of the files and the remaining disk space, these files can either be moved (by renaming) or copied. Typically, data files from **[70,20]** are reasonably large and are **moved in PIP** by:

(destination) (rename) (from)
**[73,20]*.*;*/RE=[70,20]*.*;*** (assuming that files have been limited in **PIP** by date)
or **[73,20]*.*;*/RE=[70,20]*36*.*;*** (assuming there are **N36** data files and not **DF36** files)

Usually the rest of the files are collectively small enough to copy over in **PIP** by using:

**[73,21]*.*;*/CD=[70,21]*.*;*** (you may have to specify the **.GCC** file to include it)
**[73,40]*.*;*/CD=[70,40]*.*;*** (assumes you have limited files in PIP by date)
**[73,41]*.*;*/CD=[70,41]*.*;*** (assumes you have limited files in PIP by date)
**[73,42]*.*;*/CD=[70,42]*.*;*** (assumes you have limited files in PIP by date)

**Files with extensions .MTH, .TRG, .CAL, .PKS, .RES, and .RPG ARE VERY TIME-CONSUMING TO BUILD -- DO NOT DELETE OR UPDATE INDISCRIMINATELY!**

Archive files on the TK-50 tape using **BRU** accessed from the PC as a second terminal using **SmarTerm** and its Screen Capture feature. At the > prompt, type **HELLO VG/USER2** on the PC to log on to the VG system, type **{CTRL}** and **@VT** to enter RSX mode. In SmarTerm's Screen Capture mode, type in **VGTAP**?.BRU** (where * is the tape number and ? arbitrarily designates A

through **Z** as the initial or later **BAC**kups on the tape) to capture the terminal's response on that file during tape operation. Hit {RETURN} will send you back to the **RSX** prompt of the VG system **>**. Make sure the desired TK-50 tape has been put into the tape drive (remove latch to remove a tape only when the **Green** (safe) light is on at the left of the tape drive and when the **Red** (unsafe) light is not on at the right of the tape drive). When ready, Activate the tape drive by pushing in the **Red** button and lighting the **Red** light. Make sure the Write Protect is not engaged (slide lever on right side of tape does not show **Orange**).

From the **>** prompt, first mount the tape by: **MOU MU:/FOR** {RETURN}
(wait a few seconds for the prompt)
then **BRU** {RETURN}

(for a **new** tape **only**) **/REW/DIS/VER/MOU/BAC:**{up to 11 characters} {Return}
(**append** to a **used** tape) **/APP/DIS/VER/MOU/BAC:**{up to 11 characters} {Return}

(then for either tape): It asks FROM:
(type this in): **DU:[73,20],[73,21],[73,40],[73,41],[73,42]** {Return}
**MU:** {Return}

After **BRU** has successfully archived the desired files, type {CTRL} **Z** to exit **BRU**. Then dismount the tape drive using **DMO MU:** (wait a few seconds), then type **BYE** to logoff as the second terminal and then exit the VG emulation (SmarTerm Office) by mouse-clicking the upper right corner X..

Write or type the tape number and letter at the beginning of the file **VGTAP****?.BRU** and print out to the laser printer using Wordperfect or MS Word and file it with the **PIP** printouts. Delete the undesired files or duplicate files in **PIP** by: **[73,21]*.*;*/SD** (for Selective Delete) or **[73,21]*.*;*/DE/LD** (lists deleted files).

**ECRC SOP: P481**

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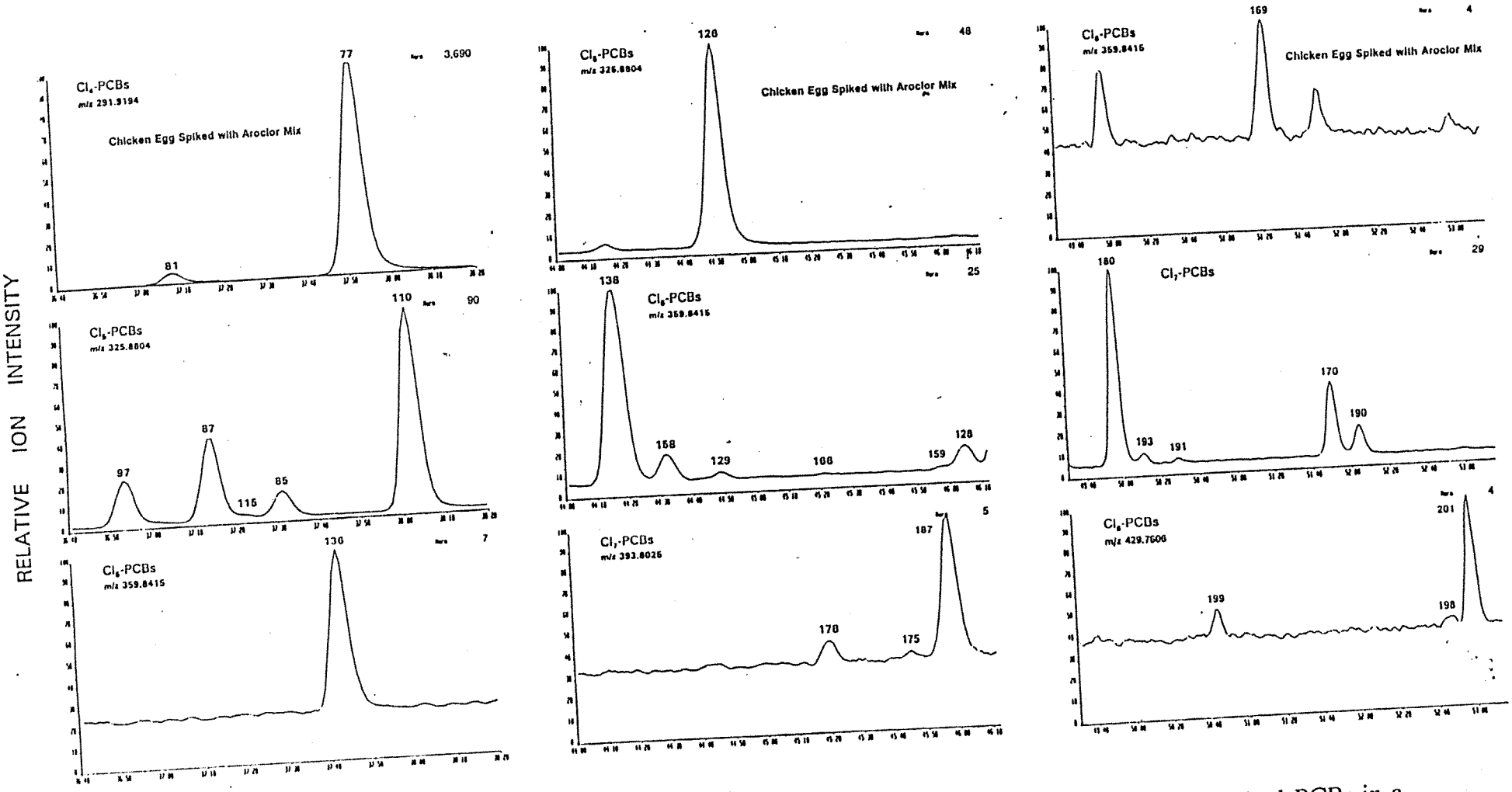


Figure 1. HRGC/HRMS Ion Chromatographic Segments Show Non-*o*-PCBs (top traces) and Other Residual PCBs in a Chicken Egg Spiked with a Mix of Aroclors 1242, 1248, 1254, and 1260.

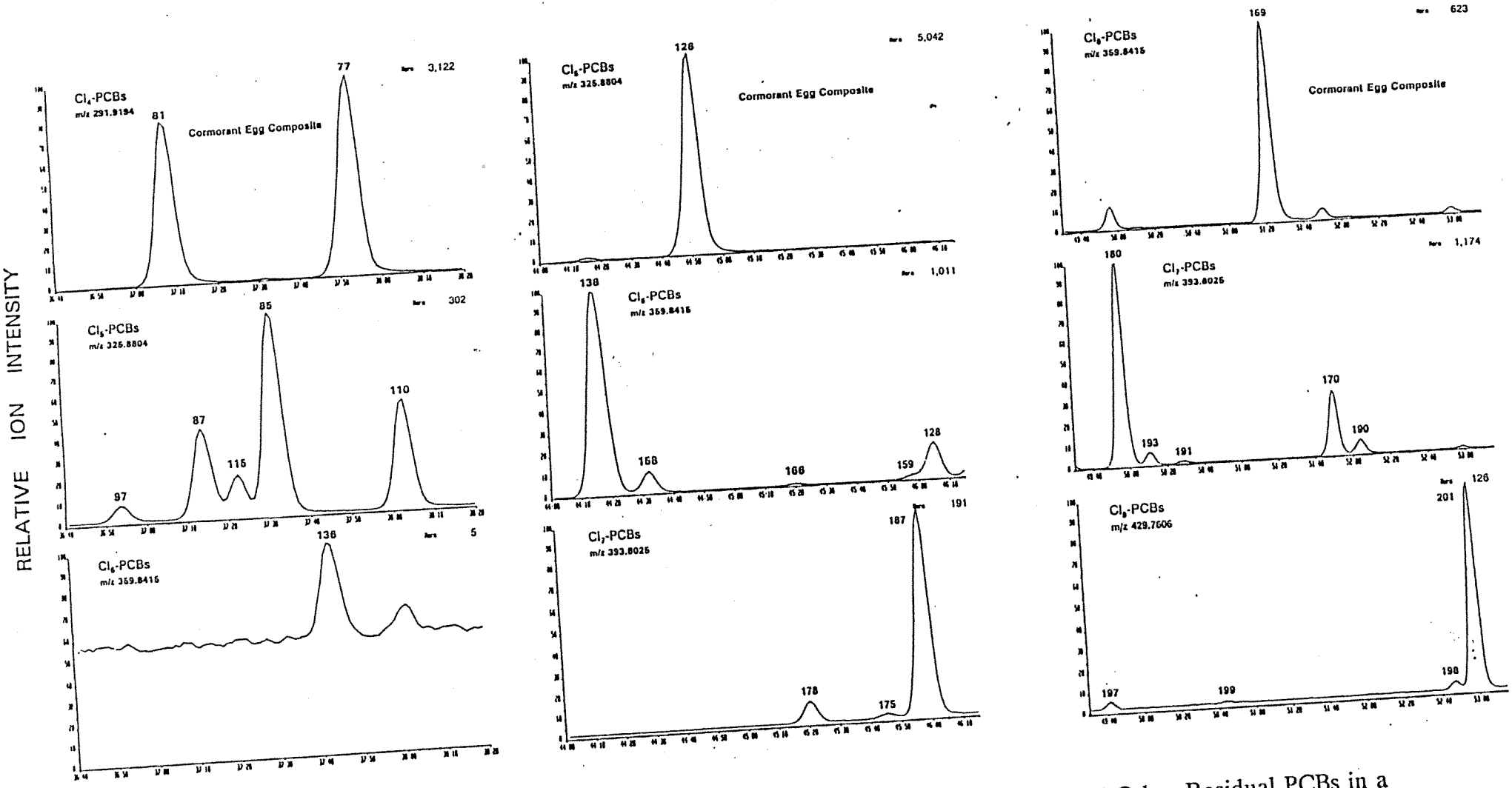


Figure 2. HRGC/HRMS Ion Chromatographic Segments Show Non-*o*-PCBs (top traces) and Other Residual PCBs in a Large-Scale Cormorant Egg Composite.

## Table 1. Spiking & Calibration Solutions (pg/μL) for Non–*o*–PCB Analysis

| Compound | $^{13}C_{12}$– Surrogate Standard[1] | Natives Spiking Standard[3] | Interfering PCBs Standard[4] | Calibration Standards in Nonane in Autosampler Vial (pg/μL, 100 μL total) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 93W | 94W | 95W | 96W | 97W | 98W | 99W |
| 3,3',4,4'–TCB (#77) | –– | 500 | –– | 0.25 | 1 | 5 | 25 | 100 | 500 | 2500 |
| 3,4,4',5–TCB (#81) | –– | 500 | –– | 0.25 | 1 | 5 | 25 | 100 | 500 | 2500 |
| 3,3',4,4',5–PeCB (#126) | –– | 500 | –– | 0.25 | 1 | 5 | 25 | 100 | 500 | 2500 |
| 3,3',4,4',5,5'–HxCB (#169) | –– | 500 | –– | 0.25 | 1 | 5 | 25 | 100 | 500 | 2500 |
| $^{13}C_{12}$–3,3',4,4'–TCB (#77) | 100 | –– | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| $^{13}C_{12}$–3,4,4',5–TCB (#81) | 100 | –– | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| $^{13}C_{12}$–3,3',4,4',5–PeCB (#126) | 100 | –– | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| $^{13}C_{12}$–3,3',4,4',5,5'–HxCB (#169) | 100 | –– | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| $^{13}C_{12}$–2,2',4,4',5–PeCB (#101)[2] | –– | –– | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 2,2',3,4,5'–PeCB (#87) | | | 250 | | | | | | | |
| 2,3,3',4',6–PeCB (#110) | | | 250 | | | | | | | |
| 2,2',3,3',4,5–HxCB (#129) | | | 250 | | | | | | | |

[1] The $^{13}C$–surrogate standard (191W–4) is spiked at 5000 pg (or 5 ng) into all samples before extraction.

[2] All calibration standards and each extract just before GC/MS analysis is spiked with 5000 pg of the internal standard (90W–2)

[3] This native–only standard (92W–2) is spiked at varying amounts into matrix spikes before extraction or a mixed Aroclors standard is used.

[4] PCB congener #87 nearly co–elutes with 81, 110 also nearly co–elutes with 77, and 129 co–elutes with 126.

Table 2. Typical VG 70-250S GC/HRMS Conditions for Non-o-PCBs

Sample Data Set: _____
Source: EI - CI _X_ or EI-Only ___

Electron Energy: 31 eV (28 set)
Repeller: (+/-) - 2.5 V
Source Temperature: 285°C (set)
Trap Current: 200 µA
Emission: (+/-) 1.4 mA
Filament Current: 4.4 A

Photomultiplier: 360 V

Energy Filter: ON
Ion Energy: 5.2
Y-Focus: 3.35

Variable Slit (on Micrometer): 13.02
α Slit Position: (0 to 4): 0

Current on HV Meter: 1.71 mA
Source VAC: $5 \times 10^{-6}$ mbar, GC at 120 °C
Analyser VAC: $1.5 \times 10^{-7}$ mbar, GC at 120 °C

Resolving Power: 10,000

Calibrant: Perfluorodecalin

SYS (SIR): NONPCB10K

SYS (GCC): PCBONCOL

Channel 7 (X Line): 300 °C
Direct Reentrant: 305 °C

Source FOCUS (Coarse):
1. 2 2. 2

Deflects:
1. -2.5 2. 2.3

ESA Pots:

| Y-Deflect: | Z-Deflect 1: | Z-Deflect 2: | Curve 1: | Rotate 1: |
|---|---|---|---|---|
| 3.2 | -1.3 | 3.0 | 0.2 | -0.2 |

| Z-Deflect: | Z-Focus 1: | Z-Focus 2: | Curve 2: | Rotate 2: |
|---|---|---|---|---|
| 0.3 | 6.3 | 6.1 | -1.8 | -1.0 |

GC Column Size/Film: 50 m x 200 µm x 0.11 µm film Ultra-1 (HP)
Length/Diameter of Retention Gap: ~2.5 m x 530 µm
Head Pressure: 45 psi
Linear Velocity: 27 cm/sec @ 120 °C.
Injector: Cool-on Column -- 2 µL Injected

Table 3. Ions Monitored for Non–*o*– and $^{13}C$–Labeled PCBs by High Resolution MS

| | More Abundant Ion | (m/z) | Less Abundant Ion | (m/z) | Ion Ratio (Less Abundant)/(More Abundant) |
|---|---|---|---|---|---|
| **GROUP 1: Ion acquisition time 35 ms each with 10 ms each interscan delay [5 ms for lock mass check]** | | | | | |
| 3,4,4'–TCB (#37, If Targeted) | A | 255.9614 | B | 257.9584 | 0.98 |
| $Cl_4$–PCNaphthalenes (PCNs) | D | 265.9038 | C | 263.9067 | 0.78 |
| 3,3',4,4'–(#77) and 3,4,4',5–TCB (#81) | F | 291.9194 | E | 289.9224 | 0.78 |
| Lock Mass/Lock Mass Check | G | 292.9825 | H | 292.9825 | --- |
| $Cl_5$–PCNs | I | 299.8648 | J | 301.8618 | 0.66 |
| $^{13}C_{12}$–3,3',4,4'–TCB (#77) | L | 303.9597 | K | 301.9626 | 0.78 |
| $Cl_4$–PCDFs – Breakthrough Check | M | 305.8987 | | | |
| 3,3',4,4',5–PeCB (#126), other $Cl_5$–PCBs | O | 325.8804 | N | 323.8834 | 0.62 |
| $Cl_6$–PCNs | P | 333.8258 | Q | 335.8229 | 0.82 |
| $^{13}C$–3,3',4,4',5–PeCB (#126), and $^{13}C$–2,2',4,5,5'–PeCB (#101)[a] | S | 337.9207 | R | 335.9236 | 0.62 |
| Interfering $Cl_6$–PCBs | T | 359.8415 | | | |
| $Cl_6$–Diphenyl ether – Carryover Check | U | 375.8364 | | | |
| Interfering $Cl_7$–PCBs | V | 393.8025 | | | |
| **GROUP 2: Ion acquisition time 60 ms each with 10 ms each interscan delay [5 ms for lock mass check]** | | | | | |
| $Cl_3$–PCTerphenyls (PCTs) | A | 333.9897 | | | |
| 3,3',4,4',5,5'–HxCB (#169) | B | 359.8415 | C | 361.8385 | 0.80 |
| $Cl_7$–PCNs | D | 367.7868 | | | |
| $Cl_4$–PCTs | E | 367.9507 | | | |
| $^{13}C_{12}$–3,3',4,4',5,5'–HxCB (#169) | F | 371.8817 | G | 373.8788 | 0.80 |
| Lock Mass/Lock Mass Check | H | 392.9760 | I | 392.9760 | |
| $Cl_7$–PCBs | J | 393.8025 | | | |
| $Cl_5$–PCTs | K | 401.9117 | | | |
| $Cl_8$–PCN | L | 401.7479 | | | |
| $Br_5$–Diphenyl Ether (M–$Br_2$) | M | 403.7870 | | | |
| $Cl_8$–PCBs | N | 429.7606 | | | |

[a] Internal Standard

# APPENDIX E-13

# CAPILLARY GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION PROCEDURE FOR CONGENER-SPECIFIC POLYCHLORINATED BIPHENYL ANALYSIS [USGS-TILLITT]

CERC SOP: P.195 (C5.154)

Date Prepared: 06/24/91

**For users other than Columbia Environmental Research Center staff, this document is for reference only. This is not a citable document.**

Date Revised: 10/14/98

# Capillary gas chromatography with electron capture detection procedure for congener specific polychlorinated biphenyl analysis

## I. General:

The procedure described here details the analytical steps for quantitative determination of 81 (or more) polychlorinated biphenyl (PCB) congeners in environmental sample solutions by capillary gas chromatography with electron capture detection (CGC/ECD). Although most steps specify exact details, some steps only give guidelines that require the analyst to select the optimum parameters within the specified range of values.

## II. Safety:

A. Since some PCB congeners may have toxicities similar to those of polychlorinated dibenzo(p)dioxins, potential routes of exposure should be eliminated by using a fume hood as much as possible, gloves and safety glasses should be worn when handling pure compounds or open solutions.

B. High resolution Capillary gas chromatography with electron capture detection (CGC/ECD) analyses require hydrogen as a carrier gas. Hydrogen is extremely flammable and only trained technicians and chemists should handle, maintain, and install components of the supply and analytical gas lines. Special efforts should be made to assure that all connections are leak-tight.

C. **CAUTION:**
**DO NOT USE use compressed hydrogen or air with the Supelco High Capacity Gas Purifier! Hydrogen and air are NOT COMPATIBLE with the reactive scavenger catalyst and contact will generate large amounts of heat adequate to melt the metal scrubber tubing!**

## III. Equipment:

A. **Gas chromatograph (GC) system - Hewlett-Packard 5890 II equipped with:**

1. an HP model 7673 autosampler with 100 sample tray, robotic arm and injector with a 10-µL 26/23 gauge needle;

2. an automated cool on-column injection port with an adapter for 0.53 µm capillary columns;

3. a capillary $^{63}$Ni-ECD with electrometer (0-1V analog output);

4. a remote control cable to maintain synchronization of: autosampling, GC oven temperature programming, and the computerized collection of the ECD analog signal. This is a "general purpose" remote cable when used together with an HP 3393A or HP 3396B integrator, or a custom "Y"-remote cable when stand alone control is employed;

5. a shielded analog signal cable;

6. a **60 m x 0.25 mm i.d. fused silica capillary column, DB-5 (0.25 µm film thickness, cross linked and bonded 5% phenyl-, 95% methylpolysiloxane, J & W Scientific) or equivalent from other vendor** connected to the on-column injector via. a ~1-3 m X 0.53 mm i.d. uncoated and deactivated (dimethyl phenyl-silane) retention gap capillary (Restek, Inc.). The retention gap capillary is connected to the DB-5 analytical column by means of a press tight union (deactivated quartz, Restek, Inc.);

7. a **P.E. Nelson 763 SB or 926 intelligent interface** connected to the output of the analog signal cable. The remote cable is also connected to the interface "Ready" terminals (a contact "open" state signifies to the HP 5890 remote control system that the interface is ready to collect analog signal data) and to the interface remote "Start" terminals (a contact closure from the HP 5890 signifies that the interface should begin collecting analog signal data). Therefore, when the PE Nelson intelligent interface is not ready to start an analysis (No data collection method loaded, another analysis is in progress, etc.) the "Ready" contact on the interface will be closed. Note: the red "not ready" light on the 5890 will be on during an analysis when both remote and INET (via. 339n integrator) control are being used. The nature of the "not ready" situation can be determined by pressing the clear key on the HP 5890 key pad. The LED should indicate that a "remote device is not ready" until the intelligent interface has collected all the data and is ready for the next analysis to begin.

8. **Optional**, HP 3393 or 3396 (339n) Integrator for INET sequence control of the automated sample injections.

B. **The PE Nelson Chromatography system**

1. An intelligent interface (see above) with 192K to 256K bytes of data storage.

2. IEEE-488 interface cable (1-16m) which connects the interface to the Ziatec IEEE-488 interface card of the host microcomputer.

3. A microcomputer with a Ziatec interface card, a mouse pointer, 0.5 Gb or larger hard drive, 20+ Mb addressable RAM, an VGA color monitor and driver, a parallel printer port, a 3.5 inch disc drive(s), PE Nelson Turbochrom version 4.1, Microsoft Windows 95, spread sheet software (Lotus or Excel) and a bubble-jet or dot matrix printer. **NOTE:** This SOP will refer to the Turbochrom Users' Guide throughout.

C. Indicating oxygen traps installed on both the hydrogen carrier gas line and the nitrogen make-up gas line (just prior to the GC) and high capacity (catalytic high temperature, Supelco or equivalent) oxygen traps installed near to the supply tank(s) for the nitrogen make-up gas.

D. GOW MAC electronic leak detector.

## IV. Materials and Reagents:

A. Target[R] amber crimp-cap 1-mL autosampler vials or equivalent. For small samples (< 200 µL) use Chromacol 0.9-mL conical tipped wide-mouthed vials. For standard and replicate injected samples use the screw cap target vials. All vials should be baked at 450°C prior to use and stored in clean glass containers with solvent washed aluminum foil covers.

B. Pasteur pipettes. Bake as for autosampler vials and store the same way.

C. SMI[R] Digital Adjust Micro/Pettor[R] Series 1200, Models D 10-60 µL and J 50-250 µL with silanized, glass micro-pipettes.

D. Solvents for dilution of samples: Hexane and isooctane (pesticide grade or equivalent).

E. Autosampler rinse solvents: solvent A is 50:50 (v/v) acetone/toluene and solvent B is isooctane or hexane (pesticide grade or equivalent).

F. High purity laboratory gases: Hydrogen - (99.999%, ultra high purity grade, Linde or equivalent) and Nitrogen - (99.9%, Medipure[R], Linde or equivalent).

G. Z-fold dot-matrix, tractor-feed printer paper (9.5 in X 11 in) and/or photocopy paper for laser printers. Avery label sheets with 80/page for printing sample labels.

## V. Procedure for CGC/ECD Instrumental Analysis:

A. **Chromatography section computer file naming conventions:** Using the file name **H15D.MTH** as an example, the naming conventions for Chromatography Method (**.MTH**), Sequence (**.SEQ**), Raw Data (**.RAW**), Report Format (**.RPT**), Results (**.RST**), and Summary Files (**.SUM, .PRN**) files are: "**H15**" designates Hewlett-Packard GC #15, "**D**" designates the fourth block of ECD analyses, and "**.MTH**" designates the file as a Method file.

B. **CGC/ECD operating conditions:** Detector Temperature is 330°C. Temperature Program (optimize if necessary) = 60/0/10/160/0/2/260/2/10/320/5 (oven equilibration time of 2 minutes) with $H_2$ carrier gas at 20-25 PSIG (constant pressure) and $N_2$ Make Up Gas. The **cool on-column injector** temperature is control by the **HP Oven Track** being turned On (the injector temperature stays 3° above oven temperature during temperature programing). The capillary column should be 70 mm into the ECD. The injector end of the retention gap column should be up against the needle guide of the injector, but not tightly pushed up to compress the spring and block the septum sweep outlet.

C. **Instrumental system setup and performance optimization:**

1. Using the Turbochrom Navigator, select **Method**, prepare and save to hard disk a) a method for collecting ECD test-mix chromatographic data (QAQC) and b) a method for collecting calibration standard and sample chromatographic data. For reference refer to the attached example methods **Attachment 1A**, H15D.MTH and refer to **Turbochrom Users' Guide**, Chapters 5-10.

2. Using Navigator, select **Sequence**, prepare a data collection sequence and save to a hard-disk file (e.g. H15D.SEQ, **Attachment 1B**). For a description of how to prepare a data collection sequence, refer to the Turbochrom Users' Guide Chapter 11. Be sure that the correct Raw data file name (including system paths) is specified. If you fail to specify the correct path the "**.RAW**" and "**.RST**" files will be stored to the default path. **Note:** You will always collect the Raw data (.RAW) files and store them to disk for subsequent data reprocessing and quantitation.

3. From the Navigator select **Setup** and "download" the appropriate data collection method and sequence to the PE Nelson interface. **NOTE:** Be

sure to download to the correct channel (A or B) and that the Sequence you are using also has the correct channel designation.

Optimize the chromatographic carrier gas and temperature parameters using QAQC solution 165W-40, an ECD test mixture containing 40 pg/µL each of OC pesticides and by using performance evaluation standards (PES) of p,p'-DDT and o,p'-DDT in isooctane to determine retention gap cleanliness by the percentage of DDT breakdown (**retention gap needs to be cut or changed if DDT breakdown ≥10%**). Standard solution 165W-40 is used to optimize the chromatographic peak shape in terms of dead volumes in capillary connections and chromatographic system activity. Using solution 165W-40, check peak width at baseline of the Δ-BHC (not to exceed 0.3 minutes), check all peak areas, calculate linear velocity (needs to be ~ **40 cm/sec**) and **Theoretical Plates,** then record the **QA/QC data** in the GC's **Maintenance Logbook.** If the **Δ-BHC** peak width exceeds 0.3 minutes then either remove 20-25 cm from front of the retention gap column (if long enough) or install a new retention gap column and quick connect. Inject the DDT PES every 10 - 15 samples.

4. An A1111 high standard is used to optimize and verify the adequacy of the chromatographic separation of individual PCB congeners. Refer to **Figure 1** (A1111 2000 ng/mL standard) for examples of optimized chromatography. Record the results of all optimization analyses in the corresponding instrument log to document this quality control procedure.

C. **Analyses:**

1. **General considerations**: Since congener-specific PCB (cPCB) analyses require highly reproducible oven temperature profiles to facilitate congener identifications, it is **imperative** that you cycle the CGC oven (to the maximum temperature used during the analysis) at least once immediately prior to beginning or restarting any block of sample analyses. This is necessary so that the GC oven will experience the same equilibration as it would following a sample analysis during the actual auto-sampling sequence. Cycling the CGC to the maximum program temperature will also effectively eliminate component ghosting (sample carry-over) when the analytical sequence is interrupted in the middle of a CGC temperature program.

2. **Congener Specific Calibration**. The responses of individual PCB congeners (~81 listed in **Table 1** and identified in **Figure 1**) are calibrated by analyzing at least three levels of the mixed Aroclor standard A1111. For preparation of Standards see **SOP P.230 (C5.198).** Typically, you

will use the six levels of mixed Aroclor standards (A1111-50, A1111-100, A1111-250, A1111-500, A1111-1000 and A1111-2000 (ng/mL individual Aroclor std)) to define a calibration. The total PCB calibration ranges between about 200 ng/mL (200 pg/µL injected) to about 8 µg/mL total PCB (8 ng/µL injected). Additional low level standards can be added for absolute detection limit determinations and for accurate quantitation of very low sample concentrations. These six levels will be analyzed from low to high at the beginning of the block of samples and standards and at the end of the block of samples. After this initial calibration, every sixth or seventh CGC/ECD analysis (auto-sampler injection) will repeat only one of these six levels, in order, from lowest to highest concentration. Thus, the entire calibration curve will be repeated about every 42 analyses until all of the samples have been analyzed. For a block of 120 CGC/ECD analyses the calibration will be repeated a total of four times.

3. **Preparation of an Analytical Sequence**. Once the total number of samples and QC samples is established the analytical sequence can be constructed around the above described calibration standards as follows:

   a) Divide all duplicate procedural and triplicate instrumental (5% to 10% of the sample analyses) analyses into two groups and randomly place the replicated samples in the sample analyses sequence.

   b) when sample cPCB results will be examined using principal components analysis, then replicate (typically duplicates) instrumental analyses of individual Aroclor standards (1.0 µg/mL A1242, A1248, A1254 and A1260) will also be included.

   c) individual samples, including QC samples (OC standards, AHH stds, PIS standards, isooctane GC blank, solvent blank, etc.) will then be randomly added to the remaining slots in the sequence. An example "**sample analytical sequence**" is given in **Attachment 1C**.

   d) in constructing the sequence include the information about dilution factors, sample weights and internal standards amounts **in the initial sequence** in order to insure the correct information is included the RAW file header as analog data is digitized and written to the hard disk.

4. **Final sample preparation for CGC/ECD analysis**. The upper limit of the ECD detector is about 80-100 pg on-column per PCB component. The

sample size usually extracted for PCBs is 10 - 25 g. Often these extracts must be diluted in order to accurately quantitate PCB congeners.

Typical final volumes for biotic sample extracts are 5 - 10 mL. Gram-equivalent concentrations for this will range from 2 to 5 g-eq/mL.

a. **IS Spiking.** Final sample extracts in isooctane are spiked with the internal standard (IS), aldrin or OCN, at a rate of 20 ng/mL or 50 ng/mL, respectively (i.e. 5 mL final volume will be spiked with 500 ng of OCN). Spiking should be witnessed and documented by a colleague.

b. **Loading Target$^R$ or Chromacol autosampler vials.** Typically, an approximate portion (1 mL or less) of each sample extract will be transferred into standard Target$^R$ autosampler vials (or 100 µL for Chromacol vials) and then capped with a crimper. It is important that the cap be crimped tight enough to prevent it from being turned while holding the vial, but not so tightly that the septum is significantly concave or convex. Undertightening will result in a loss of volatile components from the sample extract including solvent, while over tightening will tear the teflon face of the septum and cause greater exposure of the sample to the silicone rubber septum.

c. **Dilutions.** If sample extracts must be diluted for analysis use calibrated glassware, pipettes and gas tight Hamilton syringes to measure volumes. It is important to know the approximate total PCB concentration of each sample extract prior to loading the diluted extract into the Target$^R$ autosampler vial. Because of the somewhat limited range of A1111 calibration standards used, only samples with total PCB concentrations between about 200 ng/mL and about 8 µg/mL will be within the calibration range of the CGC/ECD method. Typically, we try to adjust the sample volume (by dilution or concentration) so the concentration is equal to approximately 4000 ng/mL. If extracts must be diluted more than 1:50 additional IS will have to be spiked in the dilution. Follow a) and b) above. Include the dilution factor in the sequence if diluting this much.

5. **Starting and restarting a CGC/ECD analysis block.**

a. Verify that the CGC/ECD, 7673 autosampler, 339n series integrator, and PE Nelson interface are all properly configured.

Check the CGC/ECD set points such as injection temperature, detector temperature, oven temperature program conditions, $H_2$-carrier gas pressure, ECD total flow including $N_2$-make-up gas flow, and ECD signal level and output assignment (typically signal 1 and signal 2 are both assigned to the ECD).

b. Verify that the PE Nelson interface is ready (has the correct cycle #, sequence file, method file, and the sample raw data file are downloaded from the host microcomputer). Verify that the method parameters and sequence data are accurate and appropriate for the current analysis. If the set points are incorrect make any necessary changes.

c. Prior to start of CGC/ECD analysis of a block of samples, inject A1111 standards right up to start of the analysis to eliminate any active sites on the analytical column. This minimizes drift of instrumental replicates over a set of samples.

d. When **starting a CGC/ECD analysis block under Remote Stand-Alone control** and after cycling the CGC/ECD oven temperature two times, press the 7673 "**START**" button to begin the sample analysis. Observe that the injector rinses the needle and loads the sample solution properly (1 µL). Make sure that at the injection the CGC/ECD temperature program is started and that the PE Nelson interface starts collecting data. Also verify that the CGC/ECD signal has the expected response level and polarity by viewing the screen display of the chromatogram in **Real-Time Plot** and monitor time, sample number, etc. under **Status** and in **Details**.

f. When **restarting a CGC/ECD analysis block after an interruption**, cycle the CGC/ECD oven temperature and proceed as in "5d." above.

**A REMINDER:** When restarting the CGC/ECD analysis block under **HP Remote Stand-Alone control**, the remaining samples will need to be moved back in the sample tray so that the next sample is in the "one" position. The HP 7673 autosampler always starts/restarts the injection sequence from the "one" position if under Remote Stand-Alone control.

f. After completion of the CGC analysis, remember to archive the raw data files (*.**RAW**), result files (*.**RST**), methods (*.**MTH**) and sequence (*.**SEQ**) onto 3.5" floppy disks or EVEREX Tape and record this information in the **QA/QC Archived Computer Data Logbook CERC # 119**. Store floppies or tapes in a designated place and a hard copy of what data files are archived on each floppy/tape with the original chromatograms and area reports.

## VI. CGC/ECD Data Reduction:

### A. Integration and calibration of PCB standards:

1. Create or modify a method for concentration calculations using the same method root name that was used to collect the data, (it should conform to the computer filing naming convention listed in Section V.A.) add the letter C to end of the method name to designate it as a method for concentration calculations (e.g. H15DC.MTH). This method should have all the Congeners already listed with their correct retention times, and levels of concentration. Use this method for standard reintegration and calibration. See **SOP P.483** for calibration, data processing and QC procedures for processing GC/ECD data.

2. **Data Processing: Peak Matching, Timed Events, Integration.**

   a. Data can be processed from **Graphic (Method) Edit** or **Results**. Process Raw Data from Calibration Standards in Graphic Edit. After all standard result files are processed this way perform a manual calibration update with the standard result files in **Method**. Once calibrated use method to process sample RAW files (see below). See Turbochrom 4 Users Guide Chapter 10 for details.

### B. Integration of PCB congeners in samples:

1. **Data Processing: Peak Matching, Timed Events, Integration.**

   a. Process all data files in **Reprocess-Batch** from the Navigator (TC4 Users' Guide Chapter 16) using the **calibrated method** (from above, ie. H15DC.MTH). You may need to change the sequence before processing if you renamed the method file.

   b. After batch process select individual **Result Files** for each sample and update peak assignments, and integration timed events as

necessary. See Turbochrom Users' Guide Chapter 10 for details. **SAVE RESULTS FILES AT THIS POINT.**

c. Finally, batch reprocess the sequence again, using the methods in the **RESULT File**. This will give the corrected quantification.

C. Use **Reprocess-Summary** to produce a .PRN file to read into the Lotus 123 Spreadsheet or equivalent (See TC4 Users' Guide Chapter 17). Import the PRN file into Lotus (or CSV into Excel) spreadsheet as numbers and save file. Open MACROS file behind the new file and run **MAC** to move wrap-around data correctly (sample names down/congeners across format). Once the wrap-around data is formated delete rows under the data block. Erase "Adjusted Amount" header and starting at the first value run MACRO **ZERO** to convert all label "0.00" or "---" to 0. Now you are ready to customize the spread sheet for the final report.

## VII. Documentation:

Use the instrument log books and personal daily log books to keep accurate written records of all CGC/ECD system configurational details (e.g. column serial number, length, CGC parameters, etc.), instrument maintenance and repairs, and samples and standards analyzed. Also Archive all of the Raw Data Files, pertinent reprocessed files (*.RAW, *.MTH, *.SEQ, *.RST, *.RPT, *.SUM, *.PRN, *.WK3, and *.DOC--Word Perfert); and record this information in the **Archived Computer Data Logbook CERC # 119.**

## VIII. Attachments

Table 1— PCB congeners in A1111 calibration standards (@ 4000 ng/mL level).

Figure 1— Optimized chromatogram of PCB congener analysis.

1A, 1B— Method and sequence file examples.

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Table 1. Composition of A1111 Mixture for PCB Congeners (ng/mL concentration) analyzed on DB-5 Capillary Column

| Peak No. | IUPAC No. | No. of Cl | # ortho Cl | Refs. for Assignment | Congener Integration | Description/Structure | Concentration in A1111 @ 4000 ng/mL |
|---|---|---|---|---|---|---|---|
| 1 | 001 | 1 | 1 | | NA | 2-chlorobiphenyl | |
| 2 | 004 | 2 | 2 | 1,2 | | 2,2'-dichlorobiphenyl | 49.46 |
| | 010 | 2 | 2 | 1,2 | | 2,6-dichlorobiphenyl | |
| 3 | 007 | 2 | 1 | 1,2 | | 2,4-dichlorobiphenyl | 11.12 |
| | 009 | 2 | 1 | 1,2 | | 2,5-dichlorobiphenyl | |
| 4 | 006 | 2 | 1 | 1,2 | | 2,3'-dichlorobiphenyl | 17.79 |
| 5 | 005 | 2 | 1 | 1,2 | | 2,3-dichlorobiphenyl | 82.88 |
| | 008 | 2 | 1 | 1,2 | | 2,4'-dichlorobiphenyl | |
| 6 | 019 | 3 | 3 | 1,2 | | 2,2',6-dichlorobiphenyl | 17.12 |
| 7 | 030 IS | 3 | 2 | 1 | (IS) | 2,4,6-trichlorobiphenyl | |
| 8 | 011 t | 2 | 0 | | NA | 3,3'-dichlorobiphenyl | |
| 9 | 012 t | 2 | 0 | | NA | 3,4-dichlorobiphenyl | |
| | 013 t | 2 | 0 | | NA | 3,4'-dichlorobiphenyl | |
| 10 | 018 | 3 | 2 | 1,2 | | 2,2',5-trichlorobiphenyl | 162.40 |
| 11 | 015 | 2 | 0 | 1,2 | | 4,4'-dichlorobiphenyl | 73.44 |
| | 017 | 3 | 2 | 1,2 | | 2,2',4-trichlorobiphenyl | |
| 12 | 024 | 3 | 2 | 1,2 | C | 2,3,6-trichlorobiphenyl | 4.67 |
| 13 | 027 | 3 | 2 | 1,2 | C | 2,3',6-trichlorobiphenyl | |
| 14 | 016 | 3 | 2 | 1,2 | C | 2,2',3-trichlorobiphenyl | 97.70 |
| 15 | 032 | 3 | 2 | 1,2 | C | 2,4',6-trichlorobiphenyl | |
| | (034) t | 3 | | 1,2 | NA | | |
| 16 | 029 | 3 | 1 | 1,2 | | 2,4,5-trichlorobiphenyl | 2.48 |
| 17 | 026 | 3 | 1 | 1,2 | | 2,3',5-trichlorobiphenyl | 21.39 |
| 18 | 025 | 3 | 1 | 1,2 | | 2,3',4-trichlorobiphenyl | 9.53 |
| 19 | 031 | 3 | 1 | 1,2 | | 2,4',5-trichlorobiphenyl | 76.50 |
| | 028 | 3 | 1 | 1,2 | | 2,4,4'-trichlorobiphenyl | 112.75 |
| 20 | 021 t | 3 | 1 | 1,2 | NA | 2,3,4-trichlorobiphenyl | |
| 21 | 020 | 3 | 1 | 1,2 | C | 2,3,3'-trichlorobiphenyl | 110.45 |
| | 033 | 3 | 1 | 1,2 | C | 2',3,4-trichlorobiphenyl | |
| 22 | 053 | 4 | 3 | 1,2 | C | 2,2',5,6'-tetrachlorobiphenyl | |
| 23 | 051 | 4 | 3 | 1,2 | | 2,2',4,6'-tetrachlorobiphenyl | 9.23 |
| 24 | 022 | 3 | 1 | 1,2 | | 2,3,4'-tetrachlorobiphenyl | 44.78 |
| 25 | 045 | 4 | 3 | 1,2 | | 2,2',3,6-tetrachlorobiphenyl | 28.67 |
| 26 | 046 | 4 | 3 | 1,2 | | 2,2',3,6'-tetrachlorobiphenyl | 13.55 |
| | (069) t | 4 | | 1,2 | NA | | |
| 27 | 052 | 4 | 2 | 1,2 | | 2,2',5,5'-tetrachlorobiphenyl | 177.91 |
| | 073 t | 4 | 2 | 1,2 | NA | 2,3',5',6-tetrachlorobiphenyl | |
| 28 | 043 t | 4 | 2 | 1,2 | C | 2,2',3,5-tetrachlorobiphenyl | 117.61 |
| | (038) t | 3 | | 1,2 | NA | | |
| 29 | 049 | 4 | 2 | 1,2 | C | 2,2',4,5'-tetrachlorobiphenyl | |
| 30 | 047 | 4 | 2 | 1,2 | | 2,2',4,4'-tetrachlorobiphenyl | 45.75 |
| 31 | 048 | 4 | 2 | 1,2 | | 2,2',4,5-tetrachlorobiphenyl | 56.00 |
| | (075) t | 4 | | 2 | NA | | |
| | (035) t | 3 | | 2 | NA | | |
| | 044 | 4 | 2 | 1,2 | | 2,2',3,5'-tetrachlorobiphenyl | 117.94 |
| | 037 t | 3 | | 2 | NA | | |
| | 059 t | 4 | | 2 | NA | | |
| | 071 | 4 | 2 | 2 | NA | 2,3',4',6-tetrachlorobiphenyl | |
| 33 | 042 | 4 | 2 | 1,2 | | 2,2',3,4'-tetrachlorobiphenyl | 91.43 |
| 34 | 041 | 4 | 2 | 1,2 | | 2,2',3,4-tetrachlorobiphenyl | 49.58 |
| 35 | 064 | 4 | 2 | 1,2 | | 2,3,4',6-tetrachlorobiphenyl | 54.54 |
| | (096) t | 5 | | 2 | NA | | |
| 36 | 040 | 4 | 2 | 1,2 | | 2,2',3,3'-tetrachlorobiphenyl | 26.52 |
| | 103 IS | 5 | 3 | 2 | NA | 2,2',4,5',6-pentachlorobiphenyl | |
| 37 | 067 | 4 | 1 | 1,2 | | 2,3',4,5-tetrachlorobiphenyl | 4.65 |
| | 100 t | 5 | 3 | 1,2 | NA | 2,2',4,4',6-pentachlorobiphenyl | |
| 38 | 063 | 4 | 1 | 1,2 | | 2,3,4',5-tetrachlorobiphenyl | 2.83 |
| 39 | 074 | 4 | 1 | 1,2 | | 2,4,4',5-tetrachlorobiphenyl | 61.09 |
| 40 | 070 | 4 | 1 | 1,2 | C | 2,3',4',5-tetrachlorobiphenyl | 125.69 |
| | 076 t | 4 | 1 | 1,2 | C | 2',3,4,5-tetrachlorobiphenyl | |

Table 1. Composition of A1111 Mixture for PCB Congeners (ng/mL concentration) analyzed on DB-5 Capillary Column

| Peak No. | IUPAC No. | No. of Cl | # ortho Cl | Refs. for Assignment | Congener Integration | Description/Structure | Concentration in A1111 @ 4000 ng/mL |
|---|---|---|---|---|---|---|---|
| 41 | 093 t | 5 | 3 | | NA | 2,2',3,5,6-pentachlorobiphenyl | |
| | 102 t | 5 | 3 | | NA | 2,2',4,5,6'-pentachlorobiphenyl | |
| 42 | 066 | 4 | 1 | 1,2 | | 2,3',4,4'-tetrachlorobiphenyl | 111.84 |
| 43 | 095 | 5 | 3 | 1,2 | | 2,2',3,5',6-pentachlorobiphenyl | 121.34 |
| | 088 t | 5 | 3 | 1,2 | | 2,2',3,4,6-pentachlorobiphenyl | 4.80 |
| 44 | 091 | 5 | 3 | 1,2 | | 2,2',3,4',6-pentachlorobiphenyl | 24.66 |
| | 155 IS | 6 | 4 | 2 | NA | 2,2',4,4',6,6'-hexachlorobiphenyl | |
| 45 | 056 | 4 | 1 | 1,2 | C | 2,3,3',4'-tetrachlorobiphenyl | 45.35 |
| | 060 | 4 | 1 | 1,2 | C | 2,3,4,4'-tetrachlorobiphenyl | |
| 46 | 092 | 5 | 2 | 1,2 | | 2,2',3,5,5'-pentachlorobiphenyl | 27.38 |
| 47 | 084 | 5 | 3 | 1,2 | | 2,2',3,3',6-pentachlorobiphenyl | 39.10 |
| 48 | 089 t | 5 | 3 | 2 | NA | 2,2',3,4,6'-pentachlorobiphenyl | |
| 49 | 090 t | 5 | 2 | 1,2 | C | 2,2',3,4',5-pentachlorobiphenyl | 152.68 |
| | 101 | 5 | 2 | 1,2 | C | 2,2',4,5,5'-pentachlorobiphenyl | |
| 50 | 099 | 5 | 2 | 1,2 | | 2,2',4,4',5-pentachlorobiphenyl | 59.84 |
| 51 | 119 | 5 | 2 | 1,2 | | 2,3',4,4',6-pentachlorobiphenyl | 2.68 |
| | (055) t | | | | NA | | |
| 52 | 083 | 5 | 2 | 1,2 | | 2,2',3,3',5-pentachlorobiphenyl | 8.83 |
| 53 | 097 | 5 | 2 | 1,2 | | 2,2',3',4,5-pentachlorobiphenyl | 46.71 |
| 54 | 081 t | 4 | 0 | 1,2 | NA | 3,4,4',5-tetrachlorobiphenyl | |
| 55 | 087 | 5 | 2 | 1,2 | | 2,2',3,4,5'-pentachlorobiphenyl | 69.97 |
| | (115) t | 5 | | 2 | NA | | |
| 56 | 085 | 5 | 2 | 1,2 | NA (DDE int) | 2,2',3,4,4'-pentachlorobiphenyl | |
| 57 | 136 | 6 | 4 | 1,2 | | 2,2',3,3',6,6'-hexachlorobiphenyl | 31.27 |
| 58 | 077 t | 4 | 0 | 1,2 | NA | 3,3',4,4'-tetrachlorobiphenyl | |
| 59 | 110 | 5 | 2 | 1,2 | | 2,3.3',4',6-pentachlorobiphenyl | 106.32 |
| 60 | 082 | 5 | 2 | 1,2 | | 2,2',3,3',4-pentachlorobiphenyl | 17.05 |
| 61 | 151 | 6 | 3 | 1,2 | | 2,2'3,5,5',6-hexachlorobiphenyl | 52.00 |
| 62 | 135 | 6 | 3 | 1,2 | C | 2,2',3,3',5,6'-hexachlorobiphenyl | 37.33 |
| | 144 | 6 | 3 | 1,2 | C | 2,2',3,4,5',6-hexachlorobiphenyl | |
| 63 | 124 | 5 | 1 | 1,2 | C | 2',3,4,5,5'-pentachlorobiphenyl | |
| 64 | 147 | 6 | 3 | 1,2 | NA | 2,2',3,4',5,6-hexachlorobiphenyl | |
| 65 | 107 | 5 | 1 | 1,2 | | 2,3,3',4',5-pentachlorobiphenyl | 7.14 |
| | (108) t | 5 | | 2 | NA | | |
| 66 | 123 | 5 | 1 | 1,2 | C | 2',3,4,4',5-pentachlorobiphenyl | 129.75 |
| 67 | 149 | 6 | 3 | 1,2 | C | 2,2',3,4',5',6-hexachlorobiphenyl | |
| 68 | 118 | 5 | 1 | 1,2 | | 2,3',4,4',5-pentachlorobiphenyl | 76.83 |
| 69 | 134 | 6 | 3 | 1,2 | | 2,2',3,3',5,6-hexachlorobiphenyl | 11.60 |
| 70 | 114 m | 5 | 1 | 1,2 | NA | 2,3,4,4',5-pentachlorobiphenyl | |
| 71 | 131 m | 6 | 3 | 1,2 | NA | 2,2',3,3',4,6-hexachlorobiphenyl | |
| 72 | 122 m | 5 | 1 | 1,2 | NA | 2',3,3',4,5-pentachlorobiphenyl | |
| 73 | 146 | 6 | 2 | 1,2 | | 2,2',3,4',5,5'-hexachlorobiphenyl | 21.06 |
| 74 | 153 | 6 | 2 | 2,3 | | 2,2',4,4',5,5'-hexachlorobiphenyl | 75.50 |
| 75 | 132 | 6 | 3 | 2,3 | | 2,2',3,3',4,6'-hexachlorobiphenyl | 112.90 |
| 76 | 105 | 5 | 1 | 1,2,3 | | 2,3,3',4,4'-pentachlorobiphenyl | 38.39 |
| 77 | 141 | 6 | 2 | 1,2 | | 2,2',3,4,5,5'-hexachlorobiphenyl | 38.21 |
| | 179 | 7 | 4 | 1,2 | | 2,2',3,3',5,6,6'-heptachlorobiphenyl | 14.59 |
| 78 | 130(137) | 6 | 2 | 1(2) | | 2,2',3,4,4',5-hexachlorobiphenyl | 7.25 |
| 79 | 176 | 7 | 4 | 1,2 | | 2,2',3,3',4,6,6'-heptachlorobiphenyl | 9.88 |
| 80 | 137(130) | 6 | 2 | 1(2) | | 2,2',3,3',4,5'-hexachlorobiphenyl | 9.88 |
| 81 | 138 | 6 | 2 | 1,2 | | 2,2',3,4,4',5'-hexachlorobiphenyl | 129.14 |
| | (160) t | 6 | | 2 | NA | | |
| | 163 t | 6 | 2 | 1,2 | NA | 2,3,3',4',5,6-hexachlorobiphenyl | |
| 82 | 158 | 6 | 2 | 1,2 | | 2,3,3',4,4',6-hexachlorobiphenyl | 15.65 |
| 83 | 129 | 6 | 2 | 1,2 | | 2,2',3,3',4,5-hexachlorobiphenyl | 8.56 |
| 84 | 126 t | 5 | 0 | 1,2 | NA | 3,3',4,4',5-pentachlorobiphenyl | |
| 85 | 178 | 7 | 3 | 2 | | 2,2',3,3',5,5',6-heptachlorobiphenyl | 10.04 |
| 86 | 166 t | 6 | 2 | 1 | NA | 2,3,4,4',5,6-hexachlorobiphenyl | |
| 87 | 175 | 7 | 3 | 1,2 | NA | 2,2',3,3',4,5',6-heptachlorobiphenyl | |
| 88 | 182 t | 7 | 3 | 1,2 | C | 2,2',3,4,4',5,6'-heptachlorobiphenyl | 55.24 |

Table 1. Composition of A1111 Mixture for PCB Congeners (ng/mL concentration) analyzed on DB-5 Capillary Column

| Peak No. | IUPAC No. | No. of Cl | # ortho Cl | Refs. for Assignment | Congener Integration | Description/Structure | Concentration in A1111 @ 4000 ng/mL |
|---|---|---|---|---|---|---|---|
| | 187 | 7 | 3 | 1,2 | C | 2,2',3,4',5,5',6-heptachlorobiphenyl | 29.71 |
| 89 | 183 | 7 | 3 | 1,2 | | 2,2',3,4,4',5',6-heptachlorobiphenyl | 22.66 |
| 90 | 128 | 6 | 2 | 1,2 | | 2,2',3,3',4,4'-hexachlorobiphenyl | 5.18 |
| 91 | 167 | 6 | 1 | 1,2 | | 2,3',4,4',5,5'-hexachlorobiphenyl | 5.46 |
| 92 | 185 | 7 | 3 | 1,2 | | 2,2',3,4,5,5',6-heptachlorobiphenyl | 46.25 |
| 93 | 174 | 7 | 3 | 1,2 | | 2,2',3,3',4,5,6'-heptachlorobiphenyl | 27.44 |
| 94 | 177 | 7 | 3 | 1,2 | | 2,2',3,3',4',5,6-heptachlorobiphenyl | 28.64 |
| 95 | 171 | 7 | 3 | 1,2 | C | 2,2',3,3',4,4',6-heptachlorobiphenyl | |
| | 202 t | 8 | 4 | 1,2 | C | 2,2',3,3',5,5',6,6'-octachlorobiphenyl | |
| 96 | 156 | 6 | 1 | 1,2 | C | 2,3,3',4,4',5-hexachlorobiphenyl | |
| 97 | 173 | 7 | 3 | 1,2 | NA | 2,2',3,3',4,5,6-heptachlorobiphenyl | 7.86 |
| 98 | 157 t | 6 | 1 | 1,2 | C | 2,3,3',4,4',5'-hexachlorobiphenyl | |
| | 201 | 8 | 4 | 2 | C | 2,2',3,3',4,5',6,6'-octachlorobiphenyl | |
| | 204 IS | 8 | 4 | 2 | | 2,2',3,4,4',5,6,6'-octachlorobiphenyl | 5.16 |
| 99 | 172 | 7 | 2 | 1,2 | | 2,2',3,3',4,5,5'-heptachlorobiphenyl | |
| | 197 t | 8 | 4 | 1,2 | NA | 2,2',3,3',4,4',6,6'-octachlorobiphenyl | 80.18 |
| 100 | 180 | 7 | 2 | 1,2 | | 2,2',3,4,4',5,5'-heptachlorobiphenyl | 4.82 |
| 101 | 193 | 7 | 2 | 1,2 | | 2,3,3',4,',5,5',6-heptachlorobiphenyl | 3.65 |
| 102 | 191 | 7 | 2 | 1,2 | | 2,3,3',4,4',5',6-heptachlorobiphenyl | 3.28 |
| 103 | 200 | 8 | 4 | 2 | | 2,2',3,3',4,5,6,6'-octachlorobiphenyl | |
| 104 | 169t | 6 | 0 | 1,2 | NA | 3,3',4,4',5,5'-hexachlorobiphenyl | 41.21 |
| 105 | 170 | 7 | 2 | 1,2 | C | 2,2',3,3',4,4',5-heptachlorobiphenyl | |
| | 190 m | 7 | 2 | 1,2 | C | 2,3,3',4,4',5,6-heptachlorobiphenyl | |
| 106 | 198 t | 8 | 3 | 1,2 | NA | 2,2',3,3',4,5,5',6-octachlorobiphenyl | 17.28 |
| 107 | 199 | 8 | 3 | 2 | | 2,2',3,3',4,5,5',6'-octachlorobiphenyl | 18.35 |
| 108 | 196 | 8 | 3 | 1,2 | C | 2,2',3,3',4,4',5,6'-octachlorobiphenyl | |
| | 203 | 8 | 3 | 1,2 | C | 2,2',3,4,4',5,5',6-octachlorobiphenyl | |
| 109 | 189 m | 7 | 1 | 1,2 | NA | 2,3,3',4,4',5,5'-heptachlorobiphenyl | 7.18 |
| 110 | 195 | 8 | 3 | 1,2 | C | 2,2',3,3',4,4',5,6-octachlorobiphenyl | |
| | 208 | 9 | 4 | 1,2 | C | 2,2',3,3',4,5,5',6,6'-nonachlorobiphenyl | |
| 111 | 207 m | 9 | 4 | 1,2 | NA | 2,2',3,3',4,4',5,6,6'-nonachlorobiphenyl | 15.40 |
| 112 | 194 | 8 | 2 | 1,2 | | 2,2',3,3',4,4',5,5'-octachlorobiphenyl | |
| 113 | 205 | 8 | 2 | 1,2 | NA | 2,3,3',4,4',5,5',6-octachlorobiphenyl | |
| 114 | OCN IS | | | | | (IIS) octachloronaphthalene | |
| 115 | 206 t | 9 | 3 | 1,2 | NA | 2,2',3,3',4,4',5,5',6-nonachlorobiphenyl | |
| 116 | 209 | 10 | 4 | 1,2 | | 2,2',3,3',4,4',5,5',6,6'-decachlorobiphenyl | |
| | | | | | | Total PCB = | 3977.91 |

Notes: t-trace amount; m-minor amount; IS-internal std;
C--combined; NA-not analyzed.

References: (1) Mullins etal. ES&T, 18(6), 1984; Schultz etal. ES&T, 23(7). 1989; Vetter etal. Chemosphere, 23(2), 1991.

# Chromatogram

Sample Name : A1111 @ 1000 ng/ml EACH AROCLOR
FileName : C:\TC4\LANLFISH\H15U007.RAW
Method : H15D.MTH
Start Time : 0.00 min End Time : 118.00 min
Scale Factor: 0.0 Plot Offset: 0 mV

Sample #: 7
Date : 9/9/98 02:50 PM
Time of Injection: 5/18/98 09:13 PM
Low Point : 0.00 mV High Point : 100.00 mV
Plot Scale: 100.0 mV



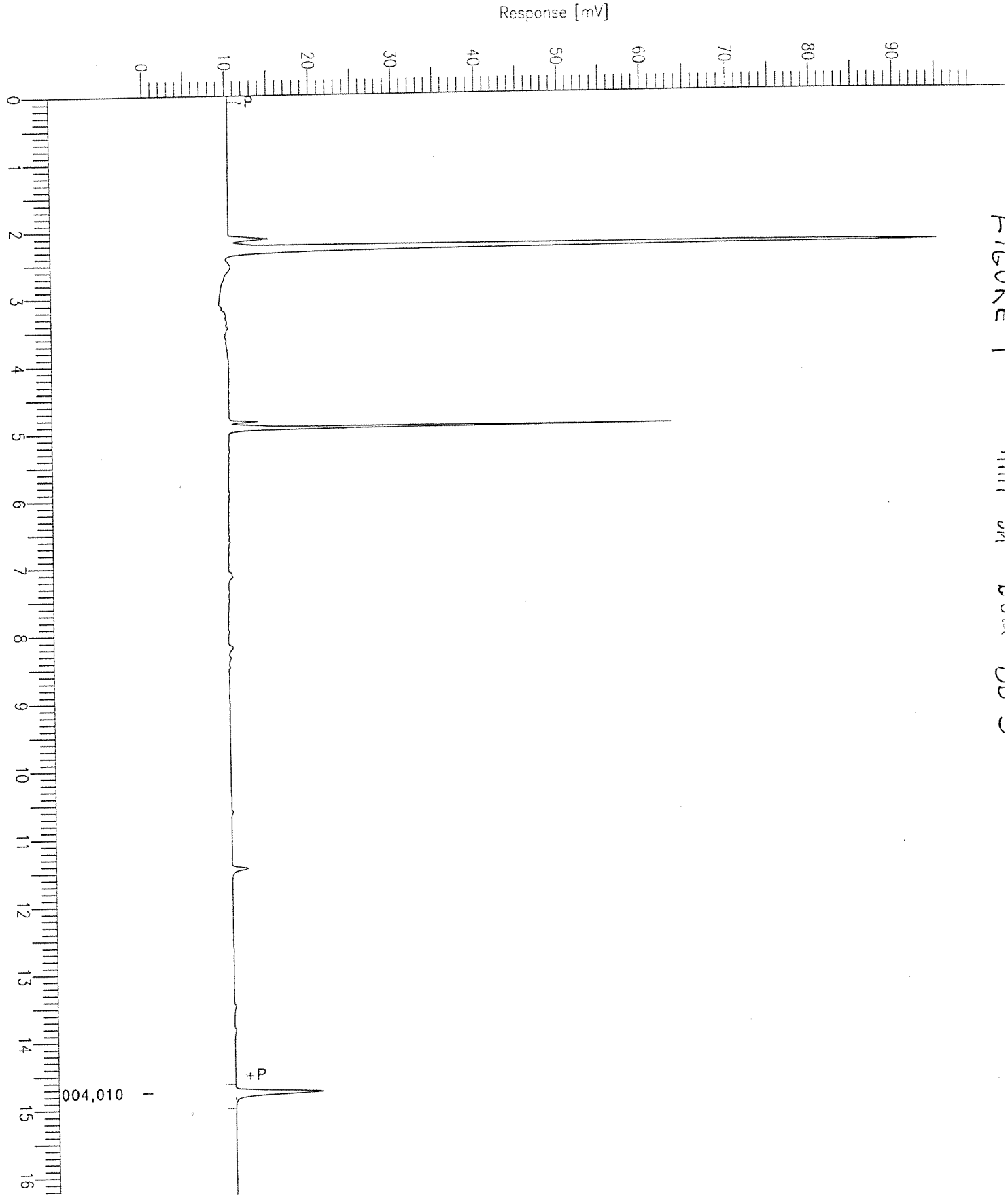
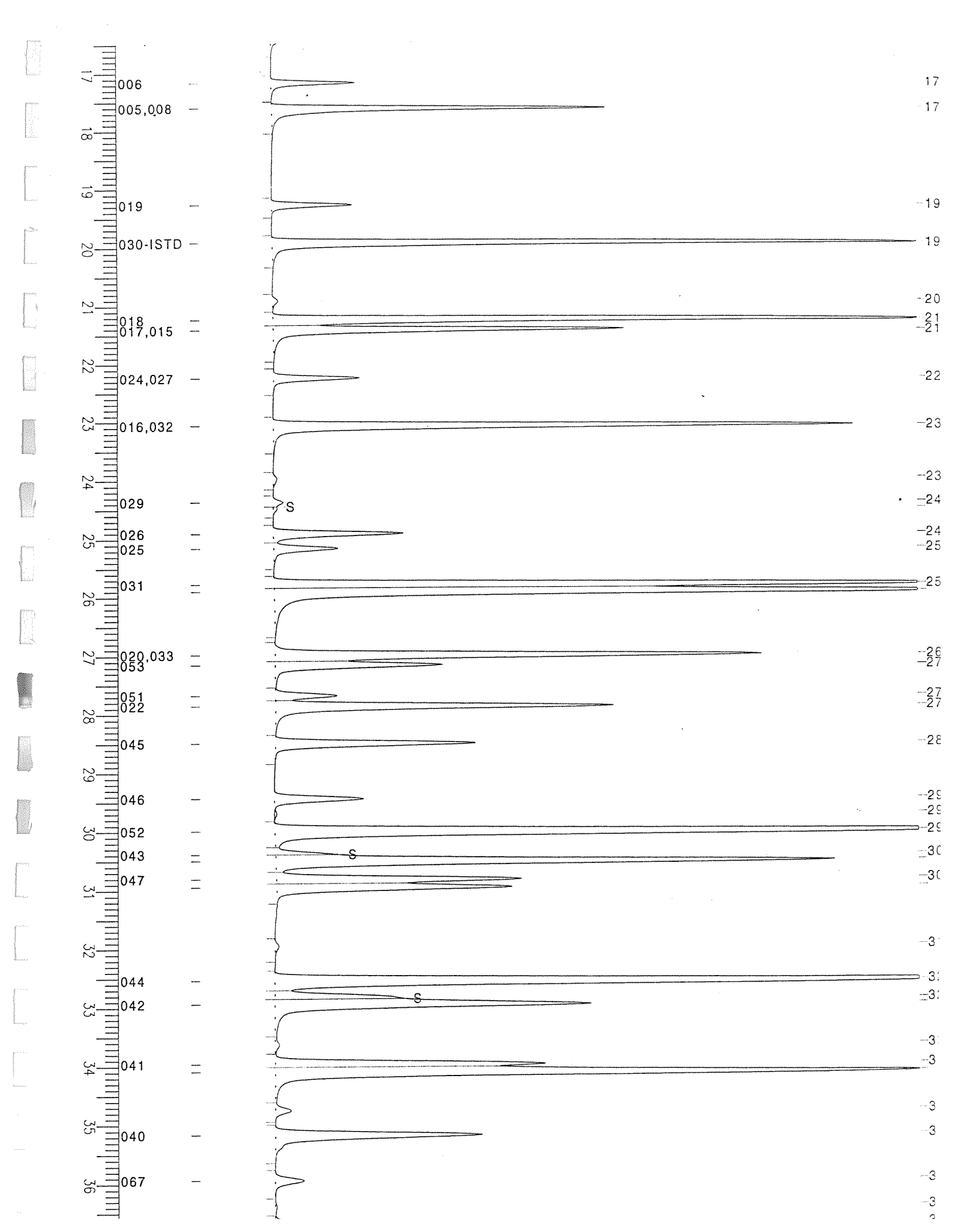



FIGURE 1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016,032
029
026
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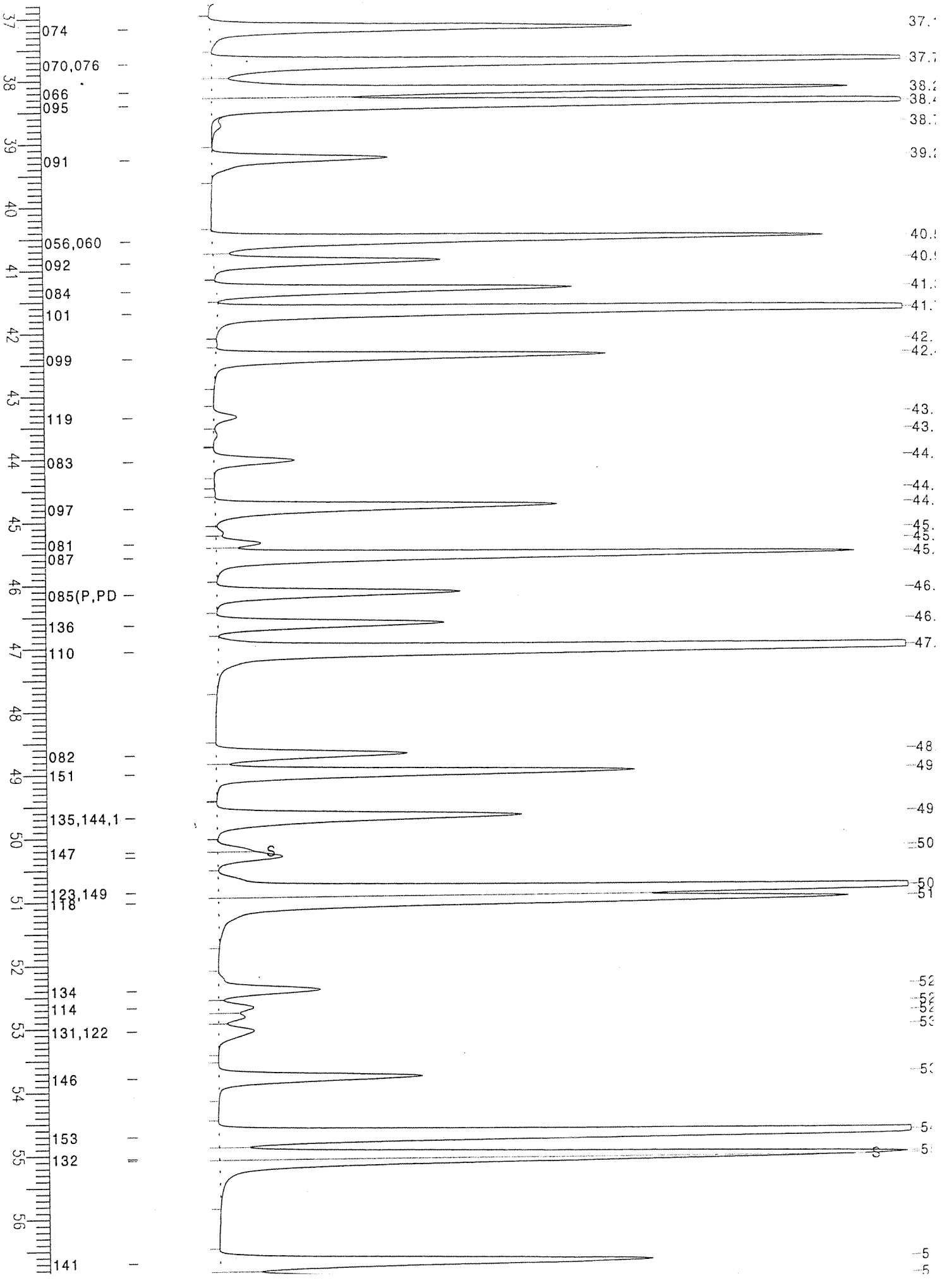
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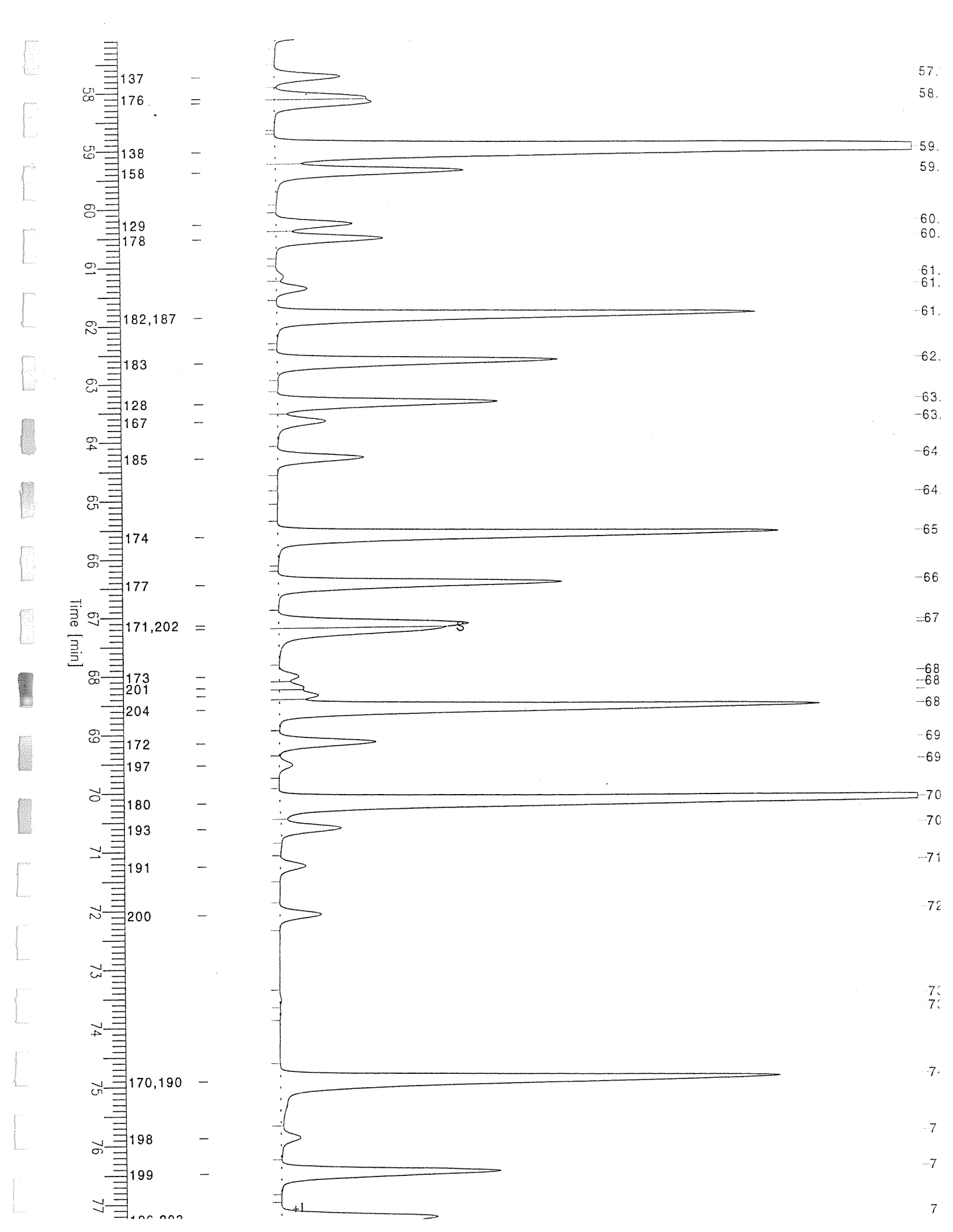
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138
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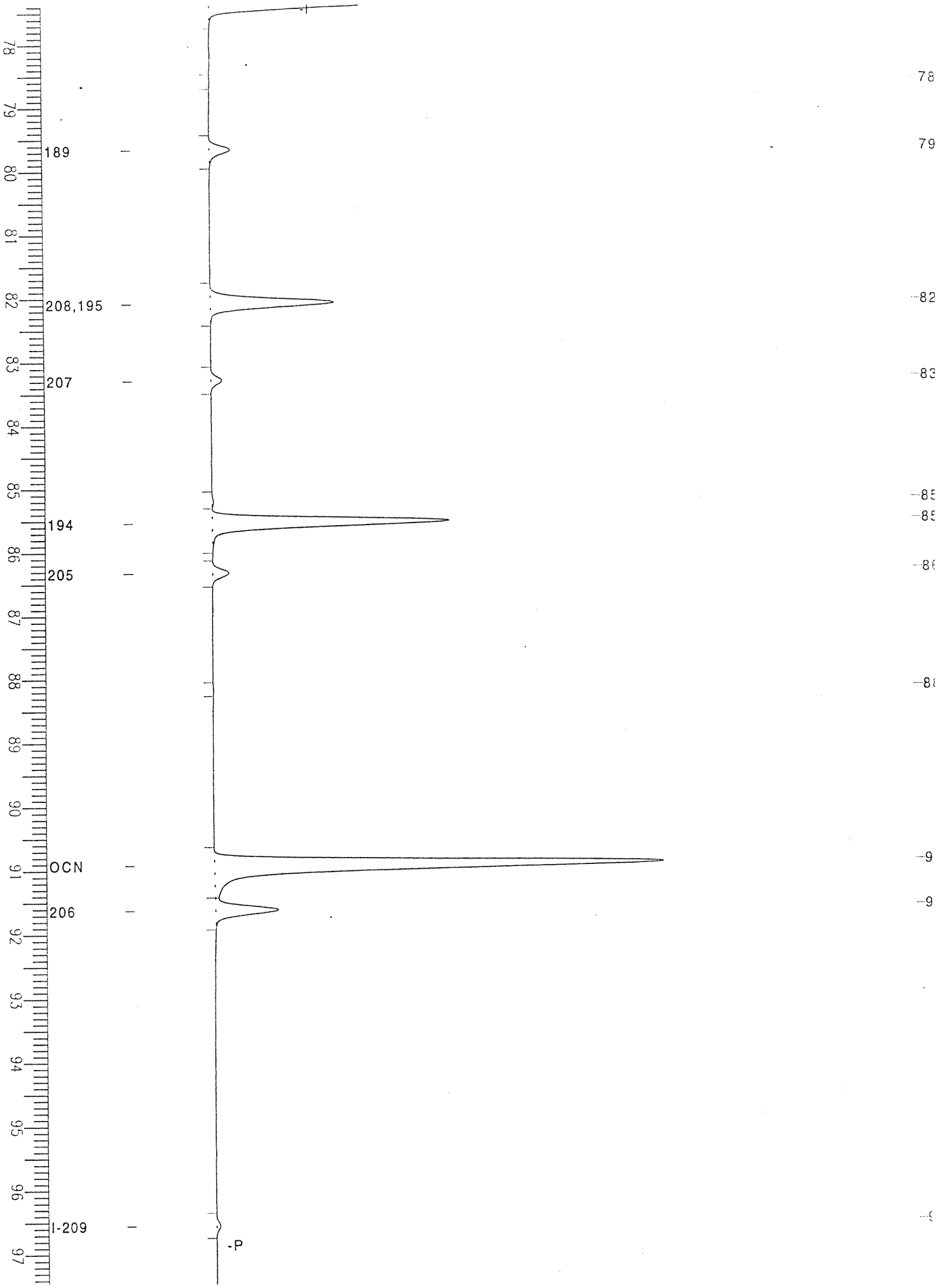
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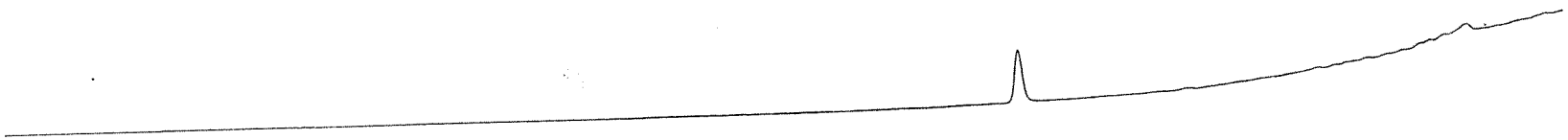

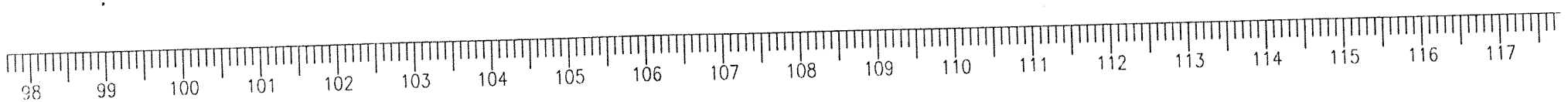
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# APPENDIX E-14

# MINIMUM QUALITY ASSURANCE STANDARDS FOR TRACE ORGANIC RESIDUE ANALYSIS [USGS-TILLITT]

Minimum Quality Assurance Standards
for Trace Organic Residue Analysis

Table of Contents

## Introduction

The following Proposal briefly summarizes the Organic trace Residue Quality Assurance Program Requirements of the United States Fish and Wildlife Service.

The Minimum Quality Assurance Standards are organized according to a general analytical strategy. Specific factors are identified which assure reliable measurements and which are common to many analytical trace residue processes. A detailed analytical protocol for each methodology is beyond the scope of this document; instead an attempt has been made to generalize Quality Assurance considerations. This attempt has resulted in these minimum acceptable criteria for obtaining and relating useful and reliable information.

These Minimum Quality Assurance Standards are designed to be applied only to routine organic trace analyses, and not to analytical research and development. Nor do these standards address the quality assurance necessary for the validation of analytical methods.

The approach taken in the development of these Standards, and the philosophy of quality assurance in scientific measurements, has been adapted from the authors cited in the bibliography.

# I. Summary of Minimum Quality Assurance Standards

## II. Sample Receipt

### A. Sample Inspection

#### A.1. Transport and Receipt

1. Sample condition must be verified upon receipt.
2. All special requirements for sample storage should be included in the accompanying sample documentation.
3. All samples must be inventoried upon receipt.

#### A.2. Chain of Custody

1. All documentation must be contained in, or refer to, the chain of custody form.
2. The sample inventory determined by the analytical facility will be verified by the submitter.
3. Each sample must be given unique identification, to be used for all subsequent tracking.

#### A.3. Field Blanks

1. Negative Control samples (Field Blanks) simulating the sample and not containing the analyte must be procured from the same source and submitter.
2. Other Negative Control samples (Synthetic or Simulated Blanks) may be substituted at the discretion of the analyst.

### A.4. Field Control Samples

Negative Control samples must be submitted to establish the significance of any determinations made at sampling sites.

### A.5. Field Spike Samples

Replicate Control samples should be spiked with analytes in the field.

## B. Invalid Sample Action

### B.1. Reporting

1. Samples judged invalid must be reported to the sample submitter in writing.
2. Samples may be included on the inventory upon resolution of conflicts,
3. All samples of unresolved validity will be designated for disposal, or returned to the submitter.

### B.2. Analysis

1. Any invalid sample may be analyzed at the discretion of the analyst and the sample submitter.
2. Results of invalid sample analyses must be clearly noted in the final report.

## C. Sample Storage

### C.1. Container Suitability

All containers used to collect, transfer, transport, and store samples must be demonstrated not to alter the matrix or analyte by:

1. Validation of container types during method development
2. Use of Field Matrix Blanks, and Field Matrix Spikes

### C.2. Storage Conditions

1. Samples must be stored under conditions specified by the accompanying sample documentation.
2. All samples must be stored in a location free from contamination by analytes or possible interferents.
3. Storage must be in a secure environment.
4. Sample storage locations must be discernable by the sample-tracking method.

### C.3. Holding Time

1. Samples must be held no longer than prescribed by the analytical method.
2. Any sample exhibiting atypical characteristics must be treated as an invalid sample.
3. All samples exceeding the holding time must be treated as invalid samples.

## D. Sample Disposal

### D.1. Archiving Samples

1. Sufficient original portions of all samples will be archived.
2. Unless otherwise stated in the protocol samples will be archived for a period of five years.

D.2. Documentation

1. At the end of the sample archival period the submitter will be notified, and requested to decide between sample disposal or return of the samples.
2. The Quality Assurance documentation is updated to indicate final disposition of the sample.

D.3. Disposal

All samples will be disposed of according to Good Laboratory Practice Guidelines and in accordance with all Federal Standards.

## III. Sample Preparation

1. Significant steps of treatments must be written as Standard Operating Procedures and strictly adhered to.
2. Each chemical or instrumental treatment a sample is subjected to must be documented.
3. Quality Assurance samples must be subjected to the same preparation steps as the analytical samples.

## IV. Analytical Measurement Processes

### A. Calibration Standards

1. Chemical standards must be of the highest purity obtainable.
2. Chemical standards must be stored under appropriate conditions to protect against loss or degradation.
3. All documentation associated with the chemical must be retained in accessible files.
4. All steps in standard preparation must be documented and the results of any calculations verified, and signed.

5. Superstock solutions of standard materials must be logged and their use tracked.
6. All stock and working solutions must refer to the superstock solution and ultimately to the standard material inventory.
7. No standard materials, stock or working solutions may be used after their expiration date without reestablishing their quality.
8. Records of preparation and dilutions of standards must be included as part of the Quality Assurance documentation.

## B. Instrument Calibration

1. The instrument must be demonstrated to be in suitable condition for performing analyses.
2. The instrument maintenance log must be updated to reflect all tests performed.
3. All instrumental calibrations must be performed at a minimum of three concentration levels.
4. Each standard will be analyzed in triplicate.
5. Suitable regression analysis of the net signal on analyte concentration will be performed to calibrate instrument response.

## C. Analytical Working Range

1. Calibration and measurements must be performed under the same instrumental and chemical conditions.
2. Calibration will be performed at the beginning of, and verified at the end of each sample block.
3. A mid-range standard must be analyzed after each tenth sample.
4. The concentration range of the analyte in the samples must be bracketed by the calibration standards.
5. No data will be reported beyond the calibration range of

the method. If inadequate sample exists for reanalysis a properly noted estimate may be reported.

D. Limits of Detection and Quantitation

1. The limit of detection (LOD) will be estimated from the standard deviation of seven low-level spiked replicate measurements.
2. The limit of quantitation (LOQ) will be estimated from the standard deviation of seven low-level spiked replicate measurements.
3. Estimates of LOD and LOQ will be included in the Quality Assurance report.

V. Quality Assurance of Chemical Measurements

A. Quality Control

1. Principles of good laboratory practice will be developed and strictly adhered to.
2. Standard operating procedures will be consistently used.
3. Carefully designed protocols for specific measurement programs will be established and strictly adhered to.

B. Quality Assessment

1. One procedural method blank must be analyzed per matrix type per sample block, or at frequency of 5% of the number of sample analyses, whichever is greater.
2. One procedural method spike of a negative field or laboratory control sample, within the range of expected analyte concentrations, must be analyzed for each matrix type per sample block, or at frequency of 5% of the number of sample analyses, whichever is greater.
3. One positive control sample, positive laboratory reference sample, or positive certified reference material must be

analyzed for each matrix type per sample block, or at frequency of 5% of the number of sample analyses, whichever is greater.

4. One sample must be analyzed in duplicate per matrix type per sample block, or at frequency of 5% of the number of sample analyses, whichever is greater.

Control charts will be maintained and regularly updated for the following Quality Assurance parameters:

1. Procedural method blank measurements
2. Procedural method spike recoveries
3. Positive control samples, positive laboratory reference samples, or positive certified reference materials
4. Duplicate samples

Separate control charts will be constructed for each analyte in each matrix. The large numbers of analytes in multicomponent analyses may limit the number analytes that can reasonably be charted. In this instance suitable analytes will be determined during method validation to represent the full suite of analytes.

Warning limits for establishing statistical control charts of the various measurements are defined by this Quality Assurance program in the following manner:

1. Measurements greater than 2 standard deviations from the mean value will be considered to be in a warning state.
2. Measurements greater than 3 standard deviations from the mean value will be considered to be out-of-control.

## VI. Reporting Requirements

The following information must be included in all final laboratory reports as appropriate:

1. Sufficient documentation to reconstruct an audit-trail
2. Description of the analytical methodologies used
3. Description of all confirmatory evidence
4. Method validation information: limits of detection and quantitation, precision or relative percent difference, and recovery data
5. Quality Assurance documentation
6. Quality Assurance Statement of measurement confidence
7. Results must be expressed in proper units
8. Appropriate numbers of significant figures must be used
9. Average values, including the number of replicates used and an estimate of precision

## VII. Quality Assurance Records

### A. Analytical Reports

The following components of the analytical process will be archived:

1. All information specifically requested in the design of the investigation's analytical protocol
2. All sample and reference material data
3. Aliquants of all remaining samples, extracts, and reference materials
4. All records of instrument maintenance, calibration, or modification

### B. Data Archiving

1. All data for the Quality Assurance program must be archived
2. Samples and Quality Assurance records must be cross-indexed to enable reconstruction of the audit-trail

C. Laboratory Maintenance Records

This documentation will include:

1. Information necessary to determine which samples are affected by any specific maintenance item
2. Sufficient detail to reconstruct the maintenance events
3. All calibration or optimization information performed on instrumentation

## II. Sample Receipt

Sampling is the most important single concept of an analytical scheme. Providing environmental samples which adequately represent the situation under study is essential for obtaining useful analytical data of sufficient quality for decision making. Insuring the adequacy of sample collection, labeling, preservation, and transport to the analytical laboratory, and training personnel in the Quality Assurance aspects of sample receipt are vital components of the analytical scheme.

### A. Sample Inspection

Exclusive of a protocol for sampling, sample management is the foremost Quality Assurance endeavor to be undertaken by the analytical facility. Development of a laboratory protocol for receiving, tracking, storing, and disposing samples critically impacts the entire analytical Quality Assurance program. The utility and quality of all analytical data used in decision-making pivots on the reliability of the sampling effort in the field and in the laboratory.

#### A.1. Transport and Receipt

Sample conditions must be verified upon receipt. The condition of samples during transport must be evaluated and conditions affecting sample integrity such as improper packing or containers, refrigeration, protection from light or air, or handling must be documented. All special requirements for sample storage should be included in the accompanying sample documentation. Sample storage from receipt until log-in should follow all recommendations of the submitter. All samples must be inventoried upon receipt and verified for agreement in number, type, identification, and condition.

When specialized sample handling will be required, the sample submitter must consult with the analytical facility and provide written guidelines prior to submitting samples.

## A.2. Chain of Custody

All documentation pursuant to the inventory, handling, storage, and analysis of samples must be contained in a chain of custody. The chain of custody will form an audit-trail based on the sample inventory produced by the sample submitter. Any discrepancies or ambiguities in the samples, accompanying inventory, or the chain of custody must be resolved upon receipt. The sample inventory, as determined by the analytical facility, will be returned to the submitter for verification and signature before sample log-in.

Each sample must be given unique identification by the analytical facility which will be used for all further tracking. The chain of custody must maintain an audit-trail for each sample at each step of the analysis specifying: analyst, location, handling and treatment; and point to the location of all analytical and quality control and quality assessment information associated with each sample.

## A.3. Field Blanks

Whenever possible Negative Control samples (Field Blanks) simulating the sample and not containing the analyte must be procured from the same source and submitter. In the event that a representative field blank is not available, a synthetic or simulated blank may be substituted at the discretion of the analyst.

## A.4. Field Control Samples

Negative Control samples must be submitted to establish the significance of any determinations made at sampling sites. Control sites, deemed to be free of the contaminants of interest yet similar in

all other respects, should be used. The importance of samples from such control sites increases as the levels of contaminants in the control and sample sites approach each other; more samples will be required to gain the higher precision necessary to distinguish smaller differences in analyte concentration. Failure to submit adequate numbers of control samples indicates flaws in the sampling design and may preclude production of meaningful analytical results.

### A.5. Field Spike Samples

Replicate samples taken from control sites and spiked with analytes are the best approximation to naturally-incurred residues. Recovery of analytes from samples spiked in the field evaluates sample handling, transport, storage, and analytical method performance. Unlike laboratory spiking, where analytes are spiked and then immediately extracted, field spiking enables estimation of analyte recovery based on longer equilibration time. The use of field spiked samples should be integrated into the design of each sampling protocol.

## B. Invalid Sample Action

Receipt of invalid samples: samples which are damaged, stored or packaged improperly, or misidentified in the accompanying inventory; requires notification of the submitter. Notification is followed by appending the suspect samples to the inventory either for analysis, or for disposal.

### B.1. Reporting

Immediately upon discovery, samples judged invalid for reasons of damage, impropriety, or misidentification, must be reported to the sample submitter in writing to await disposition. If the above conflicts can be resolved, and with the concurrence of the submitter, the samples may be included on the sample inventory. If conflicts can not be resolved or the sample submitter so requests, the samples will be designated for disposal

or returned to the submitter; and all Quality Assurance documentation emended.

### B.2. Analysis

At the discretion of the analyst any invalid sample may be analyzed. The analytical time and cost, as well as the irreplaceability, uniqueness, and costs associated with the sample must be considered. Invalid samples destined for analysis must be clearly labeled as such, and all Quality Assurance documentation must indicate the nature of the sample to preclude any later misinterpretation of the results.

## C. Sample Storage

Samples must be collected, transported, and stored in containers which are demonstrated not to alter the matrix and analyte in a manner which would affect the identification or determination of the concentration of the analyte. Any special conditions for transport or storage must be clearly indicated by the sample submitter in the accompanying sample documentation. The holding time which sample integrity can be maintained must have been determined during method development. If longer holding times are expected then samples of archived positive controls which predate any analytical samples should be analyzed regularly to increment the established holding time.

### C.1. Container Suitability

All containers used to collect, transfer, transport, and store samples must be demonstrated not to alter the matrix or analyte in any manner which would affect the identification or determination of the concentration of the analyte. Containers must be evaluated during validation of the method and shown to be suitable. Each set of sample containers must be demonstrated to be free of interferences or analytes prior to sampling. Field Matrix Blanks and Field Matrix Spiked samples will be used to demonstrate the adequacy of the sample containers.

Alternatively, sufficient numbers of containers must be provided upon sample receipt to determine whether or not the containers are in compliance, after the fact. The sample submitter must be fully cognizant of the consequences of possible failure.

### C.2. Storage Conditions

Samples must be stored under conditions specified by the accompanying sample documentation, with regard to temperature and protection from light or air, or other parameters. All samples must be stored in a location free from contamination by analytes or possible interferents. Adequate conditions for sample storage must be determined prior to sample receipt and demonstrated during validation of the analytical method.

Storage must be in a secure environment to avoid inadvertent losses, tampering, contamination, or misidentification of samples. The location of each sample during storage must be discernable by the sample-tracking methods in use.

### C.3. Holding Time

Samples must be held under conditions which adequately insure the integrity of the sample for a period no longer than that prescribed by the analytical method. This time is measured from sample collection, through transport, receipt, and up to sample preparation. In addition, any sample which exhibits atypical characteristics is suspect even during the normal holding time and must be treated as an invalid sample. All samples exceeding the holding time must be treated in a manner analogous to invalid samples.

## D. Sample Disposal

All samples will be retained after issuance of the final analytical report as part of a Quality Assurance archive of samples, data, and

quality control and assessment information. Samples will be archived for a period of five years, unless otherwise stated in the experimental protocol, and will be contingent upon the ability to maintain sample integrity. All samples will be disposed according to Good Laboratory Practice Guidelines and in accordance with all Federal Standards.

## D.1. Archiving Samples

Sufficient original portions of all samples will be archived to allow confirmatory analyses at a later date. Samples will be archived for a period of five years. There are two exceptions to this archival period: samples whose integrity can not be insured for five years will be subject to archival for the longest time it is possible to insure integrity; samples constituting part of long-term monitoring programs must be maintained as stated in the monitoring protocol.

## D.2. Documentation

At the end of the sample archival period two steps need to be completed to terminate responsibility for the sample: notification to the submitter that the samples are destined for disposal, and updating the final Quality Assurance documentation to indicate that the samples are no longer available for confirmation.

a) Notification to Submitter

At the end of the sample archival period the original sample submitters or their agents must be notified of the intent of the analytical facility to dispose of samples. A written statement must be sent to the submitter containing the sample inventory. The submitter must reply, stating whether the samples are to be returned or disposed by the analytical facility, within ninety days.

b) Closing Quality Assurance Reports

Upon receipt of the declaration from the sample submitter the samples must be verified to safeguard against misidentifications are either returned or removed for disposal. The declaration becomes a record of the final disposition of the samples and recorded in the Quality Assurance information.

### D.3. Disposal

All samples will be disposed of according to Good Laboratory Practice Guidelines and in accordance with all Federal Standards. All information necessary for proper disposal of the samples must be included in the Quality Assurance documentation.

## III. Sample Preparation

Samples often must require several physical or chemical treatments prior to making the actual analytical determination. Each chemical or instrumental treatment increases the complexity and introduces additional sources of bias, variance, contamination, and loss. Sample preparation should be planned carefully and validated to minimize the effects of handling on the analytical determination. Significant steps of treatments must be standardized enabling sample preparation to be efficiently and reproducibly performed. Each treatment must be documented for each sample to maintain an audit-trail which can be reconstructed if the need arises. Quality Assurance samples must be subjected to each of these same preparation steps.

The analyst is responsible for recognizing the risks involved with each form of treatment and must take appropriate action for each risk. Actions may include the removal of potentially detrimental steps, or alteration of Standard Operating Procedures (SOP) and larger sections of the analytical protocol. Whenever possible the analyst should strive to remove or minimize interferences. The final recourse is reporting the risks and interferences present in the analyses, documenting all steps

taken to minimize problems, and noting all pertinent quality control and assessment information necessary to make informed decisions using the analytical data.

Treatments which require a high degree of reproducibility, such as chromatographic enrichment steps, can be treated in a manner analogous to analytical measurement processes discussed below.

## IV. Analytical Measurement Processes

Analytical determinations must be performed only with validated procedures. Procedures should be selected which provide the required sensitivity and precision. Procedures should also be selected which provide maximum recoveries of analytes and which minimize random and systematic errors, as well as errors introduced by contamination. Calibration and quality control steps must be incorporated into each procedure to minimize random and systematic errors. Sufficient numbers of typical samples must be analyzed to demonstrate the competency of the laboratory and the adequacy the methodology.

Quality Assurance of analytical determinations requires that both samples and controls be processed similarly at each step of the analytical procedure. Calibration standards, field or laboratory blanks, spiked field or laboratory blanks, and reference samples (when available) must be associated with each analytical sample block. Calibration standards are requisite for quantitation of the analytes. Results of analyses of field or laboratory blanks, and spiked blanks will be used to establish that the analytical system is in a state of control; and the results of reference samples will be used to assessment the quality of the analytical data.

### A. Calibration Standards

The reliability of the materials used is critical to establishing the accuracy of calibration for analytical instrumentation. Chemical

standards must be of the highest purity obtainable and all documentation associated with the chemical must be retained in the Quality Assurance records, and readily accessible. Certification of purity, chemical lot number, and safety and handling information must as complete as possible to insure reconstruction of the Quality Assurance audit-trail. Standard material log-in, security, and tracking must be included as part of the Quality Assurance program. Chemical standards must be stored under conditions which protect against loss [by volatilization] or degradation.

Analytical standards must be carefully prepared using standard operating procedures and calibrated balances and volumetric ware. All steps in standard preparation must be documented and the results of any calculations verified, and signed. Superstock solutions of standard materials must be logged and their use tracked. All stock and working solutions must refer to the superstock solution and ultimately to the standard material. No standard materials, stock or working solutions may be used after their expiration date. Records of preparation and dilutions of standards must be included as part of the Quality Assurance program.

## B. Instrument Calibration

Prior to concentration calibration, the instrument must be demonstrated to be in suitable condition for performing analyses. The instrument maintenance log must be updated to reflect all tests performed to insure adequate introduction, separation, and detection of the analytes. Quality Assurance documentation should refer to maintenance log entries to substantiate proper functioning of the instrument. All instrumental calibrations must be performed at a minimum of three concentration levels, with each standard analyzed in triplicate. Calibration at additional concentration levels is strongly recommended. Suitable regression analysis of the net signal on analyte concentration will be performed to calibrate instrument response. Calibration must be performed under the same set of conditions which will be used for the analysis of samples. Calibration standards, instrument response data, calculations, and all other data used to establish the proper functioning

of the analytical instrument must be included in the Quality Assurance documentation.

## C. Analytical Working Range

Calibration and measurements must be performed under the same instrumental and chemical conditions. The frequency of calibration will be determined by the stability of the instrument, the accuracy required by the investigation, and by the protocol developed during the validation of the method. The expected concentration range of the analyte in the samples must be bracketed by the concentrations of the calibration standards. Calibration will be performed at the beginning and verified at the end of each sample block. A mid-range standard must be analyzed after each tenth sample, or more frequently. No data will be reported beyond the calibration range of the method.

## D. Limits of Detection and Quantitation

The limit of detection (LOD) is defined as the lowest concentration level that can be determined to be statistically different from a blank. Based on the difference between the analyte signal, the blank signal, and the standard deviation (o) of these measurements, the LOD is defined (for the purposes of these Quality Assurance standards) as 3o, corresponding to a 99% confidence level for determining that a signal from a sample is different from that of a blank. The method detection limit (MDL) is defined as the lowest concentration of an analyte that can be determined reliably in a blank or a sample and is numerically equivalent to the LOD as the blank signal approaches zero.

The limit of quantitation (LOQ) is defined as the level above which analytical results may be determined with a specified degree of confidence. The confidence associated with measurements above the LOD increases with increasing analyte signal and defining a LOQ is useful for describing the lower limit of the useful range of analytical methodologies. The LOQ is defined (for the purposes of these Quality

Assurance standards) as 10o, corresponding to an uncertainty of ±30% in the measured value at the 99% confidence level.

For the purposes of this Quality Assurance program the method limits of detection and quantitation are estimated as follows:

1. The limit of detection (LOD) will be estimated from the standard deviation of seven low-level spiked replicate measurements.

2. The limit of quantitation (LOQ) will be estimated from the standard deviation of seven low-level spiked replicate measurements.

Standards for reporting data at low levels of determination are defined as follows: signals below the LOD (3o) will be reported as "not detected", with the estimation of the LOD given in parentheses. Signals in the region of less-certain quantitation (3o to 10o) will be reported as "detected" with the LOD given in parentheses.

The quality and usefulness of data below the limit of detection have associated high uncertainties, which may approach the reported values. Confirmatory identifications of analytes at these levels are difficult. Only the selectivity of the analytical method can be used to establish the presence of the analyte. It must be stressed that quantitative interpretation, decision-making, and regulatory actions be limited to data at or above the limit of quantitation.

Limits of detection and quantitation must be determined during the validation of each analytical method for each matrix. These values depend upon the precision attainable by the laboratory and the variability of the matrix. Estimates of LOD and LOQ will be determined for each sample based on the knowledge and experience of the analyst and past history of sample behavior, integrating the effects of possible interferences, background, and random noise.

## V. Quality Assurance of Chemical Measurements

Quality Assurance incorporates essential decision-making criteria into every phase of the chemical measurement process: protocols, procedures, methods, and techniques. The outcome of an adequate Quality Assurance program is production of valid results.

The purpose of the Quality Assurance program is to reduce measurement errors to predefined limits and to assure that the analytical results have a high probability of being of acceptable quality. Quality Assurance consists of two parts: quality control, the procedures used to control measurement errors; and quality assessment, the procedures used to insure that all analytical processes are functioning within acceptable limits.

Quality Assurance of chemical measurements is limited to assuring analytical precision unless the true value of a sample is known.

Precision can be statistically treated and controlled by monitoring the degree to which measurements from the same or similar samples differ from each other.

Accuracy can only be determined using standard reference materials, chosen as well-behaved samples and certified by diverse methodologies. Unless the true value of the analyte is known only estimates of bias can be made. The quality assessment program attempts to make such estimates based on recovery of analytes from spiked samples. Though spiking does not fully simulate the conditions of naturally incurred analytes, it can pose a valuable tool for establishing the credibility of the data.

### A. Quality Control

The quality control (QC) portion of the Quality Assurance program manages mechanisms to detect and correct measurement problems and monitor reproducibility of analytical measurements.

The components of quality control are the development of and strict adherence to principles of good laboratory practice; consistent use of standard operating procedures; and establishment of and adherence to carefully designed protocols for specific measurement programs. The result of quality control is a measurement system operating in a state of statistical control, where errors have been decreased to predefined levels and have been characterized statistically.

Quality control operates at two levels: constant inspection of data to detect real or apparent problems; and compilation of data over longer times providing the information necessary for the quality assessment program.

## B. Quality Assessment

The quality assessment (QA) portion of the Quality Assurance program demonstrates the state of statistical control over all analytical processes over time.

The components of quality assessment are the development of and strict adherence to techniques used to assess the quality of the measurement process and the results. The most basic precept of quality assessment is the establishing of methods to chart the results of the same or similar samples over time.

Control charting results of analytical measurements for the same or similar samples in a systematic and well planned manner is used to determine whether statistical control has been achieved and to monitor the precision of analytical measurements on a continuing basis. The corrective action steps required when out-of-control situations arise must be included in the Quality Assurance plan and followed.

Accuracy can be assessed for an analytical methodology only after statistical control has been attained. Statistical concepts must be used

to develop a quality assessment program: the kinds of samples to be used, and the sequence used for analyzing them must be specified; and then statistical controls can be established.

Quality assessment requires measurements of procedural method blanks, spiked samples, reference materials, and replicate samples over time to establish statistical control and monitor precision of the analytical protocol. The frequencies of these various measurements are defined by this Quality Assurance program in the following manner:

1) One procedural method blank must be analyzed per matrix type per sample block, or at frequency of 5% of the number of sample analyses, whichever is greater.

2) One procedural method spike of a negative field or laboratory control sample must be analyzed for each matrix type per sample block, or at frequency of 5% of the number of sample analyses, whichever is greater. The spike should be within the range of expected analyte concentrations.

3) One positive control sample, positive laboratory reference sample, or positive certified reference material must be analyzed for each matrix type per sample block, or at frequency of 5% of the number of sample analyses, whichever is greater.

4) One sample must be analyzed in duplicate per matrix type per sample block, or at frequency of 5% of the number of sample analyses, whichever is greater.

Appropriate control charts of each of the above measurements must maintained, and regularly updated to assure the quality of all analytical data. The procedures for establishing control charts of these various measurements are defined by this Quality Assurance program in the following manner:

1) Control charts for monitoring procedural method blank measurements will be developed using the X-bar technique.

2) Control charts for monitoring procedural method spike recoveries will be developed using the X-bar technique.

3) Control charts for monitoring positive control samples, positive laboratory reference samples, or positive certified reference materials will be developed using the X-bar technique.

4) Control charts for monitoring duplicate samples will be developed using the Range-ratio technique.

Separate control charts will be constructed for each analyte in each matrix. The large numbers of analytes in multicomponent analyses may limit the number analytes that can reasonably be charted. In this instance suitable analytes will be determined during method validation to represent the full suite of analytes.

X-bar charting techniques are used for evaluating bias of the measurement process where a single result is being monitored for consistency with the mean. Departures from the mean are measured in standard deviation units.

Range-ratio charting techniques are used for evaluating precision of the measurement process where replicate measurements of the same or similar samples are involved. Departures from the mean are measured in standard deviation units.

Warning limits for establishing statistical control charts of the various measurements are defined by this Quality Assurance program in the following manner:

1) Measurements greater than 2 standard deviations from the mean value will be considered to be in a warning state.

2) Measurements greater than 3 standard deviations from the mean value will be considered to be out-of-control.

All data collected from the time the system was last demonstrated to be in a of state of statistical control, up to the time the first out of control measurement was made are suspect. All samples analyzed within this period must be re-analyzed after corrective actions are taken and the system is once again demonstrated to be in control. Laboratory guidelines must be established to determine the nature and extent of any corrective action upon lack of control and the steps necessary to demonstrate that statistical control has been re-established.

Blanks, controls, spikes, and duplicates of field samples should be obtained whenever possible as part of the sampling protocol, but their incorporation into the sampling design is beyond the scope of the analytical facility. Responsibility rests with the investigators developing the overall experimental protocol; however, given the opportunity, input from the analytical facility should reflect consideration of these Quality Assurance samples in the sampling scheme.

## VI. Reporting Requirements

Documentation for reporting analytical measurements requires that all of the information necessary to verify the prove the claims made in the analytical report. This information includes:

1. sufficient documentation to reconstruct an audit-trail of the sample—from the field to the analytical result

2. a description of the analytical methodologies used

3. a description of all confirmatory evidence pertaining to the identification and quantitation of the analyte

4. Method validation information: limits of detection and quantitation, precision or percent relative difference, and recovery data

5. Quality Assurance program description and documentation associated with the sample

6. Quality Assurance Statement discussing the confidence of the measurement

The report must contain summaries of the scope and application of the data, required Quality Assurance steps, and any other detailed information critical to interpreting the data. Instances where departures from the cited procedures have been taken must be reported, and any affect these departures have on the quality or usefulness of the data should be discussed.

The detail of the information provided must be sufficient to recreate the analytical process to verify the validity of the measurement. All necessary information must be summarized in the analytical report. Any analytical methodologies adapted from the literature must be properly cited, and any modifications thereof clearly documented and summarized. Data, data reduction, quality control, and quality assessment measurements made within the same analytical sequence as the sample must be traceable. Results and implications of the quality assessment must be presented to support the quality and validity of the measurement of that particular sample.

Conclusions concerning the detection, and confirmation of an analyte should be performed by the analyst. Uncertainties associated with each treatment of the sample, from receipt to final report, should be

assessed by the analyst routinely, as part of the Quality Assurance program.

Analytical results must be expressed in a suitable manner which will not distort casual inspection. Appropriate numbers of significant figures must be used and must be consistent with the uncertainties in the measurement process and the overall methodology. Any average values reported must contain the number of replicates used and an estimate of precision. Relationships between the analytical results, quality assessment results, and other supporting data should be clear. The manner in which the results are reported must be clearly and obviously stated: corrections for blanks, recoveries or interferences must be readily apparent.

The analyst must make all interpretation of the data, from receipt through the final reporting, clear and logical. Users of the data should not be required to reconstruct, reprocess, and reinterpret information contained in the final report to reach conclusions.

## VII. Quality Assurance Records

Proper documentation of all Quality Assurance information will be performed as part of the Good Laboratory Practice Guidelines established. This documentation will be sufficient in detail and organization to reconstruct, on paper, the analytical process from receipt to final report.

### A. Analytical Reports

The following components of the analytical process will be archived:

1. all information specifically requested in the design of the investigation's protocol

2. all sample and reference material data

3. aliquants of all remaining samples, extracts, and reference materials
4. all records of instrument maintenance, calibration, or modification

Quality Assurance records will be retained, and archived in a secure environment for the length of time determined by the experimental protocol. Components of Quality Assurance documentation : analytical reports, standard operating procedures, standard methods, data, chain of custody, quality control documentation, and quality assessment results may be archived separately provided a suitable means is available to reference and access all records related to an individual sample.

## B. Data Archiving

Any archival system for the Quality Assurance program and all analyses performed under its auspices should function both to locate all information about a particular sample, and also to locate all samples which may be affected by suspected Quality Assurance information. For example: a search for a sample will point to all of the data and records necessary to reconstruct the result and Quality Assurance information for that sample; and, a search of a discrepancy in the Quality Assurance information will be able to locate all samples which may be affected by this possible error.

## C. Laboratory Maintenance Records

Maintenance for all instrumentation and equipment related to analytical measurements must be documented. This documentation will include:

1. information necessary to determine which samples are affected by any specific maintenance item

2. sufficient detail to reconstruct the maintenance events, both the work which was done, and the order in which it was performed

3. any calibration or optimization information which could qualitatively (or quantitatively) indicate the instrument's performance

Maintenance records must be archived analogous to all other Quality Assurance records. Proper referencing should be maintained to facilitate locating maintenance records associated with any particular sample or block of samples analyzed.

Records should be maintained for all equipment used for making analytical determinations. Each step of the analytical protocol must state the maintenance, calibration, and optimization requirements of the instrumentation and equipment used. These requirements must be recorded in the Quality Assurance documentation as they are met, and finally archived.

## VIII. Appendices

A. Definition of Terms

B. Statistical Methods

## C. Bibliography


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