PCB Analysis:

Laboratory Certification Concerns

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WISCONSIN
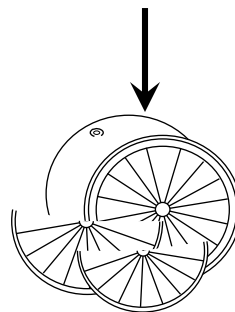DEPT. OF NATURAL RESOURCES

MDL for Total PCBs

MDL Determinations
5-pt calibration
with each Aroclor

Routine analysis
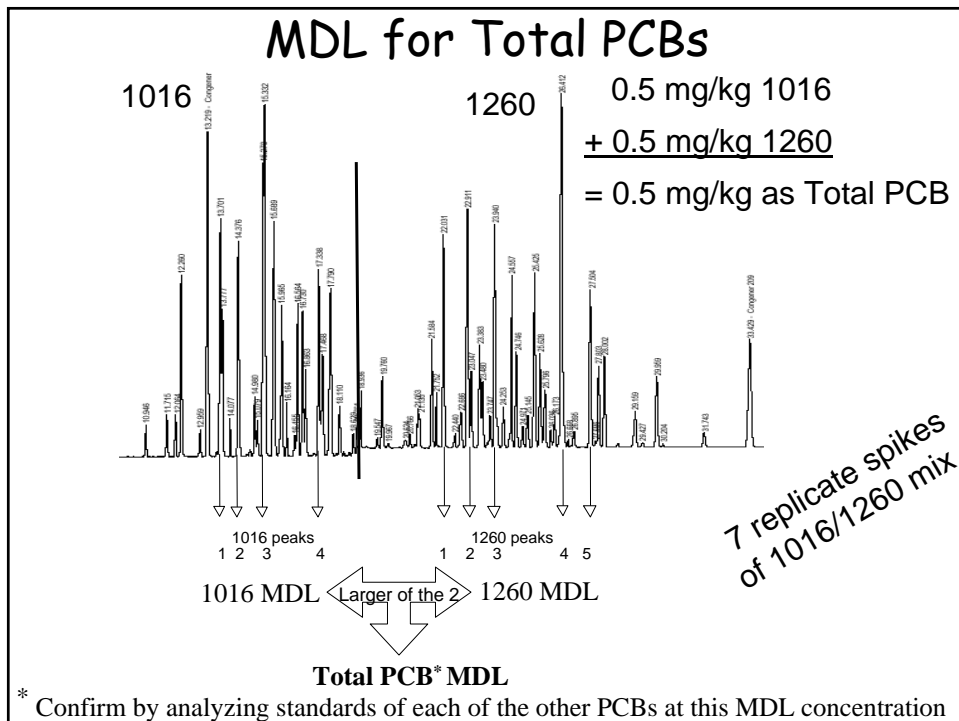5-pt calibration
with 1016/1260 mix

If no PCB pattern is detected in a sample, how to report?
< the highest (of 7) MDLs
< the lowest (of 7) MDLs
< something else

Key Question: What level could be detected, regardless of PCB

MDL for Total PCBs

- Prepare 7 replicates of a 1016/1260 mix
- *** in a biosolids matrix ****
- 1016/1260 at the same concentration, "x"
- since no overlap, "x" = concentration of "Total PCBs"
- Choose 3-5 total peaks from each of 1016/1260
- Generate a 5-point calibration for each peak.
- Quantitate each MDL vs. 1016 (for the 1016 peaks) and vs. 1260 (for the 1260 peaks).
- Use the 2 sets of 7 results to determine MDL for 1016 and one for 1260
- The Total PCB MDL will be the larger of the two MDLs
- Prepare & analyze individual standards of each of the other PCBs at the MDL level to confirm

MDL for Total PCBs

1016   1260   0.5 mg/kg 1016

+ 0.5 mg/kg 1260

= 0.5 mg/kg as Total PCB

7 replicate spikes of 1016/1260 mix

1016 peaks
1 2 3   4

1260 peaks
1 2 3   4 5

1016 MDL ◁ Larger of the 2 ▷ 1260 MDL

Total PCB* MDL

* Confirm by analyzing standards of each of the other PCBs at this MDL concentration
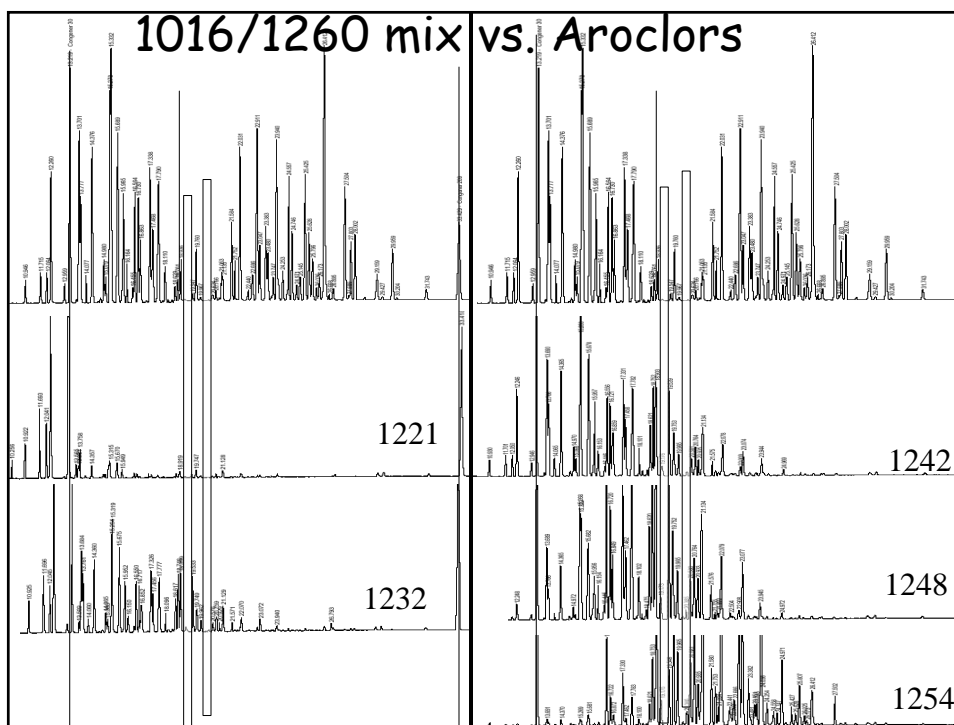
Calibration standards

5.6 Calibration standards for Aroclors

5.6.1 A standard mix of Aroclor 1016 + Aroclor 1260 will include many of the peaks represented in the other five Aroclor mixtures.

Thus... a 5-point initial calibration of 1016/1260 should be sufficient to demonstrate the linearity of detector response without performing multi-point initial calibrations for each of the seven Aroclors.

In addition, 1016/1260 mix can be used as a standard to demonstrate that a sample does not contain peaks that represent any one of the Aroclors.

This standard can also be used to determine the concentrations of either Aroclor 1016 or 1260, should they be present in a sample.

1016/1260 mix vs. Aroclors

Calibration (Aroclors)

5.6 Calibration standards for Aroclors

> 5.6.2 Single standards of each of the other five Aroclors **are required** to aid the analyst in pattern recognition.
>
> Assuming that the Aroclor 1016/1260 mix has been used to demonstrate detector linearity, these single standards of the remaining five Aroclors <u>also may be used</u> to determine the calibration factor for each Aroclor when a linear calibration model through the origin is used
>
> > Linearity thru the origin = Quantitation based on Mean RF (or CF)
>
> Prepare a standard for each of the other Aroclors. The concentrations **should** generally correspond to the mid-point of the linear range of the detector, but lower concentrations may be employed at the discretion of the analyst.
>
> 5.6.3 … If you know what PCBs are there...use standards of those particular Aroclors.
>
> See Method 8000 for information on non-linear calibration approaches.

Calibration: Internal & Surrogate Standards

Internal standard (IS)

PCBs as Aroclors : **typically not used**	PCBs as congeners: **highly recommend**
Decachlorobiphenyl (DCB)	Decachlorobiphenyl (DCB)

Surrogate standard (SS)

5.9 Surrogate standards Method performance **should** be monitored using surrogates. Surrogates are added to all samples, blanks, matrix spikes, and <u>calibration standards</u>..

5.9.1 PCBs as Aroclors: Decachlorobiphenyl [DCB] or Tetrachloro-m-xylene [TCMX] . <u>Recommend</u> spike w/ 5 mg/L in acetone.	5.9.2 PCBs as congeners: Tetrachloro-m-xylene [TCMX] may be used as a surrogate <u>Recommend</u> spike w/ 5 mg/L in acetone.
	5.9.3 If **DCB** is a target congener, 2,2',4,4',5,5'-hexabromobiphenyl may be used as an IS or an SS

DDT Analog standard

5.10 Used to determine if the commonly found DDT analogs (DDT,DDE, and DDD) elute at the same retention times as any of the target analytes (congeners or Aroclors). A single standard containing all three compounds should be sufficient. This standard need only be analyzed when the retention time windows <u>are determined</u>

Calibration

7.4.2 **If reporting PCBs as congeners, an initial multi-point calibration <u>must</u> be performed that includes standards for all the target congeners**.

7.4.3 If reporting PCBs as (Total)Aroclors, the initial calibration consists of:

7.4.3.1 a 1016/260 mix 5-pt calibration **may be used**
- **to demonstrate the linearity of the detector** and
- that a sample does not contain peaks from one of the Aroclors, and
- to quantitate either Aroclor 1016 or Aroclor 1260.

7.4.3.2 Standards of the other 5 Aroclors necessary for pattern recognition**.**
- **If calibration is a 5-pt of 1016/1260 AND quantitation is by average calibration factor (CF), these standards also used to determine a single-point calibration factor for each Aroclor**
- The standards for these five Aroclors **should** be analyzed before the analysis of any samples, and may be analyzed before or after the five 1016/1260 standards

7.4.3.3 If contaminant = specific Aroclor, 5-pt calibration of that Aroclor is OK
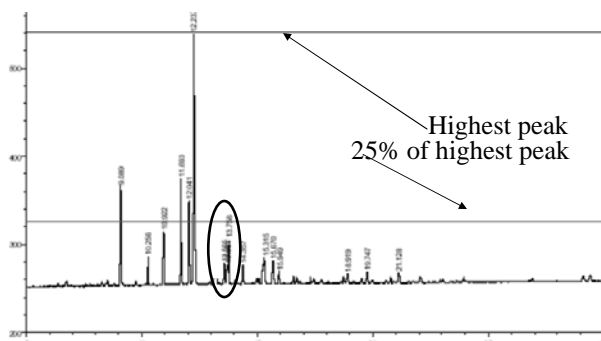
Peak Selection

7.4.6.1
A minimum of 3 peaks **<u>must</u>** be chosen for each Aroclor...
    ...and <u>preferably</u> 5 peaks.
The peaks **<u>must</u>** be characteristic of the Aroclor in question.
Choose peaks that are at least 25% of the height of the largest Aroclor peak.

Highest peak
25% of highest peak

For each Aroclor, **should** <u>include at least one peak unique</u> to that Aroclor.
Use at least 5 peaks for the Aroclor 1016/1260 mixture…
...none of which should be found in both of these Aroclors.

8000B and Calibration Method

2 Options for calibration
1. Begin with the simplest approach (linear through the origin)
    Progress through higher order functions until meet criteria
2. Use *a priori* knowledge of detector response to select a model
    (if using a linearized ECD, one would assume a linear model is in order)

Linear calibration using average calibration factor
If %RSD of calibration factors $\leq$ 20%, the model is appropriate
If %RSD of calibration factors > 20%,
  • re-analyze any "bad" standards
  • replace the "bad" standard
  • narrow the calibration range

There is an option to proceed if one or more analytes of a multi-analyte
method exceed the 20% RSD, but that does not apply here

8000B Initial Calibration

Linear Calibration using regression
If the RSD of CFs > 20%, it suggests a non-zero intercept
May also use regression based on "past experience"
If this approach is used:
  • "Y" of the "Y=mX+b" **must** be instrument response

  • Should NOT force the line through the origin

  • Do NOT include (0,0) as a 6th calibration point

  • "Weighting" is allowed...
      •…IF replicate multipoint calibrations are generated
      • (if weighting is used, $1/SD^2$ factor is suggested)

  • correlation coefficient **"r" must** be $\geq$ 0.990

8000B Ongoing Calibration Verification

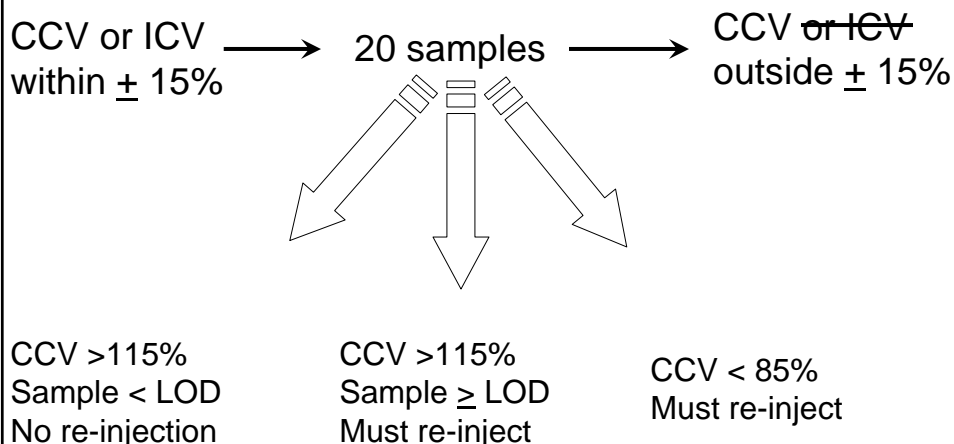**Initial calibration must be verified**
- at the beginning of each 12-hr shift...
    - ...during which samples are analyzed
- after every 20 samples (8082)
    - (more frequent checks are strongly encouraged)
- and at the end of an analysis sequence (8082)
- by the analysis of one (or more) standards (usually midpoint)
- % Difference must be $\leq$ 15%

$$\% \text{ Difference} = \frac{CF_v - \overline{CF}}{\overline{CF}} \times 100$$

8.2.2 requires samples to be bracketed by acceptable calibration verification standards [if external standard calibration]

If the standard analyzed **after** a group of samples >15%,
    ...and the analyte was not detected in any of previous samples
        ...then the sample extracts do not need to be reanalyzed,
demonstration that the analyte would have been detected were it present.


Sample Result Evaluation Scenarios

CCV or ICV within $\pm$ 15% → 20 samples → CCV ~~or ICV~~ outside $\pm$ 15%

CCV >115%
Sample < LOD
No re-injection

CCV >115%
Sample $\geq$ LOD
Must re-inject

CCV < 85%
Must re-inject

Retention Time Windows

Relative (to congener #30) Retention time		1016	1232	Δ mins.
0.8281	0.8276	10.946	10.925	-0.025
0.8862	0.8860	11.715	11.696	-0.019
0.9126	0.9124	12.064	12.045	-0.019
0.9275	0.9273	12.260 (6)	12.241 (1)	-0.019
1.0000	1.0000	13.219	13.201	-0.018
1.0365	1.0366	13.701 (3)	13.684 (6)	-0.016
1.0422	1.0424	13.777	13.761	-0.016
1.0875	1.0878	14.376 (5)	14.360 (5)	-0.016
1.1556	1.1563	15.276 (2)	15.264 (3)	-0.012
1.1598	1.1604	15.332 (1)	15.319 (2)	-0.013

[8000B] 7.6.4 The retention time window for each analyte/surrogate is:
mean ± 3 X std deviation
absolute retention time established during the [initial calibration]72-hour period.
If standard deviation is 0.00, the width of the window will be ± 0.03 minutes.

Relative retention time can often be a better identification tool

Retention Time (Rt) Windows
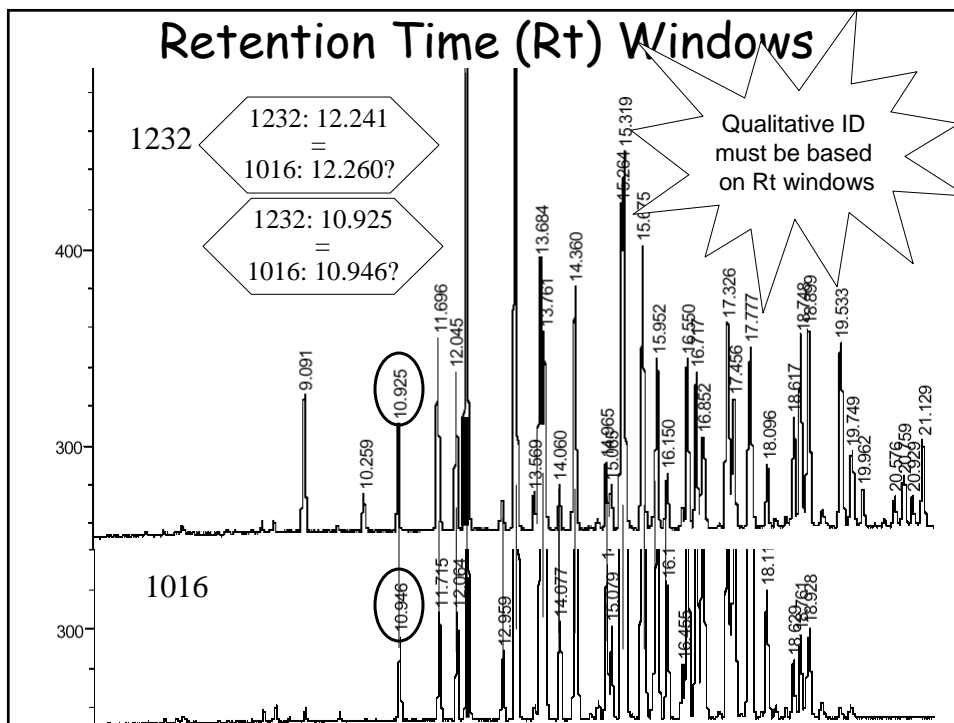
Quality Control

The laboratory **<u>must</u>** have procedures for documenting the effect of the matrix on method performance (precision and accuracy). **Minimally, this includes analysis of QC samples including**

- ☞ a method blank and
- ☞ a lab control sample (LCS) per analytical batch,
- ☞ addition of surrogates to each field and QC sample,
- ☞ routine analyses of MS and MSDs

8.4.1 To document effect of the matrix **<u>should</u>** include at least

- one MS + one DUPLICATE unspiked sample or
- one MS / MSD duplicate pair.

B **If samples are not expected to contain PCBs,** labs should use a **MS/MSD** pair, spiked with **1016/1260**

B **When specific Aroclor(s) are known/expected** the **specific Aroclor(s) should be used** for spiking.

B If samples expected to contain PCBs, then labs may use an MS/DUP vs. MS/MSD.

Quality Control

MS/MSD frequency must also meet Code requirements
NR 149 requires a spike per 10 samples
NR 149 requires a replicate (duplicate) per 20 samples
An MS/MSD every 20 samples satisfies NR 149 requirements

8.4.2 A laboratory control sample (LCS) **<u>should</u>** be included with each analytical batch.
The clean (control) matrix should be similar to the sample matrix and of the same weight or volume.
Spike with the same analytes/concentrations as a matrix spike.

The laboratory **<u>must</u>** evaluate surrogate recovery data from individual samples versus the surrogate control limits developed by the laboratory

IDC

8.3 Initial demonstration of proficiency

       8.3.1 Each laboratory **must** demonstrate initial <u>proficiency with each sample preparation and determinative method combination it utilizes</u>, by generating data of acceptable accuracy and precision for target analytes in a clean matrix.

       Must <u>repeat the following operations</u> **whenever**
- **new staff are trained or**
- **significant changes in instrumentation are made**.

8000B

8.4.3.2 Add1.0 mL of the reference sample concentrate to each of four 1-L aliquots of [clean representative matrix].

8.4.4 Analyze at least four replicate aliquots by the same procedures used to analyze actual samples.

8.4.5 Calculate

    the average recovery (x) in [relevant units], and

    the standard deviation (s) of the recovery in [relevant units],

IDC

8.3.2 The QC Reference Sample concentrate (Method 3500) **should** contain PCBs as Aroclors at 10-50 mg/L in the concentrate for water samples.

A 1-mL volume of this concentrate spiked into 1 L of reagent water will result in a sample concentration of 10-50 µg/L.

If Aroclors are not expected in samples from a particular source, then prepare the QC reference samples with a mix of Aroclors 1016 and 1260.

When specific Aroclors are known to be present or expected in samples, the specific Aroclors should be used for the QC reference sample.

8.3.3 **Calculate the average recovery and the standard deviation of the recoveries of the analytes in each of the four QC reference samples**

8000B:

8.4.9 In the absence of recommended acceptance criteria for the initial demonstration of proficiency, the laboratory should use recoveries of 70 - 130% as guidance in evaluating the results. Given that the initial demonstration is performed in a clean matrix, the average recoveries of analyte from the four replicates should generally fall within this range.

Cleanup Reminders - 3660B SULFUR

Cu IN BOILING FLASK

**Cu powder needs to be very reactive (shiny!)
Remove all traces of acid used for Cu prep to
minimize potential degradation of analytes**

Copper powder -
Remove oxides by
- treating with dilute nitric acid,
- rinse with organic-free reagent water to remove all traces of acid,
- rinse with acetone and
- dry under a stream of nitrogen.

(Copper, fine granular Mallinckrodt 4649 or equivalent)

8000B 8.2.6.4 When extracts are subjected to cleanup, the associated method blank must also be subjected to the same cleanup procedures.

FLORISIL → GPC → SILICA GEL → SULFURIC ACID → ALUMINA → GC

Cleanup Reminders - 3620 C Florisil

For sample extracts that are cleaned up using this method, the associated quality control samples must also be processed through this cleanup method.

Florisil PR is activated at 675°C and is most useful for pesticide residue analyses.
Florisil A is activated at 650°C and is generally used for other analytes.
Store Florisil in glass containers with ground-glass stoppers or foil-liner screw caps.

5.5.3 Calculate the "strength" of the NaOH solution as the mg of lauric acid neutralized per mL of NaOH solution.

Lauric acid value = 200 ÷ (titration volume in mL of NaOH) (strength of NaOH in mg LA/mL of 0.05N NaOH)

$$\frac{110}{\text{lauric acid value}} \times 20 \text{ g} = \text{Required weight of Florisil}$$

3620B ** INCORRECT** formula
$$\frac{\text{lauric acid value}}{110} \times 20 \text{ g} = \text{Required weight of Florisil}$$

8.2 The analyst **must** demonstrate that the compounds of interest are being quantitatively recovered before applying this method to actual samples.

8.2.1 This test must be conducted on each batch of Florisil following its activation (Sec. 5.4).

8.2.3 **Organo-Cl pesticides** - Check each lot of Florisil cartridges before use; perform the following in duplicate:
8.2.3.1 Mix 0.5 mL of 2,4,5-trichlorophenol solution, 1.0 mL of pesticide solution, and 0.5 mL of hexane in a vial.
8.2.3.2 Condition the cartridge and then perform the cartridge cleanup
8.2.3.3 Elute the cartridge with 9 mL of acetone/hexane (10/90, v/v) only. Reduce to 1.0 mL; analyze by 8081.
8.2.3.4 The lot is acceptable if all pesticides are recovered at 80 to 110 %, if the recovery of trichlorophenol is less than 5 %, and if no peaks interfering with the target analytes are detected.

Cleanup Reminders - 3640A Gel Permeation

For sample extracts that are cleaned up using this method, the associated quality control samples must also be processed through this cleanup method.

GPC Calibration Solution
corn oil (25,000 mg/L),
bis(2-ethylhexyl) phthalate (1,000 mg/L),
methoxychlor (200 mg/L),
perylene (20 mg/L),
sulfur (80 mg/L)

7.2.2.5 Calibration for Organochlorine Pesticides/PCBs
- Determine elution times for phthalate, methoxychlor, perylene, and sulfur.
- Choose a dump time which
    - removes >85% of the phthalate,
    - but <u>collects</u> >95% of the methoxychlor.
- Stop collection after the elution of perylene, but before sulfur elutes.

7.2.2.6 Verify flow rate.
    Collect column eluate for 10 mins in a grad. cylinder; measure volume.
    <u>Should</u> be 45-55 mL (4.5-5.5 mL/min).

Cleanup Reminders - 3640A Gel Permeation

Inject the calibration solution and obtain a UV trace showing a discrete peak for each component.
Adjust detector / recorder sensitivity to produce a UV trace similar to Figure 2 that meets the following requirements
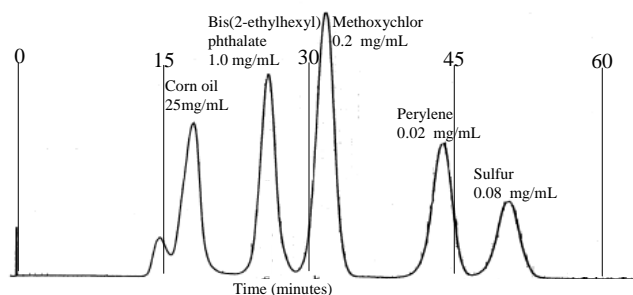
7.2.2.3.1 Peaks (for all compounds) must be observed, and should be symmetrical.
7.2.2.3.2 Corn oil & phthalate peaks must exhibit >85% resolution.
7.2.2.3.3 Phthalate & methoxychlor peaks must exhibit >85% resolution.
7.2.2.3.4 Methoxychlor & perylene peaks must exhibit >85% resolution.
7.2.2.3.5 Perylene & sulfur peaks must not be saturated & must exhibit >90%

Cleanup Reminders - 3630C Silica Gel

For sample extracts that are cleaned up using this method, the associated quality control samples must also be processed through this cleanup method.

Activate for at least 16 hr. at 130ºC in a shallow glass tray,
Deactivate to 3.3% with reagent water in a 500 mL glass jar.
Mix the contents thoroughly and allow to equilibrate for 6 hours.
Store deactivated silica gel in a sealed glass jar in a desiccator.

8.3 The analyst must demonstrate that the compounds of interest are being quantitatively recovered before applying this method to actual samples.

Method Tables [93-124% for 1016-1260] for acceptable recovery data.

For untested compounds, recovery must be > 85%.

Cleanup Reminders - 3611B Alumina

For sample extracts that are cleaned up using this method, the associated quality control samples must also be processed through this cleanup method.

The analyst should demonstrate that the compounds of interest are being quantitatively recovered before applying this method to actual samples.

5.3 **Alumina**: Neutral 80-325 MCB chromatographic grade or eq. Dry alumina overnight at 130°C prior to use.