Analysis of Polychlorinated Biphenyls and Chlorinated Pesticides by Gas Chromatography with Electron Capture Detection

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1.0 Scope and Application

This SOP is applicable to the analysis of environmental sample extracts for polychlorinated biphenyls (PCBs) as Aroclors and individual congeners and chlorinated pesticides by capillary gas chromatography with ^{63}Ni electron-capture detection.

This procedure provides typical gas chromatography (GC) conditions for the detection of trace levels of PCBs and pesticides, methods for identifying the analytes, and methods for analyte quantification using the internal standard method. Tables 1 and 2 list the most frequently analyzed compounds and formulations. However, this list may be amended to meet requirements of specific projects.

2.0 Definitions

The following terms and acronyms may be associated with this procedure:

ECD	Electron capture detector or detection
GC	Gas chromatography
PCB	Polychlorinated biphenyl
RF	Response factor
RRF	Relative response factor; response factor of analyte normalized to the response factor of the internal standard.
RSD	Relative standard deviation (%)
RT	Retention time
IS	Internal standard - compound(s) added just prior to analysis on instrument.
SS	Surrogate standard - Compound(s) added prior to extraction to assess efficiency of method.

3.0 Responsible Staff

Project Manager: A Scientist responsible for 1) administration of the project; 2) providing project specific quality control requirements to the laboratory; 3) defending the data in a Quality Assurance Audit; and 4) reporting results to client.

Laboratory Supervisor: A Technical Specialist or Scientist having expertise in the principles involved with this procedure and in the use of the GC. Responsible for 1) ensuring that analysts are trained in operation of the GC; 2) appropriate quality control samples are included with the sample analysis to monitor precision and accuracy of the analysis; 3) checking the analysts' work to ensure that data are collected and interpreted correctly; 4) making decisions regarding problems with the analysis or deviations from the SOP; 5) defending the data in a Quality Assurance Audit; and 6) reporting results to project manager or client.

Analyst: A Technician, Technical Specialist, or Scientist assigned to conduct analyses using this procedure. Responsible for 1) understanding the proper use and maintenance of the GC; 2) recording information regarding instrument use and maintenance in the appropriate log books; 3) analyzing the appropriate number of quality assurance samples for each batch of samples analyzed; 4) tabulating all sample and QC data and reviewing the quality of the data based on QC guidelines presented in this SOP and any other project-specific QC guidelines; 5) reporting results to the Project Manager; and 6) defending the data during an audit.

Table 1. PCB and Chlorinated Pesticide Analyte List

PCBs (Aroclors)	**Suggested Internal Standards**
Aroclor 1232	PCB-030
Aroclor 1248	PCB-204
Aroclor 1262	
Aldrin	**Suggested Surrogate Standards**
alpha-BHC	PCB-014
beta-BHC	PCB-065
gamma-BHC (Lindane)	PCB-166
delta-BHC	PBB-153
4,4'-DDE	
4,4'-DDD	
4,4'-DDT	
(cis)alpha-Chlordane	
(trans)gamma-Chlordane	
Tech. Chlordane	
Dieldrin	
Endosufan I	
Endosufan II	
Endrin	
Endrin Aldehyde	
Endrin ketone	
Heptachlor	
Heptachlor Epoxide	
Endosulfan Sulfate	
Hexachlorobenzene	
Mirex	
Trans-Nonachlor	
Cis-nonachlor	

Table 2. PCB Congener List

CB Number[a]	CAS Nomenclature[b]	CAS Registry Number[b]
8	2,4'-dichlorobiphenyl	34883-43-7
18	2,2',5-trichlorobiphenyl	37680-65-2
28	2,4,4'-trichlorobiphenyl	7012-37-5
29	2,4,5-trichlorobiphenyl	15862-07-4
44	2,3',3,5'-tetrachlorobiphenyl	41464-29-5
49	2,2',4,5'-tetrachlorobiphenyl	41464-40-8
50	2,2',4,6-tetrachlorobiphenyl	62796-65-8
52	2,2',5,5'-tetrachlorobiphenyl	35693-99-3
66	2,3',4,4'-tetrachlorobiphenyl	32598-10-0
77	3,3',4,4'-tetrachlorobiphenyl	32598-13-3
87	2,2',3,4,5'-pentachlorobiphenyl	38380-02-8
101	2,2',4,5,5'-pentachlorobiphenyl	37680-73-2
104	2,2',4,6,6'-pentachlorobiphenyl	56558-16-8
105	2,3,3',4,4'-pentachlorobiphenyl	32598-14-4
118	2,3',4,4',5-pentachlorobiphenyl	31508-00-6
126	3,3',4,4',5-pentachlorobiphenyl	57465-28-8
128	2,2',3,3',4,4'-hexachlorobiphenyl	38380-07-3
138	2,2',3',4,4',5-hexachlorobiphenyl	35065-28-2
153	2,2',4,4',5,5'-hexachlorobiphenyl	35065-27-1
154	2,2',4,4',5,6'-hexachlorobiphenyl	60145-55-4
170	2,2',3,3',4,4',5-heptachlorobiphenyl	35065-30-6
180	2,2',3,4,4',5,5'-heptachlorobiphenyl	35065-29-3
183	2,2',3,4,4',5',6-heptachlorobiphenyl	52663-69-1
184	2,2',3,4,4',6,6'-heptachlorobiphenyl	74472-48-3
187	2,2',3,4',5,5',6-heptachlorobiphenyl	52663-68-0
188	2,2',3,4',5,6,6'-heptachlorobiphenyl	74487-85-7
195	2,2',3,3',4,4',5,6-octachlorobiphenyl	52663-78-2
200[c]	2,2',3,3',4,5',6,6'-octachlorobiphenyl	40186-71-8
206	2,2',3,3',4,4',5,5',6-nonachlorobiphenyl	40186-72-9
209	2,2',3,3',4,4',5,5',6,6'-decachlorobiphenyl	2051-24-3

[a] Ballschmiter and Zell numbering scheme.

[b] Chemical Abstracts, Tenth Collective Inex, Index Guide, American Chemical Society, Columbus, Ohio, 1982.

[c] CB 200 in the Ballschmiter and Zell numbering scheme.

Table 3. Suggested Instrument Conditions for PCB and Chlorinated Pesticide Analysis

Injection port temperature	250°C
Detector temperature	325°C
Initital temperature	100°C
Initial hold	0 min
Ramp 1 rate	1°C/min to 265°C
Ramp 2 rate	20°C/min to 300°C
Final hold	0 min
Carrier gas flow (linear velocity)	45 cm/sec
Makeup gas flow	40 mL/min
Purge Vent	3 mL/min
Split vent	60 mL/min
Purge on after 1 min	

4.0 Procedures

4.1 GC Preparation

The GC is typically fitted with one column; a DB-5 60-m x 0.25 mm (i.d.) fused silica capillary column with a 0.1-μm film thickness (J&W Scientific, Inc.). Suggested instrumental conditions are listed in Table 3. Other columns or GC conditions may be specified in individual project plans.

4.2 Sample Collection, Preservation, and Handling

To conduct this analysis, the analyst should receive the samples as solvent extracts reduced to an appropriate volume. All organic extracts are normally analyzed within 40 days from extraction. Refer to project-specific plans or protocols for sample collection, preservation, and handling methods. If holding times have been exceeded, the Project manager should be notified immediately.

4.3 Sample Specifications

Sample preparation methods may vary depending on the sample matrix and project needs; refer to project-specific protocols. Samples and standards for analysis using this SOP should be prepared in hexane unless otherwise specified. Methylene chloride injected into the GC/ECD system should be limited, as it may damage the detector. Unless otherwise specified, standard and sample aliquots of 2-μL volumes will be injected.

4.4 Analyte Identification

Prior to sample analysis, the elution order of the analytes of interest must be determined by analyzing the analytes individually or in combination with other analytes having known or predetermined retention times.

4.5 Instrument Calibration

Before the sample is injected into the GC, the detector must be calibrated to determine the response of the detector to the analytes of interest. Demonstration of linearity of detector response is required before sample analysis. Calibration checks must be analyzed at a minimum frequency of once every 10 samples during sample analysis.

4.5.1 Initial Calibration

The initial calibration consists of the analysis of a minimum of five calibration solutions, each at different concentrations that span the expected concentration range of the samples. These standards include the analytes of interest as well as the appropriate surrogates (SS) and internal standards (IS). The concentration of the LOW standard should be approximately 2-5 times the detection limit of the instrument. The MID-range calibration standard should be near the expected concentration of the samples. The HIGH concentration standard should be approximately 4-10 times the concentration of the MID standard, or high enough to span the expected concentration range. The range of concentrations of these standards are as follows:

Pesticides	PCB Congener Mixture
1.0 - 100 ng/mL	1.0 -100 ng/mL

The concentrations of IS and SS should be the same in all calibration solutions and in the same concentration range as they are spiked in the samples, typically at a concentration 2 to 5 times below the highest calibration standard. Initial calibration standards must be analyzed prior to initiating sample analyses. An initial calibration should also be run if any GC conditions have changed. If GC conditions have not changed since the previous initial calibration, a continuing calibration standard may be analyzed and if it falls within acceptable criteria, the previous initial calibration may be used.

4.5.2 Continuing Calibration

The upper mid-level calibration solution is analyzed as a calibration check minimally every 10 samples while samples are being analyzed. All sample analyses must be bracketed by two calibration check standards that meet calibration criteria (see acceptance criteria in section 4.5.4.3, Relative Response Factors).

4.5.3 Calibration for Analysis of Aroclors and Multi-Peak Pesticides

A multilevel calibration is analyzed when samples are to be quantified for multicomponent mixtures such as Aroclors, or toxaphene. Calibration solutions are analyzed minimally at the beginning of each analysis run or sample batch unless otherwise noted in project protocols.

4.5.4 Relative Response Factors

The relative response factor (RRF) of each analyte is calculated as follows:

$RRF_A = (H_A) \times (C_{IS})/(H_{IS}) \times (C_A)$

where: H_A = *Analyte Peak Height*
H_{IS} = *Internal Standard Peak Height*
C_{IS} = *Concentration of Internal Standard*
C_A = *Concentration of Analyte*

4.5.4.1 Initial Calibration Response Factors

Individual Relative Response factors are generated for each analyte at each calibration level. A weighted average RRF (X_{RRF}) and correlation coefficient are calculated from the linear regression of the ratio of responses (H_A/H_{IS}) versus the ratio of amounts (C_A/C_{IS}) for a multipoint calibration. The correlation coefficient must be >0.95 for each individual analyte unless otherwise specified in project plans. If any correlation coefficient does not meet the acceptable criteria, the initial calibration must be repeated and all samples associated with that calibration re-run (unless otherwise specified in a specific project plan and/or documented by the Project Manager).

4.5.4.2 Response Factors for Six Mixture Components

For multi-peak analytes such as polychlorinated biphenyls, up to 110 significant peaks or specific predetermined components are chosen. An RRF is calculated for each peak as described above. A similar method is used for quantifying the technical mixture of chlordane. The acceptance criteria for the multicomponent mixture RRF linear regression is the same as stated for single peak components (r^2 >0.95).

4.5.4.3 Continuing Calibration RFs

Continuing calibration checks are considered acceptable if the % difference between the concentration of the analyte and the known calibration concentration is less than 25% for four selected medium to large peaks and less than 50% for two selected small peaks. If the newly generated concentrations are acceptable, the initial calibration is still valid and sample analysis may continue. If the percent difference exceeds the acceptable criteria, remedial action should be taken and the continuing calibration check solution should be reanalyzed. If the calibration fails again, the analyses should be terminated, remedial action taken, a new initial calibration should be performed, and the affected samples reanalyzed. The percent difference is calculated as follows:

$\%\ difference = (C_{AI} - C_A)/C_{AI} \times 100\%$

where: C_{AI} = *Concentration of the analyte from initial calibration*
C_A=*Concentration of the analyte from continuing calibration check*

4.6 Evaluation of DDT and Endrin Degradation

DDT and endrin are easily degraded in the injection port, if the injection port or front of the column is contaminated with buildup of high boiling residue from sample injection. Check for degradation problems by injecting a mid-concentration standard containing only 4,4'-DDT and endrin prior to sample analyses. Look for the degradation products of 4,4'-DDT (4,4'-DDE and 4,4'-DDD) and endrin (endrin ketone and endrin aldehyde).

If degradation of either DDT or endrin exceeds 20% or the combined breakdown of DDT and endrin is greater than 20%, then take corrective action before proceeding with calibration. Corrective action includes cleaning and deactivating the injection port, breaking off at least 0.5 m of column and remounting it. Lowering the injection port temperature may also be an option. This should be determined by the laboratory Supervisor. Calculate percent breakdown as follows:

$$\%\ \text{breakdown for 4,4'-DDT} = \frac{\text{Response (peak height) (DDE + DDD) x 100}}{\text{Response (peak height) DDT injected}}$$

$$\%\ \text{breakdown for endrin} = \frac{\text{Response (peak height) endrin aldehyde + endrin ketone x 100}}{\text{Response (peak height) endrin}}$$

$$\text{Combined \% breakdown} = \%\ \text{breakdown DDT} + \%\ \text{breakdown endrin}$$

4.7 Sample Analysis Procedure

Samples are analyzed under the same analytical conditions as the calibration standards. Samples must be bracketed by acceptable calibrations. Criteria for accepting peaks as analytes of interest are explained in Sections 4.7.1 through 4.7.2.

4.7.1 Relative Retention Time

Retention time (RT) windows for each analyte may be determined daily or by batch of samples. Relative retention time (RRT) for each analyte shall be determined from the ratio of the RT of the analyte and the RT of a time reference compound, usually the internal standard for a designated time interval. RRT for a particular analyte shall be within 1% of RRT determined during initial calibration for a peak to be identified.

4.7.2 Minimum Height

Peaks with a signal-to-noise ratio of three or less should be regarded as not detected unless otherwise noted in a specific project plan and/or documented by project management.

5.0 Data Analysis and Reporting

5.1 Data Recording

Data quantification and calculations will be performed on personal computers using commercial spreadsheet software such as *HP CHEM* version A03.01 and *Microsoft Excel*. All transfers of data to forms and data reductions (e.g., concentration calculations, means, standard deviations) will be checked by the analyst and approved by the Laboratory Supervisor. Hard copies of GC printouts of calibrations and sample data and spreadsheet reports will be kept in the GC/ECD files. A copy of the summary sheets and extraction logs will be placed in the appropriate project file in the Laboratory Supervisor's Central Files. Hard copies of chromatograms from each sample and all calibrations will be kept in the GC/ECD files unless otherwise noted in a specific project plan.

5.2 Sample Quantification

The internal standard method is used to quantify PCBs and chlorinated pesticides in environmental samples. The internal standards added to the samples prior to GC analysis are the basis for sample quantification.

5.2.1 Single-Peak Analytes

The concentration of a specific analyte in a sample is calculated as follows:

$$\text{Concentration} = [H_A \times Amt_{IS} / [H_{IS} \times X_{RRF}]] / \text{Sample Amt}$$
(ng/sample amt)

where: H_A *= Peak Height of analyte in sample*
H_{IS} *= Peak Height of IS*
X_{RRF} *= Relative response factor of the analyte based on the linear regression of the initial calibration*
Amt_{IS} *= Amount of the IS added (ng)*
Sample Amt = g (sediment) or L (water)

5.2.2 Multicomponent Analytes

The same calculation as above is used for quantifying multi-peak analytes.

Multicomponent analyses are performed as follows:

Quantification

1. Analyze samples as described previously.
2. Using RRF identify the peaks of interest in the samples.
3. Calculate concentration for each identified peak in the mixture as described above (Section 5.2.1).

5.3 Dual Column Confirmation Data

Data from the confirmation column is treated exactly the same as that obtained from the primary column as described above. QA criteria outlined in Section 4.0 also applies. Quantitative comparison of the values obtained from both columns should be performed for the single peak chlorinated pesticides. In the absence of interferences, values obtained from each column should be within approximately a factor of two of each other to be considered acceptable. If the criteria are not met, the value from the primary column is reported with a "G" flag. This criteria is only a guideline and should not be applied as an absolute rule, especially as concentration values approach detection limits. The value reported should be that obtained from the primary column unless chromatographic interferences would indicate that a more accurate value has been obtained from the confirmation column.

In addition, a number of compounds may co-elute when multi-component mixtures are present in the samples. The quantitative confirmation/comparison guidelines cannot be strictly adhered to in these cases. Likewise, if chromatographic interferences (i.e., poor peak shape, baseline drift etc.) are present on one of the columns this quantitative comparison is not required. These decisions are

based in a large part on the judgement of the analyst and any decisions made in regards to this should be noted on the analytical reports and on copies of the chromatograms.

5.4 Surrogate and Matrix Spike Recovery Calculations

Calculation of Surrogate recovery is as follows:

$$\% \text{ Surrogate Recovery} = Q_d / Q_a \times 100$$

Q_d = *Quantity determined by analysis*
Q_a = *Quantity added*

The matrix spike recovery is determined as follows:

$$\text{Matrix Spike Recovery} = (SSR/SR) \times 100/SA$$

SSR = Spike sample result
SR = Fraction surrogate recovery
SA = Spike added

Note that the matrix spike recovery is a surrogate corrected calculation.

The Relative Percent Difference (RPD) between spike and spike duplicates is calculated as follows:

$$RPD = \frac{|MSR - MSDR|}{\frac{1}{2}(MSR + MSDR)} \times 100$$

MSR = Matrix Spike % Recovery
MSDR = Matrix Spike Duplicate % Recovery

The following recovery criteria apply:

Surrogate Recoveries -	50 to 130%
Spike Recoveries -	50 to 130%
MS/MSD RPD -	≤30%

These values cover the recoveries suggested by the EPA CLP. These recovery limits are only guidelines. Data exceeding these values should be flagged as described below, however, no corrective action is advised. Frequent failure to meet the limits warrant investigation by the laboratory. Specific actions or limits may also be imposed by QAPPs associated with individual projects.

6.0 Quality Control

Most quality control consideration associated with this SOP are described in the individual sections to which they apply:

Instrument calibration	Section 4.5.1 - 4.5.3
Acceptance criteria for instrument calibration	Section 4.5.4

Acceptance criteria for sample peak recognition	Section 4.7.1 - 4.7.2
Data recording	Section 5.0
Surrogate and Spike Recovery Limits	Section 5.4

Minimum requirements for quality control samples, such as method blanks, matrix spikes, and standard reference materials (SRM), intended to monitor precision and accuracy of the analytical method, are specified in the extraction SOPs. In addition, project specific guidelines may be specified which differ from those outlined in the SOPs.

7.0 Safety

All analysts following this procedure should be aware of routine laboratory safety concerns, including the following:

1. Protective clothing and eyeglasses should be worn when appropriate
2. Proper care must be exercised when using syringes
3. Certain areas of the GC system are heated. Avoid bodily contact with these areas and use care in handling flammable solvents in and around the GC system.

8.0 Training

All analysts following this procedure will be directly supervised by the Principal investigator, qualified analyst, or laboratory supervisor until they have demonstrated to the satisfaction of the supervisor that they are capable of operating the GC independently. At a minimum, the analyst trainee should be competent in operation and maintenance of the GC. The analyst trainee should also be able to analyze and quantify a multi-point calibration and quantitate a sample of known concentration (e.g., a reference material or matrix spike) within established control limits

9.0 References

EPA Method 8000. U.S. Environmental Protection Agency (EPA). 1988. Test Methods for Evaluating Solid Wastes: Physical/Chemical Methods. EPA-600-4-79-020. 3rd Edition. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.