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Title: Determination of Chlorinated Hydrocarbons (CHCs) and Chlorinated Organophosphate Hydrocarbons (COPs) with Gel Permeation Chromatography (GPC).		
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Part I: Fat Matrix

A. INTRODUCTION

1. Theory

   Melted animal fat is dissolved in cyclopentane. Residues are purified by gel permeation chromatography (GPC), identified and measured by gas chromatography using an electron capture detector.

2. Applicability

   This method is applicable to fat and extracted fat from adipose tissue.

   a. Compounds for which method applies for quantitation:

Aldrin	p,p'-DDT	Heptachlor Epoxide B	Polychlorinated biphenyls (PCB) such as 1254 or 1260
alpha-BHC	Dieldrin	Hexachlorobenzene (HCB)	Ronnel
Chlorfenvinphos	Endosulfan II	2,2',4,4',5,5'-Hexabromobiphenyl	Stirofos
Chlorpyriphos	Endosulfan Sulfate	Lindane	p,p'-TDE
(cis) or alpha-Chlordane	Endrin	Methoxychlor	Toxaphene
trans-Chlordane	Endrin Ketone	Mirex	
Coumaphos-S	Heptachlor	trans-Nonachlor	
p,p'-DDE	Heptachlor Epoxide A	Oxychlordane	

   b. Coumpounds for which method applies for identification only.

beta-BHC	Coumaphos-O	Endosulfan I	Polybrominated biphenyls(PBB)
delta-BHC	Chlorpyriphos methyl	Halowaxes	op-TDE
Captan	o,p'-DDT	Kepone	
Carbophenothion	o,p'-DDE	Linuron	
Chlordene	Dichlorofenthion	Phosalone	

B. EQUIPMENT

Note: Equivalent instrumentation or apparatus may be substituted.

1. Apparatus
   a. Oven, able to maintain a temperature of 100 ± 5°C
   b. Class A volumetric flasks
   c. Filters, 0.45 µm pore size (Gelman, Acrodisc CR PTFE)
   d. Syringe, 10 mL, Luer lock
   e. Disposable glass culture tubes, 16 x 125 mm
   f. GPC sample input tubes
   g. Eppendorf pipettors, 10-100 µL, 50 µL, 200 µL, 100-1000 µL

2. Instrumentation
   a. GPC system, AS2000 (O.I. Analytical Sample Preparation Products Division)
   b. Optima GPC column (O.I. Analytical Sample Preparation Product Division, # 624-123)
   c. Gas chromatograph (GC): Hewlett Packard 6890 equipped with an electron capture detector (ECD, 63Ni) and a split/splitless injection port
   d. GC column, DB608 fused silica, 30 m, 0.45 mm ID, 0.7 µm film thickness, megabore column (J&W Scientific, Inc.)

C. REAGENTS AND SOLUTIONS

Equivalent reagents and solutions may be substituted.

1. Reagents
   a. Ethyl acetate, Honeywell, B&J Brand
   b. Cyclopentane, Honeywell, B&J Brand
   c. Iso-octane, Honeywell, B&J Brand

2. Solutions

   GPC mobile phase 70:30 v/v ethyl acetate/cyclopentane

   To prepare 4 L of mobile phase, measure separately 2.8 L of ethyl acetate and 1.2 L of cyclopentane in class A graduated cylinders. Combine and mix well.

D. STANDARDS

Equivalent standards may be substituted.

1. Source

   Dibutyl chlorendate (DBC), aldrin, and all pesticide standards can be ordered from Ultrascientific or ChemService.

   a. DBC stock solution 100 µg/mL in iso-octane.

   b. Aldrin stock solution 100 µg/mL in iso-octane.

   c. PCB 1254 stock solution 1000 µg/mL in iso-octane.

   d. PCB 1260 stock solution 1000 µg/mL in iso-octane.

   e. Toxaphene stock solution 100 µg/mL in iso-octane.

f. Custom mix solution of CHC/COP compounds can be ordered with the following concentrations as an example

Analytes in order of elution	**CAS #**	**Concentration (μg/mL in isooctane)**
Hexachlorobenzene	000118-74-1	50
α-BHC	000319-84-6	50
Lindane	000058-89-9	50
Heptachlor	000076-44-8	50
Ronnel	000299-84-3	50
Linuron	000330-55-2	150
Chlorpyriphos	002921-88-2	50
Heptachlor Epoxide B	001024-57-3	50
Heptachlor Epoxide A	001024-57-3	50
trans-Nonachlor	039765-80-5	50
α-Chlordane	005103-71-9	50
Chlorfenvinphos	000470-90-6	150
Dieldrin	000060-57-1	50
p,p'-DDE	000072-55-9	50
Stirofos	000961-11-5	50
Endrin	000072-20-8	50
Endosulfan II	033213-65-9	50
p,p'-TDE	000072-54-8	50
p,p'-DDT	000050-29-3	50
Carbophenothion	000786-19-6	50
Endosulfan Sulfate	001031-07-8	50
Mirex	002385-85-5	50
Endrin Ketone	053494-70-5	50
Methoxychlor	000072-43-5	50
Phosalone	002310-17-0	50
Coumaphos-O	000321-54-0	150
Coumaphos-S	000056-72-4	150
2,2',4,4',5,5'-Hexabromobiphenyl	059080-40-9	50

2. Preparation:

   a. Fortification standards

      Equivalent volumes/concentrations may be used.

      i. CHC/COP fortification solution (1.5 µg/mL linuron, chlorfenvinphos, coumaphos-O, and coumaphos-S; 0.5 µg/mL for the remaining CHC/COPs)

         Pipet 1.0 mL of the custom mix solution (D.1.f) into a class A 100 mL volumetric flask, dilute to volume with iso-octane and mix well.

      ii. PCB 1254 fortification solution(10 µg/mL)

         Pipet 100 µL of the 1000 µg/mL PCB 1254 stock solution into a 10 mL class A volumetric flask and dilute to volume with iso-octane. Mix well.

      iii. PCB 1260 fortification solution (10 µg/mL)

         Pipet 100 µL of the 1000 µg/mL PCB 1260 stock solution into a 10 mL class A volumetric flask and dilute to volume with iso-octane. Mix well.

      iv. Toxaphene fortification solution (10 µg/mL)

         Pipet 1.0 mL of the 100 µg/mL toxaphene stock solution into a 10 mL class A volumetric flask and dilute to volume with iso-octane. Mix well.

      v. Aldrin fortification solution (2.0 µg/mL)

         Pipet 2.0 mL of the 100 µg/mL Aldrin stock solution (D.1) into a class A 100 mL volumetric flask and dilute to volume with iso-octane. Mix well.

   b. GPC Diluent: 0.025 µg/mL DBC solution

      Pipet 250 µL of the DBC stock solution (D.1.a) into a class A 1000 mL volumetric flask, dilute to volume with iso-octane and shake well.

   c. GC Injection Standard (varied concentrations 0.20 ppm & 0.60 ppm)

      Fill a class-A 5 mL volumetric flask with GPC diluent (D.2.b.) Evaporate under nitrogen until approximately 1 mL has evaporated. Pipet 250 µL of the CHC/COP fortification solution (D.2.a.i) and 62.5 µL of the Aldrin fortification solution (D.2.a.v), dilute to volume with Iso-octane, and mix well.

   d. PCB 1254 GC Injection Standard (0.50 ppm)

      Fill a 5 mL class A volumetric flask with GPC diluent (D.2.b). Evaporate under nitrogen until approximately 1 mL has evaporated. Pipet 31.25 µL of the PCB 1254 fortification solution (D.2.a.ii) and 62.5 µL of the Aldrin fortification solution (D.2.a.v) and dilute to volume with Iso-octane. Mix well.

e. PCB 1260 GC Injection Standard (0.50 ppm)

Fill a 5 mL class A volumetric flask with GPC diluent (D.2.b). Evaporate under nitrogen until approximately 1 mL has evaporated. Pipet 31.25 µL of the PCB 1260 fortification solution (D.2.a.iii) and 62.5 µL of the Aldrin fortification solution (D.2.a.v) and dilute to volume with Iso-octane. Mix well.

f. Toxaphene GC Injection Standard (1.00 ppm)

Fill a 5 mL class A volumetric flask with GPC diluent (D.2.b). Evaporate under nitrogen until approximately 1 mL has evaporated. Pipet 62.5 µL of the toxaphene fortification solution (D.2.a.iv) and 62.5 µL of the Aldrin fortification solution (D.2.a.v) and dilute to volume with Iso-octane. Mix well.

3. Storage and Stability

a. Stock standards

Storage conditions and expiration dates are listed in the certificates of analysis (COA).

b. Fortification standards

Fortification standards are stable for one year if stored at < -10 °C. The fortification standards can be stored at 2-8°C for up to 3 months.

c. Injection standards

Injection standards may be stored at room temperature for three months.

E. SAMPLE PREPARATION

Place animal fat sample into a glass bottle or beaker and render sample in an oven at 100 ± 5 °C until an adequate amount of fat has been rendered.

F. ANALYTICAL PROCEDURE

1. Extraction

a. Weigh 0.5 ± 0.03 g of the liquid fat samples, including a blank fat and recovery samples, into 5 mL volumetric flasks or disposable/graduated glass culture tubes. Fortify all samples and recoveries with 50 µL of the 2.0 µg/mL Aldrin fortification solution (D.2.a.v),dilute to 5 mL with cyclopentane and mix well.

b. Fortify two recovery samples at the (1X) level with 200 µL of the CHC/COP fortification solution (D.2.a.i), equivalent to 0.20 ppm of each compound and 0.60 ppm for linuron, chlorfenvinphos, coumaphos-O, and coumaphos-S.

c. Separate recoveries for residues not in the regular fortification solution will need to be prepared if these compounds are being quantitated. Examples are PCBs and toxaphene. Two recovery samples for a violative sample have to be prepared at the level close to the finding.

d. Filter each sample through a 0.45 µm PTFE filter into GPC input tubes.

2. Load samples onto the GPC.

GPC Conditions:

Note: Conditions can be optimized as necessary.

Mobile phase flow rate	4.5 - 5.0 mL/min
Sample loop size	2.5 mL
Dump time	8.5 to 9.5 min
Evaporation time	9-10 min
Evaporation temperature	56 - 65 °C
High pressure sensor setting	1
Ultra Sonic Sensor (USS) preload time	03 (for 2.5-mL loop)
Keeper time	0 sec
Diluent addition	1 addition, 2 sec at 1 mL/sec
Mixing time	10 sec
Chamber transfer time	5 sec
Chamber rinse time	20 sec
Chamber wash temperature	55-57 °C
Vacuum pressure	250 Torr
Final reconstituted volume	2 mL in iso-octane

3. GC/ECD Conditions

Conditions can be modified to optimize the operation.

Injector temperature	250 °C
Detector temperature	325 °C
Carrier gas	Ultra High Purity Helium
Carrier gas flow rate	12-18 mL/min, constant flow
Make-up gas	Nitrogen 99.9% purity or better.
Make-up gas flow rate	25-40 mL/min
Injection mode	Splitless with 1 minute purge time

Oven Temperature Program:

Initial temperature	100 °C
Initial time	1 minute
Ramp #1	30 °C/min to 170 °C, hold for 10 minutes

Ramp #2	3.5 °C/min to 280 °C, hold for 10 minutes
Total run time	50-55 minutes
Post run time	10-15 minutes

4. General Operation

Typical retention times, relative to aldrin (minutes):

Analyte	Retention Time	Analyte	Retention Time	Analyte	Retention Time
Hexachlorobenzene	0.47	α or cis-Chlordane	1.59	Endosulfan sulfate	2.31
Alpha BHC	0.53	Chlorfenvinphos	1.65	DBC	2.54
Lindane	0.67	Dieldrin	1.76	Mirex	2.59
Heptachlor	0.82	*p,p'*-DDE	1.79	Endrin ketone	2.65
Aldrin	1.00	Stirofos	1.85	Methoxychlor	2.71
Ronnel	1.05	Captan	1.85	Phosalone	2.80
Linuron	1.24	Endrin	1.95	Coumaphos-O	3.08
Chlorpyrifos	1.30	Endosulfan II	2.06	Coumaphos-S	3.17
Oxychlordane	1.31	*p,p'*-TDE	2.10	*2,2',4,4',5,5'*-Hexabromo-biphenyl	3.59
Heptachlor ep B	1.39	*o,p'*-DDT	2.10	Toxaphene	NA
Heptachlor ep A	1.45	*p,p'*-DDT	2.24	PCB 1254	NA
trans-Nonachlor	1.51	Carbophenothion	2.27	PCB 1260	NA

Examples of co-eluting compounds are oxychlordane/chlorpyrifos; captan/stirofos; p,p'-TDE/o,p'-DDT; trans-Nonachlor/trans-chlordane; α-chlordane/Endosulfan I; Chlorfenvinphos/o,p'-DDE; Endosulfan II/o,p'-DDT; heptachlor/dichlofenthion; ronnel/chlorpyrifos; and mirex/phenylbutazone.

5. Identification and Quantitation

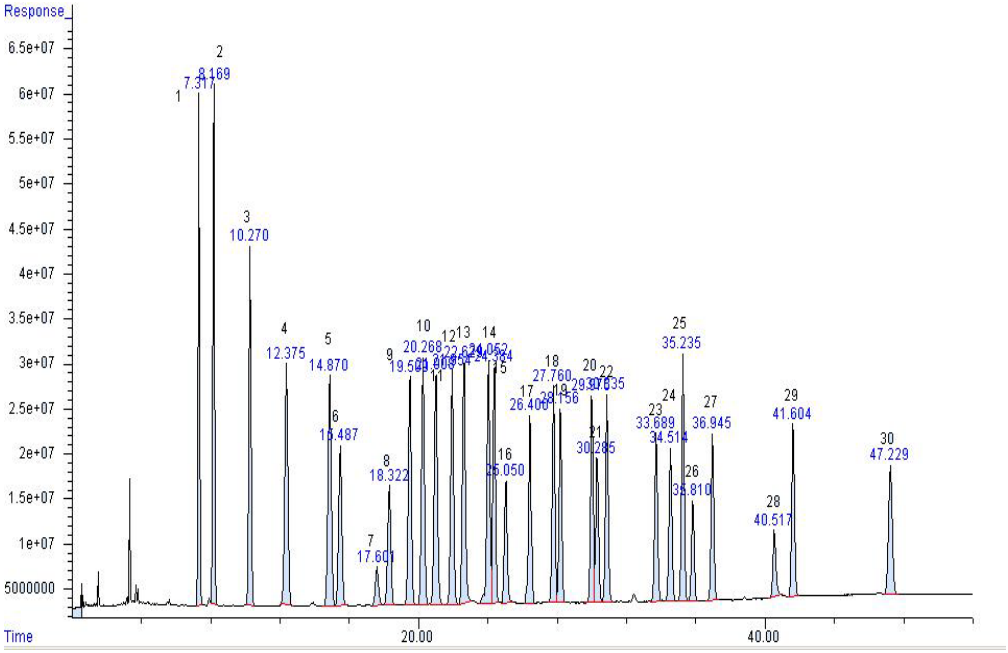
GC operators should use the appropriate calculating function depending on the peak type so that the software could identify the peak by retention time and calculating the amount. The results should be corrected for percent recovery by using the fortified recovery sample (fat) that has been carried through the entire procedure.

* Toxaphene is quantitated by summing the instrument response (using area) of peaks attributable to toxaphene in the sample and comparing them to the sum of the peaks of the standard.

* PCB residues (weathered or otherwise) are quantitated by summing the instrument responses of three to seven peaks common to the sample and to the PCB reference recovery. Use only those peaks from the sample that can be attributed to the Aroclor of interest. External standard quantitation is acceptable. The concentration of the recovery should approximate that found in the sample.

6. Sample Chromatograms

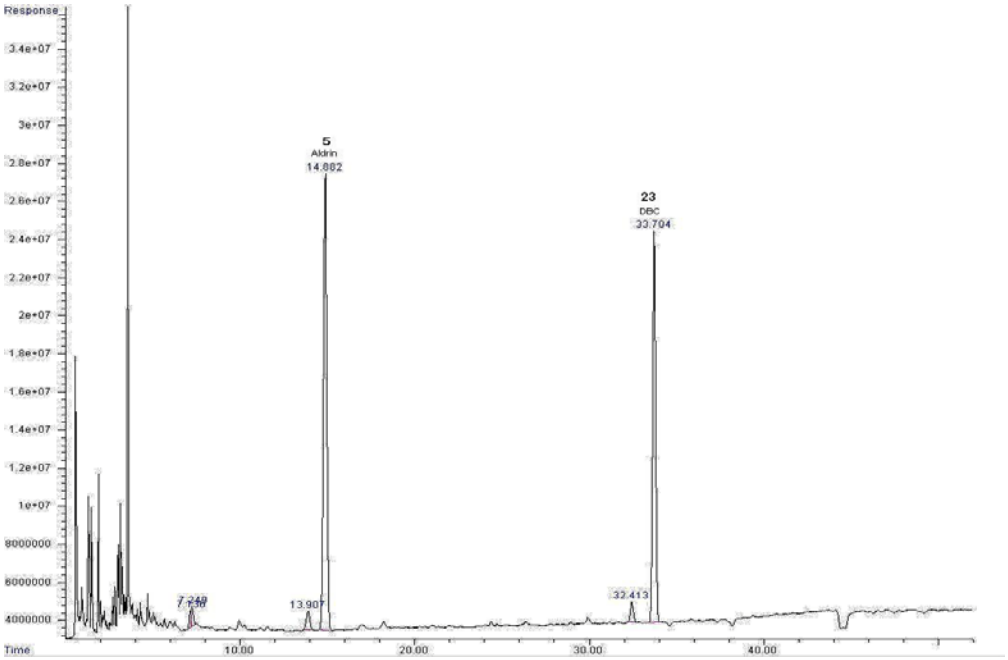
a. Figure 1: Chromatogram of theThirty-Compound Standard



Chromatographic Peak Numbers

1. hexachlorobenzene	11. trans-nonachlor	21. carbophenothion
2. α-BHC	12. cis(α) chlordane	22. endosulfan sulfate
3. lindane	13. chlorfenvinphos	23. DBC (internal standard)
4. heptachlor	14. dieldrin	24. mirex
5. aldrin	15. p,p'-DDE	25. endrin ketone
6. ronnel	16. stirofos	26. methoxychlor
7. linuron	17. endrin	27. phosalone
8. chlorpyrifos	18. endosulfan II	28. coumaphos-O
9. β-heptachlor epoxide	19. p,p'-TDE	29. coumaphos-S
10. α-heptachlor epoxide	20. p,p'-DDT	30. 2,2',4,4',5,5'- hexabromobiphenyl

a. Figure 2: Chromatogram of a Tissue Blank



G. CALCULATIONS

1. Injection Standard in D.2.c

The ppm concentration of the CHCs/COPs in the injection standard (C_{INJ}) is determined on the basis of the sample weight equivalent (SEq) in the final extract according to the following equations:

$$\text{Final extract, SEq} = \frac{\text{SMP wt.}}{\text{Initial Dil. Vol.}} \times \frac{\text{GPC SMP Loop Vol.}}{\text{Final Ext. Vol.}}$$

$$= \frac{0.5\text{ g}}{5\text{ ml}} \times \frac{2.5\text{ mL}}{2\text{ mL}} = 0.125\text{ g SMP/mL}$$

where SMP wt. = 0.5 g sample weight
Initial Dil. Vol. = 5 mL, initial dilution volume
GPC SMP Loop Vol. = 2.5 mL
Final Ext. Vol. = 2 mL, final extract volume

$$\text{ppm } C_{INJ} = \frac{C_{STD} \times \text{Vol. STD}}{\text{Dil. Vol.}} \times \frac{1}{\text{Final extract, SEq}}$$

$$= \frac{C_{STD} \times 0.250 \text{ mL}}{5 \text{ mL}} \times \frac{1 \text{ mL}}{0.125 \text{ g}}$$

where C_{STD} = concentration of the fortification standard, D.2.a.i.
Vol. STD = 0.250 mL volume of the fortification standard
Dil. Vol. = 5 mL, dilution volume

If a different dilution volume is desired, adjust the fortification standard amount proportionally. For example, use 0.5 mL fortification standard to prepare 10 mL of the injection standard. GC injection volume does not appear in the calculations because it is the same for quality samples as well as for test samples.

			Aldrin solution		Aldrin Amount in Each Soln (µg)	Concentration
		(1)	(2)	(3)		
		Weight of fat (g)	Volume (µL)	Concentration (µg/mL)	(2)x(3)	(µg Aldrin/g fat)
Before loading on GPC	5 mL of fat soln *	0.5	50	2	0.1	(2)x(3)/(1) = 0.2 µg/g
Loading onto GPC column	2.5 mL fat soln *	0.25	25	2	0.05	
After GPC clean-up	2mL final extract **	0.25	25	2	0.05	
Per mL of inj. std	1mL final extract **	0.125	12.5	2	0.025	

			CHC fortification soln		CHC Compounds Amount in Each Soln (µg)	Concentration
		(1)	(2)	(3)		
		Weight of fat (g)	Volume (µL)	Concentration (µg/mL)	(2)x(3)	(µg CHC/g fat)
Before loading on GPC	5 mL of fat soln *	0.5	200	0.5	0.1	(2)x(3)/(1) = 0.2 µg/g
Loading onto GPC column	2.5 mL fat soln *	0.25	100	0.5	0.05	
After GPC clean-up	2mL final extract **	0.25	100	0.5	0.05	
Per mL of inj. std	1mL final extract **	0.125	50	0.5	0.025	

* in cyclopentane
** in diluent (Iso-octane and DBC) the same calculation applied to CHC compounds with 1.5ug/mL or 0.60 ppm.

2. Calculating Results Using Recovery Correction

All CHC results are recovery corrected.

For each CHC/COP, the percent recovery (% Rec.), ppm amount in the sample, and recovery corrected ppm amount in the sample are calculated by using the following equations:

$$\%\ \text{Rec. CHC/COP} = R_{SPK} / R_{STD} \times 100$$

$$\text{ppm CHC/COP}_{IS} = R_{SMP} / R_{STD} \times C_{INJ}$$

$$\text{ppm (Rec. Corrected)} = (\text{ppm CHC/COP}_{IS}) / (\%\ \text{Rec. CHC/COP}) \times 100$$

where R_{SMP}, R_{STD}, and R_{SPK} are the ratios of the analyte GC/ECD responses (peak area or height) obtained for the sample, injection standard (C_{INJ}), and spiked 1X recovery (F.1.b), respectively, to the internal standard (DBC) response. C_{INJ} is the ppm concentration of the analyte in the injection standard.

3. External Standard (ES) Quantitation

For each CHC/COP, the percent recovery (% Rec.), ppm amount in the sample, and recovery corrected ppm amount in the sample are calculated by using the following equations:

$$\%\ \text{Rec. CHC/COP} = P_{SPK} / P_{STD} \times 100$$

$$\text{ppm CHC/COP}_{ES} = P_{SMP} / P_{STD} \times C_{INJ}$$

$$\text{ppm (Rec. Corrected)} = (\text{ppm CHC/COP}_{ES}) / (\%\ \text{Rec. CHC/COP}) \times 100$$

where P_{SMP}, P_{STD}, and P_{SPK} are the GC/ECD area/height responses for each analyte obtained for the sample, injection standard (C_{INJ}), and spiked recovery. C_{INJ} is the ppm concentration of the analyte in the injection standard

H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment — safety glasses, laboratory coat, and nitrile gloves.

2. Hazards

Procedure Step	*Hazard*	*Recommended Safe Procedures*
Ethyl acetate/ Cyclopentane mixture	Highly flammable; Irritating to skin and mucous tissue	Prepare mixture in fume hood. Vent GPC vapors to a fume hood.

3. Disposal Procedures

Procedure Step	*Hazard*	*Recommended Safe Procedures*
Ethyl acetate/ Cyclopentane mixture	See above	This mixture should be temporarily stored with the flammable waste solvents until disposal by the waste disposal contractor or in-house specialist. Observe all Federal, state, and local environmental laws.

I. QUALITY ASSURANCE PLAN

1. Performance Standards

a. Fortified recovery requirements

Analyte	*% Recovery*	*Repeatability (% CV)*	*Reproducibility* (% CV)*
HCB	70 - 120	17	20
alpha BHC	70 - 120	17	20
Lindane	70 - 120	17	20
Heptachlor	70 - 120	17	20
Aldrin	70 - 120	17	20
Ronnel	70 - 120	17	ND
Oxychlordane	70 - 120	17	20
Chlorpyrifos	50 - 120	17	20
Nonachlor	70 - 120	17	20
Heptachlor epoxide	70 - 120	17	20
trans-Chlordane	70 - 120	17	20
cis-Chlordane	70 - 120	17	20
Chlorfenvinphos	70 - 120	17	ND
Dieldrin	70 - 120	17	20
p,p'-DDE	70 - 120	17	20
Captan	50 - 120	17	ND

Analyte	*% Recovery*	*Repeatability (% CV)*	*Reproducibility* (% CV)*
Stirofos	50 - 120	17	ND
Kepone	50 - 120	17	ND
Endrin	70 - 120	17	20
p,p'-TDE	70 - 120	17	20
Endosulfan II	70 - 120	17	20
p,p'-DDT	70 - 120	17	20
Mirex	70 - 120	17	20
Methoxychlor	70 - 120	17	20
Coumaphos-S	70 - 120	17	ND
Toxaphene	70 - 120	12	20
PCB 1254	70 - 120	12	20
PCB 1260	70 - 120	12	20

ND – Not Determined
* Within-Lab reproducibility

a. Aldrin recovery: 70-120%

b. Chromatography: All pesticides of interest should be at least 60% resolved for quantitation

c. Blank sample: No peaks should be present at or greater than 10% of the recovery for each compound

2. Critical Control Points and Specifications

	Record	*Acceptable Control*
a.	Rendering oven temperature	100 ± 5 °C
b.	Sample weight	0.5 ± 0.03 g fat

3. Readiness To Perform

a. Familiarization

i. Phase I: Standards-External standard curve analyzed over three different

days, which will include the following:

(a) 0 Level

(b) At 0.5X Level

(c) At 1X Level

(d) At 2X Level

1X = 0.60 ppm for linuron, chlorfenvinphos, coumaphos-O, and coumaphos-S; 0.20 ppm for all other pesticides.

The correlation coefficient must be ≥ 0.995, except for linuron, chlorfenvinphos, coumaphos O & S.

ii. Phase II: Fortified samples-3 replicates spiked at the levels specified below over 3 different days. Each set includes a reagent blank and a blank fat sample.

(a) At 0.5X Level –Sample spiked with 100 µL of the fortification solution (D.2.a.i)

(b) At 1X Level –Sample spiked with 200 µL of the fortification solution(D.2.a.i)

(c) At 2X Level-Sample spiked with 400 µL of the fortification solution (D.2.a.i)

The 1X level for PCBs is 0.5 ppm. Both aldrin and DBC should be at 0.2 ppm for all standards and samples.

PCB Levels

(a) 0.5X Level-Sample spiked with 12.5 µL of the PCB fortification solution (D.2.a.ii or D.2.a.iii)

(b) 1X Level-Sample spiked with 25 µL of the PCB fortification solution (D.2.a.ii or D.2.a.iii)

(c) 2X Level-Sample spiked with 50 µL of the PCB fortification solution (D.2.a.ii or D.2.a.iii)

Phase I and Phase II may be performed concurrently.

iii. Phase III: Check samples for analyst accreditation.

(a) A total of 6 samples unknown to the analyst. At least one must be blank and the remainder must be fortified at or above the MPL (Section I.7).

(b) Report analytical findings to Quality Assurance Manager (QAM) and supervisor.

(c) Authorization by the QAM and Supervisor is required to commence official analysis.

Analyst accreditation for PCB analysis must be performed separately following the accreditation plan provided above.

b. Acceptability criteria

Refer to I. 1.

4. Intralaboratory Check Samples

a. System, minimum contents.

i. Frequency: Once per week per analyst when samples are analyzed.

i. Composition: Each intralaboratory check sample should contain three compounds: an early eluter, a late eluter and one in between. For example, alpha-BHC (early eluter), Mirex (late eluter) and p,p'-DDE (eluting in between).

ii. Records are to be maintained.

b. Acceptability criteria.

Refer to I. 1.

If unacceptable values are obtained, then:

i. Stop all official analyses by that analyst with this method.

ii. Take corrective action.

5. Sample Acceptability and Stability

a. Matrix: Fat, extracted fat from adipose tissue

b. Sample receipt size: Enough sample to obtain 0.5 g of rendered fat.

c. Condition upon receipt: Not spoiled or rancid.

d. Sample storage:

i. Time: CHCs, indefinite; organophosphates, approximately 1 month.

ii. Condition: refrigerated or frozen.

6. Sample Set

a. Blank fat

b. Standard(s)

c. Recovery:

i. Routine samples: at least two recoveries at the 1X level.

ii. Special samples (e.g. violation): two recoveries of the pesticide of interest at the level close to the finding or its tolerance level.

d. Samples

7. Sensitivity

a. Minimum proficiency level (MPL):

Pesticide Name	MPL (ppm)	Pesticide Name	MPL (ppm)
HCB	0.10	Captan	0.04
alpha BHC	0.10	Stirofos	0.06
Lindane	0.10	Kepone	0.06
Heptachlor	0.10	Endrin	0.10
Aldrin	0.10	*p,p'*-TDE	0.15
Ronnel	0.03	*o,p'*-DDT	0.15
Linuron	0.50	Endosulfan II	0.04
Oxychlordane	0.04	*p,p'*-DDT	0.15
Chlorpyrifos	0.10	Carbophenthion	0.06
Nonachlor	0.15	Mirex	0.10
Heptachlor epoxide	0.10	Methoxychlor	0.50
Endosulfan I	0.02	Phosalone	0.02
trans-Chlordane	0.30	Coumaphos-O	0.20
cis-Chlordane	0.30	Coumaphos-S	0.20
Chlorfenvinphos	0.05	Toxaphene	1.00
Dieldrin	0.10	PCB 1254	0.50
p,p'-DDE	0.10	PCB 1260	0.50

J. WORKSHEET

USDA

HEC-CHC-COP
GPC WORKSHEET

REGULATORY CHEMISTRY SECTION, USDA-FSIS WESTERN LAB 620 CENTRAL AVE., BLDG. 1 , ALAMEDA, CA 94501

Individual sample size = 0.5 g weighed, 0.25 g processed by GPC — **Start date:**

Final volume of GPC extract = 2.0 mL — **Completed date:**

Aldrin and DBC at 200 ppb for CHC and 100 ppb for HEC

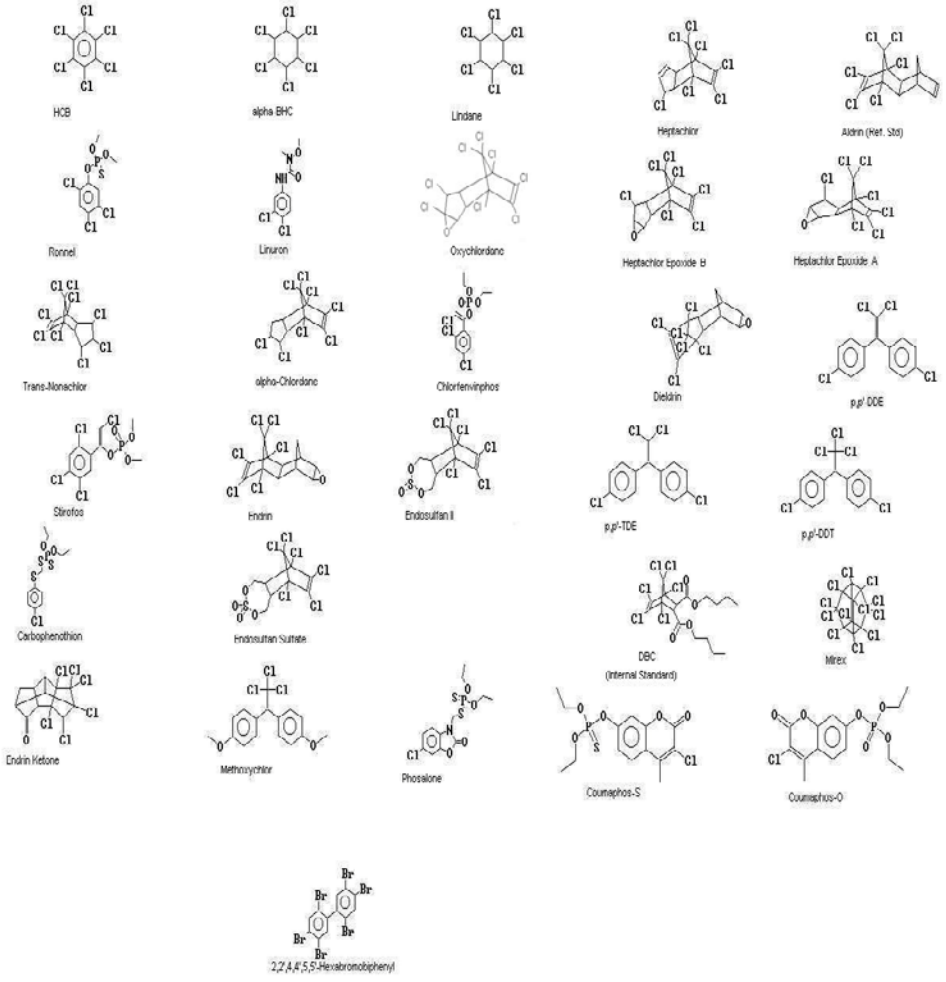
SAMPLE#	ALDRIN FOUND (ppb)	RESULTS (ppm) AND COMMENTS
BLANK		

SAMPLES WEIGHED BY: **GPC:** **GC:**
WEIGHT LOGBOOK: **PAGES:** **Date processed by GPC:**
BALANCE: **GPC MOBILE PHASE:**
REFRIGERATOR: **DILUENT:**
EPPENDORF(S): **ALDRIN FORTIFICATION STD:**
DISPENSETTE: **HC or HEC FORTIFICATION STD:**
OVEN: **CHC or HEC INJECTION STD:**
TEMPERATURE (°C): **CYCLOPENTANE:**
GENERAL COMMENTS:

HEC – Halogenated Environmental Contaminates

K. APPENDIX

1. Reference: Structures of CHC compounds



CHC Pesticides in Standard

Part II: Egg Products Matrix

A. INTRODUCTION

1. Theory

This procedure describes the extraction and quantitation of CHC-COP compounds in egg products. The GPC and GC parameters used are the same as those used for determination of CHC-COP compounds in fat. Pesticides are extracted from egg products into acetonitrile. The residues are then partitioned into cyclopentane. The cyclopentane is then diluted to 10 mL and cleaned up by GPC. Analytical determination is done by GLC-EC detection.

2. Applicability

Compounds for which this method applies for quantitation:

Aldrin	Endrin	Methoxychlor
Alpha BHC	Endrin ketone	Mirex
α or cis-chlordane	Heptachlor	*trans*-Nonachlor
Chlorpyrifos	Heptachlor epoxide a	Ronnel
p,p'-DDE	Heptachlor epoxide b	Stirophos
p,p'-DDT	*2,2',4,4',5,5'*-Hexabromobiphenyl	*p,p*'-TDE
Dieldrin	Hexachlorobenzene (HCB)	
Endosulfan II	Lindane	

B. EQUIPMENT

Note: Equivalent apparatus and instrumentation may be substituted

1. Apparatus

a. Centrifuge - IEC-Damon CU5000

b. Homogenizer - Polytron 10/35.

c. Volumetric flasks - Class A, 10 mL.

d. Graduated cylinders - Class A glass, 10 mL or 25 mL.

e. Graduated centrifuge tubes - Class A glass, 15 mL.

f. Separatory funnels - 125 mL or 250 mL.

g. Centrifuge tubes - glass or disposable polypropylene, 50 mL.

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents and solutions may be substituted.

1. Reagents

   a. Acetonitrile, Honeywell, B&J Brand,

   b. Distilled/deionized water.

   c. Cyclopentane, Honeywell, B&J Brand,

   d. Sodium sulfate (Na_2SO_4) - anhydrous, Fisher

2. Solutions

   a. 4% Na_2SO_4 solution:

      Dissolve 160 ± 10 g anhydrous Na_2SO_4 in 4 liters of distilled water.

D. STANDARDS

Refer to Part I, Section D.

E. SAMPLE PREPARATION

Process sample until homogeneous.

F. ANALYTICAL PROCEDURE

Note: Rinse all glassware used in the analysis with cyclopentane prior to use.

1. Weigh 4.0 g of liquid whole egg product into a 50 mL centrifuge tube. For products other than liquid whole eggs, weigh 4.0 g liquid whole egg equivalent (see Section K, Table 1).

2. Fortify each sample (samples, blank and recovery) with 100 µL of 2.0 µg/mL Aldrin fortification standard (Part I, Section D.2.a.v.).

3. Fortify the two recovery samples with 400 µL of 0.5 µg/mL CHC-COP fortification solution (Part I, Section D.2.a.i.).

4. Add 20 mL of acetonitrile to each sample.

5. Blend all the samples for one minute with a homogenizer. Rinse the homogenizer with acetonitrile and add rinsings to centrifuge tube. Dried egg samples and salted egg yolks may be vortexed for one minute instead of using the homogenizer.

6. Shake or vortex samples briefly to remove material adhering to the side of the tubes, and centrifuge for 15 minutes.

7. Transfer the supernatant (liquid only, avoid solids or particulate matter) from the centrifuge tube to a separatory funnel containing 80 - 100 mL 4% Na_2SO_4 solution and 6 - 10 mL cyclopentane.

8. Shake the separatory funnels vigorously for at least one minute. Allow the mixture to stand undisturbed at least one hour to ensure adequate separation of the two phases, upper = cyclopentane/pesticides. The upper layer should be clear yellow.

   If phases are not separated, add additional Na_2SO_4 solution and repeat step 8.

   Stopping point: The samples may be stored overnight at room temperature.

9. Drain the lower aqueous phase containing the acetonitrile and Na_2SO_4 and discard. Do not drain cyclopentane phase or any material near the solvent interface.

10. Add 20 mL distilled water to separatory funnels and shake 5 - 10 seconds. Drain off as much aqueous phase / particulate matter as possible without losing any cyclopentane phase. Repeat if upper layer is cloudy.

11. Transfer cyclopentane phase to clean 10 mL class A volumetric flask. Glass graduated cylinders or centrifuge tubes are acceptable for screening. Remove as much of remaining aqueous phase as possible with Pasteur pipet. There should be ~0.2 mL aqueous solution present.

12. Bring liquid in volumetric flask to 10 mL with cyclopentane and mix well. If extract is excessively cloudy, centrifuge in a glass centrifuge tube and discard extraneous material before continuing.

13. Filter approximately 5 mL of extract through a 0.45 µm filter, avoiding aqueous or solid materials if any are present.

14. Process by GPC following instructions in Part I, section F.2

G. CALCULATIONS

The same injection standards and GC temperature programs are used as found in Part I, Section F.2. The final GPC extract represents 1 g of tissue compared with 0.25 g tissue

for fat analysis. The GC calibration table values should be adjusted to ¼ the values in Section I. Fat Matrix.

Analytes in order of elution	Concentration in 0.25 g fat sample (ppm)	Concentration in one gram of egg sample (ppm)
Hexachlorobenzene	0.20	0.05
α-BHC	0.20	0.05
Lindane	0.20	0.05
Heptachlor	0.20	0.05
Ronnel	0.20	0.05
Linuron	0.60	0.15
Chlorpyriphos	0.20	0.05
Heptachlor Epoxide B	0.20	0.05
Heptachlor Epoxide A	0.20	0.05
trans-Nonachlor	0.20	0.05
α-Chlordane	0.20	0.05
Chlorfenvinphos	0.60	0.15
Dieldrin	0.20	0.05
p,p'-DDE	0.20	0.05
Stirofos	0.20	0.05
Endrin	0.20	0.05
Endosulfan II	0.20	0.05
p,p'-TDE	0.20	0.05
p,p'-DDT	0.20	0.05
Carbophenothion	0.20	0.05
Endosulfan Sulfate	0.20	0.05
Mirex	0.20	0.05
Endrin Ketone	0.20	0.05
Methoxychlor	0.20	0.05
Phosalone	0.20	0.05
Coumaphos-O	0.60	0.15
Coumaphos-S	0.60	0.15
2,2',4,4',5,5'-Hexabromobiphenyl	0.20	0.05
Aldrin	0.20	0.05
DBC	0.20	0.05

H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment — safety glasses, laboratory coat, and nitrile gloves.

2. Hazards

Procedure Step	*Hazard*	*Recommended Safe Procedures*
Cyclopentane	Highly flammable; Irritating to skin and mucous tissue	Perform extraction under well ventilated fume hood.
Acetonitrile	Flammable, absorbed through skin; Irritating mucous tissue	

3. Disposal Procedures

Procedure Step	*Hazard*	*Recommended Safe Procedures*
Acetonitrile / Na_2SO_4 solution	See above	This mixture should be temporarily stored with the flammable waste solvents until disposal by the waste disposal contractor or in-house specialist. Observe all Federal, state, and local environmental laws.

I. QUALITY ASSURANCE PLAN

1. Performance Standards

Analyte	*% Recovery*
Aldrin	60 - 120
alpha BHC	60 - 120
a-Chlordane	50 - 120
Chlorpyrifos	50 - 120
p,p'-DDE	60 - 120
p,p'-DDT	60 - 120
Dieldrin	60 - 120
Endosulfan II	60 - 120
Endrin	60 - 120

Analyte	*% Recovery*
Endrin ketone	60 - 120
Heptachlor	50 - 120
Heptachlor epoxide a	60 - 120
Heptachlor epoxide b	60 - 120
2,2',4,4',5,5'-Hexabromobiphenyl	50 - 120
Hexachlorobenzene	50 - 120
Lindane	50 - 120
Methoxychlor	60 - 120
Mirex	60 - 120
trans-Nonachlor	50 - 120
Ronnel	60 - 120
Stirofos	50 - 120
p,p'-TDE	60 - 120

J. WORKSHEET

Use GPC Worksheet in Part 1, Section G.

K. APPENDIX

TABLE 1: **LIQUID WHOLE EGG EQUIVALENTS (Products of Known Composition)**

Adapted from AMS Laboratory Guidebook for Egg Products

Product	Weight in grams
Liquid Whole Eggs	4.00 ± 0.20
Dried Whole Eggs	1.04 ± 0.04
Liquid Egg Yolks	1.60 ± 0.08
Dried Egg Yolks	0.72 ± 0.04

This table is used to determine the amount of egg product to weigh for products other than liquid whole eggs. For example, 4.00 g liquid whole eggs is equivalent to 1.04 g dried whole eggs.

Note: The analyst performing the above calculations must remember that everywhere the percent sign appears, the percentage must be converted to its decimal equivalent. For example, 25% becomes 0.25 in the above formulas.

L. APPROVALS AND AUTHORITIES

1. Approvals on file.

2. Issuing Authority: Director, Laboratory Quality Assurance Division.