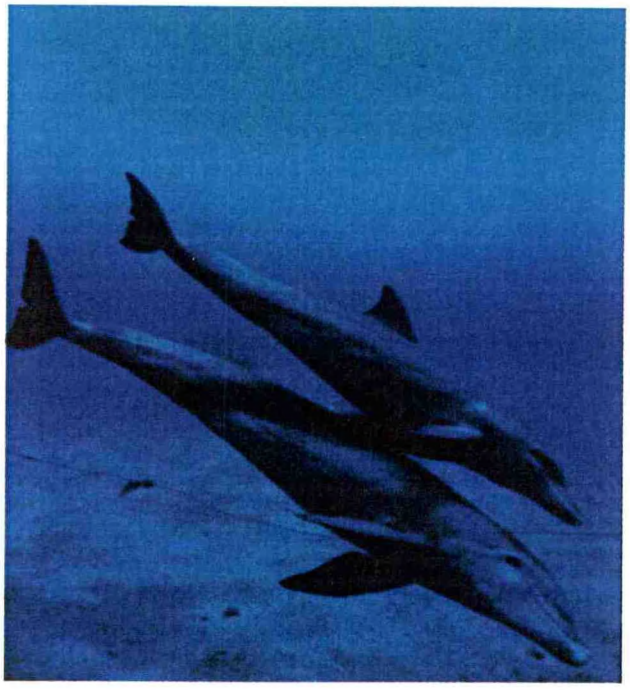
nical Memorandum NOS-NCCOS-CCEHBR-0005

Method for the Analysis of Polychlorinated Biphenyls and Chlorinated Pesticides in Marine Mammal Tissue



Gregory B. Mitchum

Charleston, SC
May 2003

U.S. Department of Commerce
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National Ocean Service
National Centers for Coastal Ocean Science
Center for Coastal Environmental Health and Biomolecular Research

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Cover: *Tursiops truncatus* (bottlenose dolphin) Mother and calf pair.

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Introduction

Polychlorinated biphenyls and chlorinated pesticides are ubiquitous marine pollutants that are bioaccumulated by marine mammals and are biomagnified through the food chain. Marine mammals, at the top of their food chain, carry very heavy body burdens of these potentially toxic anthropogenic contaminants. In cetaceans and pinnipeds there is some limited evidence that high body burdens of organochlorines may impair reproduction[1,2,3]. There is also evidence that high body burdens and increased whole blood concentrations of these compounds can cause problems with immunological response systems in marine mammals which may make them more susceptible to secondary infections[4,5]. In order to study the possible chronic toxic effects of chlorinated hydrocarbons on the health of marine mammal populations, data on levels of these compounds in tissues of stranded and healthy animals is needed.

Neither the American Oil Chemist Society, (AOCS), nor the Association of Official Analytical Chemist, (AOAC), have an official method for the determination of PCBs or pesticides in marine oils or tissues. The AOAC has first and final actions for packed column gas chromatographic methods for the determination of these compounds in fish and non-fatty foods[6,7] and the EPA has mass spectroscopy methods for the determination of these compounds in soil and sediment[8]. There is also a gel permeation first action method for organochlorine residues in poultry, beef, and swine fats [9] . The AOAC does not have methods for congener specific analysis of polychlorinated biphenyls needed for the toxicological assessment of these compounds. The gas chromatography/mass spectrometry (GC/MS) procedure described in this technical memorandum is an adaption of these methods that accommodates the matrices, congener specific analysis, sample sizes and analyte levels encountered in the analysis of marine mammal tissues.

Principle

The lipid and lipid soluble PCBs and pesticides are extracted from the tissue by Soxhlet extraction. A gel permeation column is used to separate the analytes from the lipid components based on molecular weight and is followed by Florisil adsorption chromatography to separate the analytes from other polar interferences of similar molecular weight. The isolated analytes are then analyzed on a HP 5890 GC interfaced with a HP 5971 mass spectrometer. The analytes are separated on a non-polar (5% phenyl) methylpolysiloxane (DB-5, J&W Scientific) fused silica capillary column. The mass spectrometer is operated in the selected ion monitoring (SIM) mode which allows for identification of only the ions of interest and increases the sensitivity of the analysis. Analytes are identified based on comparison of their retention times with those of known standards, and confirmed by MS using target ion and qualifier ion ratios. The individual pesticides and selected PCB isomers are quantified using internal standard/linear regression methodology. Total PCBs are calculated by comparing 5 peak areas in the sample to the corresponding peak areas of a 1254

Arochlor standard[10,11,12]. To optimize sensitivity, three separate runs are required, one each for the determination of individual chlorinated pesticides, selected PCB congeners and total PCBs. Differences in the selected ions to be monitored and optimizing dwell times to increase sensitivity predicate the need for three runs. Periodic analysis of standard reference materials (SRM) ensures accuracy of the analyses and continuing calibration checks with each sample set analyzed ensures the analyses are within control limits.

Apparatus

- Gel permeation column, 25 cm x 2.5 cm (Kontes)
- HPLC pump (Laboratory Data Control)
- Soxhlet Extraction glassware (Baxter)
- Turbo-Vap (Zymark)
- 200 ml collection/evaporation tubes (Zymark)
- Florisil column, 10 cm x 1.0 cm (Tudor)
- Gas chromatograph (Hewlett Packard 5890) with capillary direct interface to a mass spectrometer (HP 5971)
- Autosampler (Hewlett Packard 7973 or equivalent)
- DB-5 (coating) capillary column, 0.25 μm film thickness , 0.25mm ID x 30m length (J&W scientific)
- Analytical balance capable of weighing to 0.0001 g
- Autosampler vials (compatable with instrumentation)
- Vortex mixer
- Mortar and pestle (4.5" mortar and 6" pestle)
- 9" disposable Pastuer pipets
- Volumetric flasks (1,2,5,10,25,50) ml
- 250 ml round bottom flask
- Volumetric, micro pipets

Reagents

- Biobeads SX-3 (Biorad)
- Dichloromethane:cyclohexane (50:50), V:V, (organic residue grade)
- 2,2,4 Trimethylpentane (organic residue grade)
- Florisil, 60/100 mesh (Supelco)
- Petroleum ether:ethyl ether (80:20), V:V, (organic residue grade)
- Grannular anhydrous Na_2SO_4 (Baker)
- Hexane (organic residue grade)
- Helium gas, ultra-high purity
- Nitrogen gas, ultra-high purity
- Pesticide standard (custom preparation by AccuStandards of New Haven, CT) 20 ng/μl in iso-octane (Table 1)

- PCB congener standard (custom preparation AccuStandards of New Haven, CT) 20 ng/µl in iso-octane (Table 2)
- Total PCB standard (AccuStandards of New Haven CT) 100 ng/µl Tech. Mix Arochlor 1254 in toluene (Table 3)
- PCB 103 (AccuStandards of New Haven CT, 35 ng/µl in iso-octane)
- PCB 198 (AccuStandards of New Haven CT, 35 ng/µl in iso-octane)

Preparation of standards and samples

Standard preparation

1. Using the mixed PCB standard containing 20 ng/µl of each of the components listed in Table 1, prepare working standards for 5 calibration levels (0.05, 0.2, 2.0, 5.0, and 25 ng/µl), each containing 0.75 ng/µl each of PCB congeners 103 and 198 as the internal standards.

2. Using the mixed pesticide standard containing 20 ng/µl of each of the components listed in Table 2, prepare working standards for 5 calibration levels (0.20, 1.0, 2.0, 5.0, and 15 ng/µl), each containing 0.75 ng/µl each of PCB congeners 103 and 198 as internal standards.

3. Using the 100 ng/µl Tech mix Arochlor 1254 PCB standard, prepare working standards for 4 calibration levels (5.0,10.0, 15.0, and 75 ng/µl), each containing 0.75 ng/µl of PCB congeners 103 and 198 as internal standards. Compounds monitored are listed in Table 3.

4. Recovery compounds phenanthrene d_{10} and chrysene d_{12} are added to extracts just prior to instrumental analysis at 100 ng each.

Sample preparation

Sources for blubber samples are surgical biopsies, dart biopsies, or necropsies. Samples should be collected in a manner that limits the possibility of contamination, using clean teflon instruments and storage containers where possible. The samples should be frozen immediately and stored at -80°C as soon as possible. Skin is usually attached and can be removed for subsequent DNA techniques.

Lipid Extraction

1. Following the removal of skin, slice the blubber sample thinly (approximately 0 .20), grams place in a mortar and macerate with approximately 100g of Na_2SO_4. Prior to use, the Na_2SO_4 should be cleaned as follows:
   a. Place approximately 100 g of Na_2SO_4 in pyrex Soxhlet thimble. Place the thimble in the Soxhlet apparatus and extract overnight with 300 ml of hexane. After extraction store Na_2SO_4 in a 130°C oven. Allow the Na $_2SO_4$ to come to room temperature in a desiccator before use.

2. Transfer the sample to a fritted Soxhlet extraction thimble and add 100 ng each of the internal standards (PCB 103 & 198).

3. Extract in Soxhlet apparatus with 150-200 ml of hexane for approximately 12 hours.

Determination of Lipid Content

1. After completion of the extraction, reduce the volume of hexane to less than 100 ml, transfer to a 100 ml volumetric flask and bring to volume with hexane.

2. Transfer 2 ml to a tared aluminum weighing pan.

3. Dry in a drying oven at 60°C until solvent is removed.

4. After solvent has evaporated remove and cool in a desiccator.

5. Re-weigh pan to a constant weight and calculate lipid amount as % wet weight of the sample. (pan + lipid minus pan weight / 2) X 100/sample weight X 100 = % lipid)

6. Transfer the remaining extract to an evaporating tube and reduce the solvent on the Zymark Turbo-Vap.

Sample Clean-up

Gel permeation:

Gel permeation chromatography is performed on a 25 cm x 2.5 cm glass column. The eluting solvent is a 50:50 MeCl:cyclohexane mixture. A typical closed system is shown in Figure 1. In our hands a bed length of 22.5 cm will remove approximately 0.70 g of lipid. A sample with higher amount of lipid must be run through the column twice. The solvent is pumped onto the column with an HPLC pump at a flow rate of 5 ml/min. The sample is introduced via a sample injection valve with a 2 ml sample loop of Teflon tubing. Multidirectional valves permit the column effluent to be directed either to a waste jar, to a pre-calibrated fore-run catch flask (which allows collection of a known volume of solvent before sample collection begins), or to a collection-evaporation tube. Prior to using the gel permeation column, the fore-run and collection volumes must be verified as follows: Sequential 5 ml fractions are collected and analyzed to determine elution times and volumes for analytes of interest. An oil sample was run through the column and each fraction was analyzed by Thin Layer Chromatography to determine which fraction the oil first appears. A standard with the analytes of interest was also run through the column and analyzed by

GC/MS to determine in which fraction they first appear. This allows for the determination of the fore-run and collection volumes. By calculating the flow rate of the column in ml/min, these volumes can be converted to elution times.

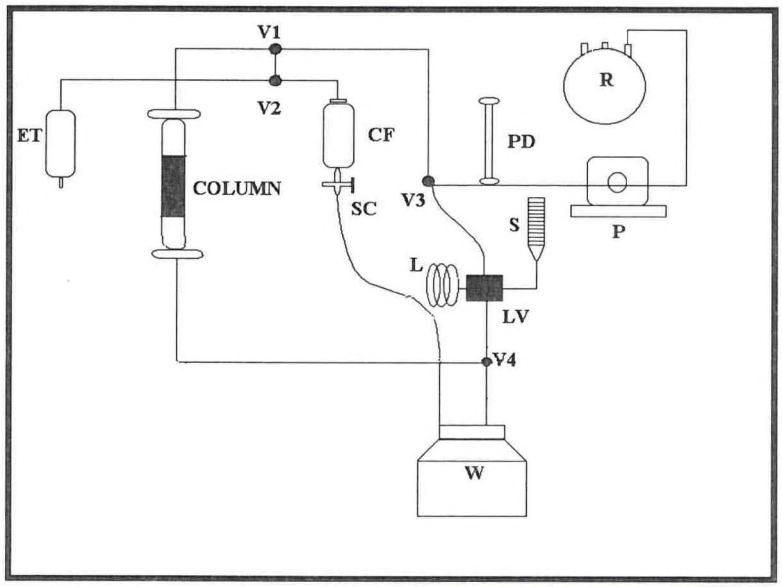

Figure 1. Gel Permeation apparatus. R=reservoir, PD= pulse damper, P= pump, S= syringe, CF= collection flask, SC= stopcock, L= loop, W= waste, ET= evaporation tube LV= loop valve and V_1-V_4= valves

1. Fill the reservoir (R) with eluting solvent. Run the pump (P) for 30 min or until the column packing is totally moistened with solvent. Set the valves so that the flow of solvent is from the reservoir through valve 3 (V3), loop valve (LV), valve 4 (V4), column, valve 1 (V1), valve 2 (V2), catch flask (CF), stopcock (SC) and to waste (W). Valves 1,2,3 and 4 are three way valves and the loop valve is a four way valve. The pulse damper (PD) allows for a constant flow through the column.

2. Turn the loop valve to the load position and load the sample syringe (S). With the loop valve in the load position the effluent end of the loop is being sent to waste. Rinse the tube with a small amount of solvent and apply it to the sample syringe taking care not to exceed the 2 ml total volume of the sample loop. After the sample is loaded onto the loop (L) switch, the loop valve to "column" position to direct the solvent flow through the loop and onto the column, while simultaneously closing the stopcock. Start timing.

3. Carry the pre-run out for the predetermined time and collect the forerun in the catch flask. Once the forerun has eluted, switch valve 2 to direct the eluting solvent to the evaporation tube (ET). Collect the fraction containing the analytes using the predetermined time. Upon completion of the elution, switch valve 2 back to its original position and open stopcock to drain the waste.

4. After sample is eluted, back-flush the column to prepare it for the next sample. Turn the pump off and wait until flow stops eluting from column. Then turn valves 1, 3, and 4 to reverse flow of the solvent through the column. Turn pump on and run for 5-10 min. After back flushing, return all valves back to their original positions. Store column with valve 4 in the closed position to keep solvent on the column.

5. Place the evaporation tubes on the Turbo-vap and reduce the solvent to 1-2 ml (water bath maintained at 38°C and nitrogen pressure is 8-12 psi). Monitor evaporation to ensure that the sample does not go to complete dryness. The samples are now ready for the Florisil column.

Florisil chromatography:

1. Fill a 10 cm x 1.0 cm glass column that has a 100 ml reservoir with 7.8 cm of Florisil and add a 1.0 cm layer of anhydrous Na_2SO_4 (the Florisil and Na_2SO_4 are kept in a 190°C oven and are removed from the oven and placed in a desiccator to cool prior to column packing).

2. *Florisil Calibration:* Because each lot of Florisil varies in its activity, each new lot of Florisil must be calibrated to determine the volume of solvent required to elute the PCBs and pesticides.
   a. Prepare a Florisil column as described in step 1.

   b. Wet the column with 80:20 petroleum ether/ethyl ether. Keep the solvent 1 cm above the Na_2SO_4. Place a 10-fold concentrated PCB and pesticide standard on the head of the column. Collect 3 x 10-ml fractions followed by 20 x 2-ml fractions. Analyze each fraction for PCBs and pesticides by GC/MS. The elution volume is determined when analytes are no longer present.

3. *Analyte Isolation*: Wet the packing with 20 ml petroleum ether/ethyl ether (80:20). Do not allow the column packing to go dry. Keep the solvent level at the top of Na_2SO_4 layer by closing the stopcock when the solvent is about 1 cm above the Na_2SO_4.

4. Transfer the sample from the concentrator tube to the column using a clean disposable Pasteur pipet.

5. Wash the sample tube with 2 x 1 ml of the predetermined elution volume.

6. Open the stopcock to begin elution of the sample back into the original sample tube. Maintain the flow rate at 4-5 ml/min; pressurizing with nitrogen if necessary.

7. When the solvent reaches the top of the Na_2SO_4 layer, add an additional 5 ml of the predetermined eluting solvent to wash the walls of the column.

8. As the solvent reaches the top of the Na_2SO_4 layer, elute with the remaining volume.

9. Place the evaporation tubes on the Turbo-vap and reduce the solvent to approximately 100 µl (maintain the water bath at 38° C and a nitrogen pressure of 8-12 psi). The sample should never be allowed to go completely to dryness.

10. Add 100 ng of the recovery compounds (phenanthrene d_{10} and chrysene d_{12}) and adjust the final volume to approximately 100-200 µl. The samples are now ready for GC/MS analysis.

GC/MSD Analysis

Instrumental Conditions

The following conditions are typical for this analysis, but must be modified for each GC/MS system, and with column age. The individual analyst must determine conditions that are best suited for their application. Separations are achieved on a DB-5 fused silica capillary column directly interfaced to the MSD. Since optimum separation and quantitation for each of the types of compounds (chlorinated pesticides, selected PCB congeners and total PCBs) is achieved at different oven parameters and by monitoring different ion groups, a separate instrument method is used for each. Selected ion monitoring (SIM) groups and ions monitored for each of the groups are given in Tables 1, 2 & 3. Oven parameters for each method are given in Tables 4, 5 & 6 . The ions selected for monitoring are chosen from known fragmentation patterns. For all separations, the injection port is maintained at 275°C and the MSD heater transfer line at 280°C. The carrier gas (helium) flow rate is maintained throughout the temperature programmed run at 1 ml/min (linear velocity of 36 -37 cm/sec) by the use of electronic pressure control. The GC is equipped with an automated sampler. A one µl sample is injected using the splitless mode and the injection port is purged 1 min. after injection to sweep away any residuals.

Calibration

Following a high-sensitivity autotune, retention times and ion ratios are determined for each analyte by the analysis of individual standards. Ion ratios may differ somewhat based on operating conditions and MS tune parameters and must be determined by the analyst. Mixed calibration standards, prepared as described previously, are analyzed and calibration curves are established for each analyte by plotting the response ratio (response of analyte/response of ISTD) *vs* amount ratios

(concentration of analyte/concentration of ISTD). Typical chromatograms of a 2 ng/µl midpoint calibration standard for each method can be found in Figures 2, 3, and 4. The analytes are listed in elution order except the internal standards 103 and 198. Analytes listed after each internal standard are quantified using that standard (Tables 1,2,3). Typical samples cover a broad range of analyte concentrations. The calibration range has been divided into a low and high concentration range. The low concentration curve for the PCB and pesticide analysis consist of the first three calibration levels for each method and the high concentration curve includes the last three levels with the 2 ng midpoint included in both curves. Analyte areas are checked to determine which curve is used for quantitation. The total PCB analysis consist of only one curve.

Analysis of samples

Samples are analyzed using identical conditions as for standards. Analytes are identified by comparison of retention times with those of standards and confirmed by qualifying ion ratios to the target ions. Samples in which the area counts are outside the high concentration calibration range for certain analytes are diluted and re-analyzed. The target ion /confirmation ions ratios are determined for each compound from pure standards. The ion ratios percent relative uncertainty is set ± 25 %. The identification of analytes is accepted if they meet the qualifying criteria and match the retention time of analytes in the calibration samples. If identification criteria are met, the analyte amount is calculated as follows:

Calculations

1. PCB congeners and pesticides are calculated by the MS software as follows:

- A calibration curve is constructed for each analyte by plotting concentration ratio vs response ratio for the different concentration levels.
  Response ratio = response of analyte/response of internal standard in a given level.
  Concentration ratio = concentration of analyte/concentration of internal standard for that level.
- A equation for the curve through the calibration points is calculated using the curve fit type and origin handling specified in method.
- An unknown sample is run and a response ratio is determined.
  Response ratio = response of analyte/response of internal standard added to the unknown.
- A corrected concentration of the unknown is calculated using the curve fit equation.
- The corrected concentration ratio is multiplied by the concentration of the internal standard added to the unknown to determine the concentration of the analytes in the unknown.

2. Total PCBs: Total PCBs are calculated using the sum of the area amounts of the 5 peaks in the standard (Table 3) as compared to the sum of the analytes in the sample.

$$x(ng\ /\ g) = \frac{a \times B}{C} \times D$$

a = total area of the 5 peaks in sample/ area of internal standard in sample
B = amount of analytes in standard (ng)/ internal standard amount in standard (ng)
C = total area of the 5 peaks in the standard/internal standard peak area in the standard
D = amount of internal standard (ng) /sample weight (g)

3. Percent recovery of internal standard

$$x(\%) = \left(\frac{e \times f}{g} \times h \right) \times 100$$

e = area of internal standard (sample)/area of recovery compound (sample)
f = amount of internal standard (standard) /amount of recovery compound (standard)
g = area of internal standard (standard)/area of recovery compound (standard)
h = amount of recovery compound added to sample (ng)/amount of internal standard added to the sample (ng)

QA/QC

A solvent blank, a 2.0 ng/µl midpoint pesticide and PCB congener standard, and a 15 ng/µl total PCB standard are run with each set of samples. The analyte amount in the pesticide standard and the PCB congener standard are calculated as samples at both high and low concentration range for use as continuing calibration checks. The amount measured for each compound should not exceed ±20% of the known concentration. If any one analyte falls outside this range three consecutive times, further analysis should be terminated until the problem is solved. NIST SRM 1945, Organics in Whale Blubber, and a pesticide spiked oil (menhaden oil), are analyzed with every set of 12 samples. The values obtained for the SRM 1945 and pesticide spiked oils should be within ± 30 % of the calculated mean value, determined from the initial method validation.

Validation and Method Level of Detection

The selected PCB congener method was validated using NIST SRM 1945 (Organics in Whale Blubber). A set of 8 samples was analyzed. Amounts of each analyte were calculated for each sample as well as standard deviations and recoveries. The method detection limit for each analyte

was set at 3 times the standard deviation. The instrumental detection limit was set at 10 times signal to noise ratio. Since no standard reference material (SRM) is available for the complete list of pesticides analyzed, the validation was conducted using a menhaden oil spiked with 100 ng of each pesticide (Table 7). An initial set of 16 samples (8 SRMs and 8 spiked oils) was run through the above analytical process under the same conditions as the calibration standards. The standard deviations and recoveries were calculated and method and instrumental detection limits were set as described above.

Although not used in the calculation of analyte amounts, recovery of the internal standard in each validation sample was calculated using the recovery compound phenanthrene d_{10}. Acceptable recovery of the ISTD was set at > 50%. This same criteria is used in analyzing the unknown samples. Data from the validation sets are used to determine analytical accuracy and precision and to establish mean values for comparison to subsequent analyses. The standard deviation and recovery information can be found in Table 7. Similar validation procedures should be conducted by each laboratory prior to use of this method.

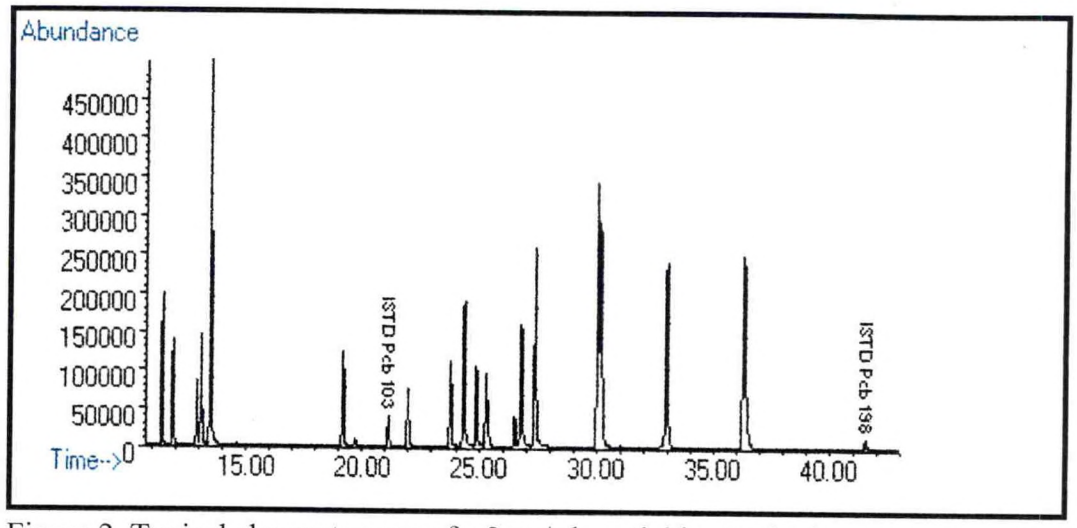

Figure 2. Typical chromatogram of a 2 ng/µl pesticide standard.

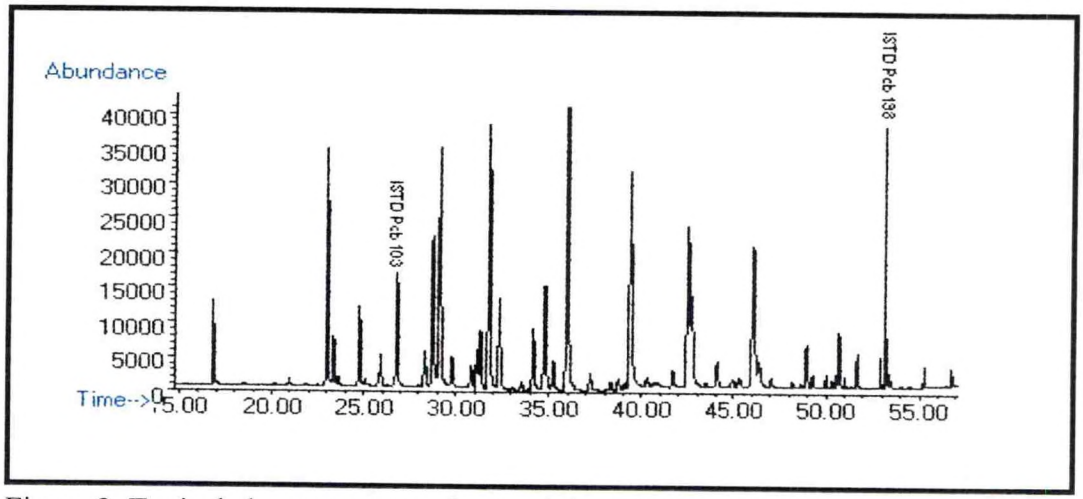

Figure 3. Typical chromatogram of a 5 ng/µl total PCB standard.

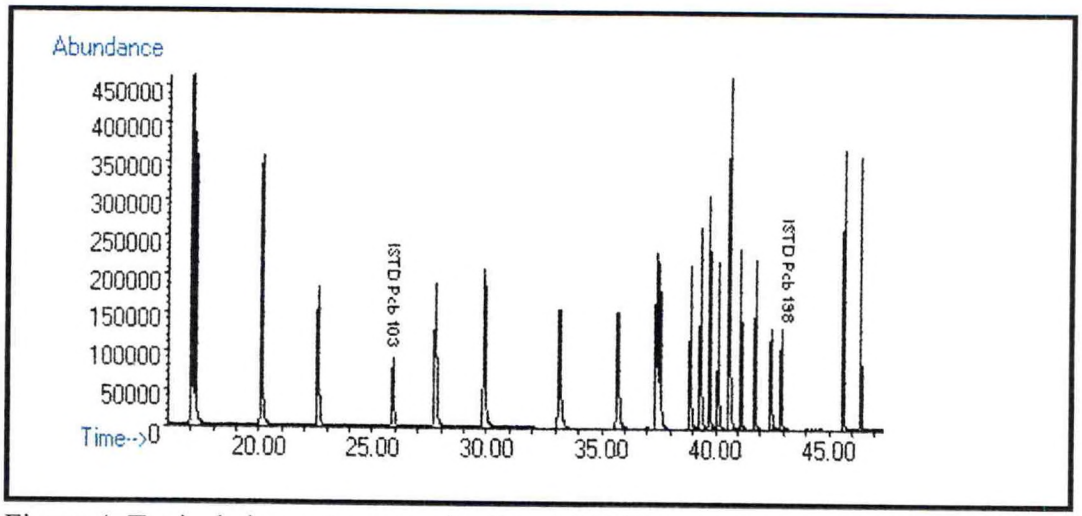

Figure 4. Typical chromatogram of a 2 ng/µl PCB congener standard.

Table 1. Analytes, internal standards (ISTD) and recovery compounds included in PCB congener calibration standards and the ions monitored for each group.

SIM Group	Analytes (listed by elution order in SIM groups)	Ions Monitored
Group 1	ISTD PCB 103	324, 326, 328
	Phenenthrene d_{12}	188,189
	18	186, 256, 258
	28	186, 256, 258
	52	290, 292, 294
	66	290, 292, 294
	101	324, 326, 328
	77	290, 292, 294
	correction ions	254, 288,322
Group 2	118	324, 326, 328
	153	358, 360, 362
	105	324, 326, 328
	138	358, 360, 362
	126	324, 326, 328
	correction ions	322, 356
Group 3	187	391.8, 293.8, 395.8
	128	358, 360, 362
	Chrysene d_{12}	240, 241
	156	358, 360, 362
	180	391.8, 293.8, 395.8
	correction ions	356
Group 4	169	358,360, 362
	ISTD PCB 198	427.8, 429.8, 431.8
	206	461.8, 463.7, 465.7
	209	495.7, 497.7, 499.7
	correction ions	356

Table 2. Analytes, internal standards and recovery compounds included in pesticide calibration standards and the ions monitored for each group.

SIM Group	Analytes (listed by elution order in SIM groups)	Ions Monitored
Group 1	ISTD PCB 103	326, 328
	α- BHC	181, 183, 219
	HCB	282, 284, 286
	β- BHC	181, 183, 219
	lindane	181, 183, 219
	phenenthrene d_{10}	188, 189
Group 2	Aldrin	66, 263, 265
	heptachlor epoxide	53, 355
	γ-Chlordane	373, 375, 377
	2,4′- DDE	246, 248, 318
	Chrysene d_{12}	240, 241
	α-Chlordane	373, 375, 377
	Transnonachlor	407, 409, 411
	Dieldrin	263, 237
	4,4′- DDE	246, 248, 318
	*2,*4′- DDD	165, 235, 237
Group 3	4,4′′-DDD	165, 235, 237
	ISTD PCB 198	428, 430
	*2,*4′-DDT	165, 235, 237
	4,4′- DDT	165, 235, 237

Table 3. Analytes, internal standards and recovery compounds included in total PCB calibration standards and the ions monitored for each group.

SIM Group	Analytes (listed by elution order in SIM groups)	Ions Monitored
Group 1	ISTD PCB 103 52 correction ion	324, 326, 328 290, 292, 294 288, 322
Group 2	101 correction ion	324, 326, 328 322
Group 3	118 138 128 ISTD PCB 198 correction ions	324, 326, 328 358, 360, 362 358, 360, 362 427.8, 429.8, 431.8 322, 356

Table 4. GC oven program for the analyses of PCB congeners.

Initial Temperature: 80°C		*Initial Time: 1.00 min*	
Ramp	Rate (°C/min)	Final Temperature (°C)	Final Time (Min.)
1	10.00	165	1.00
2	1.50	200	1.00
3	8.00	320	10.00

Table 5. GC oven program for the analyses of pesticides.

Initial Temperature: 80°C		*Initial Time: 1.00 min*	
Ramp	Rate (°C/min)	Final Temperature (°C)	Final Time (Min.)
1	30.00	160	1.00
2	2.00	250	2.00
Level	50.00	320	10.00

Table 6. GC oven program for the analyses of total PCBs

Initial Temperature: 80°C		*Initial Time: 1.00 min*	
Ramp	Rate (°C/min)	Final Temperature (°C)	Final Time (Min.)
1	10.00	165	1.00
2	1.00	200	1.00
3	8.50	320	5.00

Table 7. Mean concentrations (± SD) of 16 pesticides measured in a spiked marine oil and 15 PCB congeners measured in SRM 1945, and recovery for each analyte (% of spiked or certified amount).

Pesticides	µg/kg			PCB congener	µg/kg		
	Mean (n = 8)	SD	% Recovery		Mean (n = 8)	SD	% Recovery
α-BHC	101	11	101	18	7.67	1	161
HCB	77	16	77	28	18.42	2	112
β-BHC	90	14	90	52	40.94	2	97
Lindane	119	12	119	66	20.16	1	85
Aldrin	127	8	127	101	79.46	8	116
Heptachlor epoxide	113	6	113	118	85.86	7	120
γ-Chlordane	103	4	103	153	226.9	13	106
2,4′-DDE	101	1	101	105	27.74	2	93
α-Chlordane	101	6	101	138	174.99	15	130
Transnonachlor	98	7	98	187	120.32	9	120
Dieldrin	110	11	110	128	21.56	2	94
4,4′- DDE	104	4	104	156	10.37	1	103
2,4′- DDD	116	8	116	180	123.22	10	113
4,4′- DDD	123	11	123	206	47.68	5	160
2,4′- DDT	107	15	107	209	18.99	2	190
4,4′-DDT	126	34	126				

References:

1. Reijnders, P. J. H. 1986. Reproductive Failure of Common Seals Feeding on Fish from Polluted Waters. Nature 324: 456-457.

2. Martineau, D., Beland, P., Desjardins, C. and Lagace, A. 1987. Levels of Organochlorine Chemicals in Tissues of Beluga Whales, (*Delphinapterus leucas*), from the St. Lawrence Estuary, Quebec, Canada. Archives of Environmental Contamination and Toxicology 16: 137-147.

3. Subramanian, A. N. , Tanabe, S., Tatsukawa, R., Saito, S., and Miyazaki, N. 1987. Reduction in the Testosterone levels by PCBs and DDE in Dall's Porpoises of the North-Western North Pacific. Marine Pollution Bulletin 18(12): 643-646.

4. De Guis, S., Martineau, D., Beland, P., and Fournier, M. 1998. Effects of *in vitro* Exposure of Beluga Whale Leucocytes to Selected Organochlorines. Journal of Toxicology and Environmental Health. 55: 479-493.

5. Martineau, D., Lagace, A., Beland, P., Higgins R., Armstrong, D., and Shugart, L., R. 1988. Pathology of Stranded Beluga Whales, (Delphinapterus leucas), from the St. Lawrence Estuary, Quebec, Canada. Journal of Comparative Pathology. 98: 287-311.

6. AOAC. 1985. Organochlorine Residues, Organochlorine Pesticide and Polychlorinated Biphenyl Residues in Fish, Gas Chromatographic Method (#29.029-29.036, Final action). p. 385. *In* Changes in Official Methods of Analysis, 1st Supplement to Official Methods of Analysis of AOAC 14th edition, S. Williams (ed.). Association of Official Analytical Chemists Arlington, VA.

7. AOAC. 1986. Organochlorine Residues, Organochlorine Pesticide and Polychlorinated Biphenyl Residues (in high moisture foods), Gas Chromatographic Method (#29.A01-29.A04, final action). p. 365. *In* Changes in Official Methods of Analysis, 2nd Supplement to Official Methods of Analysis of AOAC, 14th edition, S. Williams (ed.). Association of Official Analytical Chemists Arlington, VA.

8. EPA. 1985. Method 680. Determination of Pesticides and PCBs in Water and Soil/Sediment by Gas Chromatography/Mass Spectrometry.

9. AOAC. 1985. Organochlorine Pesticide Residues in Poultry Fat, Gel Permeation Chromatographic Method (#29.037-29.043, Final Action). p. 385. *In* Changes in Official Methods of Analysis, 1st Supplement to Official Methods of Analysis of AOAC, 14th edition, S. Williams (ed.). Association of Official Analytical Chemists Arlington, VA.

10. Chau, A.S.Y. and Sampson, C.J. 1975. Electron Capture Gas Chromatographic Methodology for the Quantitation of Polychlorinated Biphenyls: Survey and Compromise. Environmental Letters. 8(2): 89-102.

11. Schwartz, T.R., Stalling, D.L. and Rice, C.L. 1987. Are Polychlorinated Biphenyl Residues Adequately Described by Aroclor Mixture Equivalents? Isomer - Specific Principal Components Analysis of Such Residues in Fish and Turtles. Environmental Science and Technology. 21: 72-76.

12. Morris, R.J., Law, R.J., Allchin, C.R., Carole, K.A. and Fileman, C.F. 1989. Metals and Organochlorines in Dolphins and Porpoises of Cardigan Bay, West Whales. Marine Pollution Bulletin, 20(10): 512-523.

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