$C_{12}H_{10-x}Cl_x$ (x = 1 to 10) MW: 188 to 498 CAS: Varies RTECS: Varies

METHOD: 8004, Issue 2 **EVALUATION: PARTIAL** **Issue 1: 15 February 1984**
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BIOLOGICAL INDICATOR OF: exposure to PCB.

SYNONYMS: Aroclor; PCB; chlorodiphenyl

BIOLOGICAL SAMPLING		MEASUREMENT	
SPECIMEN:	serum	**TECHNIQUE:**	GAS CHROMATOGRAPHY, ECD
VOLUME:	10 mL	**INJECTION VOLUME:**	3 µL, Grob type splitless
PRESERVATIVE:	none	**COLUMN:**	capillary, 15 m x 0.2-mm ID, WCOT SE-54
SHIPMENT:	polyethylene shippers @ 10 °C	**CARRIER GAS:**	5% methane in argon, 1 mL/min
STABILITY:	indefinite if serum is kept frozen	**TEMPERATURE-INJECTOR:**	260 °C
CONTROLS:	collect 3 specimens from unexposed populations per study or 1 per 10 unknowns for studies exceeding 30 specimens	**-COLUMN:**	100 °C for 2 min; 20 °C/min to 260 °C for 4 min
BULK:	submit a bulk sample of material to which workers were exposed	**-DETECTOR:**	350 °C
		CALIBRATION:	hexane solutions of Aroclors 1016, 1221, 1232, 1242, 1248, 1254 and 1260
		QUALITY CONTROL:	pooled or spiked serum
		RANGE:	0.005 to 1.0 µg/mL
		ESTIMATED LOD:	0.001 µg/mL
		RECOVERY:	greater than 80%
		PRECISION ($\bar{S}_r$):	0.16
		ACCURACY:	> ± 30%

APPLICABILITY: Since PCBs are rapidly absorbed from the lung, GI tract, and skin, this procedure is useful for estimating acute and chronic exposures to PCB, assuming (a) no metabolism of PCB, (b) all isomers of PCB are extracted with equal efficie ncy, and (c) similar ECD response for all PCBs. PCBs have high biological and chemical stability. They accumulate in adipose tissue and may present a serious latent health threat. Metabolism involves hydroxylation and conjugation with glucose and sulfa tes.

INTERFERENCES: Chlorinated hydrocarbons, phthalate based plasticizers, dibenzofurans, and chlorinated naphthalenes interfere.

OTHER METHODS: This replaces P&CAM 329 [1]. Other methods include a screening method for Aroclor 1254 in whole blood [2].

REAGENTS:

1. Methanol, hexane, ethyl ether, and acetone (pesticide grade).
2. Potassium hydroxide, KOH.
3. Calibration stock solutions, 0.1 mg/mL.* Dissolve 10 mg of the bulk sample or other suitable standard, e.g., Aroclor 1016, 1221, 1232, 1242, 1248, 1254, and 1260 in 100 mL n-hexane (standards available from EPA).
4. Silica gel, activity grade I.
   a. Heat to 130 °C for 24 h.
   b. Cool and add 3 g water per 100 g silica gel. Mix for 2 h in a sealed container.
5. Sodium sulfate (anhydrous).
6. 1,1-Dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) solution, 50 ng/mL, in hexane.
7. Methanol KOH, 2% (w/v). Dissolve 2 g KOH in methanol to make 100 mL solution.
8. Methanol-water, 1:1 (v/v).
9. Hexane-ethyl ether, 1:1 (v/v).
10. Nitrogen, compressed, filtered.

* See SPECIAL PRECAUTIONS.

EQUIPMENT:

1. Gas-liquid chromatograph equipped with an electron capture detector.
2. Syringes, glass, 30-mL.**
3. Culture tubes, 16- x 150-mm, organic-free, with PTFE-lined screw caps.**
4. Rotary mixer, variable speed.
5. Centrifuge.
6. Kuderna-Danish concentrator tubes, 25-mL.**
7. Micro-Synder Columns.**
8. Tube heater.
9. Chromatography column, 7-mm ID x 200 mm, with 50-mL reservoir and PTFE stopcock.**
   a. Put a small plug of glass wool in the bottom of the column.
   b. Pour a suspension of 3 g silica gel in 50 mL hexane into the column.
   c. Let the suspension settle and add 5 to 7 g sodium sulfate.
10. Syringes, 10-µL, glass.
11. Graduated cylinder, 25-mL.**
12. Pipet, Pasteur.

** Clean all glassware, including that used in sampling as follows: Wash in detergent. Rinse with tap water. Soak in chromic acid. Rinse with, in order, tap water, distilled water, acetone and hexane.

SPECIAL PRECAUTIONS: Samples of blood collected from humans pose a real health risk to laboratory workers who collect and handle these samples. These risks are primarily due to personal contact with infective biological samples and can have serious health consequences, such as infectious hepatitis, and other diseases. There is also some risk from the chemical content of these samples, but this is much less. Those who handle blood specimens should wear protective gloves, and avoid aerosolization of the samples. Mouth pipetting, of course, must be avoided.

PCBs have potential carcinogenicity in humans. They are microsomal enzyme inducers and liver toxins; therefore, use extreme caution when handling these substances [3].

SAMPLING:

1. Collect 20 to 25 mL whole blood samples by venipuncture via 30-mL glass syringe.
2. After the blood has clotted, centrifuge for 10 min at 2000 rpm. Transfer the serum to a 16 x 150-mm culture tube with a PTFE-lined screw cap.
3. Ship the serum in an insulated container with bagged refrigerant to keep the samples at 4 °C.
4. Freeze the samples upon arrival at the laboratory.

SAMPLE PREPARATION:

5. Pipet 5 mL serum into a clean culture tube containing 4 mL methanol.
6. Cap the tube and mix 4 min on a rotary mixer.
7. Add 5 mL hexane-ethyl ether (1:1, v/v).
8. Mix on a rotary mixer for 15 min.
9. Centrifuge at 2000 rpm for 5 min.
10. Transfer the upper solvent layer by pipet into a 25-mL Kuderna-Danish concentrator tube.
11. Repeat steps 7 through 10 twice, combining the extracts in the concentrator tube.
12. Concentrate the extract to 0.5 mL under a gentle stream of dry organic-free nitrogen.
13. Add 2 mL methanolic KOH to the concentrator tube. Add a piece of hollow glass (end of a Pasteur pipet) to prevent bumping.
14. Attach a micro-Synder column and bring the contents to a gentle boil with of a tube heater and reduce the volume to 0.3 mL.
    NOTE: If a precipitate forms, add a few drops of methanolic KOH solution and warm gently with a tube heater until it dissolves.
15. Cool the solution slightly and add 2 mL 1:1 methanol:water.
16. Add 2 mL n-hexane when the solution reaches room temperature.
17. Stopper and shake the tube vigorously.
18. Rinse the prepared chromatography column (EQUIPMENT, 9.) with 20 mL hexane. As the hexane settles into the sodium sulfate bed, add the sample.
19. Place a 25-mL graduated cylinder below the chromatographic column.
20. Rinse the concentrator tube and the micro-Synder column with 1 mL hexane.
21. As the saponified extract enters the sodium sulfate layer, add the concentrator tube and micro-Synder column rinses to the chromatographic column.
22. As the rinses settle in the sodium sulfate layer, add 25 mL hexane.
23. Collect 25 mL eluate from the column in the graduated cylinder.
24. Rinse the walls of the graduated cylinder with 2 mL hexane.
25. Concentrate to 1 mL under a gentle stream of nitrogen. Add 10 mL DDE solution and analyze (steps 29 and 30).

CALIBRATION AND QUALITY CONTROL:

26. Analyze the bulk sample for PCB content [4].
27. Calibrate daily with at least five working standards over the range 0.005 to 1 mg/mL PCB.
    a. Add known amounts of calibration stock solution to n-hexane in 10-mL volumetric flasks and dilute to the mark with n-hexane.
    b. Analyze the working standards together with samples and blanks (steps 29 and 30).
    c. Prepare calibration graph (concentration of each PCB standard vs. the sum of PCB peak areas).
28. Analyze at least three pooled or spiked serum samples.

MEASUREMENT:

29. Set gas chromatograph according to manufacturer's instructions and conditions on page 8004-1. Inject 2-µL sample together with 1 mL DDE solution.
30. Measure the PCB peak areas. Calculate the retention time of the analyte peaks relative to that of DDE.

CALCULATIONS:

31. Compare sample retention times (relative to DDE) with those of the working standards and bulk sample to ascertain the valid PCB peaks.

32. Read the concentration of PCB in the hexane solution (µg/mL) from the calibration graph.
33. Calculate the serum PCB concentration, C (µg/mL), by dividing the PCB concentration found in the extract, C_e, by 5 (5 mL serum yields 1 mL extract):

$$C = \frac{C_e}{5}, \ \mu g/mL.$$

GUIDES FOR INTERPRETATION:

PCBs are present in the biosphere. Baselt [5] indicates a level 2 to 4 µg/L of plasma is indicative of significant exposure. Finklea [6] reported 57% of individuals tested had levels below 5 µg/L. Most control populations have PCB levels below 20 µg/L serum. What levels present a health hazard is not documented; however, a 200 µg/L as an upper limit has been suggested [7]. If a serious exposure is suspected, then the serum PCB levels should be augmented by liver battery and triglyceride tests plus a complete medical evaluation to ascertain the employee health risk.

EVALUATION OF METHOD:

Eighty-nine analyses of four spiked human serum for the Aroclors 1242 and 1254 with a concentration range of 0.025 to 0.400 µg/L indicated recoveries greater than 80% with precision, $\bar{S}_r$, of 0.16 [8].

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