PENTACHLOROPHENOL in blood 8001

C_6HCl_5O MW:266.34 CAS: 87-86-5 RTECS: SM6300000

METHOD: 8001, Issue 2 **EVALUATION: FULL** **Issue 1: 15 May 1989**
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BIOLOGICAL INDICATOR OF: exposure to pentachlorophenol.

SYNONYMS: PCP; Penta

SAMPLING	MEASUREMENT
SPECIMEN: whole blood in 5-mL tubes	**TECHNIQUE:** GAS CHROMATOGRAPHY, ECD
VOLUME: 5 mL	**ANALYTE:** pentachloroanisole (PCP methyl ether)
PRESERVATIVE: none	**INJECTION VOLUME:** 5 µL
SHIPMENT: polyethylene shippers with sample container kept at 10 °C	**COLUMN:** 1.8 m x 4-mm ID glass, 4% SE-30, 6% OV-210 on 80/100 mesh silanized support
ANTICOAGULANT: EDTA	**CARRIER GAS:** 5% methane in argon, 60 mL/min
SAMPLE STABILITY: unknown	**TEMPERATURE-INJECTOR:** 220 °C **-COLUMN:** 190 °C **-MANIFOLD:** 250 °C **-DETECTOR:** 300 °C
CONTROLS: collect at least 3 blood specimens from unexposed individuals per study or one control per 10 samples for studies exceeding 30 samples	**CALIBRATION:** solutions of pentachloroanisole in hexane
	QUALITY CONTROL: spiked pooled blood
	RANGE: 0.01 to 1 µg PCP/mL blood
	ESTIMATED LOD: 0.001 to 1 µg PCP/mL blood
	RECOVERY: 90%
	PRECISION ($\bar{S}_r$): 0.02
	ACCURACY: ± 13.9%

APPLICABILITY: This procedure measures free PCP. It is useful for monitoring PCP exposures via skin contact, inhalation, and ingestion. Blood PCP exposures are good indicators of acute short-term exposures because blood PCP values reach maximum 4 h after exposure [1]. Chronic exposures can best be monitored using Method 8303, Pentachlorophenol in urine.

INTERFERENCES: Chloronaphthalenes, polychlorinated biphenyl and Diuron are also hexane-extractable but are separated from PCP by column chromatography.

OTHER METHODS: This method is based on that of Borsetti [2].

REAGENTS:

1. Hexane, acetone, and benzene* (pesticide grade).
2. 20% benzene in hexane (v/v);
   10% benzene in hexane (v/v).
3. Sulfuric acid, 6 M.
4. Sodium bisulfite.
5. Sodium sulfate, anhydrous.
6. Alumina (acid-washed), Brockman Activity I, 80/200 mesh
7. Pentachloroanisole (available from EPA Analytical Reference Lab).
8. Calibration stock solution equivalent to 100 mg/L PCP. Weight 0.0105 g pentachloroanisole; dilute to 100 mL with hexane in a volumetric flask. Stable 2 months if stored in refrigerator.
9. Diazomethane* prepared from Diazald kit according to manufacturer's instructions [3].
10. 5% methane in argon.

* See SPECIAL PRECAUTIONS.

EQUIPMENT:

1. Blood-sampling tubes, 5-ml, with 7.5 mg Na_2EDTA anticoagulant (e.g., "lavender-top" tubes).
2. Gas chromatograph with a ^{63}Ni electron capture detector, electronic integrator, and column (page 8001-1).
3. Culture tubes, 16 x 150-mm and 16 x 125-mm, with PTFE-lined screw caps.**
4. Mixer, rotary, variable speed.
5. Centrifuge (refrigerated, optional).
6. Chromatography column, 7-mm ID x 200 mm, with a 50-mL reservoir and PTFE stopcock.**
7. Syringe, 10-µL, glass.
8. Pipets, 10- to 1000-µL.**
9. Flasks, volumetric, 10-, 25-, and 100-mL.**
10. Pipets, Pasteur, disposable.
11. Tubes, centrifuge, graduated, glass-stoppered, 15-mL.**
12. Waterbath, 100 °C.

**Wash with detergent; clean with chromic acid; rinse with 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. Benzene is a documented human carcinogen and must be handled in compliance with 29 CFR 1910.1005.

Diazomethane and its precursor, n-Methyl-n-nitroso-p-toluene sulfonamide, are potent mutagens. Handle the bulk precursor in a glove box. Generate diazomethane behind an explosion shield in a hood.

SAMPLING:

1. Collect 5-mL whole blood samples by venipuncture in tubes containing 7.5 mg Na_2EDTA anticoagulant, about 4 h after suspected exposure.
2. Ship the blood samples in an insulated container with refrigerant to keep the temperature at about 10 °C. Do not freeze.

SAMPLE PREPARATION:

3. Add 2 mL whole blood to a 16 x 150-mm culture tube. Add cautiously and slowly 5 mL 6 M sulfuric acid. Cool to room temperature.
4. Add 6 mL hexane to the culture tubes in a hood; cap and place in a boiling waterbath for 45 min with shaking every 15 min.
5. Cool the culture tubes to room temperature and centrifuge at 2000 to 2500 rpm.
6. Remove the hexane layer into a 16 x 125-mm culture tube.

7. Extract twice more with 2 mL hexane for 2 min. Combine the extracts.
8. Concentrate the combined extracts to approximately 0.6 mL under a gentle stream of nitrogen.
9. Add 10 mL diazomethane reagent to the culture tube in a hood. Let stand in a hood for 1 h.
10. Concentrate to approximately 0.6 mL under a gentle stream of nitrogen in a hood.
11. Add 4 mL hexane. Evaporate the hexane solution to approximately 0.6 mL.
12. Add 4 g acid-washed alumina to a 7-mm ID x 200 mm chromatography column. Cap with 1.6 g anhydrous sodium sulfate. Rinse the column with 20% benzene in hexane. Let the column air dry and place it in an oven overnight at 130 °C. Cool the column to room temperature before use.
13. Wet the column with 5 mL hexane in a hood.
14. Add the concentrated derivatized extract when the solvent layer reaches the top of the sodium sulfate layer.
15. Rinse the culture tube three times with 0.5 mL hexane. Add the rinsings to the column.
16. Add 3.5 mL hexane to the column. Discard the hexane eluate.
17. Elute pentachloroanisole, the PCP derivative, with 20 mL 10% benzene in hexane.
18. Concentrate the sample to 2.0 mL in a graduated centrifuge tube.

CALIBRATION AND QUALITY CONTROL:

19. Prepare at least 6 working standards in the range 0.01 to 1 µg/mL PCP by dilution of the calibration stock solution with hexane.
20. Prepare a calibration graph (peak area vs. µg/mL PCP).
21. Maintain standardization by injecting a working standard after each five sample injections.
22. Run a spiked pooled control blood sample every ten samples, or if less than ten determinations are required, run at least three spiked pooled blood samples per study.
    NOTE: All blood that will be utilized for spiked pooled control blood samples must be analyzed for PCP before use because PCP is ubiquitous.

MEASUREMENT:

23. Set up the gas chromatograph according to manufacturer's instructions and to the conditions on page 8001-1.
24. Inject 5 µL sample extract (from step 18). Measure peak area of analyte.
    NOTE: The retention time for pentachloroanisole is about 4.7 to 5.0 min.

CALCULATIONS:

25. Determine the concentration, C (µg/mL), of PCP in blood by comparison of peak areas of samples with the calibration graph.

GUIDES TO INTERPRETATION:

Blood PCP levels for unexposed populations range between 0.016 to 0.32 µg/mL PCP [4,5,6]. The Biological Exposure Index is 5 mg/L for free PCP in end-of-shift plasma [7].

EVALUATION OF METHOD:

The precision for three sets of three samples of spiked whole blood was 2% relative standard deviation at levels of 0.05 to 1 µg/mL PCP whole blood, with 88 to 93% recovery.

REFERENCES:

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[2] Borsetti, A.P. Determination of Pentachlorophenol in Milk and Blood of Dairy Cattle, J. Agric. Food Chem., 28, 710-714 (1980).

[3] Diazald, Cat. No. 02800-0, Aldrich Chem. Co. Inc., Milwaukee, WI.

[4] Dougherty, R.C. Human Exposure to Pentachlorophenol in Human Blood and Urine, Dept. of Chemistry, Florida State University, Tallahassee, FL.

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[6] Klemmer, H.W., Wang, L., Sato, M.M., Reichert, E.L., Korsak, R.J. and Rashad, N.M. Clinical Findings in Workers Exposed to Pentachlorophenol, Arch. Environ. Contam. Toxicol, 9, 715-725 (1980).

[7] 1993-1994 Threshold Limit Values and Biological Exposure Indices, American Conference of Governmental Industrial Hygienists, Cincinnati, OH (1993).

METHOD REVISED BY:

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