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ICS 67.120.30

## **EAST AFRICAN STANDARD**

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**Test methods for fish and fishery products — Part 8: Determination of organochlorides, pesticides, PCBS, and PCB congeners**

**EAST AFRICAN COMMUNITY**

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## Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in East Africa. It is envisaged that through harmonized standardization, trade barriers which are encountered when goods and services are exchanged within the Community will be removed.

In order to meet the above objectives, the EAC Partner States have enacted an East African Standardization, Quality Assurance, Metrology and Test Act, 2006 (EAC SQMT Act, 2006) to make provisions for ensuring standardization, quality assurance, metrology and testing of products produced or originating in a third country and traded in the Community in order to facilitate industrial development and trade as well as helping to protect the health and safety of society and the environment in the Community.

East African Standards are formulated in accordance with the procedures established by the East African Standards Committee. The East African Standards Committee is established under the provisions of Article 4 of the EAC SQMT Act, 2006. The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the procedures of the Community.

Article 15(1) of the EAC SQMT Act, 2006 provides that "Within six months of the declaration of an East African Standard, the Partner States shall adopt, without deviation from the approved text of the standard, the East African Standard as a national standard and withdraw any existing national standard with similar scope and purpose".

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

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## Introduction

In the preparation of this East African Standard, the following sources were consulted extensively:

AOAC Official Method 983.21:1985, *Organochlorine Pesticide and Polychlorinated Biphenyl Residues in Fish — Gas Chromatographic Method*

CAC/RCP 52:2003(Rev. 4:2008), *Code of practice for fish and fishery products*

IS 4303-1:1975, *Code of hygienic conditions for fish industry — Part 1: Pre-processing stage*

IS 4303-2:1975, *Code of hygienic conditions for fish industry — Part 2: Canning stage*

Codex Alimentarius website: [http://www.codexalimentarius.net/mrls/vetdrugs/jsp/vetd\\_q-e.jsp](http://www.codexalimentarius.net/mrls/vetdrugs/jsp/vetd_q-e.jsp)

USDA Foreign Agricultural Service website: <http://www.mrlidatabase.com>

USDA Agricultural Marketing Service website: <http://www.ams.usda.gov/AMSV1.0/Standards>

European Union: [http://ec.europa.eu/enterprise/sectors/pharmaceuticals/veterinary-use/maximum-residue-limits/index\\_en.htm](http://ec.europa.eu/enterprise/sectors/pharmaceuticals/veterinary-use/maximum-residue-limits/index_en.htm)

Assistance derived from these sources is hereby acknowledged.

*Draft for comments only — Not to be cited as East African Standard*

## Test methods for fish and fishery products — Part 8: Determination of organochlorides, pesticides, PCBS, and PCB congeners

### 1 Scope

This method is applicable to fish and fishery products as well as other biological tissues.

### 2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

CAC/GL 21, *Principles for the establishment and application of microbiological criteria for foods*

CAC/RCP 1, *Recommended international code of practice — General principles of food hygiene*

CAC/GL 30, *Principles and guidelines for the conduct of microbiological risk assessment*

CAC/GL 31, *Guidelines for the sensory evaluation of fish and shellfish in laboratories*

CAC/GL 48, *Model certificate for fish and fishery products*

CAC/RCP 52:2003(Rev. 4:2008), *Code of practice for fish and fishery products*

CAC/GL 53, *Guidelines on the judgement of equivalence of sanitary measures associated with food inspection and certification systems*

EAS 38, *Labelling of prepackaged foods — Specification*

### 3 Principle

Chlorinated pesticides and polychlorinated biphenyls (PCBs) are extracted from prepared fish test portion with petroleum ether, cleaned up on Florisil column, and determined by GC against reference standards.

### 4 General apparatus, reagents, and techniques

#### 4.1 General reagents

Solvents must be purified and final distillation conducted in all-glass apparatus.

Solvent purity test. — Electron capture GC requires absence of substances causing detector response as indicated by following test: Place 300 ml solvent in Kuderna-Danish concentrator fitted with 3-ball Snyder column and calibrated collection vessel, and evaporate to 5 ml. Inject 5 µl concentrate from 10 µl syringe into gas chromatograph, using conditions described in H(c). Concentrate must not cause recorder deflection >1 mm from baseline for 2–60 min after injection.

- (a) Acetonitrile — See solvent purity test. Purify technical CH<sub>3</sub>CN as follows: To 4 L CH<sub>3</sub>CN add 1 ml H<sub>3</sub>PO<sub>4</sub>, 30 g P<sub>2</sub>O<sub>5</sub>, and boiling chips, and distil in all-glass apparatus at 81–82°C. Do not exceed 82°C.

Some lots of reagent grade CH<sub>3</sub>CN are impure and require distillation. Generally vapors from such lots will turn moistened red litmus paper blue when held over mouth of storage container. Pronounced amine odor is detectable.

- (b) Acetonitrile saturated with petroleum ether.—Saturate  $\text{CH}_3\text{CN}$ , (a), with redistilled petroleum ether, (m).
- (c) Alcohol.—USP, reagent grade, or methanol, ACS.
- (d) Alcoholic alkali solution.—2%. Dissolve 2 g KOH in alcohol, and dilute to 100 mL.
- (e) Eluting solvent, 6%.—Dilute 60 mL ethyl ether, (h), to 1 L with redistilled petroleum ether, (m).
- (f) Eluting solvent, 15%.—Prepare as in (e), using 150 mL ethyl ether.
- (g) Eluting solvent, 50%.—Prepare as in (e), using 500 mL ethyl ether.
- (h) Ethyl ether.—See solvent purity test. Redistilled at 34–35°C, and stored under  $\text{N}_2$ . Add 2% alcohol (c). Must be peroxide-free by test in Definitions of Terms and Explanatory Notes.
- (i) Florisil.—60/100 PR grade, activated at 675°C (1250°F), available from Floridin Co., 3 Pennsylvania Center, Pittsburgh, PA 15235 USA. When 675°C activated Florisil is obtained in bulk, transfer immediately after opening to ca 500 mL (1 pt) glass jars, or bottles, with glass-stoppered or foil-lined, screw-top lids, and store in dark. Heat  $\geq 5$  h at 130°C before use. Store at 130°C in glass-stoppered bottles or in air-tight desiccator at room temperature and reheat at 130°C after 2 days.

Prepare mixed pesticide standard solution in hexane containing 1, 4, 1, 2, 1, 2, and 4  $\mu\text{g}/\text{ml}$ , respectively, of ronnel, ethion, heptachlor epoxide, parathion, dieldrin, endrin, and malathion.

Test each batch of activated Florisil by placing 1 mL mixed pesticide standard on prepared column and eluting as in Cleanup, O. Concentrate eluates from Florisil column to 10 mL. Inject aliquot (see H) of each eluate into gas chromatograph and determine quantitative recovery of each compound as in R. Florisil that quantitatively elutes heptachlor epoxide, ronnel, and ethion in 6% eluate; dieldrin, endrin, and parathion in 15% eluate; and malathion in 50% eluate, is satisfactory.

Adsorptivity of lots of Florisil may be tested with lauric acid and size of column adjusted to compensate for variation in adsorptivity [JAOAC 51, 29(1968)]. Test adjusted column before use by performing elution test above.

- (j) Hexane.—See solvent purity test. Redistilled in all-glass apparatus.
- (k) Magnesium oxide.—Adsorptive magnesia (Fisher Scientific Co. No. S-120). Treat as follows: Slurry ca 500 g with  $\text{H}_2\text{O}$ , heat on steam bath ca 30 min, and filter with suction. Dry overnight at 105–130°C and pulverize to pass No. 60 sieve. Store in closed jar.
- (l) Magnesia–Celite mixture.—Mix treated  $\text{MgO}$  (k) with Celite 545, 1 + 1 by mass. Petroleum ether extract of Celite should be free of electron-capturing substances.
- (m) Petroleum ether.—See solvent purity test. Redistilled in all-glass apparatus at 30–60°C.
- (n) Sodium sulfate.—Anhydrous, granular.

#### 4.2 General apparatus

- (a) High-speed blender.—Waring, or equivalent.
- (b) Chromatographic tubes — With Teflon stopcocks and coarse fritted plate or glass wool plug; 22 mm id  $\times$  300 mm.
- (c) Chromatographic tubes without stopcocks — 22 mm id  $\times$  300 or 400 mm.

- (d) Filter tubes.—Ca 22 mm id  $\times$ 200 mm with short delivery tube and coarse fritted plate or glass wool plug.
- (e) Kuderna–Danish concentrators.— 500 and 1000 ml with Snyder distilling column and 5 or 10 ml plain, volumetric, and graduated receiving flasks (Kontes Glass Co. No. 570000, 621400, and 570050, or equivalent).
- (f) Separators.—1000 and 125 mL with Teflon stopcocks.
- (g) Micro-Snyder column.—2-ball (Kontes Glass Co. No. 569001, or equivalent).
- (h) Micro-Vigreux column.—Kontes Glass Co. No. 569251, or equivalent.

### 4.3 Apparatus for Gas Chromatography

GC system when operated with column, (b), and approximate conditions described in Gas Chromatography, R, should be capable of producing ca  $\frac{1}{2}$  scale deflection for 1 ng heptachlor epoxide by electron capture detection and for 2 ng parathion by KCl–thermionic detection, and should resolve mixture of heptachlor, aldrin, heptachlor epoxide, ethion, and carbophenothion into separate peaks. Retention time for aldrin should be ca 4.5 min. Compounds of interest must not be degraded by any part of GC system, e.g., endrin should exhibit single peak.

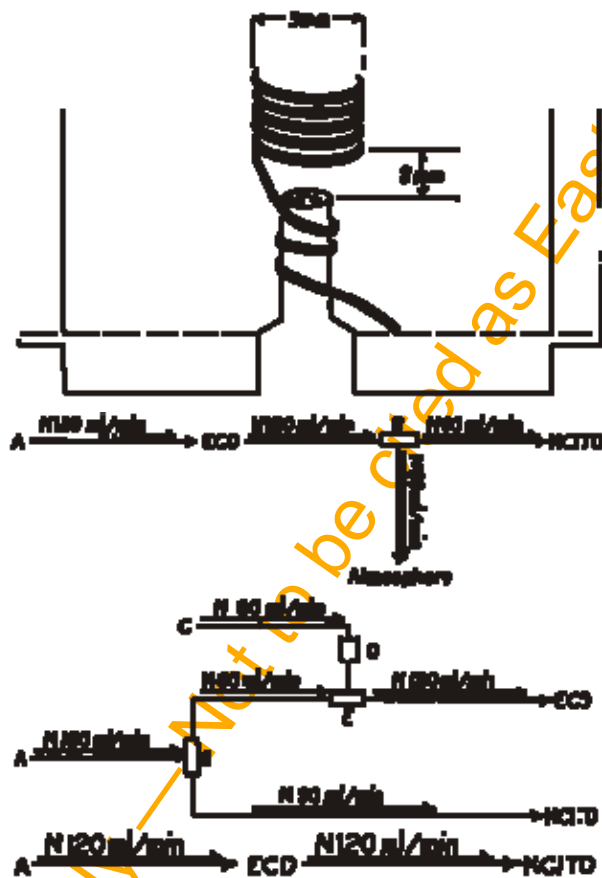
- (a) Gas chromatograph — Instrument consisting of on-column injection system, all-glass column in oven controlled to  $\pm 0.1^\circ\text{C}$ , electron capture and thermionic detectors, each with independent power supply, electrometer, and appropriate mV recorder
- (b) Column — Glass, 1.85 m (6 ft)  $\times$ 4 mm id packed with 10% DC-200 (w/w) on solid support:
  - (1) 80–100 mesh Chromosorb W HP (Manville Filtration and Minerals, manufacturer, but available through many GC distributors);
  - (2) 80–100 mesh Gas-Chrom Q (Alltech-Applied Science Laboratories, Inc.);
  - (3) 80–90 mesh Anakrom ABS (Analabs, Inc.). DC-200 may be replaced by OV-101 (available from many GC distributors).

Weigh 2 g Dow Corning 200 silicone fluid (12 500 centistokes) or OV-101 into beaker. Dissolve in  $\text{CHCl}_3$  and transfer to 300 ml Morton-type flask, using total of ca 100 ml  $\text{CHCl}_3$ . Add 18 g solid support, (1), (2) or (3), to flask. Swirl, and let stand ca 10 min. Place flask on rotary evaporator and remove solvent slowly with intermittent rotation, using  $50^\circ\text{C}$   $\text{H}_2\text{O}$  bath and slight vacuum. (Foaming may occur initially.) When solids appear damp, increase vacuum. Remove last traces of  $\text{CHCl}_3$  without rotation or by air drying. Use only free-flowing material to fill column. Use care at all stages of column preparation to prevent fracturing solid support. Condition column at  $250\text{--}260^\circ\text{C}$  with  $\text{N}_2$  flow of ca 100 ml/min  $\geq 48$  h or until endrin exhibits single peak.

- (c) Electron capture detector (ECD; Surplus 1995).—Concentric design, for use with dc voltage supply and  $^3\text{H}$  source (ca 150 mCi ( $5.55 \times 10^6$  Bq)  $^3\text{H}$ , U.S. Nuclear Regulatory Commission license is required.)

Surplus 1995.—Determine detector operating characteristics as follows: Apply dc voltage to detector. After system becomes stable (overnight), determine current-voltage relationship at various voltages between 200 and 0 V. (Current measurements at voltages of 200, 150, 100, 75, 50, 40, 30, 25, 20, 15, 10, 8, 6, 4, 2, and 1 provide points for smooth curve.) Slightly lower, stable, standing current may be obtained after detector has been at operating temperature several hours. This is probably due to loss of some easily removed radioactive material. Determine and plot response-voltage relationship at  $1 \times 10^{-9}$  A full scale sensitivity for 1 ng injections of heptachlor epoxide at same voltages used in obtaining current-voltage curve. Select as operating voltage that voltage at which heptachlor epoxide causes ca 40–50% full scale recorder deflection. Check linearity of system from 0.2–2.0 ng heptachlor epoxide.

Other electron capture detectors may be substituted for dc voltage concentric design  $^3\text{H}$  detector which is no longer marketed. Constant current, variable frequency  $^{63}\text{Ni}$  electron capture detectors are acceptable substitutes when operated at conditions to produce stable, reproducible, linear responses. Optimum conditions may produce more sensitive response than from  $^3\text{H}$  detector. To maintain same method limit of quantitation of  $^3\text{H}$  detector, inject proportionately smaller equivalent test portion weight into  $^{63}\text{Ni}$  detector system. The  $^{63}\text{Ni}$  electron capture detector may provide different relative responses for pesticides than those obtained with  $^3\text{H}$  electron capture detector. Use of  $\text{Ar-CH}_4$  carrier gas, as recommended for most  $^{63}\text{Ni}$  detectors, precludes use of KCITD dual detection system, (d), (h)–(j).



(d) Potassium chloride thermionic detector (KCITD; Surplus 1995).—Flame ionization detector modified to incorporate coil with KCl coating prepared as in (1) or (2). Detector voltage is 300 V dc. Use in dual arrangement with electron capture detector. All dual detector systems described are capable of comparable performance. In-series, (h), arrangement is preferred because of simplicity and ease of operation.

(1) Coil with potassium chloride for in-series dual detector.—See Figure 970.52A (may be used with all detector arrangements). Wind Pt-Ir wire (B&S gage 26) on 7 mm diameter rod into 2 turn helix so that turns are touching. Ca 5 mm below helix, continue to wind wire on 3 mm rod, or rod with same outside diameter as flame jet, making 3-turn spiral. Cut wire so that 7 mm helix is supported 4 mm above flame jet when 3 mm spiral is slipped over jet. Fill 30 mL tall-form Pt crucible ca 1/2 full with KCl (ACS). Heat with Meker burner until all salt melts. Continue heating until bottom of crucible glows red, imparting pink glow to melt. Remove heat and begin dipping the 2-turn helix of coil into melt at 5 s intervals as melt cools. (Make sure only 2-turn helix touches melt and do not raise coil above top of crucible.) When melt is at proper temperature, salt clings to coil. Remove coil from melt. Place probe in center of coil while salt is molten. This causes crystallization around probe tip. Remove center of

coil. Remove any rough edges on coil coating by holding coil in burner flame 1 s; id of properly coated coil is 5 mm. Position coil over flame jet.

(2) Coil with potassium chloride for parallel and in-series split dual detectors.—See Figure **970.52B**. Wind Pt-Ir wire (B&S gage 26) on 5 mm diameter rod into 5-turn helix so that turns are close together or touching. Continue to wind wire on 3 mm rod, or rod having same outside diameter as flame jet, making 3-turn spiral. Cut wire so that 5 mm helix is supported 2 mm above flame jet when 3 mm spiral is slipped over jet. Grasp formed wire by end opposite 5 mm helix with forceps. Dip 5 mm helix into saturated KCl (recrystallized twice from H<sub>2</sub>O) solution, or apply KCl solution with dropper. Fuse in flame. (Caution: Use safety glasses; spattering occurs.) Repeat application of KCl solution 3–4 times until helix is coated with fused KCl. Coating should appear almost crystal clear. Position coil over flame jet.

(e) Hydrogen (Surplus 1995).—From generator or cylinder of compressed H<sub>2</sub> gas (cylinder preferred). Equip cylinder with pressure drop of stainless steel capillary tubing (0.020 in (0.051 mm)) to restrict H<sub>2</sub> flow to ca 30 mL/min at 20 lb (9 kg) delivery pressure. Place H<sub>2</sub> source close to detector and use gas lines with minimum dead volume to reduce outgassing time in lines. (For fine precise control of H<sub>2</sub> flow, insert Nupro Fine Metering Valve, "S" series (Swagelok Co., 31400 Aurora Rd, Solon, OH 44138; Part number B-1S) between exit end of capillary tubing pressure drop and inlet of detector H<sub>2</sub> line. (Caution: Do not use Nupro valve as shut-off valve. Repeated tightening damages needle.) Use Swagelok fittings for all connections.

(f) Air (Surplus 1995).—Minimum air requirement for thermionic detector is 300 mL/min. Cylinder of compressed air or aquarium air pump is recommended.

(g) Capillary T-tube (Surplus 1995).—(See Figures **970.52C** and **D**.) Prepare 1:1 stream splitter (B) for parallel and in-series split dual detection systems. Fit two 4.5 cm lengths of stainless steel capillary tubing, 0.010 in id, 1/16 in (0.254 id, 1.588 od mm), into 1 cm length of standard wall, 1/8 in (3.175 mm) stainless steel tubing. Fit 1 in length of No. 16 hypodermic tubing at right angles in hole drilled into the piece of 1/8 in (3.175 mm) tubing. Silver braze all connections. Prepare capillary T-tube (e) for introducing purge gas to parallel system. Fit two 2.5 cm lengths of No. 16 hypodermic tubing into 1 cm length of standard wall, 1/8 in (3.175 mm) stainless steel tubing. Fit 1 cm length of No. 16 hypodermic tubing at right angles in hole drilled into piece of 1/8 in (3.175 mm) tubing. Silver braze as above.

(h) Assembly of in-series dual detection system (Surplus 1995).—Assemble as in Figure **970.52E**. Introduce column effluent (A) of 120 mL/min directly to ECD inlet. Connect ECD outlet directly to KCITD inlet, using No. 16 standard wall Teflon tubing. (Note: For in-series, (h), and in-series split, (i), operation, thoroughly check ECD for gas leaks, particularly at Teflon insulator.)

(i) Assembly of in-series split dual detection system (Surplus 1995).—Assemble as in Figure **970.52C**. Introduce column effluent (A) of 120 mL/min directly to ECD inlet. Connect 1:1 stream splitter (B) between ECD outlet and KCITD inlet so that only 60 mL N<sub>2</sub>/min enters KCITD and remaining 60 mL N<sub>2</sub>/min exits to atmosphere. Use No. 16 standard wall Teflon tubing for all connections. See Note in (h).

(j) Assembly of parallel dual detection system (Surplus 1995).—Assemble as in Figure **970.52D**. Split column effluent (A) of 120 mL/min by passing through 1:1 stream splitter (B) so that each detector receives 60 mL effluent/min. Increase flow to ECD by introducing 60 mL N<sub>2</sub>/min from second N<sub>2</sub> source (C) through capillary T-tube (e). Preheat N<sub>2</sub> from (C) by passing through stainless steel capillary tube (D: 0.040 in id [1.016 mm id] ) which extends 120 cm into column bath and returns to detector bath where additional 35 cm of tubing is coiled into small helix. Connect capillary tubes and splitters to detectors with No. 16 standard wall Teflon tubing. Measure flow at each end of splitter (B) to ensure exact 1:1 split.

(k) Potassium chloride thermionic detector operation (Surplus 1995).—Zero recorder with zero control before detector flame is ignited (no signal). Turn on H<sub>2</sub> (ca 30 mL/min) and ignite flame. Adjust H<sub>2</sub> with flame burning to give baseline current (BLC) of 0.2–0.8 × 10<sup>-8</sup> A. (Sensitivity to P compounds is directly related to KCl temperature, which depends on H<sub>2</sub> concentration in flame.) Select operational electrometer setting and adjust H<sub>2</sub> concentration to obtain 40–50% full scale recorder deflection for 2 ng parathion entering detector. When baseline has stabilized, measure BLC precisely, at electrometer

setting of  $1 \times 10^{-8}$  A full scale. Return to operational electrometer setting and zero recorder pen, using current balance control to "buck out" current generated by detector. Check linearity of system from 0.4–4.0 ng parathion. Monitor BLC frequently during operation. If drift occurs, readjust H<sub>2</sub> concentration to maintain same BLC. For accurate quantitation, BLC must be identical during chromatography of sample and standard.

### Concentration Techniques

#### 4.4 Purified Extracts

(Never evaporate purified extracts to dryness.)

(a) Ca  $\geq$  5 mL.—Evaporate on steam bath in K–D concentrator fitted with 3-ball Snyder column and volumetric flask or graduated collection tube; 20-mesh boiling chip is necessary.

(b)  $<$  5 mL.—Evaporate to ca 5 mL as in (a). Remove collection tube from concentrator and fit tube with 2-ball micro-Snyder or micro-Vigreux column. Evaporate to slightly less than desired volume, permit condensate to drain into tube, and remove column. Minimum attainable volume is 0.2–0.4 mL.

#### 4.5 Extracts Containing Fats, Oils, or Plant Extractives

(a) Concentrator.—Fitted with 3-ball Snyder column and volumetric flask or graduated collection tube. Use on steam bath.

(b) Flash evaporator.—Keep flask in H<sub>2</sub>O bath at room temperature.

(c) Beaker.—Evaporate in beaker in H<sub>2</sub>O bath at 35–40°C under stream of clean, dry air. Remove from heat and air stream as soon as last of solvent evaporates. Let residual H<sub>2</sub>O evaporate spontaneously. Solvents may be evaporated from fats on steam bath for short periods.

### 5 Apparatus

(a) Gas chromatograph — With on-column injection system, 6 ft (1.8m) glass column (4 mm id), packed with 10% DC-200 on 80–100 mesh Chromosorb WHP, and electron capture detector. Other liquid phases such as 5% OV-101 on suitable supports may be substituted if known to give adequate resolution for compounds present in test samples.

Linearized <sup>63</sup>Ni detector capable of producing  $\frac{1}{2}$  scale deflection for 1 ng heptachlor epoxide is suggested; however, other equivalent electron capture detectors may be used. Operate GC in accordance with manufacturer's directions, adjusting to provide necessary response and resolution.

(b) Chromatographic tube — 10 id  $\times$  300 mm column with Teflon stopcock, coarse fritted disk, standard taper 24/40 top joint (Kontes Glass Co. 420550, or equivalent).

(c) Kuderna–Danish (K–D) concentrators.—Snyder distilling column (Kontes 503000-0121); 125 mL K-D flask (Kontes 570001-9010) (special item) standard taper 19/22 lower joint; 10 mL concentrator tube (Kontes K-570050-1025).

(d) Micro Snyder column.—Kontes 569251, standard taper 19/22.

### 6 Reagents

(a) Florisil.—PR grade, 60–80 mesh (Floridin Co.). Must meet [970.52B\(i\)](#) (see 10.1.01) specifications.

(b) Solvents.—Petroleum ether, ethyl ether, hexane, and alcohol, known to be suitable for pesticide residue determination.

(c) Glass wool (Pyrex).—Must be free of interference with electron capture detection.

- (d) Sodium sulfate.—Anhydrous, granular, reagent grade, free of interference with electron capture detection.

## 7 Extraction

Weigh 20 g thoroughly ground and mixed test portion into metal blender cup. Moisten 40 g granular  $\text{Na}_2\text{SO}_4$  with petroleum ether and add to sample. Mix test portion, using stirring rod, let stand 20 min, and mix again. Add 100 mL petroleum ether to test portion and blend in centrifuge bottle 1–2 min. Centrifuge balanced bottle 1–2 min at ca 2000 rpm to obtain clear petroleum ether extract. Place glass wool plug in funnel, overlay with 20 g granular  $\text{Na}_2\text{SO}_4$ , and place funnel in 250 mL volumetric flask. Decant petroleum ether extract through  $\text{Na}_2\text{SO}_4$  into volumetric flask. Mix test portion again with stirring rod, add 100 mL petroleum ether, and extract as before. Repeat using 70 mL petroleum ether. Dilute to volume with petroleum ether.

Transfer 25 mL aliquot to tared 100 mL flat bottom extraction flask. Place flask on steam bath to evaporate solvent, leave additional 30 min on steam bath, remove, and cool. Weigh flask and determine % fat in fish.

For fish containing <10% fat, transfer 25 mL aliquot to 125 mL K–D concentrator. For fish containing >10% fat, take aliquot containing not >200 mg fat. Add several granules of 20–30 mesh carborundum and concentrate to ca 3 mL on steam bath. Let cool and remove Snyder column. Rinse concentrator with two 1 mL portions of petroleum ether and, using only current of air, concentrate sample to 3 mL for transfer to Florisil column.

## 8 Florisil cleanup

Use 4 g Florisil adjusted for lauric acid value [JAOAC 51, 29(1968)]. Add Florisil to 300 ×10 mm id chromatographic tube and add  $\text{Na}_2\text{SO}_4$  to height 2 cm above Florisil. Completely open stopcock, tap tube to settle adsorbent, and mark tube 1 cm above  $\text{Na}_2\text{SO}_4$  layer.

Add 20–25 mL petroleum ether wash to Florisil column; as solvent level reaches mark, place 125 mL K–D flask under column. Using disposable Pasteur pipet, transfer 3 mL extract to column, and wash tube with 1 mL petroleum ether and add wash to column. Solvent level must not go below mark. Temporarily close stopcock if necessary. Add 35 mL petroleum ether–ether mixture (94 + 6) and elute PCBs and DDT and its analogs. When solvent level reaches mark, change K–D flask, and add 35 mL petroleum ether–ether (85 + 15) to elute compounds such as dieldrin and endrin. Add several granules of carborundum to first concentrator, attach Snyder column, and carefully concentrate on steam bath. Let concentrator cool, remove Snyder column and evaporate solvent under air to appropriate volume for GC determination. Fractions containing mixture of PCBs and chlorinated compounds such as DDE may require additional separation techniques.

## 9 Additional cleanup

Often additional cleanup is required for second fraction (85 + 15) to prevent deterioration of GC column. Concentrate petroleum ether–ether (85 + 15) fraction under current of air to 2 mL, add 1 mL 2% alcoholic KOH, attach micro-Snyder column, and carefully reduce to  $\leq 1$  mL on steam bath. Reflux test solution 15 min, remove, and cool. Add 2 mL alcohol– $\text{H}_2\text{O}$  (1 + 1) and 5 mL hexane, and shake 1 min. Centrifuge to separate layers. Transfer as much hexane layer as possible to second tube, using disposable Pasteur pipet, and repeat extraction with 5 mL hexane. Concentrate combined hexane to appropriate volume for GC analysis.

## 10 Gas Chromatography

Inject suitable aliquot (3–8  $\mu\text{L}$ ) of concentrated eluate from Florisil or  $\text{MgO}$ –Celite column containing amount of compound within linear range into gas chromatograph, H, using 10  $\mu\text{L}$  syringe. Tentatively identify residue peaks on basis of retention times. Measure area or height of residue peak(s) and determine residue amount by comparison to peak area or height obtained from known amount of appropriate reference material(s). To ensure valid measurement of residue amount, size of peaks

from residue and reference standard should be within  $\pm 25\%$ . Chromatograph reference material(s) immediately after test portion.

Measure PCB residues by comparing total area or height of residue peaks to total area or height of peaks from appropriate Aroclor(s) reference materials. Measure total area or height response from common baseline under all peaks. Use only those peaks from test portion that can be attributed to chlorobiphenyls. These peaks must also be present in chromatogram of reference material. Mixture of Aroclors may be required to provide best match of GC patterns of test portion and reference.

Alternatively, determine PCB residue by individual peak area comparisons using Aroclor reference material weight factors in Table 970.52B. Calculate each PCB peak against appropriate individual reference peak with exactly same absolute retention. Sum individual peak values to obtain total  $\mu\text{g/g}$  PCB. (This method is recommended for PCB residue with chromatographic patterns which are altered extensively from that of any Aroclor reference.)

(a) *Recommended operating conditions for 10% DC-200 or OV-101 column (Surplus 1995).*—Glass column, 1.8 m (6 ft)  $\times$  4 mm id. Temperatures: injector, 225°C; column 200°C;  $^3\text{H}$  electron capture detector,  $\leq 210^\circ\text{C}$ ; carrier gas flow,  $>120$  mL  $\text{N}_2/\text{min}$ .

(b) *Electron capture detection (ECD; Surplus 1995).*—(Use for determination of organochlorine pesticides in fruits, vegetables, and food containing fats and for determination of PCBs in foods and paperboard.) Select for  $^3\text{H}$  electron capture detector operating voltage that voltage (ca 50 V dc) at which 1 ng heptachlor epoxide produces 40–50% full scale recorder deflection at 1 or 3  $\times 10^{-9}$  A full scale sensitivity.

Operate  $^{63}\text{Ni}$  electron capture detector to produce stable, reproducible, linear response, and adjust amount of injected sample to accommodate differences in instrument sensitivity.

(c) *Potassium chloride thermionic and electron capture dual detection (Surplus 1995).*—[Use one of the 3 dual detection systems specified in H(h)–(j), for determination of organophosphorus and organochlorine pesticides and PCBs. In-series system, (h), is preferred because of simplicity and ease of operation.] (1) *In-series dual detection.*—Operate ECD as in (b). For KCITD, adjust  $\text{H}_2$  flow producing 0.2–0.8  $\times 10^{-8}$  A baseline current and select electrometer setting at which 2 ng parathion produces 40–50% full scale recorder deflection. (2) *In-series split dual detection.*—Same as (3), *Parallel*, except ECD receives entire injection and KCITD receives  $\frac{1}{2}$  amount injected into column. (3) *Parallel dual detection.*—Same as (1), *In-series dual*, except column effluent is split; therefore, inject twice as much sample to obtain desired limit of quantitation.

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