AOAC Official Method 992.32
Chlorinated Acidic Pesticide Residues in Finished Drinking Water
Gas Chromatographic Method Using Electron Capture Detector

First Action 1992
Final Action 1995

[Applicable to quantitative determination of residues of 12 chlorinated acidic pesticides, in low ppb range, in (ng/mL) finished drinking water.]

See Table **992.32A** for the results of interlaboratory study. Method is acceptable for quantitative determination of residue levels of bentazon, 2,4-D, 2,4-DB, 3,5-dichlorobenzoic acid, DCPA-diacid, dicamba, dichlorprop, 5-hydroxydicamba, pentachlorophenol, picloram, 2,4,5-T, and 2,4,5-TP.

A. Principle

Laboratory samples are treated with sodium hydroxide to hydrolyze esters of analytes, washed with solvent to remove extraneous organic material, and acidified. Chlorinated acids are ether extracted and converted to methyl esters using diazomethane. Excess derivatizing reagent is removed by solvent wash and activated magnesium silicate cleanup column. Esters are determined by capillary column gas chromatography (GC) using electron capture detector (ECD).

B. Apparatus

(**a**) *Laboratroy sample bottles*.—1 L borosilicate glass, graduated, with TFE-fluorocarbon-lined screw caps. Rinse liners with methanol before use.

(**b**) *Separatory funnel*.—2 L borosilicate glass, with TFE-fluorocarbon stopcock, and ground-glass or TFE-fluorocarbon stopper.

(**c**) *Tumbler bottles*.—1.7 L low-extractable borosilicate glass, with TFE-fluorocarbon-lined screw caps. Cut liners to fit from TFE-fluorocarbon sheets. Rinse liners with methanol before use.

(**d**) *Kuderna–Danish (K–D) apparatus*.—(*1*) *Concentrator tube*.—10 or 25 mL borosilicate glass, graduated, standard taper 19/22. Check calibration of concentrator tubes by accurately weighing 5.00 g H_2O into tubes and verifying 5 mL mark. Use only accurately calibrated tubes. Use ground-glass stoppers to prevent evaporation of extracts. (*2*) *Evaporation flask*.—500 mL borosilicate glass, standard taper 24/40 top, standard taper 19/22 bottom, capable of attachment to concentrator tube with springs. (*3*) *Snyder columns*.—3-ball macro, 218 mm, standard taper 24/40 and 2-ball micro, 170 mm, standard taper 19/22.

(**e**) *Flask, round-bottom*.—500 mL borosilicate glass, standard taper 24/40.

(**f**) *Vials*.—5–10 mL borosilicate glass, with TFE-fluorocarbon-lined screw caps. Rinse liners with methanol before use.

(**g**) *Disposable pipets*.—5 mL, borosilicate glass, sterile, plugged.

(**h**) *Separatory funnel shaker*.—(Optional) Capable of holding 2 L separatory funnels and shaking them with rocking motion to thoroughly mix funnel contents.

(**i**) *Tumbler*.—Capable of turning tumbler bottles end-over-end at 30 rpm.

(**j**) *Boiling stones*.—Teflon, PTFE chips or carborundum, No. 12 granules (heat granules 30 min at 400°C in muffle furnace, cool, and store in desiccator).

(**k**) *Water bath*.—Heated, capable of control ±2°C. Use bath in hood.

(**l**) *Balance*.—Analytical, capable of accurately weighing to nearest 0.0001 g.

(**m**) *Diazomethane generator*.—Using three 20×150 mm tubes, Neoprene rubber stoppers to fit tubes, 5 mm od×3 mm id glass tubing, and nitrogen (ultra high purity) as in Figure **992.32A** or **B**. Cool diazomethane collector in ice bath or cryogenically cooled vessel.

(**n**) *Glass wool*.—Acid wash and heat 4 h at 450°C.

(**o**) *GC system*.—Temperature-programmable system for use with capillary columns, including syringes, analytical columns, gases, detector, and strip chart recorder. Data system is recommended for measuring peak areas. Primary column: 30 m × 0.25 mm id 5% phenyl–95% methylpolysiloxane fused-silica capillary column, 0.25 μm film thickness. Confirmation column: 30 m × 0.25 mm id 14% cyanopropylphenyl–86% methylpolysiloxane fused-silica capillary column, 0.25 μm film thickness. Operating conditions: injection volume 2 μL splitless with 45 s delay; He carrier gas 30 cm/s linear velocity; injector 250°C; detector 320°C; oven programmed from 60–300°C at 4°C/min; ECD. *See* Table **992.32B** for typical rentention time.

C. Reagents

(**a**) *Solvents*.—Acetone, methanol, methylene chloride (CH_2Cl_2), methyl *tert*-butyl ether (MTBE). Distilled-in-glass quality, or equivalent.

(**b**) *Ethyl ether*.—Unpreserved, nanograde, redistilled in glass. Free of peroxides as indicated by peroxide test strips (EM Quant Test Strips, EM Industries, Inc., Gibbstown, NJ USA, are suitable).

(**c**) *Reagent Water*.—Water determined to be free of interfering contaminants. Confirm water quality using columns and conditions described in **B**(**o**).

(**d**) *Acidified sodium sulfate*.—Place granular Na_2SO_4 in shallow tray and heat in muffle furnace >4 h at 450°C to remove interfering organic substances. Slurry 100 g sodium sulfate with enough ethyl ether, (**b**), to just cover solids. Add 0.1 mL concentrated sulfuric acid; mix thoroughly. Remove ether under vacuum. Mix 1 g resulting solid with 5 mL water. Measured pH must be <4. Store at 130°C.

(**e**) *Sodium thiosulfate*.—Granular, anhydrous.

(**f**) *Sodium hydroxide solution*.—6M. Dissolve 216 g NaOH pellets in 900 mL reagent water.

(**g**) *Sulfuric acid solution*.—6M. Slowly add 335 mL concentrated sulfuric acid to 665 mL reagent water.

(**h**) *Potassium hydroxide solution*.—37% (w/v). Dissolve 37 g KOH pellets in reagent water. Dilute to 100 mL.

(**i**) *Carbitol*.—2-(2-Ethoxyethoxy)ethanol (Aldrich Chemical Co., Inc., Milwaukee, WI USA, is suitable source).

(**j**) *Diazald*.—*N*-methyl-*N*-nitroso-*p*-toluene-sulfonamide (Aldrich Chemical Co. is suitable source).

(**k**) *Diazald solution*.—Add 10 g Diazald to 100 mL ethyl ether–carbitol (1 + 1, [v/v]). Solution is stable for 1 month. Store at 4°C in amber bottle using screw cap with PTFE liners.

(**l**) *Sodium chloride (NaCl)*.—Crystal. Heat in muffle furnace >4 h in shallow tray at 450°C to remove interfering organic substances.

Table 992.32A Interlaboratory study results for chlorinated acids in water and drinking water, gas chromatographic method

		Reagent water				Finished drinking water			
Analyte	C[a]	*N*	X[b]	s_R[c]	s_r[d]	*N*	X[b]	s_R	s_r[d]
Bentazon	0.40	4	0.40	0.01	0.09	7	0.52	0.25	0.12
	0.60	6	0.55	0.10		6	0.67	0.15	
	2.39	5	2.03	0.10	0.22	6	1.91	0.87	0.88
	2.98	6	1.79	0.50		6	2.98	0.55	
	7.97	6	6.51	0.94	0.98	7	7.31	1.48	0.95
	9.96	6	8.38	1.98		6	8.49	2.35	
2,4-D	0.40	7	0.38	0.13	0.09	7	0.61	0.33	0.11
	0.60	7	0.53	0.08		7	0.83	0.23	
	2.39	6	2.18	0.07	0.33	6	2.85	0.33	0.42
	2.98	7	2.89	0.38		7	3.28	0.70	
	7.95	7	7.45	1.46	0.82	7	7.20	0.77	0.56
	9.94	7	8.50	1.28		6	8.64	0.64	
2,4-DB	8.00	7	8.17	1.21	1.61	7	7.78	1.99	1.42
	12.00	7	11.54	1.98		7	12.15	2.33	
	20.00	7	20.33	2.98	1.39	7	20.22	6.55	4.36
	24.00	7	23.63	3.66		7	24.57	3.24	
	32.00	7	33.81	7.01	4.58	7	28.56	5.70	5.41
	40.00	7	37.56	4.46		7	31.63	8.46	
3,5-Dichlorobenzoic acid	0.12	6	0.10	0.06	0.02	6	0.14	0.06	0.09
	0.18	7	0.16	0.08		6	0.24	0.12	
	0.49	7	0.44	0.18	0.04	6	0.44	0.18	0.09
	0.61	7	0.54	0.23		6	0.62	0.16	
	1.22	6	1.05	0.16	0.09	6	1.12	0.18	0.08
	1.58	6	1.50	0.45		6	0.26	1.42	
DCPA-diacid	0.40	6	0.28	0.11	0.13	4	0.34	0.06	0.06
	0.60	6	0.43	0.20		5	0.50	0.23	
	1.00	5	0.72	0.06	0.05	5	0.54	0.27	0.16
	1.20	6	0.91	0.24		5	0.84	0.10	
	1.60	6	1.35	0.29	0.18	5	1.30	0.30	0.29
	2.00	6	1.83	0.46		5	1.54	0.34	
Dicamba	0.16	8	0.14	0.05	0.03	6	0.19	0.02	0.03
	0.24	7	0.25	0.03		7	0.26	0.05	
	0.96	7	0.95	0.11	0.08	8	0.97	0.10	0.12
	1.21	7	1.18	0.13		8	1.25	0.15	
	3.22	8	3.09	0.27	0.15	8	3.15	0.50	0.34
	4.02	6	4.02	0.12		8	3.46	0.56	
Dichlorprop	0.80	6	0.84	0.09	0.13	7	1.12	0.42	0.28
	1.19	6	1.17	0.22		7	1.57	0.42	
	2.00	6	2.20	0.11	0.29	7	2.13	0.61	0.33
	2.40	6	2.44	0.34		6	2.69	0.06	
	3.19	6	3.08	0.48	0.28	7	3.51	0.46	0.46
	3.99	5	3.60	0.61		7	3.71	0.75	
5-Hydroxydicamba	0.08	5	0.11	0.03	0.02	5	0.30	0.26	0.08
	0.12	5	0.16	0.03		6	0.26	0.22	
	0.32	5	0.32	0.08	0.06	6	0.39	0.25	0.20
	0.40	5	0.44	0.11		5	0.63	0.32	
	0.80	5	0.85	0.24	0.17	5	1.16	0.40	0.23
	1.04	5	0.91	0.37		5	1.54	0.68	

Table 992.32A (*continued*)

Analyte	C[a]	Reagent water				Finished drinking water			
		N	X[b]	s_R[c]	s_r[d]	*N*	X[b]	s_R	s_r[d]
Pentachlorophenol	0.20	6	0.20	0.01	0.03	5	0.19	0.04	0.04
	0.30	7	0.29	0.05		5	0.24	0.06	
	0.50	7	0.47	0.06	0.07	5	0.45	0.02	0.03
	0.60	7	0.57	0.11		5	0.58	0.04	
	0.80	7	0.67	0.13	0.04	5	0.67	0.13	0.04
	1.00	7	0.81	0.17		5	0.78	0.16	
Picloram	0.27	6	0.23	0.07	0.02	7	0.38	0.07	0.11
	0.40	6	0.35	0.07		7	0.57	0.19	
	1.06	7	1.17	0.37	0.28	7	1.26	0.59	0.26
	1.33	6	1.45	0.69		7	1.66	0.36	
	2.66	7	2.83	0.96	0.38	7	3.03	1.02	0.54
	3.46	7	3.33	0.85		7	3.93	1.71	
2,4,5-T	0.16	7	0.19	0.06	0.03	6	0.18	0.04	0.03
	0.24	7	0.22	0.04		5	0.28	0.01	
	0.97	7	0.89	0.04	0.09	6	0.98	0.26	0.23
	1.21	6	1.20	0.10		6	1.25	0.20	
	3.23	7	2.97	0.59	0.33	6	2.84	0.45	0.57
	4.04	6	3.66	0.18		6	3.04	0.70	
2,4,5-T(Silvex)	0.38	7	0.42	0.07	0.06	7	0.50	0.09	0.23
	0.58	7	0.59	0.10		7	0.79	0.28	
	1.54	7	1.64	0.22	0.13	7	1.56	0.52	0.32
	1.92	7	2.03	0.28		6	2.06	0.17	
	3.84	7	3.64	0.46	0.30	7	3.65	0.66	0.25
	4.99	7	4.42	0.71		7	4.71	0.87	

[a] Spike concentration, µg/L.

[b] Mean recovery, µg/L.

[c] Overall standard deviation, µg/L.

[d] Single-analyst standard deviation, µg/L.

(**m**) *Preservative (bacteriocide).*—10 mg/mL mercuric chloride ($HgCl_2$) in reagent water. (*Caution*: Dispose of extractions containing mercuric chloride as hazardous waste.) If another preservative is shown to maintain sample integrity 7 days at 4°C with no interference in chlorinated acids analysis, it may be substituted.

(**n**) *Silicic acid.*

(**o**) *Activated magnesium silicate.*—60–100/PR mesh (Florisil, Aldrich Chemical Co., is suitable). Heat activate 24–48 h in shallow container at 150°C.

(**p**) *Standard solutions.*—Prepare standards of test compounds (purity >96%): bentazon, 2,4-D, 2,4-DB, 3,5-dichlorobenzoic acid, DCPA-diacid, dicamba, dichlorprop, 5-hydroxydicamba, pentachlorophenol, picloram, 2,4,5-T, and 2,4,5-TP(Silvex), at stock concentration 1 mg/mL in MTBE. Commercially prepared stock standards may be used if certified by U.S. Environmental Protection Agency or independent source. Store solutions at room temperature and protect from light. Replace stock standards after 2 months, or sooner if comparison with laboratory control standards indicates degradation.

(**q**) *Internal standard solution.*—Dissolve 0.0010 g 4,4′-dibromooctafluorobiphenyl (DBOB; 99% purity; Aldrich Chemical Co., is suitable source) in 10 mL MTBE. Store in vial, **B**(**f**), at room temperature. Replace when control chart indicates degradation of area counts or peak height >50% from initial preparation.

(**r**) *Surrogate standard solution.*—Dissolve 0.0010 g 2,4-dichlorophenylacetice acid (DCAA; 99% purity; Aldrich Chemical Co., is suitable source.) in 10 mL MTBE. Store in vial, **B**(**f**), at room trremperature. Replace when DCAA percent recovery exceeds control chart limits.

(**s**) *Instrument performance check solution.*—Dilute 10 µL dinoseb standard solution, (**p**), to volume with MTBE in 10 mL volumetric flask. Add 40 µL diluted dinoseb solution, 16 µL 4-nitrophenol standard solution, (**p**), 6 µL 3,5-dichlorobenzoic acid standard solution, (**p**), 50 µL surrogate standard solution (**r**), 25 µL internal standard solution (**q**), and 250 µL of methanol to 5 mL volumetric flask; dilute to volume with MTBE.

D. Preparation of Laboratory Sample Bottles

Add 1 mL preservative, **C**(**m**), to laboratory sample bottle. Add 80 mg sodium thiosulfate to laboratory sample bottle before collection if residual chlorine is expected.

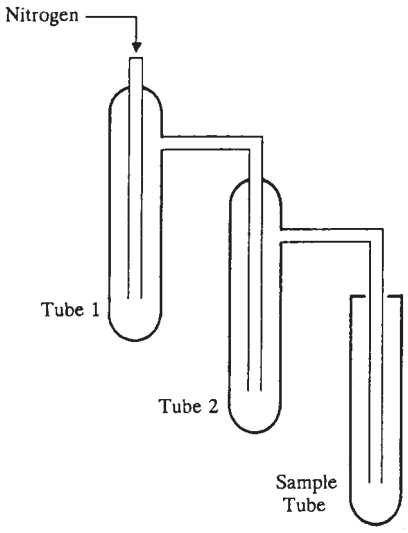

Figure 992.32A—Gaseous diazomethane generator, *see* H(a).

E. Laboratory Sample Collection

Collect 1 L grab laboratory samples, by conventional sampling practices, in laboratory sample bottles, prepared as in **D**. Do not prerinse bottles with water before collection. Seal bottles and shake vigorously 1 min. Refrigerate laboratory samples until extracted. Protect from light. Extract laboratory samples within 7 days of collection.

F. Laboratory Sample Preparation

Perform either manual or mechanical hydrolysis/extraction method below:

(**a**) *Manual hydrolysis/extraction method.*—Add preservative, **C**(**m**), to laboratory samples (blanks, quality control check samples, and test portions) not previously preserved. Mark water meniscus on side of bottle for later determination of laboratory sample volume. Pour entire laboratory sample into 2 L separatory funnel. Fortify with 50 µL of surrogate standard solution (surrogate concentration in laboratory sample = 5 µg/L; final concentration in extract = 0.5 mg/L). Add 250 g NaCl to solution, seal, and shake to dissolve salt. Add 17 mL 6M NaOH to solution, seal, and shake. Check solution pH. If necessary, adjust pH to ≥12 with 6M NaOH. Let solution sit 1 h at room temperature, shaking funnel periodically.

Rinse laboratory sample bottle with 60 mL CH_2Cl_2, transfer rinse to separatory funnel, and extract by shaking 2 min with periodic venting. Let layers separate >10 min. If emulsion interface between layers is more than ⅓ volume of solvent layer, complete phase separation by mechanical techniques such as stirring, filtration of emulsion through glass wool, or centrifugation. Discard CH_2Cl_2 phase. Add second 60 mL volume of CH_2Cl_2 to laboratory sample bottle, rinse, transfer, and repeat extraction procedure in same separatory funnel, discarding CH_2Cl_2 layer. Perform third extraction in same manner.

Cautiously add 17 mL 6M H_2SO_4 to solution in separatory funnel, seal, and shake; pH must be ≤2. If necessary, adjust pH using 6M H_2SO_4. Add 120 mL ethyl ether, **C**(**b**), to solution in separatory funnel, seal, and extract solution by shaking 2 min with periodic venting. Let layers separate >10 min. If emulsion interface between layers is more than ⅓ volume of solvent layer, employ mechanical techniques to complete phase separation.

Remove aqueous phase to 2 L Erlenmeyer flask. Collect ethyl ether phase in 500 mL round-bottom flask containing ca 10 g acidified anhydrous sodium sulfate, **C**(**d**). Shake extract and drying agent periodically, keeping extract in contact with sodium sulfate 2 h. Return aqueous phase to separatory funnel, add 60 mL ethyl ether, and repeat extraction procedure second time, combining ethyl ether phases in 500 mL Erlenmeyer flask. Perform third extraction with 60 mL ethyl ether in same manner. Discard aqueous phase.

Determine original laboratory sample volume by refilling bottle with water to mark and transferring water to 1 L graduated cylinder. Record laboratory sample volume to nearest 5 mL.

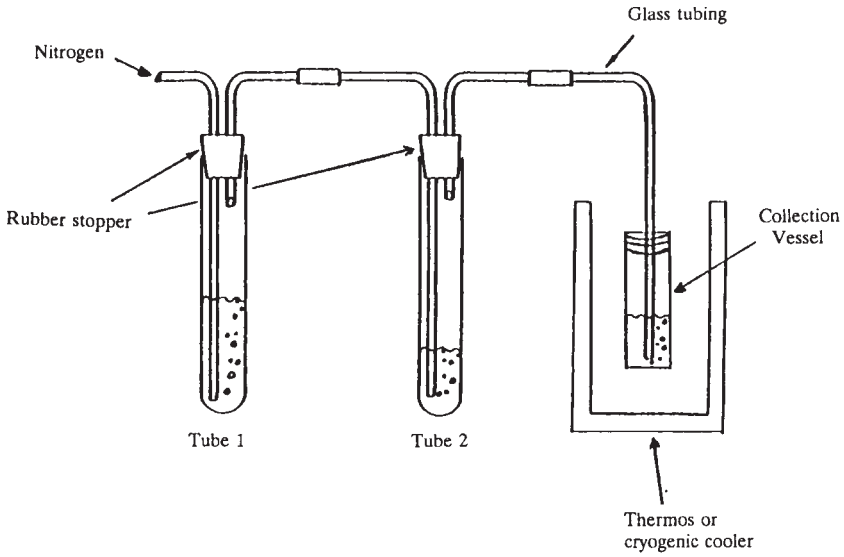

Figure 992.32B—Diazomethane solution generator, *see* H(b).

Table 992.32B Retention times for method analytes

Analyte	CAS No.	Retention time,[a] min	
		Primary	Confirmation
3,5-Dichlorobenzoic acid	51-36-5	18.6	17.7
DCAA (surrogate)	19719-28-9	22.0	14.9
Dicamba	1918-00-9	22.1	22.6
Dichlorprop	120-36-5	25.0	25.6
2,4-D	94-75-7	25.5	27.0
DBOB (int. standard)	10386-84-2	27.5	27.6
Pentachlorophenol	87-86-5	28.3	27.0
2,4,5-TP	93-72-1	29.7	29.5
5-Hydroxydicamba	7600-50-2	30.0	30.7
2,4,5-T	93-76-5	30.5	30.9
2,4-DB	94-82-6	32.2	32.2
Bentazon	25057-89-0	33.3	34.6
Picloram	1918-02-1	34.4	37.5
DCPA acid metabolites[b]		35.8	37.8

[a] Columns and analytical conditions are described in method section, **B**(**o**).

[b] DCPA monoacid and diacid metabolites included in method scope; DCPA diacid metabolite used for validation studies.

(**b**) *Mechanical hydrolysis/extraction method*.—Add preservative, **C**(**m**), to laboratory samples (blanks, quality control check samples, and test portions) not previously preserved. Mark water meniscus on side of bottles for later determination of laboratory sample volumes. Pour each entire laboratory sample into 2 L separatory funnel if mechanical separatory funnel shaker is used or into tumbler bottle if mechanical tumbler is used. Fortify extract with 50 µL of surrogate standard solution (surrogate concentration in extract = 5 µg/L; final concentration in extract = 0.5 mg/L). Add 250 g NaCl to extract, seal, and shake to dissolve salt. Add 17 mL 6M NaOH to extract, seal, and shake. Check extract pH. If necessary, adjust pH ≥12 by adding 6M NaOH. Shake extract for 1 h using appropriate mechanical mixing device.

Rinse laboratory sample bottle with 300 mL CH_2Cl_2, transfer rinse to separatory funnel or tumbler bottle, seal, and shake for 10 s, venting periodically. Repeat shaking and venting until pressure release is not observed during venting. Reseal and place separatory funnel or tumbler bottle in appropriate mechanical mixing device. Shake or tumble sample 1 h at speed which provides complete and thorough mixing of organic and aqueous phases in 2 min.

Remove test portion container from mixing device. If tumbler is used, pour contents of tumbler bottle into 2 L separatory funnel. Allow layers to separate >10 min. If emulsion interface between layers is more than ⅓ volume of solvent layer, complete phase separation by mechanical techniques such as stirring, filtration of emulsion through glass wool, or centrifugation. Discard CH_2Cl_2 phase.

If tumbler is used, return aqueous phase to tumbler bottle. Cautiously add 17 mL of 6M H_2SO_4 to test portion sample, seal, and shake. If necessary, adjust pH ≤2 using 6M H_2SO_4. Add 300 mL ethyl ether, **C**(**b**), to separatory funnel or tumbler bottle, seal, and shake 10 s, venting periodically. Repeat shaking and venting until pressure release is not observed during venting. Reseal and place test portion container in appropriate mechanical mixing device. Shake or tumble test portion for 1 h. Complete and thorough mixing of organic and aqueous phases should be observed in 2 min. Remove test portion container from mixing device. If tumbler is used, pour contents of tumbler bottle into 2 L separatory funnel. Allow layers to separate >10 min. If emulsion interface between layers is more than ⅓ volume of solvent layer, analyst must employ mechanical techniques to complete the phase separation. Drain and discard aqueous phase.

Collect extract in 500 mL round-bottom flask containing 10 g acidified anhydrous sodium sulfate, **C**(**d**). Shake extract and drying agent periodically, keeping extract in contact with sodium sulfate 2 h.

Determine original laboratory sample volume by refilling laboratory sample with water to mark and transferring water to 1 L graduated cylinder. Record laboratory sample volume to nearest 5 mL.

G. Extract Concentration

Assemble K–D apparatus, **B**(**d**), by attaching 10 mL concentrator tube to 500 mL evaporative flask. Pour dried extract, **F**, through funnel plugged with acid-washed glass wool, and collect extract in K–D concentrator. Use glass rod to crush caked sodium sulfate during transfer. Rinse round-bottom flask and funnel with 20–30 mL ethyl ether, **C**(**b**), to complete quantitative transfer.

Add 1 or 2 clean boiling stones, **B**(**j**), to K–D apparatus, **B**(**d**), and attach macro-Snyder column. Prewet top of Snyder column with 1 mL ethyl ether. Place K–D apparatus on 60–65°C water bath so concentrator tube is partially immersed in hot water and entire lower rounded surface of flask is bathed with hot vapor. At proper rate of distillation, column balls will actively chatter but chambers will not flood. When apparent volume of liquid reaches 1 mL, remove K–D apparatus. Let column drain and cool ≥10 min.

Remove Snyder column, rinsing flask and lower joint with 1–2 mL ethyl ether into concentrator tube. Add 2 mL MTBE and fresh boiling stone. Attach micro-Snyder column to concentrator tube and prewet top of column by adding ca 0.5 mL ethyl ether. Place micro K–D apparatus on water bath so concentrator tube is partially immersed in hot water. Adjust vertical position of apparatus and water temperature as required to complete concentration in

Table 992.32C Acceptance limits for the analysis of a laboratory quality control test portion, as percent of spiked value

Analyte	Spike level[a], µg/L	Mean recovery[b], %	Overall standard deviation	Acceptance limits,[c] %
Bentazon	3	2.36	0.41	37–120
2,4-D	3	2.75	0.36	55–128
2,4-DB	40	39.7	6.34	52–147
3,5-Dichlorobenzoic acid	1	0.90	0.28	7–173
DCPA-diacid	2	1.66	0.34	32–134
Dicamba	1	0.98	0.09	70–126
Dichlorprop	4	3.90	0.54	57–138
5-Hydroxydicamba	1	0.99	0.29	11–187
Pentachlorophenol	1	0.87	0.18	34–140
Picloram	2	2.10	0.72	0–213
2,4,5-T	1	0.93	0.10	62–124
2,4,5-TP (Silvex)	3	2.93	0.41	57–139

[a] Spike level is 10–15 times estimated method detection limit, µg/L.

[b] Estimated method statistics calculated from the regression equations for mean recovery and overall standard deviation for reagent water matrix.

[c] Acceptance limits defined as (mean recovery ± 3 standard deviation)/spike level.

5–10 min. When apparent volume of liquid reaches 0.5 mL, remove micro K–D from bath, let column drain, and cool. Remove micro-Snyder column and add 250 µL methanol to concentrator tube. If gaseous diazomethane procedure will be used for esterification of chlorinated acids, rinse walls of concentrator tube while adjusting volume to 5.0 mL with MTBE. If diazomethane solution will be used for esterification of chlorinated acids, rinse walls of concentrator tube while adjusting volume to 4.5 mL with MTBE.

H. Esterification Procedure

Perform esterification of chlorinated acids using gaseous diazomethane or diazomethane solution as described below:

(**a**) *Esterification of acids using gaseous diazomethane*.—Assemble diazomethane generator (Figure **992.32A**) in hood. (*Caution*: Wear appropriate safety equipment to minimize skin and inhalation hazards.) Add 5 mL ethyl ether, **C**(**b**), to tube 1. Add 1 mL ethyl ether, 1 mL carbitol, 1.5 mL 37% KOH solution, and 0.2 g Diazald, **C**(**j**), to tube 2. Immediately place exit tube into concentrator tube (tube 3) containing extract. Apply nitrogen flow (10 mL/min) to bubble diazomethane through extract 1 min. Extracts should turn yellow after addition of diazomethane and remain yellow for >2 min. Repeat methylation procedure if necessary. Remove concentrator tube containing extract. Rinse tip of diazomethane generator with ethyl ether after methylation of each extract. Bubble diazomethane through concentrator tube containing next extract 1 min. Prepare fresh diazomethane reaction mixture in tube 2 for each 2 additional test portion extracts. Seal concentrator tubes with stoppers. Store at room temperature in hood 30 min. Destroy unreacted diazomethane by adding 0.1–0.2 g silicic acid to concentrator tubes. Let stand until evolution of nitrogen gas ceases (ca 20 min). Adjust volume to 5.0 mL with MTBE.

(**b**) *Esterification of acids using diazomethane solution*.—Assemble diazomethane solution generator (Figure **992.32B**) in hood. Collection vessel is 10 or 15 mL vial equipped with Teflon-lined screw cap and maintained at 0–5°C. (*Caution*: Wear appropriate safety equipment to minimize skin contact and inhalation hazards.) Add sufficient ethyl ether, **C**(**b**), to tube 1 to cover first impinger. Add 5 mL MTBE to collection vial. Set nitrogen flow at 5–10 mL/min. Add 2 mL Diazald solution, **C**(**k**), and 1.5 mL 37% KOH solution to tube 2. Connect tubing as in Figure **992.32B** and let nitrogen flow purge diazomethane from reaction vessel into collection vial 30 min. (*Caution*: Avoid heat. Diazomethane is explosive near 90°C). Cap vial when collection is complete and store at 0–5°C. Diazomethane solution in collection vial is stable 48 h. Add 0.5 mL diazomethane solution to each concentrator tube containing extract or standard. Extracts should turn yellow after addition of diazomethane solution and remain yellow >2 min. Repeat methylation procedure if necessary. Seal concentrator tubes with stoppers. Store at room temperature in hood 30 min. Destroy unreacted diazomethane by adding 0.1–0.2 g silicic acid to concentrator tubes. Let stand until evolution of nitrogen gas ceases (ca 20 min). Adjust volume to 5.0 mL with MTBE.

(**c**) *Cleanup*.—Place small plug of glass wool into 5 mL disposable glass pipet. Tare pipet and measure 1 g activated Florisil, **C**(**o**), into pipet. Add 5 mL 5% methanol in MTBE to pipet to just cover Florisil. (*Note*: In this and subsequent steps, when liquid level just reaches top of Florisil, apply next rinse. Never let Florisil go dry.) Discard eluate. Add 5 mL methylated extract from (**a**) or (**b**) to Florisil, leaving silicic acid in extract tube. Collect eluate in K–D concentrator tube. Rinse extract container walls with 1 mL 5% methanol in MTBE and transfer rinse to Florisil column, leaving silicic acid in tube. Collect eluate in K–D tube. Repeat with 1 mL and 3 mL aliquots 5% methanol in MTBE, collecting eluates in K–D tube. If necessary, adjust final eluate volume to 10 mL with 5% methanol in MTBE. Add 25 µL internal standard solution to 10 mL extract (concentration of internal standard in extract = 0.25 mg/L). Seal vial and store at 4°C until analysis. Analyze by GC–ECD.

I. Calibration

Establish GC operating conditions, **B**(**o**). GC system may be calibrated using either internal standard technique or external standard technique, below.

Prepare calibration standards at 5 concentration levels for each analyte of interest, add aliquots from one or more standard solutions, **C**(**p**), and 250 µL methanol to 5 mL volumetric flask, and dilute with MTBE. Calibration standards should bracket extracts ana-

lyzed. Esterify calibration standards with diazomethane, **H**(**a**) or **H**(**b**).

(**a**) *Internal standard calibration procedure*.—Add 25 µL internal standard solution, **C**(**q**), to 10 mL esterified standard. Analyze each calibration standard and tabulate relative response factors using peak height or peak area. If relative response factor,

$$\text{RRF} = \frac{(\text{area}_{\text{standard}})(\text{concentration}_{\text{internal standard}})}{(\text{area}_{\text{internal standard}})(\text{concentration}_{\text{standard}})}$$

for each analyte is constant (<20% RSD) across calibration range, average RRF can be used for calculations. Alternatively, plot calibration curve of analyte response ratio ($A_{\text{standard}}/A_{\text{internal standard}}$), where A is area counts or peak height) versus concentration of calibration standard. Before each working shift, verify calibration curve or RRF by measurement of one or more calibration standards. If response for any analyte varies from predicted response more than ±20%, repeat test using fresh calibration standard. If repetition also fails, generate new calibration curve for that analyte using freshly prepared standards.

(**b**) *External standard calibration procedure*.—Prepare 5-level calibration using standard solution for each analyte and surrogate compound by adding volumes of one or more standard solutions and 250 µL methanol to 5 mL volumetric flask; dilute to volume with MTBE. Esterify acids with diazomethane, **H**. Analyze each calibration standard and plot response (peak height or area) versus standard concentration. Alternatively, if ratio of response to concentration (calibration factor) is constant over working range (< 20% RSD), linearity through origin can be assumed and average ratio or calibration factor can be used in place of calibration curve. Each working day, verify working calibration curve or calibration factor at 2 different concentration levels by measurement of at least 2 calibration check standards, at beginning and end of analysis day. For extended periods of analysis (>8 h), it is strongly recommended that check standards be interspersed with extracts at regular intervals. If response for any analyte varies from predicted response more than ±20%, test must be repeated using fresh calibration standard. If results still do not agree, generate new calibration curve.

J. Calculations

If internal standard calibration procedure is used, analyte concentration, C, in laboratory sample may be determined from calibration curve or calculated as follows:

$$C, \mu\text{g/L} = \frac{A_s \times I_s}{A_{is} \times \text{RRF} \times V_o}$$

where A_s = response for analyte measured; A_{is} = response for internal standard; I_s = µg internal standard added to each extract; V_o = volume, in L, laboratory sample extracted; and RRF = relative response factor from **I**(**a**).

If external standard calibration procedure is used, analyte concentration, C, in laboratory sample may be determined from calibration curve or calculated as follows:

$$C, \mu\text{g/L} = \frac{W \times V_t}{V_i \times V_s}$$

where W = ng analyte injected; V_i = µL extract injected; V_t = µL total extract; and V_s = mL laboratory sample extracted.

K. Quality Control

(**a**) *Minimum quality control requirements*.—(*1*) Demonstrate laboratory capability prior to analyses by extracting four 1 L aliquots of spiked reagent water at concentration indicated in Table **992.32C** for each analyte of interest. For each spiked reagent water test portion, percent recovery of analytes must be within acceptance limits presented in Table **992.32C** and percent relative standard deviation should be <20%. (*2*) Analyze method blank daily as contamination check. (*3*) Analyze instrument performance check solution, **C**(**s**), daily. Acceptance criteria: detection of 0.004 µg/mL dinoseb with signal-to-noise ratio >3; peak Gaussian factor (PGF) for 4-nitrophenol at 1.6 µg/mL between 0.70 and 1.05, where PGF = 1.83(W/Z) when W = peak width at half height and Z = peak width at 1/10 height; and resolution between 3,5-dichlorobenzoic acid (0.6 µg/mL) and 4-nitrophenol (1.6 µg/mL) is >0.40, where resolution = t/W, when t is difference in elution times between 2 peaks and W is average peak width, at baseline, of the 2 peaks. (*4*) Measure recovery of surrogate standard in every extract. Percent recovery of surrogate from all extracts must be 70–130%. (*5*) Monitor internal standard (IS) response (peak height or area) in every extract as system performance check. IS response in extract must not deviate from daily calibration check standard response by more than 30%. (*6*) Analyze one laboratory fortified blank test portion and one spiked matrix test portion with every 20 test portions samples at levels presented in Table **992.32C**. Percent recovery from all fortified test portions must be within acceptance limits presented in Table **992.32C**. If matrix test portion contains background concentrations of analytes in excess of 100% of spike value, acceptance limits in Table **992.32C** may not be suitable to adequately judge spike recovery.

(**b**) *Recommended quality control practices*.—(*1*) Analyze one U.S. Environmental Protection Agency certified quality control sample quarterly. (*2*) Participate in external performance evaluation study annually.

Reference: *J. AOAC Int.* **76,** 1098(1993).

CAS-25057-89-0 (bentazon)
CAS-94-75-7 (2,4-D)
CAS-94-82-6 (2,4-DB)
CAS-1918-00-9 (dicamba)
CAS-51-36-5 (3,5-dichlorobenzoic acid)
CAS-120-36-5 (dichlorprop)
CAS-7600-50-2 (5-hydroxy dicamba)
CAS-87-86-5 (pentachlorophenol)
CAS-1918-02-1 (pichloram)
CAS-93-76-5 (2,4,5-T)
CAS-93-72-1 (2,4,5-TP)

Revised: March 1998