

ANALYTES: Figure 1 FORMULA: Table 1 MW: Table 1 CAS: Table 1 RTECS: Table 1

METHOD: 9201, Issue 1		EVALUATION: PARTIAL		Issue 1: 15 January 1998	
OSHA: Table 1		PROPERTIES:		Table 1	
NIOSH: Table 1					
ACGIH: Table 1					
NAMES:	Alachlor Cyanazine	Atrazine Metolachlor	2,4-D acid Simazine	2,4-D, 2-ethylhexyl ester 2,4-D, 2-butoxyethyl ester	
SAMPLING			MEASUREMENT		
SAMPLER:	DERMAL PATCH (Polyurethane Foam [PUF] pads, 10 x 10-cm, 3 to 4 mm thick)		TECHNIQUE:	GAS CHROMATOGRAPHY, ELECTRON CAPTURE DETECTOR, (GC/ECD)	
PASSIVE EXPOSURE:	place pad in an aluminized card holder with 7.6-cm diameter circle cut in one side. Affix to worker's clothing or skin.		ANALYTE:	Table 1	
SHIPMENT:	transfer pads to 120-mL wide-mouthed glass jars with PTFE-lined caps.		DESORPTION:	40 mL isopropanol (containing diazomethane)	
SAMPLE STABILITY:	at least 30 days at 4 °C [1]		INJECTION VOLUME:	2 µL	
BLANKS:	2 to 10 field blanks per set		TEMPERATURE-INJECTION:	270 °C	
			-DETECTOR:	300 °C	
			-COLUMN:	90 °C for 1 min; ramp to 160 °C at 35 °C/min; ramp to 200 °C at 5°C/min; ramp to 230 °C at 3 °C/min; hold 9 min. Total run time = 30 min.	
ACCURACY			CARRIER GAS:	He, mL/min	
RANGE STUDIED:	not determined		COLUMN:	capillary, fused silica, 30 m X 0.25-mm ID, 0.25-µm film, 50% phenyl, 50% methyl silicone, DB-17 or equivalent. See Table 2	
BIAS:	not determined		CALIBRATION:	standard solutions of herbicides in isopropanol	
OVERALL PRECISION ($\hat{S}_r$):	not determined		RANGE:	Table 3	
ACCURACY:	not determined		ESTIMATED LOD:	Table 3	
			PRECISION ($\hat{S}_r$):	Table 3	
APPLICABILITY: The working ranges are listed in Table 3. These cover from the LOQ to approximately 30X the LOQ [1]. This method may be applicable to the determination of many other thermally stable organonitrogen; aryl and alkyl acidic; and phenolic pesticides after evaluation for desorption efficiency, sample stability, and precision and accuracy.					
INTERFERENCES: Because of the great sensitivity of the ECD, there are many potential interferences. Among those observed are plasticizers (e.g., dibutyl phthalate), methylated fatty acids (give negative responses), phenols, and antioxidants and other additives (e.g., BHT), any volatile or semivolatile halogenated or nitrated organic, organophosphorous compounds, and other pesticides. Agricultural spray additives can present serious interferences. Such additives include solvents, emulsifiers, wetting agents, breakdown products, and fertilizers (e.g., fatty acids and urea). Second-column confirmation is very desirable. Poorly precleaned pads and poor technique present significant interferences at low levels. These include phthalate plasticizers and PUF manufacturing additives and monomers.					
OTHER METHODS: Other patch methods allowing simultaneous analysis of organonitrogen and acid compounds and their esters are unknown.					

REAGENTS:

1. Analytes* listed in Table 1.
2. Isopropanol, pesticide analytical grade.
3. Silicic Acid, 100-mesh. Aldrich Chemical Co. or equivalent.
4. Diazomethane* derivatizing reagent. (See APPENDIX)
5. *Diazald*[®] (N-methyl-N-nitroso-p-toluenesulfonamide*), Aldrich Chemical Co. or equivalent.
6. Herbicide stock solutions. Prepare individual standard stock solutions of each herbicide of interest in isopropanol.
NOTE: All herbicides in Table 1 were found to be soluble to at least 1 mg/mL, except simazine, which is soluble to 0.5 mg/mL.
7. Calibration stock solution. Dilute the appropriate volume of herbicide stock solution to a known volume with isopropanol.
NOTE: Spiking solutions may contain more than one analyte.
8. Purified gases: Helium, 5% methane in Argon, or nitrogen.

* See SPECIAL PRECAUTIONS.

EQUIPMENT:

1. Polyurethane foam (PUF) patch. 10 x 10 x 0.6-cm, contained in a aluminized card holder, 10 x 10 cm, which has a 7.6-cm-diameter circle in one side. (SKC Inc., Eighty Four, PA 15330 or equivalent.)
NOTE: Before use, clean the PUF patch by soxhlet extraction for 20 hours in acetone/methanol 88/12, v/v, followed by three hexane rinses.
2. Gas chromatograph, electron capture detector, integrator, and column (see Page 9201-1 and Table 2).
3. Syringes, 2-, 5-mL, and 10-, 50-, and 100- μ L for making standard solutions and GC injections
4. Syringes, luer lock, 1-, 2.5-, or 5-mL for sample filtering.
5. PTFE syringe filters, 0.45- μ m pore size. (Gelman Acrodisc[®] CR or equivalent).
6. Volumetric flasks, 2-, 5-, 10-, 25-, 50-, and 100-mL for working standard preparation and solutions..
7. Jars, glass, 120-mL, PTFE-lined cap.
8. Vials, glass, 2-mL, GC autosampler, with PTFE-lined caps.
9. Test tubes, glass screw-top, with PTFE seals, 16 x 125 mm, 16-mL volume.
10. Forceps.
11. Platform shaker.

SPECIAL PRECAUTIONS: Diazomethane has been cited as a carcinogen. It is extremely toxic and highly irritating. Diazomethane may explode under some conditions. Do not heat above 90 °C. Avoid rough surfaces: fire-polish glass tubing, or use Teflon[®]. Do not expose solutions to strong light. Keep dilute solutions at 0 °C. Prepare in a hood [2]. *Diazald*[®] is far more convenient to use on a large scale than the alkyl-nitrosoguanidines. Avoid skin contact with *Diazald*[®] and herbicides. Avoid skin contact and open flame with solvents. Use in a hood.

SAMPLING:

1. Dermal Patch
 - a. Insert a cleaned PUF patch into patch holder. Secure to desired location on clothing or skin.
 - b. At the conclusion of the sampling period, remove PUF patch from holder with solvent-washed (acetone or isopropanol) forceps and place in a 120-mL jar; cap securely.
 - c. Label and pack for shipment.

SAMPLE PREPARATION:

2. Add 20 mL of isopropanol to PUF in jar. Add an additional 20 mL diazomethane derivatizing reagent to jar. Cap securely, and using platform shaker rotate 5 to 10 RPM for a minimum of 1.5 hours.
3. Transfer a 10-mL sample aliquot into a screw-top test tube with a PTFE-lined cap. Add approximately 10 mg of silicic acid to the solution. Allow to stand an additional hour.
4. Filter an aliquot through a 0.45- μ m PTFE filter into a 2-mL GC vial. Label.
NOTE: It is important to filter the samples because the silicic acid fines can build-up in the chromatographic system and cause deterioration of analytical column performance.

CALIBRATION AND QUALITY CONTROL:

5. Calibrate daily with at least six working standards covering the analytical range of the method for individual analytes.
 - a. Add known amounts of calibration solution to the diazomethane derivatizing reagent in a volumetric flask. Let stand for 1 hour. Include a calibration blank of unspiked diazomethane derivatizing reagent solution.
 - b. Add 10 mg silicic acid to each flask and let stand for another hour.
 - c. Filter through a 0.45 PTFE syringe filter into a GC vial.
 - d. Analyze together with field samples, field blanks, and laboratory control samples (steps 8 and 9).
 - e. Prepare calibration graph (peak height or area vs. $\mu\text{g/mL}$ analyte).
6. Determine desorption efficiency (DE) at least once for each lot of PUF samplers used for sampling in the calibration range (step 5).
 - a. Prepare three samples at each of six levels plus three media blanks.
 - b. Place PUF patch in a 120-mL glass jar. Apply a known amount of calibration stock to patch. Replace lid on jar and allow to stand over night.
 - c. Desorb the samples (steps 2 through 4) and analyze together with standards and blanks (steps 8 and 9).
 - d. Prepare a graph of DE vs. μg analyte recovered.
7. Analyze three quality control blind spikes and three analyst spikes to ensure that the calibration and DE graphs are in control.

MEASUREMENT:

8. Set gas chromatograph according to manufacturer's recommendations and to conditions listed in Table 2. Inject sample aliquot manually using solvent flush technique or with autosampler. See Table 4 for retention times of selected analytes.
NOTE: If peak height is greater than the range of the working standards, dilute the sample with isopropanol and reanalyze. Apply the appropriate dilution factor in calculations.
9. Measure peak height of analyte.

CALCULATIONS:

10. Determine the concentration, $\mu\text{g/mL}$, (corrected for DE) of the respective herbicide found on the patch sample (C_p) and in the media blank (B_b) from the calibration graph.
11. Calculate the mass of herbicide, M (μg), on patch in the desorption volume, V (mL):

$$M = (C_p V_p - B_b V_b), \mu\text{g}$$

CONFIRMATION:

Whenever the identity of an analyte is uncertain, confirmation may be achieved by analysis on a column of different polarity. If primary analysis was performed using a nonpolar or weakly polar column (e.g., DB-1 or DB-5), confirmation should be accomplished by reanalyzing on a polar column (e.g., DB-17 or DB-1701). See Table 4 for approximate retention times for each column type. For positive identification of high-level analytes (1 to 10 $\mu\text{g/mL}$ or greater), GC/MS may be used. Table 5 gives notes on the analytical characteristics of the chlorinated and organonitrogen herbicides.

EVALUATION OF METHOD:

This method was evaluated over the ranges specified in Table 3. For the method evaluation, the GC analyses were done using the DB-17 column. See Table 2 and Table 4 for chromatographic conditions. The upper limits of the analytical range were set at approximately the upper ranges studied. Also presented in Table 3 are the LOD, $\bar{S}_{r1}$ (pooled relative standard deviation), and sample storage stability for these eight

compounds. To determine the LOD and LOQ values, a series of media-spiked standards was prepared in triplicate, analyzed, and fitted with a quadratic curve. The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were estimated with Burkart's Method [3]. Actual LOD and LOQ results were inconsistent owing to periodic low level interferences in the media. Therefore, higher arbitrary LODs and LOQs were selected in order to circumvent the problem. The higher LODs were assumed to be more realistic and were used for evaluation of the method. A set of samples for long-term storage study was prepared and analyzed after 1, 30, and 120 days. The results are summarized in Table 3. The day 1 results show that the compounds, except for 2,4-D acid and the 2,4-D-butoxy ester, give 90% or better recovery based on the amount spiked onto the samplers. When compared to day 1, the day 30 sample results were all 92%. The PUF patch samples were shown to be stable for at least 30 days when refrigerated at 4°C.

REFERENCES:

- [1] NIOSH [1995]. Back-up data report for chlorinated organonitrogen and carboxylic acid herbicides. Prepared under NIOSH Contract 200-88-2618 (unpublished).
- [2] Black TH [1983]. The preparation and reactions of diazomethane. *Aldrichimica Acta* 16(1).
- [3] Burkart JA [1986]. General procedures for limit of detection calculations in the industrial hygiene chemistry laboratory, *Appl Ind Hyg* 1(3):153-155.

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APPENDIX. DIAZOMETHANE GENERATOR

The diazomethane generator (see Figure 2) consists of two 40-mL test tubes, each fitted with a two-hole rubber stopper. A glass tube which extends to within one centimeter of the bottom of the first test tube is inserted in one hole of the stopper. The other end of the glass tube is connected to a supply of nitrogen. A short piece of PTFE tubing is placed in the second hole and is directed to the bottom of the second tube through the second stopper. A third piece of PTFE tubing leads from the second tube into the receiving flask. The first test tube contains a small quantity of diethyl ether. Nitrogen bubbled through the ether is led to the second tube containing 3 mL of a 37% (w/v) KOH/water solution and 4 mL "Diazald Reagent," which is prepared by dissolving 10 g Diazald in 100 mL of 1/1 Ethyl ether/Carbitol. (Nitrogen becomes saturated with diethyl ether vapor in the first tube in order that nitrogen does not deplete the second tube of diethyl ether; diethyl ether stabilizes diazomethane through adduct formation.) The resulting diazomethane gas is swept into a flask of chilled (0 °C) isopropanol (maximum volume, 500 mL) by a flow of nitrogen gas.

NOTE: The KOH solution (37% w/v) will become weaker over time from the absorption of atmospheric carbon dioxide. Under such circumstances diazomethane generation will be considerably slower.

TABLE 1. SYNONYMS, FORMULA, MOLECULAR WEIGHT, PROPERTIES

<u>Name / Synonym</u>	<u>Empirical Formula</u>	<u>Molecular Weight</u>	<u>Physical Properties</u>	<u>Solubility in Water (mg/L)</u>	<u>LD50 mg/kg</u>	<u>TWA (mg/m3)</u>
Alachlor 2-Chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide CAS #15972-60-8 RTECS AE1225000	C ₁₄ H ₂₀ ClNO ₂	269.77	Colorless crystals; d 1.133 g/mL @ 25°C; MP 39.5-41.5°C; VP 0.0029 Pa (2.2 x 10 ⁻⁵ mm Hg) @ 25°C	140 @ 23°C	1200	
Atrazine 6-Chloro-N-ethyl-N'-isopropyl-1,3,5-triazine-2,4-diamine CAS # 1912-24-9 RTECS XY5600000	C ₈ H ₁₄ ClN ₅	215.68	Colorless crystals; MP 173-175°C; VP 4 x 10 ⁻⁵ Pa (3.0 x 10 ⁻⁷ mm Hg) @ 20°C	70 @ 25°C	1780	NIOSH 5 ACGIH 5
Cyanazine 2[[4-chloro-6-(ethylamino)-1,3,5-triazin-2-yl]amino]-2-methylpropionitrile CAS #21725-46-2 RTECS UG1490000	C ₉ H ₁₃ ClN ₆	240.69	White crystalline solid; MP 167.5-169°C; VP 2.1 x 10 ⁻⁷ Pa (1.6 x 10 ⁻⁹ mm Hg) @ 20°C	171 @ 25°C	182	
2,4-D acid 2,4-Dichlorophenoxyacetic acid CAS #94-75-7 RTECS AG6825000	C ₈ H ₆ Cl ₂ O ₃	221.04	White powder; MP 140.5°C; <10 ⁻⁵ Pa (<7.5 x 10 ⁻⁸ mm Hg) @ 25°C	almost insoluble	375	NIOSH 10 ACGIH 10 OSHA 10
2,4-D, ME 2,4-Dichlorophenoxyacetic acid, methyl ester CAS #1928-38-7	C ₉ H ₈ Cl ₂ O ₃	235.07				
2,4-D, BE 2,4-Dichlorophenoxyacetic acid, 2-butoxyethyl ester CAS #1929-73-3 RTECS AG7700000	C ₁₄ H ₁₈ Cl ₂ O ₄	321.20			150	
2,4-D, EH 2,4,2,4-Dichlorophenoxyacetic acid, 2-ethylhexylester CAS #1928-43-4	C ₁₆ H ₂₂ Cl ₂ O ₃	333.25			300 - 1000	
Metolachlor 2-Chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide CAS #51218-45-2 RTECS AN3430000	C ₁₅ H ₂₂ Cl ₂ O ₃	283.80	Odorless tan liquid; 0.0017 Pa (1.3 x 10 ⁻⁵ mm Hg) @ 20°C	530 @ 20°C	2780	
Simazine 6-Chloro-N,N'-diethyl-1,3,5-triazine-2,4-diamine CAS #122-34-9 RTECS XY5250000	C ₇ H ₁₂ ClN ₅	201.66	Crystals; MP 225 -227°C; 8.1 x 10 ⁻⁷ Pa (6.1 x 10 ⁻⁷ mm Hg) @ 20°C	3.5 @ 20°C	5000	

TABLE 2. USEFUL GAS CHROMATOGRAPHIC COLUMNS AND CONDITIONS⁽¹⁾

Parameter	Conditions								
Column Parameters:									
Stationary phase ⁽²⁾	DB-1	DB-5	DB-5ms	DB-17 ⁽³⁾	DB-1701 ⁽⁴⁾	DB-210 ⁽⁴⁾	DB-225 ⁽⁴⁾	DB-WAX	
Length (meters)	30	30	30	30	30	30	30	30	
I.D. (millimeters)	0.25	0.32	0.32	0.25	0.53	0.32	0.32	0.32	
Film thickness (μm)	0.25	0.50	1.00	0.25	1.00	0.25	0.25	0.50	
Oven Temperatures:									
Initial temperature (°C)	120	50	90	90	90	140	140	160	
Initial temperature hold time (min.)	0	1	1	1	0.5	0	0	0	
First temperature ramp (°C/min.)	5	10	35	35	15	3	5	5	
First intermediate temperature (°C)			160	160	180				
Second temperature ramp (°C/min.)			5	5	2				
Second intermediate temperature (°C)			200	200	210				
Third temperature ramp (°C/min.)			3	3	10				
Final temperature (°C)	250	290	230	230	235	215	220	250	
Final temperature hold time (min.)	4	5	9	9	10	5	15	20	
Mobile Phase and Injection conditions:									
Carrier Gas	Helium	Helium	Helium	Helium	Helium	Helium	Helium	Helium	
Head pressure (p.s.i.)	10	10	12	12	3.5	10	10	10	
Injection volume (μL)	2-4	2-4	2-4	2-4	2	2-4	2-4	2-4	
Injection mode	splitless	splitless	splitless	splitless	splitless	splitless	splitless	splitless	

(1) Actual column and conditions may vary depending on analyte, interferences, and analytical objectives. The conditions given correspond to Table 4.

(2) Other types of fused silica capillary columns also may work well.

(3) Column and conditions used for method evaluation. Good column for separation of atrazine and simazine.

(4) Useful columns for separating cyanazine from other listed analytes.

TABLE 3. APPLICABLE WORKING RANGE AND ESTIMATED LOD

Compound	Applicable working range		LOD ⁽¹⁾		$\bar{S}_{r1}$	Storage Stability		
	(µg/mL)	(µg/sample)	(µg/mL)	(µg/sample)		Day 1 ⁽²⁾	Day 30 ⁽³⁾	Day 120 ⁽³⁾
						% Recovery	% Recovery	% Recovery
1 Alachlor	0.0075 - 0.25	0.30 - 10	0.0025	0.1	0.0247	90.8	103.9	97.5
2 Atrazine	0.0525 - 50	2.10 - 200	0.0175	0.7	0.0267	101.8	92.5	95.6
3 Cyanazine	0.0098 - 0.25	0.39 - 10	0.0025	0.1	0.0789	112.0	97.1	101
4 2,4-D acid	0.0075 - 0.25	0.30 - 10	0.0025	0.1	0.0453	75.1	97.3	79.5
5 2,4-D, BE	0.035 - 0.25	1.40 - 10	0.0025	0.1	0.0424	88.0	113.8	97.6
6 2,4-D, EH	0.0085 - 0.25	0.34 - 10	0.0025	0.1	0.0354	92.8	104.2	95.4
7 Metolachlor	0.0075 - 0.25	0.30 - 10	0.0025	0.1	0.0254	107.8	95.6	86.1
8 Simazine	0.0525 - 50	0.33 - 200	0.0175	0.7	0.0189	100.3	93.9	95.7

(1) Limit of Detection

(2) Based on mass of analyte spiked

(3) Based on Day 1 results

TABLE 4. APPROXIMATE RETENTION TIMES OF SELECTED CHLORINATE COMPOUNDS⁽¹⁾

Compound (by retention time on)		Retention Times in Minutes (Capillary Column by Approximate Increase in Polarity)							
	Capillary Column:	DB-1	DB-5	DB-5ms	DB-17	DB-1701	DB-210	DB-225	DB-WAX
1	CDA		14.37						
2	2,4-D, ME⁽²⁾			10.13	10.25	12.25			
3	Dicamba, ME⁽²⁾		16.72						
4	2,4-D, IPE⁽³⁾		19.20						
5	Simazine	12.90	19.42	12.02	12.91	16.52	7.59	16.90	18.62
6	Atrazine	12.96	19.50	12.18	12.59	16.34	7.79	15.93	17.17
7	Propazine		19.61						
8	2,4-DB, ME⁽²⁾				14.03				
9	Metribuzin	13.89	21.10		17.51		9.72	22.01	23.08
10	Dimethenamid		21.13						
11	Acetochlor		21.18		14.66				
12	Alachlor	14.37	21.44	15.24	15.19	19.78	12.95	17.45	14.95
13	Cyanazine	14.97	22.23	17.17	19.99	27.07	19.67	30.00	36.00
14	Metolachlor	15.11	22.26	16.96	16.67	22.17	14.85	19.43	15.96
15	Pendimethalin		22.98		18.67				
16	2,4-D, BE⁽⁴⁾	17.01	23.73	21.46	20.60	26.25	16.79	25.86	20.50
17	2,4-D, EH⁽⁵⁾	17.70	24.38	22.73	20.12	26.71	17.17	23.49	18.55

(1) Actual retention times will vary with individual columns and chromatographic conditions.

Column conditions are given in Table 2. Data is from Backup Data Report [1].

(2) ME = Methyl ester. Methyl ester formed by reaction of the free acid form with diazomethane.

(3) IPE = Isopropyl ester.

(4) BE = 2-Butoxyethyl ester.

(5) EH = 2-Ethylhexyl ester.

TABLE 5. NOTES ON ANALYTICAL CHARACTERISTICS OF CHLORINATED AND ORGANONITROGEN HERBICIDES

	Compound (alphabetically)	A. CHEMICAL AND PHYSICAL	B. SAMPLE PREPARATION	C. GAS CHROMATOGRAPHIC
1	Alachlor		3	1
2	Atrazine		3	2,3
3	Cyanazine		1,3	2,4
4	2,4-D, acid		2 (methyl ester)	
5	2,4-D, BE	1	1,2	5
6	2,4-D, EH	1	2	
7	Metolachlor		3	1
8	Simazine		3	2,3

A. CHEMICAL AND PHYSICAL

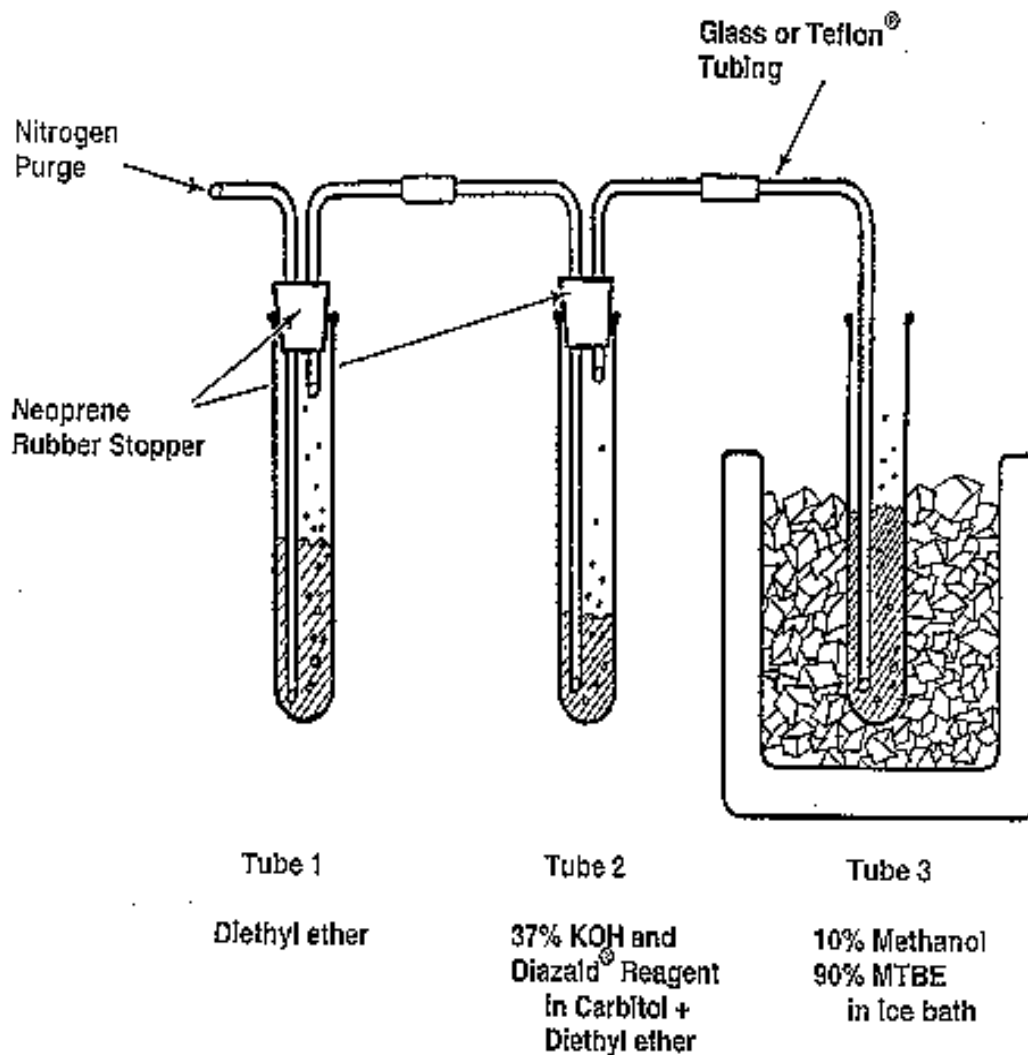
1. Esters may hydrolyze to the free acid. Free acid may also be present in formulations.

B. SAMPLE PREPARATION

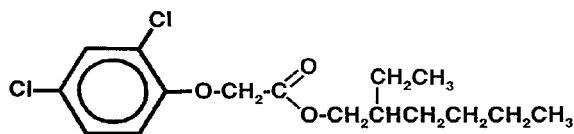
1. Generally high recoveries from PUF patches compared to liquid standards for reasons unknown.
2. The esters are not affected by the diazomethane reagent provided the solutions are quenched within one hour with silicic acid. Otherwise, recoveries for all esters will diminish. This makes possible the speciation of 2,4-D esters and of the free acid in one analysis.
3. Analytes were not affected by the diazomethane reagent.

C. GAS CHROMATOGRAPHIC

1. Very good peak shape.
2. Analyte has tendency to tail on most column phases. Columns and injection ports must be clean and in good condition.
3. The s-triazines, simazine, atrazine, and propazine, elute very closely in that order on the non-polar columns DB1 and DB-5. The order is reversed on most polar columns.
4. Cyanazine is very polar and tends to tail and to elute very late on highly polar columns. It has extremely unpredictable behavior. Peak areas either diminish or increase noticeably in subsequent injections. This behavior appears to be associated with the cyano group.
5. 2,4-D BE behaves similarly to cyanazine chromatographically (see C.4.), although to a much lesser extent.



4. 2,4-D, 2-BUTOXYETHYL ESTER



5. 2,4-D, 2-ETHYLHEXYL ESTER

STRUCTURES OF CHLORINATED ORGANONITROGEN and ACID HERBICIDES

Figure 1.

Figure 2. Diazomethane Generator