

CHLORINATED AND ORGANONITROGEN HERBICIDES (HAND WASH) 9200

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

METHOD: 9200, 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	Simazine 2,4-D acid	2,4-D, 2-ethylhexyl ester 2,4-D, 2-butoxyethyl ester	
SAMPLING			MEASUREMENT		
SAMPLER:	POLYETHYLENE BAG 12" x 18", polyethylene bag containing 150 mL isopropanol		TECHNIQUE:	GAS CHROMATOGRAPHY/ ELECTRON CAPTURE (GC/ECD)	
SHIPMENT:	transfer wash to 250-mL wide-mouthed glass jars with PTFE-lined caps.		ANALYTE:	listed above or Table 1	
SAMPLE STABILITY:	at least 30 days at 5 °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; then ramp to 230 °C at 3 °C/min; hold for 9 min.	
			CARRIER GAS:	He at 1 mL/min	
ACCURACY			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	
RANGE STUDIED:	not studied		CALIBRATION:	standard solutions of herbicides in isopropanol	
ACCURACY:	not determined		RANGE:	Table 3	
BIAS:	not determined		ESTIMATED LOD:	Table 3	
OVERALL PRECISION ($\hat{S}_{r,r}$):	not determined		PRECISION ($\hat{S}_{r,r}$):	Table 3	

APPLICABILITY: The working ranges are listed in Table 3. These cover from the LOQ to approximately 30 x LOQ. This method may be applicable to the determination of many other thermally stable organonitrogen, aryl and alkyl acidic, and phenolic pesticides after evaluation for sample stability and precision.

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 organics, organophosphorous compounds, and other pesticides. Agricultural spray additives can contribute 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.

OTHER METHODS: Other hand wash methods allowing simultaneous determination 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.
4. Diazomethane* derivatizing reagent. (See APPENDIX)
5. Diazald® (N-methyl-N-nitroso-p-toluene-sulfonamide).
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. Bags, polyethylene, 12 in x 8", 4-mil wall thickness. (Scienceware®, or equivalent).
NOTE: Test bags from each lot and supplier for interfering contaminants before use.
2. Gas chromatograph, electron-capture detector, integrator, and column (page 9200-1 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, 250-mL, PTFE-lined caps.
8. Vials, 2-mL, GC autosampler vials with PTFE-lined crimp caps.
9. Test tubes, glass, screw-top, with PTFE seals, 16 x 125 mm, 16-mL volume.

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 alkylnitrosoguanidines. Avoid skin contact with Diazald® and herbicides. Avoid skin contact and open flame with solvents. Wear appropriate protective clothing and work with these compounds in a well ventilated hood.

SAMPLING:

1. Pour 150 mL of isopropanol into the polyethylene bag.
2. Insert hand into bag. Wrap the top of the bag securely around the forearm, several inches above the wrist.
NOTE: Allergies to alcohols are extremely rare. Before conducting the handwash, check to see if the person has ever had a reaction to alcohols, or is taking medications that could react with the alcohol. Some skin conditions, such as cuts, abrasions, and eczema can be irritated by contact with alcohols. If the person's hand seems "dried-out" after the handwash, hand lotion can be applied.
3. Shake hand in alcohol with constant back and forth motion for 30 seconds. Remove hand from bag and dry with paper towel.
4. Transfer solution to glass jar, cap securely.
5. Label and pack for shipment.
6. For field blank, add 150 mL isopropanol to a clean bag, shake for 30 seconds, then transfer the solvent to a glass jar for shipment.

SAMPLE PREPARATION:

7. Pipette 10 mL of sample into a screw-top test tube with a PTFE-lined cap.
8. Add 0.5 mL of diazomethane derivatizing reagent. Mix and allow to stand for one hour.
9. Add approximately 10 mg of silicic acid to the solution. Mix and allow to stand an additional hour.

10. Filter an aliquot through a 0.45- μm 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:

11. Calibrate daily with at least six working standards covering the analytical range of the method for individual analytes. Three standards (in duplicate) should cover the range from LOD to LOQ.
 - a. Add known amounts of calibration stock solution to isopropanol in a 10-mL volumetric flask. Add 0.5 mL of the diazomethane derivatizing reagent. Let stand for 1 hour.
 - b. Add 10 mg silicic acid to each standard vial and let stand for another hour.
 - c. Filter through a 0.45- μm filter into a GC vial.
 - d. Include a calibration blank of unspiked diazomethane derivatizing reagent solution.
 - e. Analyze together with field samples and blanks (steps 13 and 14).
 - f. Prepare calibration graph (peak height or area vs. μg analyte).
12. Analyze three quality control blind spikes and three analyst spikes to ensure that the calibration graphs are in control.

MEASUREMENT:

13. Set gas chromatograph according to manufacturer's recommendations and to conditions listed in Table 3. Inject 2- μL 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 with isopropanol and reanalyze. Apply the appropriate dilution factor in calculations.

14. Measure peak height of analyte.

CALCULATIONS:

15. Determine the concentration, C ($\mu\text{g}/\text{mL}$), of the herbicide found in the sample from the calibration graph.
16. Calculate the mass of herbicide, M (μg), found in the volume, V (mL), of hand wash solution.

$$M = C \cdot V, \mu\text{g}$$

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 reanalysis 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 $\mu\text{g}/\text{mL}$ or greater), GC/MS may be used. Table 5 provides 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. These ranges represent 3 x LOQ to 30 x LOQ for each of the compounds. The analytical conditions used in the evaluation are listed in Table 2 for the DB-5ms column. Humidity did not seem to have an effect on the analyte recoveries from the samplers. Measurement precision (\bar{S}_r), LOD, LOQ, and storage stability data are also presented in Table 3. The LOD and LOQ were determined by taking a series of liquid standards, prepared in triplicate, analyzed, and the data fitted to a quadratic curve. The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were estimated with Burkart's Method [3]. A long-term storage study was done at the 10 x LOQ level. Handwash solutions were stored at $4 \pm 2^\circ\text{C}$ for 30, 60, or 120 days in glass containers sealed with caps having PTFE-faced liners. The stored samples were analyzed on the DB-17 column using the conditions listed in Table 2. The results are summarized in Table 3. The 30-Day storage samples had acceptable recoveries of nearly 100%, with the extremes being simazine and alachlor having recoveries of 127.7% and 87.1%, respectively. Day

60 and Day 120 storage samples had significantly lower recoveries.

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 DERIVATIZING REAGENT [2]

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 "Diaza[®] 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 by a flow of nitrogen gas into a flask of chilled (0 °C) isopropanol (maximum volume, 500 mL).

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

Name / Synonym	Empirical Formula	Molecular Weight	Physical Properties	Solubility in Water (mg/L)	LD50 mg/kg	TWA (mg/m ³)
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, 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 ClNO ₂	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	30
I.D. (millimeters)	0.25	0.32	0.32	0.25	0.53	0.32	0.32	0.32	0.32
Film thickness (µm)	0.25	0.50	1.00	0.25	1.00	0.25	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	Helium
Head pressure (p.s.i.)	10	10	12	12	3.5	10	10	10	10
Injection volume (µL)	2-4	2-4	2-4	2-4	2	2-4	2-4	2-4	2-4
Injection mode	splitless	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 above correspond to Table 4.
- (2) Other types of fused silica capillary columns may also 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. METHOD EVALUATION

Compound	Applicable working range			Storage Stability							
	(µg/mL)	(µg/sample)	\bar{S}_r	LOD ⁽¹⁾ (µg/mL)	LOQ ⁽²⁾ (µg/mL)	30 Days		60 days		120 days	
						%Recovery	S_r	%Recovery	S_r	%Recovery	S_r
Alachlor	0.0015 - 0.2	0.22 - 30	0.028	0.001	0.004	103.0	0.027	87.1	0.149	95.4	0.137
Atrazine	0.088 - 1.1	13 - 160	0.024	0.010	0.037	100.9	0.041	86.0	0.123	89.4	0.075
Cyanazine	0.0099 - 0.2	1.5 - 30	0.094	0.002	0.006	105.3	0.120	88.1	0.197	87.7	0.167
2,4-D acid	0.0017 - 0.11	0.26 - 16	0.035	0.001	0.004	101.9	0.019	108.4	0.169	105.5	0.111
2,4-D, BE	0.0019 - 0.12	0.28 - 18	0.084	0.001	0.004	103.5	0.071	79.3	0.175	76.5	0.159
2,4-D, EH	0.0075 - 0.05	1.1 - 7.5	0.040	0.0005	0.002	104.4	0.069	85.6	0.135	81.7	0.108
Metolachlor	0.0039 - 0.22	0.58 - 33	0.030	0.002	0.007	102.8	0.025	87.1	0.145	89.8	0.103
Simazine	0.082 - 1.1	12 - 160	0.031	0.010	0.037	127.7	0.045	83.4	0.140	91.4	0.2

(1) Limit of Detection

(2) Limit of Quantitation

TABLE 4. APPROXIMATE RETENTION TIMES OF SELECTED CHLORINATED AND ORGANONITROGEN COMPOUNDS⁽¹⁾

	Compound (by retention time on Capillary Column:	Retention Times in Minutes (Capillary Column by Approximate Increase in Polarity)							
		DB-1	DB-5	DB-5ms	DB-17	DB-1701	DB-210	DB-225	DB-WAX
1	CDAA		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 give 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		3	2,4
4	2,4-D, acid		1	
5	2,4-D, BE	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.

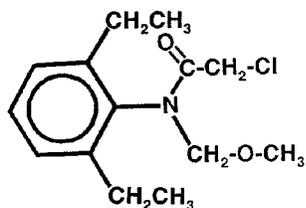
B. SAMPLE PREPARATION

1. Methylation via diazomethane is possible in isopropanol having up to 1% water present. The isopropanol must be anhydrous, and at least 99% pure.
2. The esters are not affected by the diazomethane reagent provided the solutions are quenched within one hour with silicic acid. Recoveries for all esters diminish otherwise. 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.

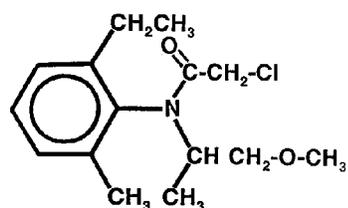
C. GAS CHROMATOGRAPHIC

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 close in that order on the non-polar columns DB-1 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 very polar columns. It has very unpredictable behavior and 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.), though to a much lesser extent.

ACETANILIDES:

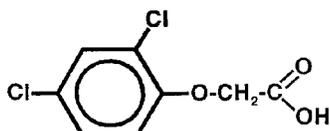


1. ALACHLOR

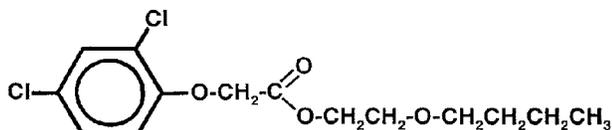


2. METOLACHLOR

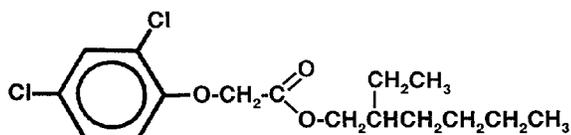
PHENOXYALKANOIC ACID AND ESTERS:



3. 2,4-D, ACID

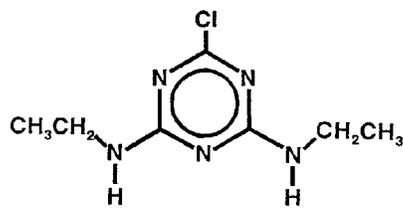


4. 2,4-D, 2-BUTOXYETHYL ESTER

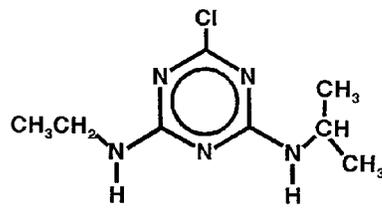


5. 2,4-D, 2-ETHYLHEXYL ESTER

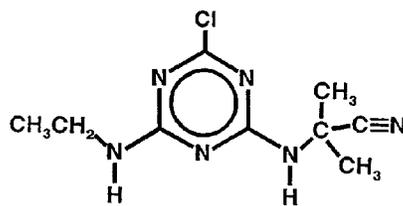
s-TRIAZINES:



6. SIMAZINE



7. ATRAZINE



8. CYANAZINE

STRUCTURES OF CHLORINATED ORGANONITROGEN and ACID HERBICIDES

Figure 1.

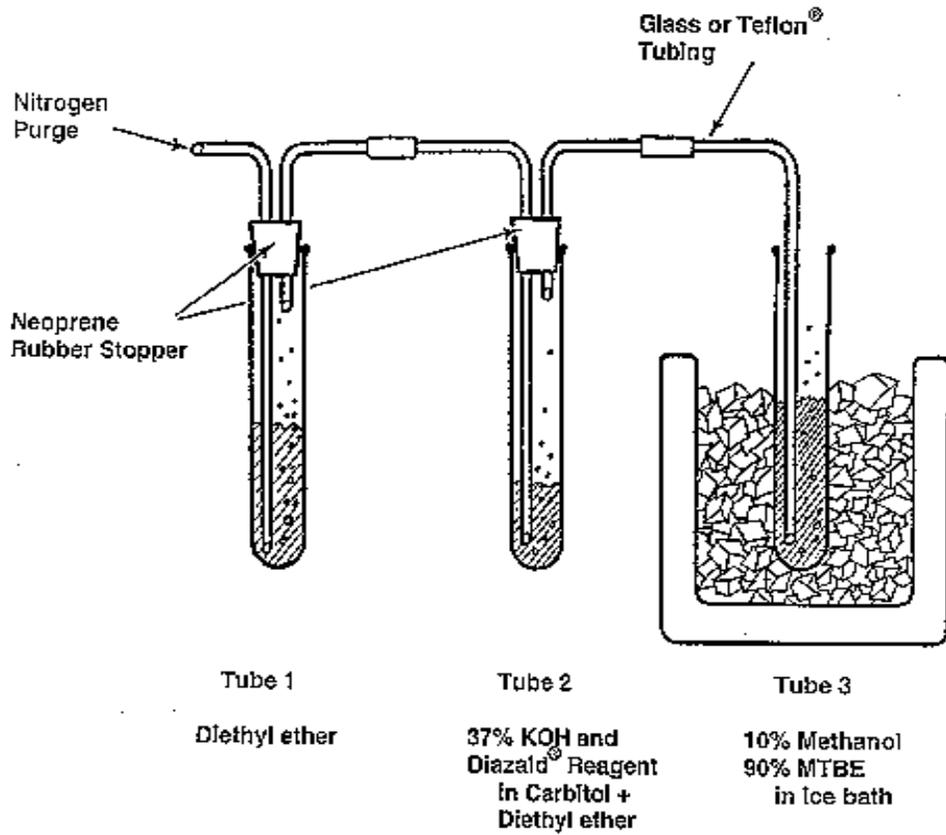


Figure 2. Diazomethane generator