# METHOD 515.2

# DETERMINATION OF CHLORINATED ACIDS IN WATER USING LIQUID-SOLID EXTRACTION AND GAS CHROMATOGRAPHY WITH AN ELECTRON CAPTURE DETECTOR

**Revision 1.1**

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R.C. Dressman and J.J. Lichtenberg - EPA 600/4-81-053, Revision 1.0 (1981)

J.W. Hodgeson - Method 515, Revision 2.0 (1986)

T. Engel (Battelle Columbus Laboratories) - National Pesticide Survey Method 3, Revision 3.0 (1987)

R.L. Graves - Method 515.1, Revision 4.0 (1989)

J.W. Hodgeson - Method 515.2, Revision 1.0 (1992)

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WITH AN ELECTRON CAPTURE DETECTOR**

## 1.0    SCOPE AND APPLICATION

1.1    This is a gas chromatographic (GC) method applicable to the determination of certain chlorinated acids in ground water and finished drinking water.  The following compounds can be determined by this method:

| Analyte | Chemical Abstract Services Registry Number |
|---|---|
| Acifluorfen | 50594-66-6 |
| Bentazon | 25057-89-0 |
| 2,4-D | 94-75-7 |
| 2,4-DB | 94-82-6 |
| Dacthal Acid Metabolites[a] | |
| Dicamba | 1918-00-9 |
| 3,5-Dichlorobenzoic Acid | 51-36-5 |
| Dichlorprop | 120-36-5 |
| Dinoseb | 88-85-7 |
| 5-Hydroxydicamba | 7600-50-2 |
| Pentachlorophenol (PCP) | 87-86-5 |
| Picloram | 1918-02-1 |
| 2,4,5-T | 93-76-5 |
| 2,4,5-TP (Silvex) | 93-72-1 |

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

1.2    This method is applicable to the determination of salts and esters of analyte acids.  The form of each acid is not distinguished by this method.  Results are calculated and reported for each listed analyte as the total free acid.

1.3    Single laboratory accuracy and precision data and method detection limits (MDLs) have been determined for the analytes above (Section 13.0).  Observed detection limits may vary among water matrices, depending upon the nature of interferences in the sample matrix and the specific instrumentation used.

1.4    This method is restricted to use by or under the supervision of analysts experienced in the use of GC and in the interpretation of gas chromatograms.  Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 9.3.

1.5     Analytes that are not separated chromatographically, (i.e., have very similar retention times) cannot be individually identified and measured in the same calibration mixture or water sample unless an alternative technique for identification and quantitation exists (Section 11.7).

1.6     When this method is used to analyze unfamiliar samples for any or all of the analytes above, analyte identifications should be confirmed by analysis on a second gas chromatographic column or by gas chromatography/mass spectrometry (GC/MS).

## 2.0     SUMMARY OF METHOD

2.1     A 250 mL measured volume of sample is adjusted to pH 12 with 6 N sodium hydroxide for one hour to hydrolyze derivatives.

**Note:**  Since many of the analytes contained in this method are applied as a variety of esters and salts, it is vital to hydrolyze them to the parent acid prior to extraction.

Extraneous organic material is removed by a solvent wash.  The sample is acidified, and the chlorinated acids are extracted with a 47 mm resin based extraction disk.  The acids are converted to their methyl esters using diazomethane or alternatively, trimethylsilyldiazomethane (TMSD).  Excess derivatizing reagent is removed, and the esters are determined by capillary column GC using an electron capture detector (ECD).  Analytes are quantitated using procedural standard calibration (Section 3.14).

## 3.0     DEFINITIONS

3.1     Internal Standard (IS) -- A pure analyte(s) added to a sample, extract, or standard solution in known amount(s), and used to measure the relative responses of other method analytes and surrogates that are components of the same sample or solution.  The IS must be an analyte that is not a sample component.

3.2     Surrogate Analyte (SA) -- A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction or other processing, and is measured with the same procedures used to measure other sample components.  The purpose of the SA is to monitor method performance with each sample.

3.3     Laboratory Duplicates (LD1 and LD2) -- Two aliquots of the same sample taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4     Field Duplicates (FD1 and FD2) -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures.  Analyses of FD1 and FD2 give a

measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.5     Laboratory Reagent Blank (LRB) -- An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples.  The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.6     Field Reagent Blank (FRB) -- An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation and all analytical procedures.  The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.7     Instrument Performance Check Solution (IPC) -- A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.8     Laboratory Fortified Blank (LFB) -- An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory.  The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.9     Laboratory Fortified Sample Matrix (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory.  The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot, and the measured values in the LFM corrected for background concentrations.

3.10    Stock Standard Solution (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using  assayed reference materials or purchased from a reputable commercial source.

3.11    Primary Dilution Standard Solution (PDS) -- A solution of several analytes prepared in the laboratory from stock standard solutions, and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.12    Calibration Standard (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes.  The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.13 Quality Control Sample (QCS) -- A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.14 Procedural Standard Calibration -- A calibration method where aqueous calibration standards are prepared and processed (e.g., purged,extracted, and/or derivatized) in <u>exactly</u> the same manner as a sample. All steps in the process from addition of sampling preservatives through instrumental analyses are included in the calibration. Using procedural standard calibration compensates for any inefficiencies in the processing procedure.

## 4.0  INTERFERENCES

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under analytical conditions by analyzing laboratory reagent blanks as described in Section 9.2.

   4.1.1 Glassware must be scrupulously cleaned.[1] Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with dilute acid, tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for one hour. Do not heat volumetric glassware. Thermally stable materials such as PCBs might not be eliminated by this treatment. Thorough rinsing with acetone may be substituted for the heating. After glassware is dry and cool, store it in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

   4.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

   **Warning:** When a solvent is purified, stabilizers and preservatives added by the manufacturer are removed, thus potentially making the solvent hazardous and reducing the shelf life.

4.2 The acid forms of the analytes are strong organic acids which react readily with alkaline substances and can be lost during sample preparation. Glassware and glass wool must be acid-rinsed with 1 N hydrochloric acid and the sodium sulfate must be acidified with sulfuric acid prior to use to avoid analyte losses due to adsorption.

4.3 Organic acids and phenols, especially chlorinated compounds, cause the most direct interference with the determination. Alkaline hydrolysis and subsequent

extraction of the basic sample removes many chlorinated hydrocarbons and phthalate esters that might otherwise interfere with the electron capture analysis.

4.4    Interferences by phthalate esters can pose a major problem in pesticide analysis when using the ECD. Phthalates generally appear in the chromatogram as large peaks. Common flexible plastics contain varying amounts of phthalates, that are easily extracted or leached during laboratory operations. Cross-contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Interferences from phthalates can best be minimized by avoiding the use of plastics in the laboratory. Exhaustive purification of reagents and glassware may be required to eliminate background phthalate contamination.[2,3]

4.5    Interfering contamination may occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. Between-sample rinsing of the sample syringe and associated equipment with methyl-tert-butyl-ether (MTBE) can minimize sample cross- contamination. After analysis of a sample containing high concentrations of analytes, one or more injections of MTBE should be made to ensure that accurate values are obtained for the next sample.

4.6    Matrix interferences may be caused by contaminants that are coextracted from the sample. Also, note that all analytes listed in the Scope and Application Section are not resolved from each other on any one column, i.e., one analyte of interest may interfere with another analyte of interest. The extent of matrix interferences will vary considerably from source to source, depending upon the water sampled. The procedures in Section 11.0 can be used to overcome many of these interferences. Analyte identifications should be confirmed (Section 11.7).

4.7    Gas chromatographic background is significantly increased when TMSD is used as the derivatizing reagent instead of the generated diazo-methane. Although no method analyte is affected by this increased background, the recommended surrogate, 2,4-dichloro-phenylacetic acid, is masked by an interfering peak. This renders the surrogate useless at 1 μg/L or lower. Any compound found suitable when TMSD is used is acceptable as a surrogate.

4.8    It is important that samples and working standards be contained in the same solvent. The solvent for working standards must be the same as the final solvent used in sample preparation. If this is not the case, chromatographic comparability of standards to sample extracts may be affected.

## 5.0    SAFETY

5.1    The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. Accordingly, exposure to these chemicals must be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe

handling of the chemicals specified in this method.  A reference file of material safety data sheets should also be made available to all personnel involved in the chemical analysis.  Additional references to laboratory safety are available and have been identified[5-7] for the information of the analyst.

5.2     Diazomethane -- A toxic carcinogen which can explode under certain conditions. The following precautions must be followed:

5.2.1     Use the diazomethane generator behind a safety shield in a well ventilated fume hood.  Under no circumstances can the generator be heated above 90°C, and all grinding surfaces such as ground glass joints, sleeve bearings, and glass stirrers must be avoided.  Diazomethane solutions must not be stored.  Only generate enough for the immediate needs.  The diazomethane generator apparatus used in the esterification procedure (Section 11.4) produces micromolar amounts of diazomethane in solution to minimize safety hazards.  If the procedure is followed exactly, no possibility for explosion exists.

5.3     Methyl-Tert-Butyl Ether -- Nanograde, redistilled in glass, if necessary.  Must be free of peroxides as indicated by EM Quant test strips (available from Scientific Products Co., Cat. No. Pl126-8, and other suppliers).

**Warning:**  When a solvent is purified, stabilizers added by the manufacturer are removed, thus potentially making the solvent hazardous.

**6.0     EQUIPMENT AND SUPPLIES** (All specifications are suggested.  Catalog numbers  are included for illustration only.)

6.1     Kontes Filter Funnels -- Fisher Cat. No. 953755-0000 or equivalent.

6.2     Vacuum Flasks -- 1000 mL with glass side arm

6.3     Vacuum Manifold -- The manifold should be capable of holding six to eight filter flasks in series with house vacuum.  Commercial manifolds are available from a number of suppliers, e.g., Baker, Fisher, and Varian.

6.4     Culture Tubes (25 x 200 mm) with Teflon-lined Screw Caps -- Fisher Cat. No. 14-933-1C, or equivalent.

6.5     Pasteur Pipets -- Glass disposable (5 mL)

6.6     Large Volume Pipets -- Disposable, Fisher Cat. No. 13-678-8 or equivalent.

6.7     Balance -- Analytical, capable of weighing to .0001 g.

6.8     pH Meter -- Wide range capable of accurate measurements in the pH = 1-12 range.

6.9     Diazomethane Generator -- See Figure 1 for a diagram of an all glass system custom made for these validation studies. A micromolar generator is also available from Aldrich Chemical.

6.10    Analytical Concentrator -- Six or 12 positions, Organomation N-EVAP Model No. 111-6917 or equivalent.

6.11    Gas Chromatography -- Analytical system complete with gas chromatograph equipped with ECD, split/splitless capillary injector, temperature programming, differential flow control and all required accessories. A data system is recommended for measuring peak areas. An autoinjector is recommended to improve precision of analysis.

6.12    GC Columns and Recommended Operating Conditions

        6.12.1  Primary -- DB-5 or equivalent, 30 m x .32 mm ID, 0.25 μm film thickness. Injector Temp. = 200°C, Detector Temp. = 280°C, Helium linear velocity is 30 cm/sec. at 200°C and 10 psi, 2 μL splitless injection with purge on three minutes. Program: Hold at 60°C one minute, increase to 260°C at 5°C/min. and hold five minutes.

        6.12.2  Confirmation -- DB-1701 or equivalent, 30 m x .32 mm ID, 0.25 μm film thickness. Injector Temp. = 200 °C, Detector Temp. = 280°C, Helium linear velocity is 30 cm/sec. at 200°C and 10 psi, 2 μL splitless injection with purge on three minutes. Program: Hold at 60°C one minute, increase to 260°C at 5°C/min. and hold five minutes.

6.13    Glass Wool -- Acid washed with 1N HCl and heated at 450°C for four hours.

6.14    Short Range pH Paper (pH=0-3).

6.15    Volumetric Flasks -- 50 mL, 100 mL, and 250 mL.

6.16    Micro-Syringes -- 25 μL, 50 μL, 100 μL, 250 μL, and 500 μL.

6.17    Amber Bottles -- 15 mL, with Teflon-lined screw caps.

6.18    Graduated Cylinder -- 250 mL.

6.19    Separatory Funnel -- 500 mL.

6.20    Graduated Centrifuge Tubes -- 15 mL or 10 mL Kuderna Danish Concentrator tubes.

## 7.0     REAGENTS AND STANDARDS

7.1     Extraction Disks, 47 mm -- Resin based polystyrenedivinylbenzene.

7.2     Reagent Water -- Reagent water is defined as a water in which an interference is not observed at the MDL of each analyte of interest.

   7.2.1   A Millipore Super-Q water system or its equivalent may be used to generate deionized reagent water. Distilled water that has been passed through granular charcoal may also be suitable.

   7.2.2   Test reagent water each day it is used by analyzing according to Section 11.0.

7.3     Methanol -- Pesticide quality or equivalent.

7.4     Methyl-Tert-Butyl Ether (MTBE) -- Nanograde, redistilled in glass if necessary. Ether must be demonstrated to be free of peroxides. One test kit (EM Quant Test Strips), is available from EM Science, Gibbstown, NJ. Procedures for removing peroxides from the ether are provided with the test strips. Ethers must be periodically tested (at least monthly) for peroxide formation during use. Any reliable test kit may be used.

7.5     Sodium Sulfate, ACS Granular, Anhydrous -- Heat in a shallow tray at 400°C for a minimum of four hours to remove phthalates and other interfering organic substances. Alternatively, extract with methylene chloride in a Soxhlet apparatus for 48 hours. After cleaning, store in a glass (not plastic) bottle.

   7.5.1   Sodium sulfate drying tubes -- Plug the bottom of a large volume disposable pipet with a minimum amount of acidified glass wool (Supelco Cat. No. 20383 or equivalent). Fill the pipet halfway (3 g) with acidified sodium sulfate (See Section 7.9).

7.6     Sulfuric Acid, Reagent Grade.

   7.6.1   Sulfuric acid, 12 N -- Slowly add 335 mL concentrated sulfuric acid to 665 mL of reagent water.

7.7     Sodium Hydroxide, ACS Reagent Grade or Equivalent.

   7.7.1   Sodium hydroxide 1 N -- Dissolve 4.0 g reagent grade sodium hydroxide in reagent water and dilute to 100 mL in volumetric flasks.

   7.7.2   Sodium hydroxide 6 N.

7.8     Ethyl Ether, Unpreserved -- Nanograde, redistilled in glass if necessary. Must be free of peroxides as indicated by EM Quant test strips (available from Scientific Products Co., Cat. No. PI126-8, and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips.

7.9     Acidified Sodium Sulfate -- Cover 500 g sodium sulfate (Section 7.5) with ethyl ether (Section 7.8). While agitating vigorously, add dropwise approximately

0.7 mL concentrated sulfuric acid. Remove the ethyl ether overnight under vacuum and store the sodium sulfate in a 100°C oven.

7.10 Carbitol, ACS Grade -- Available from Aldrich Chemical.

7.11 Diazald, ACS Grade -- Available from Aldrich Chemical.

7.12 Diazald Solution -- Prepare a solution containing 10 g Diazald in 100 mL of a 50:50 by volume mixture of ethyl ether and carbitol. This solution is stable for one month or longer when stored at 4°C in an amber bottle with a Teflon-lined screw cap.

7.13 Trimethylsilyldiazomethane (TMSD) -- Available from Aldrich Chemical Co. as a two-molar solution in hexane. TMSD is stable during storage in this solution.

7.14 4,4'-dibromooctafluorobiphenyl (DBOB), 99% purity -- For use as internal standard.

7.15 2,4-dichlorophenylacetic Acid (DCAA), 99% purity -- For use as surrogate standard.

7.16 Potassium Hydroxide, ACS Reagent Grade or Equivalent.

    7.16.1 Potassium hydroxide solution, 37% -- Using extreme caution, dissolve 37 g reagent grade potassium hydroxide in reagent water and dilute to 100 mL.

7.17 Stock Standard Solutions (SSS) (1.00-2.00 µg/µL) -- Stock standard solutions may be purchased as certified solutions or prepared from pure standard materials using the following procedure:

    7.17.1 Prepare stock standard solutions by accurately weighing approximately 0.0100-0.0200 g of pure material. Dissolve the material in methanol and dilute to volume in a 10 mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

    7.17.2 Transfer the stock standard solutions into 15 mL TFE-fluorocarbon-sealed screw cap amber vials. Store at 4°C or less when not in use.

    7.17.3 Stock standard solutions should be replaced after two months or sooner if comparison with laboratory fortified blanks, or QC samples indicate a problem.

    7.17.4 Primary Dilution Standards -- Prepare two sets of standards according to the sets labeled A and B in Table 1. For each set, add approximately

25 mL of methanol to a 50 mL volumetric flask. Add aliquots of each stock standard in the range of approximately 20-400 μL and dilute to volume with methanol. Individual analyte concentrations will then be in the range of 0.4-8 μg/mL (for a 1.0 mg/mL stock). The minimum concentration would be appropriate for an analyte with strong electron capture detector (ECD) response, e.g., pentachlorophenol. The maximum concentration is for an analyte with weak response, e.g., 2,4-DB. The concentrations given in Table 2 reflect the relative volumes of stock standards used for the primary dilution standards used in generating the method validation data. Use these relative values to determine the aliquot volumes of individual stock standards above.

7.18    Internal Standard Solution -- Prepare a stock internal standard solution by accurately weighing approximately 0.050 g of pure DBOB. Dissolve the DBOB in methanol and dilute to volume in a 10 mL volumetric flask. Transfer the DBOB solution to a TFE-fluorocarbonsealed screw cap bottle and store at room temperature. Prepare a primary dilution standard at approximately 1.00 μg/mL by the addition of 20 μL of the stock standard to 100 mL of methanol. Addition of 100 μL of the primary dilution standard solution to the final 5 mL of sample extract (Section 11.0) results in a final internal standard concentration of 0.020 μg/mL. Solution should be replaced when ongoing QC (Section 9.0) indicates a problem.

Note: DBOB has been shown to be an effective internal standard for the method analytes, but other compounds may be used if the QC requirements in Section 9.0 are met.

7.19    Surrogate Analyte Solution -- Prepare a surrogate analyte stock standard solution by accurately weighing approximately 0.050 g of pure DCAA. Dissolve the DCAA in methanol and dilute to volume in a 10 mL volumetric flask. Transfer the surrogate analyte solution to a TFE-fluorocarbon-sealed screw cap bottle and store at room temperature. Prepare a primary dilution standard at approximately 2.0 μg/mL by addition of 40 μL at the stock standard to 100 mL of methanol. Addition of 250 μL of the surrogate analyte solution to a 250 mL sample prior to extraction results in a surrogate concentration in the sample of 2 μg/L and, assuming quantitative recovery of DCAA, a surrogate analyte concentration in the final 5 mL extract of 0.1 μg/mL. The surrogate standard solution should be replaced when ongoing QC (Section 9.0) indicates a problem. DCAA has been shown to be an effective surrogate standard for the method analytes, but other compounds may be used if the QC requirements in Section 9.0 are met.

7.20    Instrument Performance Check Solution -- Prepare a diluted dinoseb solution by adding 10 μL of the 1.0 μg/μL dinoseb stock solution to the MTBE and diluting to volume in a 10 mL volumetric flask. To prepare the check solution, add 40 μL of the diluted dinoseb solution, 16 μL of the 4-nitrophenol stock solution, 6 μL of the 3,5-dichlorobenzoic acid stock solution, 50 μL of the surrogate standard solution, 25 μL of the internal standard solution, and 250 μL of methanol to a 5 mL volumetric flask and dilute to volume with MTBE. Methylate sample as

described in Section 11.4.  Dilute the sample to 10 mL in MTBE.  Transfer to a TFE-fluorocarbon-sealed screw cap bottle and store at room temperature. Solution should be replaced when ongoing QC (Section 9.0) indicates a problem.

## 8.0    SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1    Grab samples should be collected in 250 mL amber glass containers. Conventional sampling practices[7] should be followed; however, the bottle must not be prerinsed with sample before collection.

8.2    Sample Preservation and Storage

8.2.1    If residual chlorine is present, add 80 mg of sodium thiosulfate (or 50 mg of sodium sulfite) per liter of sample to the sample bottle prior to collecting the sample.  Demonstration data in Section 17.0 of this method was obtained using sodium thiosulfate.

8.2.2    After the sample is collected in the bottle containing the dechlorinating agent, seal the bottle and mix to dissolve the thiosulfate.

8.2.3    Add hydrochloric acid (diluted 1:1 in reagent water) to the sample at the sampling site in amounts to produce a sample pH $\leq 2$.  Short range (0-3) pH paper (Section 6.14) may be used to monitor the pH.

**Note:**  Do not attempt to mix sodium thiosulfate and HCl in the sample bottle prior to sample collection.

8.2.4    The samples must be iced or refrigerated at 4°C away from light from the time of collection until extraction.  Preservation study results indicate that the sample analytes (measured as total acid), except 5-hydroxy-dicamba, are stable in water for 14 days when stored under these conditions (Tables 8 and 9).  The concentration of 5-hydroxydicamba is seriously degraded over 14 days in a biologically active matrix.  However, analyte stability will very likely be affected by the matrix; therefore, the analyst should verify that the preservation technique is applicable to the samples under study.

8.3    Extract Storage

8.3.1    Extracts should be stored at 4°C or less away from light.  Preservation study results indicate that most analytes are stable for 14 days (Tables 8 and 9); however, the analyst should verify appropriate extract holding times applicable to the samples under study.

## 9.0    QUALITY CONTROL

9.1    Minimum QC requirements are initial demonstration of laboratory capability, determination of surrogate compound recoveries in each sample and blank,

monitoring internal standard peak area or height in each sample and blank, analysis of laboratory reagent blanks, laboratory fortified matrices, laboratory fortified blanks, and QC samples. A MDL for each analyte must also be determined.

9.2     Laboratory Reagent Blanks (LRB) -- Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is extracted or reagents are changed, a LRB must be analyzed. If within the retention time window of any analyte the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

9.3     Initial Demonstration of Capability

9.3.1   Select a representative fortified concentration (about 10-20 times MDL, or a mid-point in the calibration range (see Table 4) for each analyte. Prepare a primary dilution standard containing each analyte at 1000 times selected concentration. With a syringe, add 250 μL of the concentrate to each of four to seven 250 mL aliquots of reagent water, and analyze each aliquot according to procedures beginning in Section 11.0.

9.3.2   For each analyte the recovery value for all of these samples must fall in the range of ±40% of the fortified concentration. The RSD of the measurements must be 30% or less. For compounds failing this criteria, this procedure must be repeated using fresh samples until satisfactory performance has been demonstrated for all analytes.

9.3.3   For each analyte, determine the MDL. Prepare a minimum of seven LFBs at a low concentration. Fortification concentration in Table 2 may be used as a guide, or use calibration data obtained in Section 10.0 to estimate a concentration for each analyte that will produce a peak with a three to five times signal to noise response. Extract and analyze each replicate according to Sections 11.0 and 12.0. It is recommended that these LFBs be prepared and analyzed over a period of several days, so that day to day variations are reflected in the precision measurement. Calculate mean recovery and standard deviation for each analyte. Use the equation given in Section 13.0 to calculate the MDL.

9.3.4   The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. As laboratory personnel gain experience with this method the quality of data should improve beyond those required here.

9.4     The analyst is permitted to modify GC columns, GC conditions, concentration techniques (i.e., evaporation techniques), internal standard or surrogate compounds. Each time such method modifications are made, the analyst must repeat the procedures in Section 9.3.

9.5     Assessing Surrogate Recovery

9.5.1   When surrogate recovery from a sample or a blank is <60% or >140%, check calculations to locate possible errors, fortifying solutions for degradation, contamination, and instrument performance. If those steps do not reveal the cause of the problem, reanalyze the extract.

9.5.2   If a blank extract reanalysis fails the 60-140% recovery criteria, the problem must be identified and corrected before continuing.

9.5.3   If sample extract reanalysis meets the surrogate recovery criteria, report only data for the reanalyzed extract. If sample extract continues to fail the recovery criteria, report all data for that sample as suspect.

9.6     Assessing the Internal Standard (IS)

9.6.1   When using the IS calibration procedure, the analyst must monitor the IS response (peak area or peak height) of all samples during each analysis day. The IS response for any sample chromatogram should not deviate from the daily calibration check standard IS response by more than 30%.

9.6.2   If >30% deviation occurs with an individual extract, optimize instrument performance and inject a second aliquot of that extract.

        9.6.2.1  If the reinjected aliquot produces an acceptable internal standard response, report results for that aliquot.

        9.6.2.2  If a deviation of greater than 30% is obtained for the reinjected extract, analysis of the samples should be repeated beginning with Section 11.0, provided the sample is still available. Otherwise, report results obtained from the reinjected extract, but annotate as suspect.

9.6.3   If consecutive samples fail the IS response acceptance criteria, immediately analyze a medium calibration standard.

        9.6.3.1  If the standard provides a response within 20% of the predicted value, then follow procedures itemized in Section 9.6.2 for each sample failing the IS response criterion.

        9.6.3.2  If the check standard provides a response which deviates more than 20% of the predicted value, then the analyst must recalibrate as specified in Section 10.0.

9.7     Assessing Laboratory Performance -- Laboratory Fortified Blank (LFB)

9.7.1   The laboratory must analyze at least one LFB sample with every 20 samples or one per sample set (all samples extracted within a 24-hour

period) whichever is greater.  The concentration of each analyte in the LFB should be approximately the same as in Section 9.3.1.  Calculate percent recovery ($X_i$).  If the recovery of any analyte falls outside the control limits (See Section 9.7.2), that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.7.2    Until sufficient data become available, usually a minimum of results from 20-30 analyses, each laboratory should assess laboratory performance against the control limits in Section 9.3.2 that are derived from the data in Table 2.  When sufficient internal performance data become available, develop control limits from the mean percent recovery ($\overline{X}$) and standard deviation (S) of the percent recovery.  These data are used to establish upper and lower control limits as follows:

UPPER CONTROL LIMIT = $\overline{X}$ + 3S
LOWER CONTROL LIMIT = $\overline{X}$ - 3S

After each five to 10 new recovery measurements, new control limits should be calculated using only the most recent 20-30 data points.  These calculated control limits should <u>not</u> exceed those established in Section 9.3.2.

9.7.3    At least quarterly, analyze a QCS (Section 3.13) from an outside source.

9.8    Assessing Analyte Recovery -- Laboratory Fortified Sample Matrix (LFM)

9.8.1    Each laboratory must analyze a LFM for 10% of the samples or one sample concentration per set, whichever is greater.  The concentration should not be less then the background concentration of the sample selected for fortification.  Ideally, the concentration should be the same as that used for the laboratory fortified blank (Section 9.7).  Over time, samples from all routine sample sources should be fortified.

9.8.2    Calculate the percent recovery, P, of the concentration for each analyte, after correcting the measured concentration, X, from the fortified sample for the background concentration, b, measured in the unfortified sample.

P = 100 (X - b) / fortified concentration,

and compare these values to control limits appropriate for reagent water data collected in the same fashion.  Acceptance criteria are the same as those in Section 9.7 for LFBs.

9.8.3    If the recovery of any such analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Section 9.7), the recovery problem encountered with the fortified sample is judged to be matrix related, not system related.  The result for that

analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.9 Assessing Instrument System/instrument Performance Check (IPC) Sample -- Instrument performance should be monitored on a daily basis by analysis of the IPC sample. The IPC sample contains compounds designed to monitor instrument sensitivity, column performance (primary column) and chromato-graphic performance. IPC sample components and performance criteria are listed in Table 11. The sensitivity requirements are set based on the MDLs published in this method. If the laboratory MDLs differ from those demonstrated here, the amount of dinoseb in the IPC sample should be adjusted accordingly.

9.10 The laboratory may adopt additional QC practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements or field reagent blanks may be used to assess contamination of samples under site conditions, transportation, and storage.

## 10.0 CALIBRATION AND STANDARDIZATION

10.1 Establish GC operating parameters equivalent to those indicated in Section 6.12. This calibration procedure employs procedural calibration standards, i.e., fortified aqueous standards which are processed through the method (Section 11.0). The GC system is calibrated by means of the internal standard technique (Section 10.2).

**Note:** Calibration standard solutions must be prepared such that no unresolved analytes are mixed together (See Table 1).

10.2 Internal Standard Calibration Procedure -- To use this approach, the analyst must select one or more internal standards compatible in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measure-ment of the internal standard is not affected by method or matrix interferences. DBOB (Section 7.14) has been identified as a suitable internal standard.

10.2.1 Prepare aqueous calibration standards at a minimum of three (five are recommended) concentration levels for each method analyte as follows: for each concentration, fill a 250-mL volumetric flask with 240 mL of reagent water at pH 1 and containing 50 g of dissolved sodium sulfate. Add an appropriate aliquot of the primary dilution standard (Section 7.17.4) and dilute to 250 mL with the same reagent water. Guidance on the number of standards is as follows: A minimum of three calibration standards are required to calibrate a range of a factor of 20 in concentration. For a factor of 50 use at least four standards, and for a factor of 100 at least five standards. The lowest standard should represent analyte concentrations near, but above, their respective MDLs. The remaining standards should bracket the analyte concentrations expected in the sample extracts, or

should define the working range of the detector. Process each aqueous calibration sample through the analytical procedure beginning with Section 11.1.2. The internal standard is added to the final 5 mL extract as specified in Sections 11.4.3 or 11.5.9.

10.2.2  Analyze each calibration standard according to the procedure beginning in Section 11.1.2. Tabulate response (peak height or area) against concentration for each compound and internal standard. Calculate the response factor (RF) for each analyte and surrogate using the following equation.

$$ RF = \frac{(A_s)\ (C_{is})}{(A_{is})\ (C_s)} $$

where: $A_s$ = Response for the analyte to be measured
$A_{is}$ = Response for the internal standard
$C_{is}$ = Concentration of the internal standard (µg/L)
$C_s$ = Concentration of the analyte to be measured (µg/L)

10.2.3  If the RF value over the working range is constant (30% RSD or less) the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios ($A_s/A_{is}$) vs. $C_s$. A data station may be used to collect the chromatographic data, calculate response factors and generate linear or second order regression curves.

10.2.4  The working calibration curve or RF must be verified on each working shift (not to exceed 12 hours) by the measurement of one or more calibration standards. It is highly recommended that a calibration verification be performed at the beginning and at the end of every extended period of instrument operation so that field sample extracts are bracketed by calibration standards. It is also recommended that more that one standard concentration be analyzed so that the calibration is verified at more than one point. New calibration standards need not be derivatized each day. The same standard extract can be used up to 14 days. If the response for any analyte varies from the predicted response by more than ±30%, the test must be repeated using a fresh calibration standard. If the repetition also fails, a new calibration curve must be generated for that analyte using freshly prepared standards. For those analytes that failed the calibration verification, results from field samples analyzed since the last passing calibration should be considered suspect. Reanalyze sample extracts for these analytes after acceptable calibration is restored.

10.2.5  Verify calibration standards periodically, at least quarterly is recommended, by analyzing a standard prepared from reference material

obtained from an independent source.  Results from these analyses must be within the limits used to routinely check calibration.

## 11.0   **PROCEDURE**

11.1   Manual Hydrolysis and Separation of Interferences

11.1.1   Remove the sample bottles from cold storage and allow them to equilibrate to room temperature.  Acidify and add sodium thiosulfate to LFBs, LRBs and QCSs as specified in Section 8.0.

11.1.2   Measure a 250 mL aliquot of each sample with a 250 mL graduated cylinder and pour into a 500 mL separatory funnel.  Add 250 µL of the surrogate primary dilution standard (Section 7.19) to each 250 mL sample.  The surrogate will be at a concentration of 2 µg/L.  Dissolve 50 g sodium sulfate in the sample.

11.1.3   Add 4 mL of 6 N NaOH to each sample, seal, and shake.  Check the pH of the sample with pH paper or a pH meter; if the sample does not have a pH greater than or equal to 12, adjust the pH by adding more 6 N NaOH.  Let the sample sit at room temperature for one hour, shaking the separatory funnel and contents periodically.

**Note:**  Since many of the herbicides contained in this method are applied as a variety of esters and salts, it is vital to hydrolyze them to the parent acid prior to extraction.  This step must be included in the analysis of all extracted field samples, LRBs, LFBs, LFMs, and QCS.

11.1.4   Use 15 mL methylene chloride to rinse the sample bottle and the graduated cylinder.   Then transfer the methylene chloride to the separatory funnel and extract the sample by vigorously shaking the funnel for two minutes with periodic venting to release excess pressure.  Allow the organic layer to separate from the water phase for a minimum of 10 minutes.  If the emulsion interface between layers is more than ⅓ the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation.  The optimum technique depends upon the sample, but may include stirring, filtration through glass wool, centrifugation, or other physical methods.   Discard the methylene chloride phase (Sections 14.0 and 15.0).

11.1.5   Add a second 15 mL volume of methylene chloride to the separatory funnel and repeat the extraction procedure a second time, discarding the methylene chloride layer.  Perform a third extraction in the same manner.

11.1.6   Drain the contents of the separatory funnel into a 500 mL beaker.  Adjust the pH to 1.0 ±0.1 by the dropwise addition of concentrated sulfuric acid with constant stirring.  Monitor the pH with a pH meter (Section 6.8) or short range (0-3) pH paper (Section 6.14).

11.2    Sample Extraction

    11.2.1    Vacuum Manifold -- Assemble a manifold (Section 6.3) consisting of six to eight vacuum flasks with filter funnels (Sections 6.1 and 6.2).  Individual vacuum control, on-off and vacuum release valves and vacuum gauges are desirable.  Place the 47 mm extraction disks (Section 7.1) on the filter frits.

    11.2.2    Add 20 mL of 10% by volume of methanol in MTBE to the top of each disk without vacuum and allow the solvent to remain for two minutes.  Turn on full vacuum and draw the solvent through the disks, followed by room air for five minutes.

    11.2.3    Adjust the vacuum to approximately 5 in. (mercury) and add the following in series to the filter funnel (a) 20 mL methanol (b) 20 mL reagent water (c) sample.  Do not allow the disk to dry between steps and maintain the vacuum at 5 in.

    11.2.4    After all the sample has passed through the disk, apply maximum vacuum and draw room air through the disks for 20 minutes.

    11.2.5    Place the culture tubes (Section 6.4) in the vacuum tubes to collect the eluates.  Elute the disks with two each 2 mL aliquots of 10% methanol in MTBE.  Allow each aliquot to remain on the disk for one min before applying vacuum.

    11.2.6    Rinse each 500 mL beaker (Section 11.1.6) with 4 mL of pure MTBE and elute the disk with this solvent as in Section 11.2.5.

    11.2.7    Remove the culture tubes and cap.

11.3    Extract Preparation

    11.3.1    Pre-rinse the drying tubes (Section 7.5.1) with 2 mL of MTBE.

    11.3.2    Remove the entire extract with a 5 mL pipet and drain the lower aqueous layer back into the culture tube.  Add the organic layer to the sodium sulfate drying tube (Section 7.5.1).  Maintain liquid in the drying tube between this and subsequent steps.  Collect the dried extract in a 15 mL graduated centrifuge tube or a 10 mL Kuderna-Danish tube.

    11.3.3    Rinse the culture tube with an additional 1 mL of MTBE and repeat Section 11.3.2.

    11.3.4    Repeat step Section 11.3.3 and finally add a 1 mL aliquot of MTBE to the drying tube before it empties.  The final volume should be 6-9 mL.  In this form the extract is esterified as described below.

11.4    Extract Esterification with Diazomethane -- See Section 11.5 for alternative procedure.

11.4.1    Assemble the diazomethane generator (Figure 1) in a hood.

11.4.2    Add 5 mL of ethyl ether to Tube 1. Add 4 mL of Diazald solution (Section 7.12) and 3 mL of 37% KOH solution (Section 7.16.1) to the reaction Tube 2. Immediately place the exit tube into the collection tube containing the sample extract. Apply nitrogen flow (10 mL/min.) to bubble diazo-methane through the extract. Each charge of the generator should be sufficient to esterify four samples. The appearance of a persistent yellow color is an indication that esterification is complete. The first sample should require 30 seconds to one minute and each subsequent sample somewhat longer. The final sample may require two to three minutes.

11.4.3    Cap each collection tube and allow to remain stored at room temperature in a hood for 30 minutes. No significant fading of the yellow color should occur during this period. Fortify each sample with 100 µL of the internal standard primary dilution solution (Section 7.18) and reduce the volume to 5.0 mL with the analytical concentrator (Section 6.10), a stream of dry nitrogen, or an equivalent concentration technique.

**Note:** The excess diazomethane is volatilized from the extract during the concentration procedure.

11.4.4    Cap the tubes and store in a refrigerator if further processing will not be performed immediately. Analyze by GC-ECD.

11.5    Extract Esterification with Trimethylsilyldiazomethane (TMSD) -- Alternative procedure. It should be noted that the gas chromatographic background is significantly increased when TMSD is used as the derivatizing reagent instead of the generated diazomethane. Although no method analyte is affected by this increased background, the recommended surrogate, 2,4-dichloro-phenylacetic acid, is masked by an interfering peak. This renders the surrogate useless at 1 µg/L or lower. Any compound found suitable when TMSD is used is acceptable as a surrogate.

11.5.1    Carry out the hydrolysis, clean-up, and extraction of the method analytes as described up to Section 11.2.4.

11.5.2    Elute the herbicides from the disk by passing two 2 mL aliquots of methyl tertiary butyl ether (MTBE) through the disk into the collection tube. Rinse the sample container with 4 mL of MTBE and pass it through the disk into the tube.

11.5.3 Transfer the MTBE extract from the collection tube into an anhydrous sodium sulfate drying tube which has been pre-wetted with 1 mL MTBE. Be sure to discard any water layer.

11.5.4 Before the extract passes completely through the sodium sulfate, add an additional 2 mL of MTBE as a rinse.

11.5.5 Concentrate the dried extract to approximately 4 mL. Add methanol (approx. 1 mL) to the extract to yield a 20% (v/v) methanol in MTBE solution. Adjust the volume to 5 mL with MTBE. (TMSD produces the most efficient methylation of the herbicides in a 20% methanol, 80% MTBE solution.)

11.5.6 Add 50 µL of the 2 M TMSD solution to each 5 mL sample extract.

11.5.7 Place the tube containing the extract into a heating block at 50°C and heat the extract for one hour.

11.5.8 Allow the extract to cool to room temperature, then add 100 µL of 2 M acetic acid in methanol to react with any excess TMSD.

11.5.9 Fortify the extract with 100 µL of the internal standard solution (See Section 7.18) to yield a concentration of 0.020 µg/mL.

11.5.10 Proceed with the identification and measurement of the analytes using GC/ECD according to the procedures described in Section 11.6.

11.6 Gas Chromatography

11.6.1 Section 6.12 summarizes the recommended GC operating conditions. Included in Table 1 are retention times observed using this method. Figures 2A and 2B illustrate the chromatographic performance of the primary column (Section 6.12.1) for Groups A and B of the method analytes. Other GC columns or chromatographic conditions, may be used if the requirements of Section 9.3 are met.

11.6.2 Calibrate or verify the existing calibration daily as described in Section 10.0.

11.6.3 Inject 2 µL of the sample extract. Record the resulting peak size in area units.

11.6.4 If the response for any sample peak exceeds the working range of the detector, dilute the extract and reanalyze. Add additional IS, so that the IS amount in the extract will be the same as in the calibration standards.

11.7 Identification of Analytes

11.7.1 Identify a sample component by comparison of its retention time to the retention time of a reference chromatogram. If the retention time of an unknown compound corresponds, within limits, to the retention time of a standard compound, then an analyte is considered to be identified.

11.7.2 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in interpretation of chromatograms.

11.7.3 Identification requires expert judgment when sample components are not resolved chromatographically. When GC peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima, or any time doubt exists over the identification of a peak in a chromatogram, appropriate alternative techniques to help confirm peak identification need to be employed. For example, more positive identification may be made by the use of an alternative detector which operates on a chemical/physical principle different from that originally used, e.g., mass spectrometry, or the use of a second chromatography column. A suggested alternative column is described in Section 6.12.2.

## 12.0   DATA ANALYSIS AND CALCULATIONS

12.1   Calculate analyte concentrations in the sample from the response for the analyte using the calibration procedure described in Section 10.0. Use the multi-point calibration for each analyte to make all calculations. Do not use the daily calibration verification data to quantitate analytes in samples.

12.2   Calculate the concentration (C) in the sample using the response factor (RF) determined in Section 10.2.2 and the following equation, or determine sample concentration from the calibration curve (Section 10.2.3).

$$C \ (\mu g/L) \ = \ \frac{(A_s) \ (I_s)}{(A_{is}) \ (RF) \ (V_o)}$$

where: $A_s$ = Response for the analyte to be measured
$A_{is}$ = Response for the internal standard
$I_s$ = Amount of internal standard added to each extract ($\mu$g)
$V_o$ = Volume of water extracted (L)

## 13.0   METHOD PERFORMANCE

13.1 All data shown in Section 17.0 of this method were obtained with the diazomethane esterification option.

13.2 In a single laboratory, analyte recoveries from reagent water were determined at three concentration levels, Tables 2-4. Results were used to determine the analyte MDLs[8] listed in Table 2. The calculation for determining MDL is:

$$MDL = S\ t_{(n-1,\ 1-alpha\ =\ 0.99)}$$

where: $t_{(n-1,1-alpha\ =\ 0.99)}$ = Student's t value for the 99% confidence level with
                       n-1 degrees of freedom
       n = number of replicates
       S = standard deviation of replicate analyses

13.3 In a single laboratory, analyte recoveries from dechlorinated tap water were determined at two concentrations, Tables 5 and 6. In addition, analyte recoveries were determined at two concentrations from an ozonated surface (river) water, Tables 7 and 8, and at one level from a high humectant surface (reservoir) water, Table 10. Finally, a holding study was conducted on the preserved, ozonated surface water and recovery data are presented for Day 1 and Day 14 of this study, Tables 8 and 9. The ozonated surface water was chosen as the matrix in which to study analyte stability during a 14-day holding time because it was very biologically active.

## 14.0 POLLUTION PREVENTION

14.1 This method utilizes liquid-solid extraction technology which requires the use of very small quantities of organic solvents. This feature eliminates the hazards involved with the use of large volumes of potentially harmful organic solvents needed for conventional liquid-liquid extractions. Also, mercuric chloride, a highly toxic and environmentally hazardous chemical, has been replaced with hydrochloric acid as the sample preservative. These features make this method much safer and a great deal less harmful to the environment.

14.2 For information about pollution prevention that may be applicable to laboratory operations, consult "Less is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

## 15.0 WASTE MANAGEMENT

15.1 Due to the nature of this method, there is little need for waste management. No large volumes of solvents or hazardous chemicals are used. The matrices of concern are finished drinking water or source water. However, the Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations, and that laboratories protect the air, water,

and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel", also available from the American Chemical Society at the address in Section 14.2.

## 16.0   REFERENCES

1.    ASTM Annual Book of Standards, Part 11, Volume 11.02, D3694-82, "Standard Practice for Preparation of Sample Containers and for Preservation", American Society for Testing and Materials, Philadelphia, PA, p. 86, 1986.

2.    Giam, C.S., H.S. Chan, and G.S. Nef.   "Sensitive Method for Determination of Phthalate Ester Plasticizers in Open-Ocean Biota Samples", Analytical Chemistry, 47, 2225 (1975).

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4.    "Carcinogens - Working with Carcinogens", Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August 1977.

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8.    Glaser, J.A., Foerst, D.L., McKee, G.D., Quave, S.A., and Budde, W.L.   "Trace Analyses for Wastewaters", Environ. Sci. Technol. 1981, 15, 1426-1435.

9.    40 CFR, Part 136, Appendix B.

## 17.0   TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

### TABLE 1.  RETENTION DATA

| Analyte | Group[a] | Retention Time, min.[b] Primary | Retention Time, min.[b] Confirmation |
|---|---|---|---|
| 3,5-Dichlorobenzoic Acid | A | 16.72 | 18.98 |
| 2,4-Dichlorophenylacetic Acid | A,B | 19.78 | 22.83 |
| Dicamba | B | 20.18 | 23.42 |
| Dichlorprop | A | 22.53 | 25.90 |
| 2,4-D | B | 23.13 | 27.01 |
| 4,4'-Dibromoctafluorobiphenyl (IS) | A,B | 24.26 | 26.57 |
| Pentachlorophenol | A | 25.03 | 27.23 |
| Silvex | B | 25.82 | 29.08 |
| 5-Hydroxydicamba | B | 26.28 | 30.18 |
| 2,4,5-T | A | 26.57 | 30.33 |
| 2,4-DB | B | 27.95 | 31.47 |
| Dinoseb | A | 28.03 | 33.02 |
| Bentazon | B | 28.70 | 33.58 |
| Picloram | B | 29.93 | 35.90 |
| Dacthal Diacid Metabolite | A | 31.02 | 34.32 |
| Acifluorfen | B | 35.62 | 40.58 |

[a]Analytes were divided into two groups during method development to avoid chromatographic overlap.

[b]Columns and chromatographic conditions are described in Section 6.12.

**TABLE 2.  SINGLE LABORATORY RECOVERY, PRECISION DATA AND METHOD DETECTION LIMIT WITH FORTIFIED REAGENT WATER - LEVEL 1**

| Analyte | Fortified Conc. µg/L | Mean[a] Recovery % | Relative Std. Dev. % | MDL µg/L |
|---|---|---|---|---|
| Acifluorfen | 0.50 | 78 | 21 | 0.25 |
| Bentazon | 2.50 | 70 | 11 | 0.63 |
| 2,4-D | 0.25 | 96 | 38 | 0.28 |
| 2,4-DB | 2.50 | 79 | 12 | 0.72 |
| Dacthal Diacid Metabolite | 0.25 | 96 | 16 | 0.13 |
| Dicamba | 0.75 | 109 | 11 | 0.28 |
| 3,5-Dichlorobenzoic Acid | 1.25 | 126 | 24 | 1.23 |
| Dichlorprop | 0.25 | 106 | 15 | 0.13 |
| Dinoseb | 0.50 | 87 | 22 | 0.28 |
| 5-Hydroxydicamba | 0.75 | 90 | 12 | 0.25 |
| Pentachlorophenol | 0.25 | 103 | 18 | 0.16 |
| Picloram | 0.75 | 95 | 15 | 0.35 |
| 2,4,5-T | 0.25 | 116 | 18 | 0.16 |
| 2,4,5-TP | 0.25 | 98 | 9 | 0.16 |

[a]Based on the analyses of seven replicates.

**TABLE 3. SINGLE LABORATORY RECOVERY AND PRECISION DATA FOR
FORTIFIED REAGENT WATER - LEVEL 2**

| Analyte | Fortified Conc. μg/L | Mean[a] Recovery % | Relative Std. Dev. % |
|---|---|---|---|
| Acifluorfen | 0.80 | 61 | 27 |
| Bentazon | 4.0 | 81 | 8 |
| 2,4-D | 0.40 | 96 | 38 |
| 2,4-DB | 4.0 | 90 | 13 |
| Dacthal Diacid Metabolite | 0.40 | 96 | 16 |
| Dicamba | 1.20 | 109 | 11 |
| 3,5-Dichlorobenzoic Acid | 2.00 | 126 | 24 |
| Dichlorprop | 0.40 | 76 | 21 |
| Dinoseb | 0.80 | 87 | 22 |
| 5-Hydroxydicamba | 1.20 | 90 | 12 |
| Pentachlorophenol | 0.40 | 66 | 26 |
| Picloram | 1.20 | 68 | 21 |
| 2,4,5-T | 0.40 | 116 | 18 |
| 2,4,5-TP | 0.40 | 105 | 7 |

[a]Based on the analyses of six to seven replicates.

| Analyte | Fortified Conc. μg/L | Mean[a] Recovery % | Relative Std. Dev. % |
|---|---|---|---|
| Acifluorfen | 2.0 | 59 | 13 |
| Bentazon | 10.0 | 68 | 8 |
| 2,4-D | 1.0 | 90 | 20 |
| 2,4-DB | 10.0 | 74 | 6 |
| Dacthal Diacid Metabolite | 1.0 | 60 | 10 |
| Dicamba | 3.0 | 75 | 9 |
| 3,5-Dichlorobenzoic Acid | 5.0 | 62 | 18 |
| Dichlorprop | 1.0 | 97 | 17 |
| Dinoseb | 2.0 | 63 | 10 |
| 5-Hydroxydicamba | 3.0 | 77 | 8 |
| Pentachlorophenol | 1.0 | 69 | 11 |
| Picloram | 3.0 | 66 | 9 |
| 2,4,5-T | 1.0 | 64 | 15 |
| 2,4,5-TP | 1.0 | 68 | 8 |

[a]Based on the analyses of six to seven replicates.

**TABLE 5. SINGLE LABORATORY RECOVERY AND PRECISION DATA FOR FORTIFIED, DECHLORINATED TAP WATER - LEVEL 1**

| Analyte | Fortified Conc. μg/L | Mean[a] Recovery % | Relative Std. Dev. % |
|---|---|---|---|
| Acifluorfen | 0.50 | 117 | 21 |
| Bentazon | 2.50 | 96 | 12 |
| 2,4-D | 0.25 | 59[b] | 55 |
| 2,4-DB | 2.50 | 112 | 15 |
| Dacthal Diacid Metabolite | 0.25 | 101 | 10 |
| Dicamba | 0.75 | 91 | 14 |
| 3,5-Dichlorobenzoic Acid | 1.25 | 103 | 15 |
| Dichlorprop | 0.25 | 218[c] | 37 |
| Dinoseb | 0.50 | 134 | 10 |
| 5-Hydroxydicamba | 0.75 | 90 | 14 |
| Pentachlorophenol | 0.25 | 91 | 8 |
| Picloram | 0.75 | 76 | 28 |
| 2,4,5-T | 0.25 | 118 | 16 |
| 2,4,5-TP | 0.25 | 99 | 10 |

[a]Based on the analyses of six to seven replicates.
[b]2,4-D background value was 0.29 μg/L.
[c]Probable interference.

**TABLE 6.  SINGLE LABORATORY RECOVERY AND PRECISION DATA FOR FORTIFIED, DECHLORINATED TAP WATER - LEVEL 2**

| Analyte | Fortified Conc. µg/L | Mean[a] Recovery % | Relative Std. Dev. % |
|---|---|---|---|
| Acifluorfen | 2.0 | 150 | 7 |
| Bentazon | 10.0 | 112 | 9 |
| 2,4-D | 1.0 | 90 | 16 |
| 2,4-DB | 10.0 | 111 | 10 |
| Dacthal Diacid Metabolite | 1.0 | 118 | 8 |
| Dicamba | 3.0 | 86 | 10 |
| 3,5-Dichlorobenzoic Acid | 5.0 | 111 | 5 |
| Dichlorprop | 1.0 | 88 | 30 |
| Dinoseb | 2.0 | 121 | 6 |
| 5-Hydroxydicamba | 3.0 | 96 | 6 |
| Pentachlorophenol | 1.0 | 96 | 6 |
| Picloram | 3.0 | 132 | 12 |
| 2,4,5-T | 1.0 | 108 | 10 |
| 2,4,5-TP | 1.0 | 115 | 7 |
| 2,4-Dichlorophenylacetic Acid[b] | 1.0 | 120 | 19 |

[a]Based on the analyses of six to seven replicates.
[b]Surrogate analyte.

**TABLE 7. SINGLE LABORATORY RECOVERY AND PRECISION DATA FOR FORTIFIED, OZONATED SURFACE WATER - LEVEL 1**

| Analyte | Fortified Conc. μg/L | Mean[a] Recovery % | Relative Std. Dev. % |
|---|---|---|---|
| Acifluorfen | 0.50 | 172 | 14 |
| Bentazon | 2.50 | 92 | 22 |
| 2,4-D | 0.25 | 127 | 13 |
| 2,4-DB | 2.50 | 154 | 19 |
| Dacthal Diacid Metabolite | 0.25 | 113 | 17 |
| Dicamba | 0.75 | 107 | 13 |
| 3,5-Dichlorobenzoic Acid | 1.25 | 100 | 17 |
| Dichlorprop | 0.25 | 115 | 20 |
| Dinoseb | 0.50 | 134 | 28 |
| 5-Hydroxydicamba | 0.75 | 89 | 13 |
| Pentachlorophenol | 0.25 | 110 | 22 |
| Picloram | 0.75 | 109 | 27 |
| 2,4,5-T | 0.25 | 102 | 19 |
| 2,4,5-TP | 0.25 | 127 | 8 |
| 2,4-Dichlorophenylacetic Acid[b] | 0.25 | 72 | 31 |

[a]Based on the analyses of six to seven replicates.
[b]Surrogate analyte.

**TABLE 8.  SINGLE LABORATORY RECOVERY AND PRECISION DATA FOR FORTIFIED, OZONATED SURFACE WATER - LEVEL 2, STABILITY STUDY DAY 1[b]**

| Analyte | Fortified Conc. μg/L | Mean[a] Recovery % | Relative Std. Dev. % |
|---|---|---|---|
| Acifluorfen | 2.0 | 173 | 11 |
| Bentazon | 10.0 | 122 | 7 |
| 2,4-D | 1.0 | 126 | 10 |
| 2,4-DB | 10.0 | 130 | 7 |
| Dacthal Diacid Metabolite | 1.0 | 116 | 11 |
| Dicamba | 3.0 | 109 | 9 |
| 3,5-Dichlorobenzoic Acid | 5.0 | 115 | 11 |
| Dichlorprop | 1.0 | 116 | 11 |
| Dinoseb | 2.0 | 116 | 9 |
| 5-Hydroxydicamba | 3.0 | 121 | 9 |
| Pentachlorophenol | 1.0 | 118 | 10 |
| Picloram | 3.0 | 182 | 14 |
| 2,4,5-T | 1.0 | 112 | 9 |
| 2,4,5-TP | 1.0 | 122 | 10 |
| 2,4-Dichlorophenylacetic Acid[b] | 1.0 | 110 | 26 |

[a]Based on the analyses of six to seven replicates.
[b]Samples preserved at pH = 2.0.
[c]Surrogate analyte.

**TABLE 9. SINGLE LABORATORY RECOVERY AND PRECISION DATA FOR FORTIFIED, OZONATED SURFACE WATER - LEVEL 2, STABILITY STUDY DAY 14[b]**

| Analyte | Fortified Conc. µg/L | Mean[a] Recovery % | Relative Std. Dev. % |
|---|---|---|---|
| Acifluorfen | 2.0 | 151 | 18 |
| Bentazon | 10.0 | 97 | 9 |
| 2,4-D | 1.0 | 84 | 11 |
| 2,4-DB | 10.0 | 128 | 10 |
| Dacthal Diacid Metabolite | 1.0 | 116 | 7 |
| Dicamba | 3.0 | 103 | 9 |
| 3,5-Dichlorobenzoic Acid | 5.0 | 81 | 12 |
| Dichlorprop | 1.0 | 107 | 11 |
| Dinoseb | 2.0 | 118 | 7 |
| 5-Hydroxydicamba | 3.0 | 20 | 14 |
| Pentachlorophenol | 1.0 | 94 | 7 |
| Picloram | 3.0 | 110 | 32 |
| 2,4,5-T | 1.0 | 113 | 8 |
| 2,4,5-TP | 1.0 | 113 | 11 |
| 2,4-Dichlorophenylacetic Acid[b] | 1.0 | 87 | 6 |

[a]Based on the analyses of six to seven replicates.
[b]Samples preserved at pH = 2.0.
[c]Surrogate analyte.

**TABLE 10. SINGLE LABORATORY RECOVERY AND PRECISION DATA FOR FORTIFIED, HIGH HUMIC CONTENT SURFACE WATER**

| Analyte | Fortified Conc. μg/L | Mean[a] Recovery % | Relative Std. Dev. % |
|---|---|---|---|
| Acifluorfen | 2.0 | 120 | 13 |
| Bentazon | 10.0 | 87 | 11 |
| 2,4-D | 1.0 | 59 | 7 |
| 2,4-DB | 10.0 | 80 | 14 |
| Dacthal Diacid Metabolite | 1.0 | 100 | 6 |
| Dicamba | 3.0 | 76 | 9 |
| 3,5-Dichlorobenzoic Acid | 5.0 | 87 | 4 |
| Dichlorprop | 1.0 | 110 | 22 |
| Dinoseb | 2.0 | 97 | 6 |
| 5-Hydroxydicamba | 3.0 | 82 | 9 |
| Pentachlorophenol | 1.0 | 70 | 5 |
| Picloram | 3.0 | 124 | 9 |
| 2,4,5-T | 1.0 | 101 | 4 |
| 2,4,5-TP | 1.0 | 80 | 6 |

[a]Based on the analyses of six to seven replicates.

**TABLE 11.  LABORATORY PERFORMANCE CHECK SOLUTION**

| Test | Analyte | Conc. µg/mL | Requirements |
|---|---|---|---|
| Sensitivity | Dinoseb | 0.004 | Detection of analyte; S/N >3 |
| Chromatographic performance | 4-Nitrophenol | 1.6 | |
| | | | 0.70 < PGF <1.05[a] |
| Column performance | 3,5-Dichlorobenzoic Acid | 0.6 | |
| | 4-Nitrophenol | 1.6 | Resolution >0.04[b] |

[a]PGF -- peak Gaussian factor.  Calculated using the equation:

$$PGF = \frac{1.83 \text{ x } W(1/2)}{W(1/10)}$$

where:  W(1/2) = the peak width at half height in seconds
W(1/10) = the peak width in seconds at 10th height

[b]Resolution between the two peaks as defined by the equation:

$$R = \frac{t}{W}$$

where:  t = the difference in elution times between the two peaks
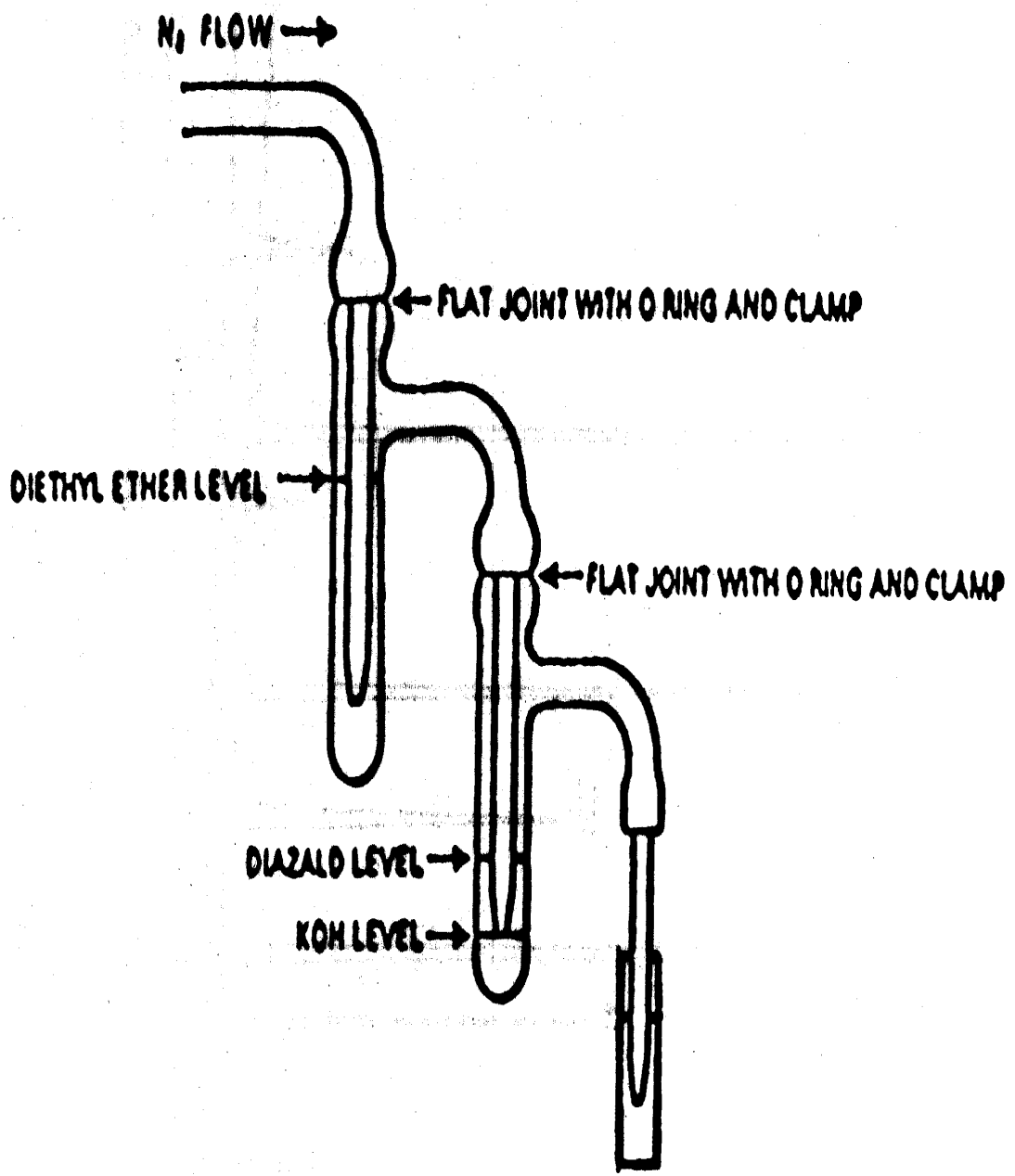W = the average peak width, at the baseline, of the two peaks

**FIGURE 1. DIAZOMETHANE GENERATOR**
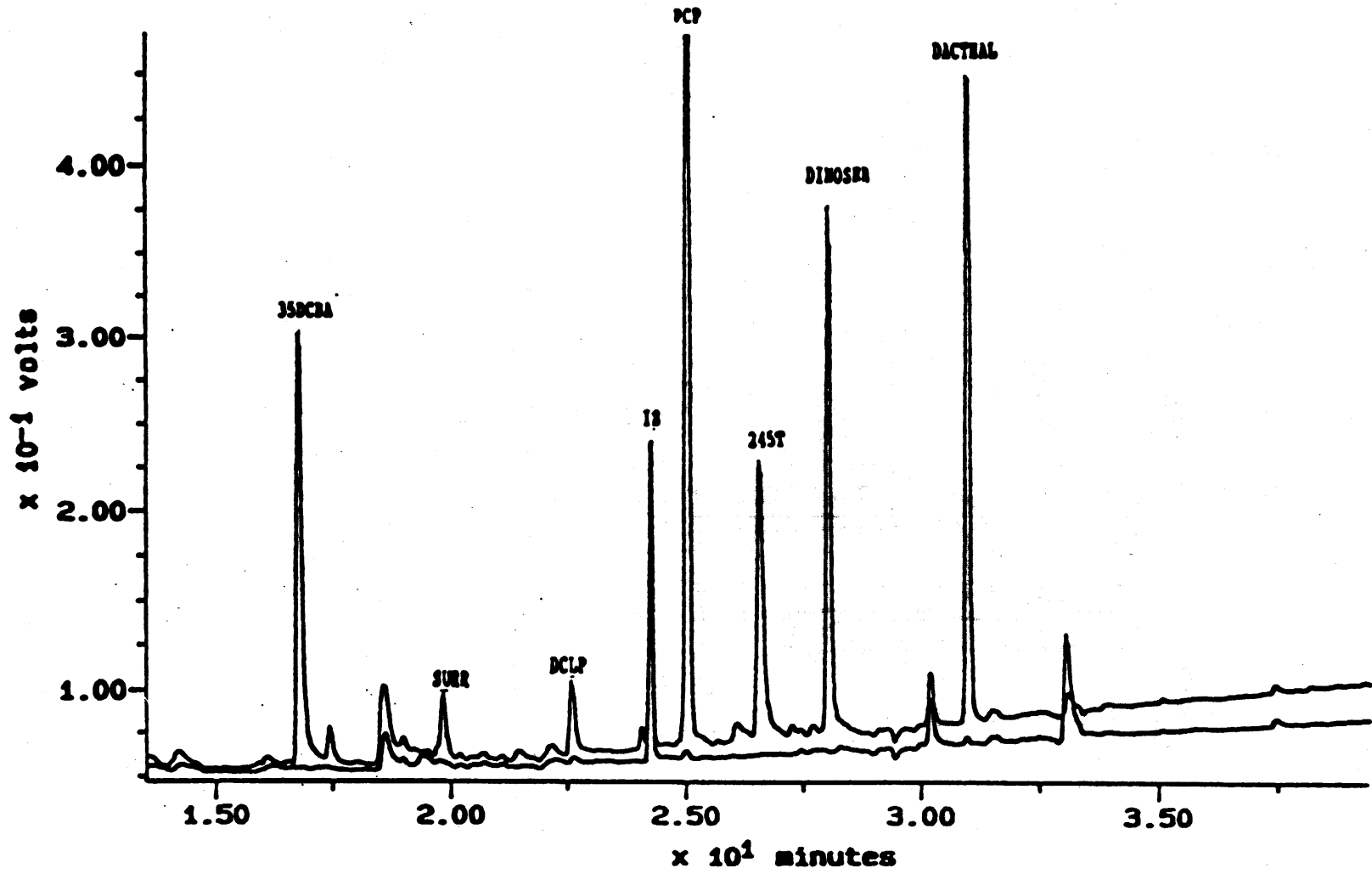
Figure 2A. Chromatogram of Group A Analytes Extracted From
Ozonated Surface Water (bottom chromatogram is
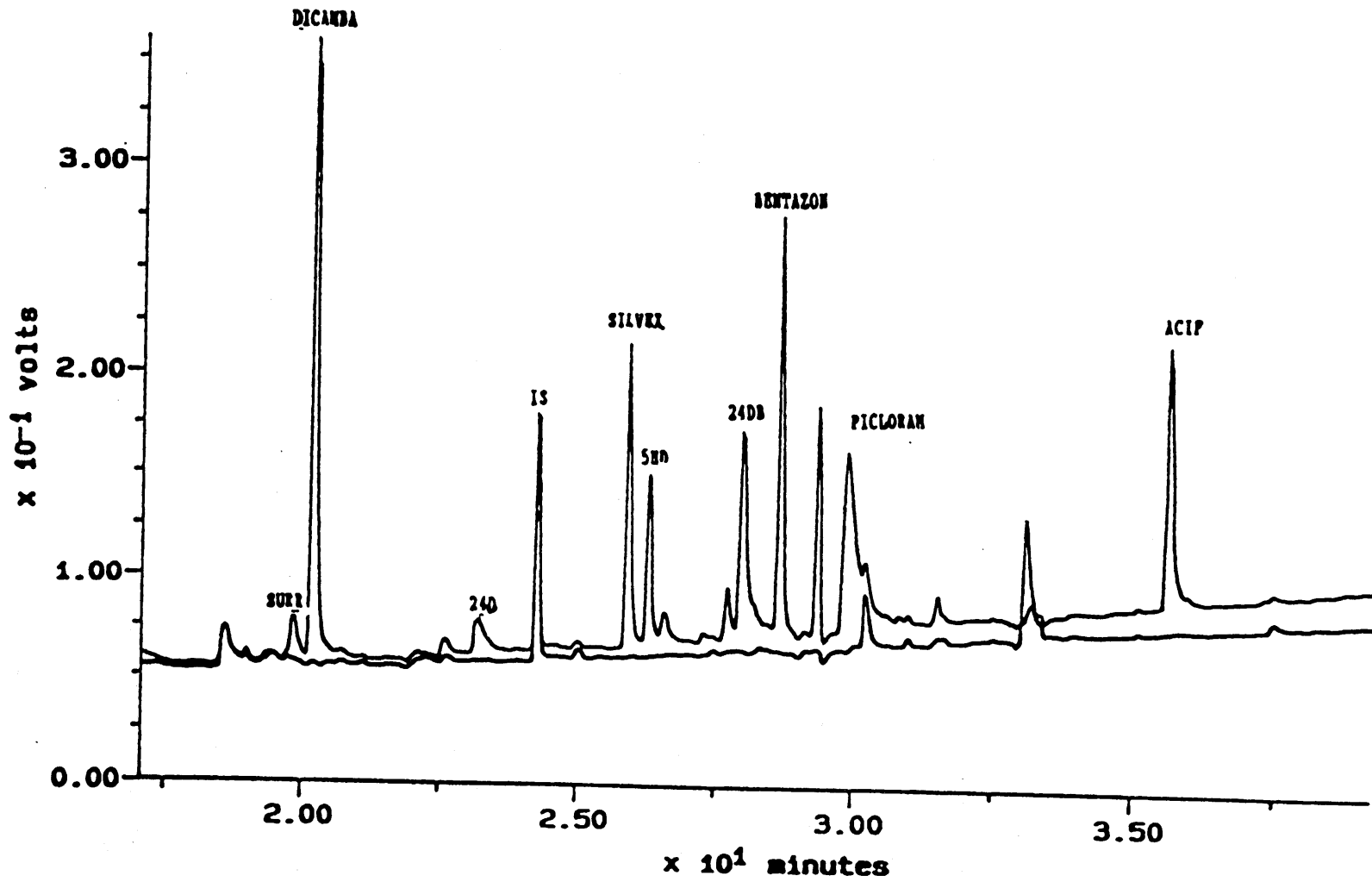the laboratory reagent blank)

Figure 2B. Chromatogram of Group B Analytes Extracted From
Ozonated Surface Water (bottom chromatogram is
the laboratory reagent blank)

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