# Method 515.2: Determination of Chlorinated Acids in Water Using Liquid-Solid Extraction and Gas Chromatography with an Electron Capture Detector


## UCT Part Numbers

**ECDVB156**
6 mL cartridge with 500 mg
styrene divinylbenzene

**ECUNIDVB500**
83 mL cartridge with 500 mg
styrene divinylbenzene

## Method Summary:

250 mL of water is adjusted to pH 12 with 6 N NaOH for one hour to hydrolyze derivatives. Extraneous organic material is removed during a solvent wash. The sample is acidified, and the chlorinated acids are extracted using a cartridge containing styrene divinylbenzene. Acid analytes are converted to their methyl esters using diazomethane or with trimethylsilyldiazomethane (TMSD). Excess derivatizing reagent is removed, and the esters are determined by capillary column GC equipped with an electron capture detector (ECD). As many of the analytes contained in this method are used as a variety of esters and salts and it is necessary to hydrolyze them to the parent acid prior to extraction.

This is a gas chromatographic (GC) method applicable to the determination of certain chlorinated acids in ground and finished drinking water. It is applicable to the determination of the salts and esters of these analyte acids.

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

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| | |
|---|---|
| **Acifluorfen** | 50594-66-6 |
| **Bentazon** | 25057-89-0 |
| **2,4-D** | 94-75-7 |
| **2,4-DB** | 94-82-6 |
| **Dacthal Acid Metabolites**[a] | NA |
| **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 |

## Sample Collection, Preservation, and Storage

- Collect grab samples in 250 mL amber glass containers
- Do not prerinse bottle before collection
- To remove residual chlorine, add 80 mg of sodium thiosulfate or 50 mg of sodium sulfite per liter. Mix to dissolve
- Add hydrochloric acid (diluted 1:1 in reagent water) to the sample at the sampling
- site to produce a sample pH 2

  **Note:** Do not attempt to mix sodium thiosulfate and HCl in the sample bottle prior to sample collection
- Samples should be iced or refrigerated at 4 °C away from light from the time of collection until extraction. Samples may be stored up to 14 days. The concentration of 5-hydroxydicamba is seriously degraded over 14 days in a biologically active matrix
- Extracts should be stored at 4 °C or less away from light

## Interferences

**Careful attention to interference details will ensure better analysis**

- Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing. Use high purity reagents and solvents
- All reagents and apparatus must be demonstrated to be free from interferences under analytical conditions by analyzing laboratory reagent blanks
- Glassware must be scrupulously cleaned
- Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used. Follow by washing with hot water and detergent then thoroughly rinse with dilute acid, tap and reagent water. Heat in an oven or muffle furnace at 400°C for one hour
- Do not heat volumetric glassware. Rinsing with acetone may be used instead of heating
- The acid forms of the analytes are strong organic acids which react readily with alkaline substances and can be lost during sample preparation. Do not use soda lime glassware
- Glassware and glass wool must be acid-rinsed with 1 N HCl. $Na_2SO_4$ must be acidified with $H_2SO_4$ prior to use to avoid analyte losses due to adsorption on the glass
- Organic acids and phenols, especially chlorinated compounds, cause the most interference. Alkaline hydrolysis and subsequent extraction of the basic sample removes many chlorinated hydrocarbons and phthalate esters that might interfere with the electron capture analysis
- Interferences by phthalate esters can pose a major problem in pesticide analysis when using ECD. Phthalates generally appear in the chromatogram as large peaks. Common flexible plastics contain varying amounts of phthalates that are easily extracted during laboratory operations. Cross-contamination of clean glassware occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Avoid the use of plastics in the laboratory if possible
- Extensive purification of reagents and glassware may be required. Interfering contamination may occur when a sample

containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes

- Rinse the sample syringe and associated equipment between analyses with methyl-tert-butyl-ether (MTBE) to 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
- Matrix interferences may be caused by contaminants that are coextracted from the sample. Not all analytes are resolved from each other on any one column
- Generated diazo-methane is preferred for derivatization. GC background is significantly increased if TMSD is used
- Do not use carbon black or neoprene stoppers. Carbon black can absorb diazomethane and neoprene can contaminate extracts
- The recommended surrogate, 2,4-dichloro-phenylacetic acid, can be 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
- 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

## Safety

**Diazomethane is a toxic carcinogen which may explode under certain conditions. The following precautions must be followed:**

### Diazomethane

- 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
- The diazomethane generator apparatus used in the esterification procedure produces micromolar amounts of diazomethane in solution to minimize safety hazards. If the procedure is followed exactly, no explosion hazard exists
- Diazomethane solutions must not be stored. Only generate enough for immediate need

### Methyl-Tert-Butyl Ether

- Use nanograde purity. Solvent must be free of peroxides as indicated by EM Quant test strips (Scientific Products Co., Cat. No. Pl126-8, or equivalent). Redistill in glass if necessary

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

### Stock Standard Solutions (SSS) 1.00-2.00 µg/µL

Stock standard solutions may be purchased as certified solutions or prepared in the lab
Prepare from pure standard materials using the following procedure:

- 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
- If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard
- Transfer the stock standard solutions into 15 mL fluorocarbon-sealed screw cap amber vials. Store at 4 °C or less
- Stock standard solutions should be replaced after two months or sooner

### Primary Dilution Standards

- Prepare two sets of standards according to the sets labeled A and B in Table 1

## Retention Data

Analytes were divided into two groups during method development to avoid chromatographic overlap

| Retention Time (minutes)[a] | | | |
|---|---|---|---|
| **Analyte** | **Group** | **Primary** | **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'-ibromoctafluorobiphenyl (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] Columns and chromatographic conditions are shown in Chromatographic Analysis Section below

- 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
- 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 ECD response, i.e., pentachlorophenol. The maximum concentration is for an analyte with weak response, i.e., 2, 4-DB

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## Internal Standard Solution (ISS)

- Prepare an ISS solution by accurately weighing approximately 0.050 g of pure 4,4'-dibromooctafluorobiphenyl (DBOB). Dissolve the DBOB in methanol and dilute to volume in a 10 mL volumetric flask. Store at room temperature in a fluorocarbon sealed screw cap bottle
- Prepare a primary dilution standard (PDS) 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 PDS solution to the final 5 mL of sample extract results in a final internal standard concentration of 0.020 µg/mL
  **Note:** DBOB has been shown to be an effective internal standard for the method analytes

## Surrogate Analyte Solution

- Prepare a surrogate analyte stock standard solution by accurately weighing approximately 0.050 g of pure 2, 4-dichlorophenylacetic acid (DCAA). Dissolve the DCAA in methanol and bring to volume in a 10 mL volumetric flask. Store in a fluorocarbon-sealed screw cap bottle at room temperature
- Prepare a primary dilution standard of approximately 2.0 µg/mL by addition of 40 µL at the stock standard to 100 mL of methanol
- Add 250 µL of the surrogate analyte solution to 250 mL sample prior to extraction provides a surrogate concentration of 2 µg/L

# SPE Procedure:

### 1. Manual Hydrolysis and Separation of Interferences

**Note:** See Section Sample Collection, Preservation, and Storage above for acidification and sodium thiosulfate addition to LFB's, LRB's and QCS's

a) Measure a 250 mL aliquot of each water sample and pour into a 500 mL separatory funnel
b) Add 250 µL of the surrogate **PDS** to each sample
c) Add 50 g sodium sulfate anhydrous
d) Add 4 mL of 6 N NaOH to each sample, seal, and shake
e) Check the pH. If the sample does not have a pH greater than or equal to 12, adjust the pH by adding more 6 N NaOH
f) Let the sample sit at room temperature for one hour, occasionally shaking the separatory funnel contents

**Note:** Many of the herbicides contained in this method are applied as a variety of esters and salts. It is required 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

g) Use 15 mL methylene chloride to rinse the sample bottle and the graduated cylinder
h) Then transfer the methylene chloride to the separatory funnel. Extract the sample by vigorously shaking the funnel for two minutes
i) Allow the organic layer to separate from the water phase for a minimum of 10 minutes
j) Discard the methylene chloride phase
k) Add a second 15 mL volume of methylene chloride to the separatory funnel and repeat the extraction procedure a second time
l) Discard the methylene chloride layer
m) Perform a third extraction in the same manner as above discarding the methylene chloride
n) Drain the contents of the separatory funnel into a 500 mL beaker
o) Adjust the pH to 1.0 ±0.1 by the dropwise addition of concentrated sulfuric acid with constant stirring

## 2. Sample Extraction

a) Assemble a vacuum manifold system (**ECUCTVAC1**, **ECUCTVAC3**, **ECUCTVAC6**, 1,3,6 stations or equivalent)
b) Place **ECDVB156** or **ECUNIDVB500** extraction cartridge(s) on the manifold
c) With vacuum turned off add 20 mL of 10% by volume of methanol in methyl-tbutyl ether (MTBE) to the top of each cartridge
d) Allow the solvent to remain for two minutes in the cartridge
e) Turn on full vacuum and draw the solvent through the cartridge(s), followed by room air for five minutes
f) Adjust the vacuum to approximately 5 in. Hg or less and add the following in series to the cartridge:
    a. 20 mL methanol
    b. 20 mL reagent water
    c. sample

**Note:** Do not allow the cartridge to dry between steps. Maintain vacuum at 5 in. of Hg
g) After all the sample has passed through the cartridge, apply maximum vacuum and draw room air through the cartridges for 20 minutes
h) Place the culture tubes in the manifold to collect eluates
i) Elute the cartridge(s) with two each 2 mL aliquots of 10% methanol in MTBE
j) Allow each aliquot to remain in the cartridge for one minute before applying vacuum
k) Rinse the beaker(s) with 4 mL of pure MTBE and add to the cartridge
l) Remove the culture tubes from the manifold and cover

## 3. Extract Preparation

a) Pre-rinse the sodium sulfate drying tubes with 2 mL of MTBE
b) Remove the entire extract with a 5 mL pipette and drain the lower aqueous layer back into the culture tube
c) Add the organic layer to a sodium sulfate drying tube
d) Maintain liquid in the drying tube between this and subsequent steps
e) Collect the dried extract in a 15 mL graduated centrifuge tube or a 10 mL Kuderna-Danish tube
f) Rinse the culture tube with an additional 1 mL of MTBE and repeat step 3) b)
g) Repeat step 3) f) and then add a 1 mL aliquot of MTBE to the drying tube before it empties. The final volume should be 6-9 mL.
h) In this form the extract is esterified as described below

## 4. Extract Esterification with Diazomethane

a) Assemble the diazomethane generator as shown in diagram below in a hood
b) Add 5 mL of ethyl ether to Tube 1
c) Add 4 mL of Diazald solution* and 3 mL of 37% KOH solution to the reaction
**Tube 2:** *Add 10 g Diazald (Sigma-Aldrich D28000 or other supplier) in 100 mL of a 50:50 by volume mixture of ethyl ether and Carbitol (2-(2-ethoxyethoxy) ethanol). Solution is stable for one month or longer when stored at 4°C in a Teflon-lined screw cap amber bottle
d) Immediately place the exit tube into the collection tube containing the sample extract
e) Turn on $N_2$ at 10 mL/min to bubble diazo-methane through the extract. Each charge of the generator should be sufficient to esterify four samples
f) The appearance of a persistent yellow color indicates 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
g) Cover each collection tube and store at room temperature in a hood for 30 minutes. No signifi cant fading of the yellow color should occur during this time
h) Fortify each sample with 100 µL of the internal standard primary dilution solution and reduce the volume to 5.0 mL with the analytical concentrator equivalent concentration technique using a stream of dry $N_2$
i) Cover tubes and store in a refrigerator if further analysis will not be performed immediately
**Note:** The excess diazomethane is volatilized from the extract during the concentration procedure
j) Samples are ready to analyze by GC-ECD

**Extract Esterification with Trimethylsilyldiazomethane (TMSD)**

# Alternative Procedure:

**Note:** GC background is significantly increased when TMSD is used as the derivatizing reagent instead of diazomethane. While no method analyte is affected by this increased background, the recommended surrogate, 2, 4-dichloro-pheny lacetic acid, is masked by an interfering peak. Any compound found suitable when TMSD is used is acceptable as a surrogate

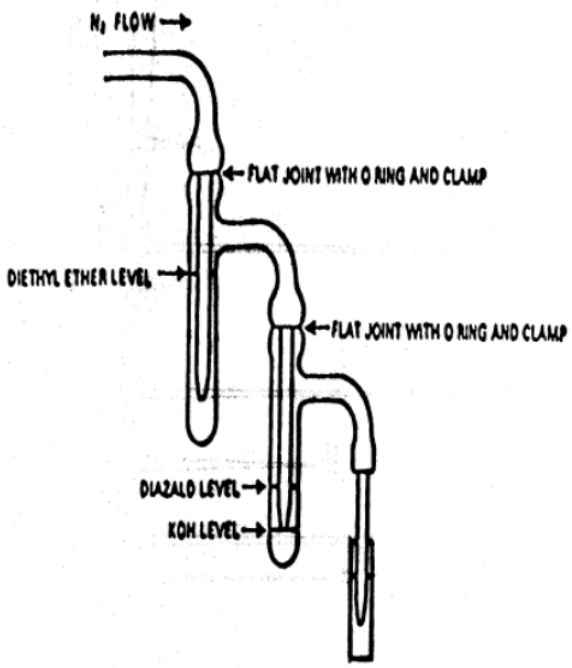
a) Conduct the hydrolysis, clean-up, and extraction of the method analytes as described up to steps 2) g) above
b) Elute the herbicides from the cartridge by passing two 2 mL aliquots of MTBE through the cartridge into the collection tube
c) Rinse the sample container with 4 mL of MTBE and pass it through the cartridge into the tube
d) Pre-wet an anhydrous sodium sulfate drying tube with 1 mL MTBE
e) Transfer the MTBE extract from the collection tube discarding any water layer
f) Before the extract passes completely through the sodium sulfate, rinse with an additional 2 mL of MTBE
g) Concentrate the dried extract to approximately 4 mL
h) Add about 1 mL of methanol to the extract to yield a 20% (v/v) methanol in MTBE solution
i) Adjust the volume to 5 mL with MTBE. (TMSD produces the most efficient methylation of the herbicides in a 20% methanol, 80% MTBE solution)
j) Add 50 µL of the 2 M TMSD solution to each 5 mL sample extract
k) Place the tube containing the extract into a heating block at 50 °C
l) Heat the extract for one hour then allow the extract to cool to room temperature
m) Add 100 µL of 2 M acetic acid in methanol to react with any excess TMSD
n) Fortify the extract with 100 µL of ISS to yield a concentration of 0.020 µg/mL
o) Sample(s) are ready for GC/ECD analysis

## Gas Chromatography Analysis

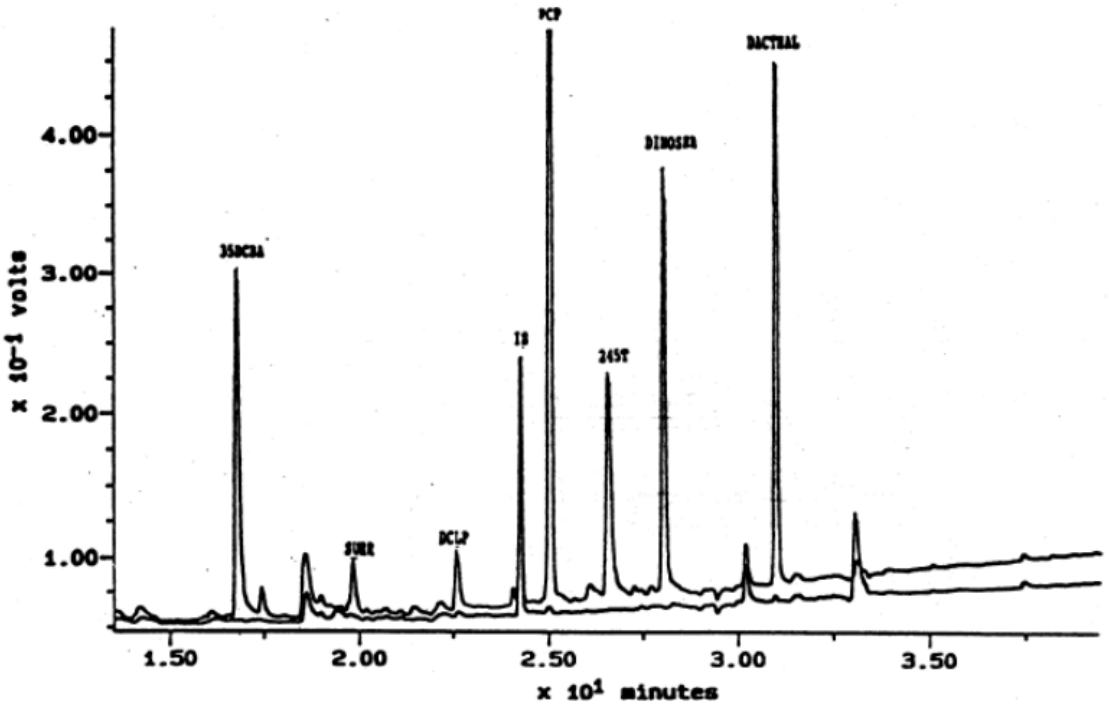
Analytical system complete with gas chromatograph and data system equipped with ECD, split/splitless capillary injector, temperature programming, differential flow control and all required accessories. An autoinjector is recommended to improve precision of analysis.

| GC Columns and Operating Conditions | |
|---|---|
| **Primary Column** | 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** | 30 cm/sec. at 200°C and 10 psi<br>**2 µL splitless injection**, purge on three minutes |
| **Program** | Hold at 60°C one minute, increase to 260°C at 5°C/min., hold five minutes |
| **Confirmation Column** | 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** | 30 cm/sec. at 200°C and 10 psi<br>**2 µL splitless injection**, purge on three minutes |
| **Program** | Hold at 60°C one minute, increase to 260°C at 5°C/min., hold five minutes |

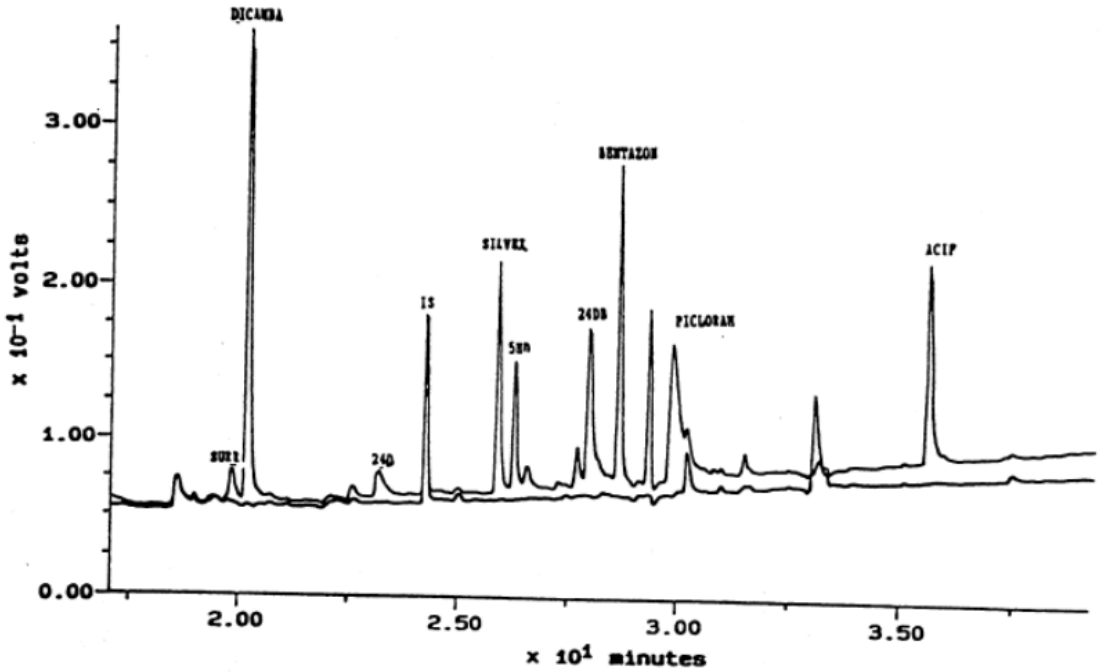
### Diazomethane Generator



## Chromatogram of Group A analytes extracted from ozonated surface water. Bottom chromatogram is laboratory blank




## Chromatogram of Group B analytes extracted from ozonated surface water. Bottom chromatogram is laboratory blank



## References:

[1] Summarized from, Method 515.2, "Determination Of Chlorinated Acids In Water Using Liquid-Solid Extraction And Gas Chromatography With An Electron Capture Detector", Revision 1.1, 1995, National Exposure Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH

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UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 800.385.3153 • 215.781.9255
www.unitedchem.com Email: methods@unitedchem.com



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