**2,4-D**  
\[ \text{C}_8\text{H}_6\text{Cl}_2\text{O}_3 \]  
MW: 221.04  
CAS: 94-75-7  
RTECS: AG6825000

**METHOD:** 5001, Issue 2  
**EVALUATION:** FULL  
**Issue 1:** 15 February 1984  
**Issue 2:** 15 August 1994

**OSHAA:** 10 mg/m³  
**NIOSH:** 10 mg/m³  
**ACGIH:** 10 mg/m³  
**PROPERTIES:** solid; MP 138 °C; VP not significant

**SYNONYMS:** 2,4-D: (2,4-dichlorophenoxy)acetic acid; hedonal; Trinoxol

## SAMPLING

<table>
<thead>
<tr>
<th><strong>SAMPLER:</strong></th>
<th>FILTER (glass fiber, binderless)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FLOW RATE:</strong></td>
<td>1 to 3 L/min</td>
</tr>
<tr>
<td><strong>VOL-MIN:</strong></td>
<td>15 L @ 10 mg/m³</td>
</tr>
<tr>
<td><strong>-MAX:</strong></td>
<td>200 L</td>
</tr>
<tr>
<td><strong>SHIPMENT:</strong></td>
<td>routine</td>
</tr>
<tr>
<td><strong>SAMPLE STABILITY:</strong></td>
<td>at least 1 week @ 25 °C</td>
</tr>
<tr>
<td><strong>BLANKS:</strong></td>
<td>2 to 10 field blanks per set</td>
</tr>
</tbody>
</table>

## MEASUREMENT

<table>
<thead>
<tr>
<th><strong>TECHNIQUE:</strong></th>
<th>HPLC, UV DETECTION</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ANALYTE:</strong></td>
<td>2,4-D anion</td>
</tr>
<tr>
<td><strong>DESORPTION:</strong></td>
<td>15 mL CH₃OH; stand 30 min</td>
</tr>
<tr>
<td><strong>INJECTION VOLUME:</strong></td>
<td>50 µL</td>
</tr>
<tr>
<td><strong>ELUENT:</strong></td>
<td>0.001 M NaClO₄-0.001 M Na₂B₄O₇</td>
</tr>
<tr>
<td><strong>FLOW RATE:</strong></td>
<td>1.7 mL/min</td>
</tr>
<tr>
<td><strong>DETECTOR:</strong></td>
<td>UV @ 284 nm</td>
</tr>
<tr>
<td><strong>COLUMN:</strong></td>
<td>stainless steel, 50 cm x 2-mm ID, packed with Zipax SAX (DuPont) ambient temperature; 6900 kPa (1000 psi)</td>
</tr>
<tr>
<td><strong>CALIBRATION:</strong></td>
<td>solutions of analyte in methanol</td>
</tr>
<tr>
<td><strong>RANGE:</strong></td>
<td>0.15 to 2 mg per filter</td>
</tr>
<tr>
<td><strong>ESTIMATED LOD:</strong></td>
<td>0.015 mg per filter [1]</td>
</tr>
<tr>
<td><strong>PRECISION (S_r):</strong></td>
<td>0.01 [1]</td>
</tr>
<tr>
<td><strong>ACCURACY:</strong></td>
<td>± 10.21%</td>
</tr>
</tbody>
</table>

**APPLICABILITY:** This method determines 2,4-D, 2,4,5-T, and their salts, but not their esters. The working range is 1.5 to 20 mg/m³ of either compound for a 100-L air sample.

**INTERFERENCES:** High concentrations of esters of either compound do not interfere but require the use of a pre-column to prevent degradation of the HPLC column.

**OTHER METHODS:** This method combines and replaces Methods S279 [3] and S303 [3] which are the same except for eluent composition and UV detector wavelength.
REAGENTS:

1. 2,4-dichlorophenoxyacetic acid.*
2. 2,4,5-trichlorophenoxyacetic acid.*
3. Methanol, HPLC grade.
4. LC eluent:
   a. 2,4-D: 0.001 N NaClO₄ and 0.001 N Na₂B₄O₇. Add 0.122 g NaClO₄ and 0.381 g Na₂B₄O₇·10H₂O to a 1-L volumetric flask. Bring to volume with distilled water. Mix, filter and de-gas the solution.
   b. 2,4,5-T: 0.003 M NaClO₄ and 0.001 M Na₂B₄O₇·10H₂O. Add 0.366 g NaClO₄ and 0.381 g Na₂B₄O₇·10H₂O to a 1-L volumetric flask. Bring to volume with distilled water. Mix, filter and de-gas the solution.
5. Compressed, filtered air or nitrogen for drying syringes.
7. Acetone.
8. Calibration stock solution, 400 µg/mL. Dissolve 0.400 g 2,4-D or 2,4,5-T in methanol and dilute to 1 L with methanol.
9. Recovery stock solution:
   a. Dissolve 0.248 g 2,4-D in ethanol. Dilute to 10 mL with ethanol.
   b. Dissolve 0.250 g 2,4,5-T, triethylamine salt, in acetone (or 0.250 g 2,4,5-T in methanol). Dilute to 10 mL with acetone.
   NOTE: Use the same form (e.g., acid or salt) of 2,4,5-T as in the air sample. Recovery may vary with the chemical form.

* See SPECIAL PRECAUTIONS.

EQUIPMENT:

1. Sampler: filter, glass fiber, binderless, in a 37-mm polystyrene two-piece cassette filter holder (Gelman type AE or equivalent).
2. Personal sampling pump, 1 to 3 L/min, with flexible connecting tubing.
3. High pressure liquid chromatograph, UV detector at 284 nm (2,4-D) and 289 nm (2,4,5-T), integrator and column (page 5001-1).
4. Filter, PTFE, 5-µm, 13-mm diameter in Swinny stainless (13-mm) filter holder.
5. Tweezers.
6. Syringes, 20-mL luer-lock.*
7. Vials, glass, 20-mL.*
8. Volumetric flasks, convenient sizes for preparing standard solutions.*

* Wash all glassware with detergent, thoroughly rinse with tap water and distilled water.

SPECIAL PRECAUTIONS: 2,4-D and 2,4,5-T are suspected animal carcinogens [4]. 2,3,7,8-Tetrachlorodibenzo-1,4-dioxin has been identified as an impurity in 2,4,5-T. Avoid any contact with these substances.
SAMPLE PREPARATION:

4. Remove the filter from the cassette with clean tweezers and place it in a 20-mL vial.
5. Add 15 mL methanol and mix by swirling. Allow to stand at least 30 min.
6. Filter the sample.
   a. Pour the sample solution into a 20-mL syringe which is fitted with a 5-µm PTFE filter.
   b. Filter the sample into a clean vial.
   c. Clean the PTFE filter by backflushing with methanol. Rinse the syringe and plunger with methanol. Dry with air or nitrogen.

CALIBRATION AND QUALITY CONTROL:

7. Calibrate daily with at least five working standards.
   a. Dilute aliquots of calibration stock solution to 10 mL with methanol in volumetric flasks.
   b. Analyze working standards (steps 9 and 10).
   c. Prepare calibration graph (peak area vs. mg 2,4-D or mg 2,4,5-T).
8. Check recovery with at least four spiked media blanks at each of four levels.
   a. Add aliquot of recovery stock solution to media blank.
   b. Analyze using standards prepared from the recovery stock solution.
   c. Calculate R (mg recovered/mg added).

MEASUREMENT:

9. Establish chromatographic conditions listed on page 5001-1 for either 2,4-D or 2,4,5-T.
10. Inject 50 µL of sample in duplicate. Rinse and dry the syringe between samples.
    NOTE 1: The analyte is the chlorinated phenoxyacetate, whether the air sample contained salts or free acid forms of 2,4-D and 2,4,5-T.
    NOTE 2: Esters of 2,4-D and 2,4,5-T will not elute from the HPLC column and may, if present in large amounts, degrade the HPLC column. Protect the main column with a precolumn of Zipax SAX if esters are known to be present. The sample preparation conditions are sufficiently mild so as to preclude hydrolysis of the esters.

CALCULATIONS:

11. Read the mass of analyte, mg (corrected for recovery), in the sample (W) and average media blank (B) from the calibration curve.
12. Calculate the concentration, C (mg/m³), of 2,4-D or 2,4,5-T in air volume, V (L), taken:
    \[ C = \frac{(W - B) \times 10^3}{V}, \text{mg/m}^3. \]

EVALUATION OF METHOD:

Methods S279 (2,4-D) and S303 (2,4,5-T) were issued on February 17, 1978, and March 17, 1978, respectively [3], and validated using 100-L air samples [1,2,5]. Atmospheres were generated using 2,4-D dimethylamine salt for S279 and Weedar Amine BK (Amchem; equal parts of 2,4-D dimethylamine salt and 2,4,5-T triethylamine salt) for S303. Overall precision and recovery for 100-L samples were as shown, representing non-significant bias in each method:
### REFERENCES:


### METHOD REVISED BY:

Robert W. Kurimo, NIOSH/DPSE; originally validated under NIOSH Contract No. 210-76-0123.