**ALACHLOR in Air**

C\(_{14}\)H\(_{20}\)O\(_2\)NCl  
MW: 269.6  
CAS: 15972-60-8  
RTECS: AE1225000

**METHOD:** 5603, Issue 1  
**EVALUATION:** FULL  
**Issue 1: 15 January 1998**

**OSHA:** no PEL  
**NIOSH:** no REL  
**ACGIH:** no TLV  

**PROPERTIES:** colorless crystalline solid; d 1.133 g/mL @ 25 °C; MP 39.5 to 41.5 °C; VP 0.0029 Pa (2.2 x 10\(^{-5}\) mm Hg) @ 25 °C; solubility in water 242 mg/L @ 25 °C.

**SYNONYMS:** 2-Chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide; Lasso; Alanex; Alanox; Chimiclor

**SAMPLING**

| **SAMPLER:** | SOLID PHASE EXTRACTION (SPE) DISK  
(Styrene divinyl benzene [SDB]-xc disk) |
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>FLOW RATE:</strong></td>
<td>1 L/min</td>
</tr>
<tr>
<td><strong>VOL-MIN:</strong></td>
<td>70 L</td>
</tr>
<tr>
<td><strong>-MAX:</strong></td>
<td>1750 L</td>
</tr>
<tr>
<td><strong>SHIPMENT:</strong></td>
<td>routine</td>
</tr>
</tbody>
</table>
| **SAMPLE STABILITY:** | at least 7 days at 25 °C  
at least 21 days at 4 °C |
| **BLANKS:** | 2 to 10 field blanks per set |

**MEASUREMENT**

<table>
<thead>
<tr>
<th><strong>TECHNIQUE:</strong></th>
<th>ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ANALYTE:</strong></td>
<td>Alachlor</td>
</tr>
<tr>
<td><strong>EXTRACTION:</strong></td>
<td>2 mL methanol</td>
</tr>
<tr>
<td><strong>WAVELENGTH:</strong></td>
<td>450 nm</td>
</tr>
<tr>
<td><strong>CALIBRATION:</strong></td>
<td>standard solutions of alachlor in ≤1% methanol in water</td>
</tr>
<tr>
<td><strong>RANGE:</strong></td>
<td>20 to 500 ng per sample [1]</td>
</tr>
<tr>
<td><strong>ESTIMATED LOD:</strong></td>
<td>9 ng per sample [1]</td>
</tr>
<tr>
<td><strong>PRECISION ((\delta_r)):</strong></td>
<td>0.072 [1]</td>
</tr>
</tbody>
</table>

**ACCURACY**

| **RANGE STUDIED:** | 0.24 to 120 µg per sample |
| **BIAS:** | 0.0368 |
| **OVERALL PRECISION (\(\delta_r\)):** | 0.078 (12 µg to 48 µg) [1] |
| **ACCURACY:** | ± 17.4% |

**APPLICABILITY:** This method provides a quantitative evaluation of alachlor in air utilizing ELISA technology designed to determine the concentration of alachlor in water. The limit of quantitation for the overall method is 20 ng/sample because of the need to negate the interferences of methanol in the ELISA. The assay is simple and adaptable enough that it may be used on site in the field as a screening method.

**INTERFERENCES:** 2-[(2,6-diethyl-phenyl)(methoxymethyl)amino]-2-oxoethane sulfonic acid (ESA) cross reacts with alachlor in the ELISA. ESA is not detected by standard HPLC or GC [2]. Alachlor related acetanilides may interfere with the determination of alachlor, such as acetochlor, metolachlor, and metalaxyl. The ELISA is also sensitive to the concentration of organic solvent remaining in the assay from solid phase extraction.

**OTHER METHODS:** Other ELISA kits may be used in a similar manner. Alachlor also is included in Method 5602, Chlorinated and Organonitrogen Herbicides in Air by GC/ECD [3].
REAGENTS:

1. Alachlor*
2. Methanol*, pesticide analytical grade
3. Deionized water
4. Alachlor plate kit, EnviroGard™, or equivalent (e.g., Ohmicron RaPID Assay® or Ensys RIS®).
5. Alachlor stock solution, 1000 µg/mL. Add 10 mg pure alachlor in 10 mL of pesticide free methanol. Store in freezer.

* See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Sampler: Solid phase extraction (SPE) disk, 25-mm, SDB-xc (3M Empore™, or equivalent), cellulose backup pad in 3-piece cassette with 50-mm extension.
2. Personal sampling pump, 1 L/min, with flexible connecting tubing.
3. Microtiter plate reader, 450 nm, 650 nm backup.
4. Glass bottles, wide-mouth, ~1-oz. (diameter large enough to lay 25-mm disk flat), with PTFE-lined caps.
5. Vials, glass, 4-mL, with PTFE-lined caps.
6. Platform orbital shaker.
7. Timer.
9. Pipettor, positive displacement.
11. Aerosol resistant tips (optional).
13. Forceps
15. Laboratory towels.
16. Parafilm

SPECIAL PRECAUTIONS: NIOSH researchers have classified alachlor as a Group I compound, which is indicative of its adverse acute health effects from exposure to low concentrations. Handle carefully. Wear gloves and protective clothing. Methanol is flammable and moderately toxic by ingestion and inhalation.

SAMPLING:

1. Assemble each sampler in a clean environment.
2. Calibrate each personal sampling pump with a representative sampler in line.
3. Sample at an accurately known flow rate of ~1 L/min. The sampling period depends on suspected concentration.
4. Seal both ends of the sampler with plugs and pack securely for shipment, including the appropriate number of blanks.

SAMPLE PREPARATION:

5. Using forceps, remove the SDB disk from the sampler and place in a glass bottle with an ID large enough to allow the filter to lie flat.
   a. Add 2.0 mL of pesticide free methanol to the SDB disk, cap, and shake on a platform orbital shaker for 15 minutes.
   b. Transfer the methanol solution to a 4-mL sample vial with a PTFE-lined cap.
   c. Using a positive displacement pipettor or syringe, remove an aliquot of the sample and dilute it a minimum of 1:100 with deionized water.

   NOTE: If concentrations are higher than 0.5 µg/sample, further dilutions are needed to meet the target range of the assay. Multiple dilutions may be done if the concentration range is unknown.
d. Wrap remainder of the samples with parafilm and store refrigerated.
e. Run the diluted samples in the assay.

6. Follow the general instructions of the EnviroGard Alachlor plate kit [4]. Adjust as needed to accommodate sample needs.
   a. Determine the number of microtiter plate wells needed to run in duplicate the kit negative control and 3 kit standards, the matrix negative control and 3 matrix standards, the samples, and an alachlor fortified control for each set of 3 strips. Remove the number of well strips needed (twelve wells per strip). Refrigerate the unused strips in the resealable plastic bag containing a desiccant provided in the kit.
   b. Bring the well strips and the remaining contents of the ELISA assay to ambient temperature before beginning the assay.
   c. Using a pipettor with aerosol resistant tips, in an orderly fashion, add 80 µL aliquots of the standards, samples, and controls to the microtiter plate wells in duplicate.
      NOTE: The ELISA assay is a time dependent matrix. Add reagents in a consistent, uninterrupted manner to avoid variability and a drift effect over the plate.
   d. In the same order the standards and samples were added to the plate, add 2 drops of enzyme conjugate from the enzyme conjugate bottle.
      NOTE: If more than 3 rows of wells are to be employed, use a multiwell pipettor. If the dropper is used, ensure that the drops are free falling and do not touch the sides of the well.
e. Carefully mix the contents of the wells with circular motions.
   f. Cover the plate with parafilm. Incubate for 1 hour at ambient temperature with mechanical mixing on an orbital mixer or microtiter plate shaker. If necessary, sedentary incubation may be used.
   g. Wash the plate with tap or deionized water 6 times with a microtiter plate washer or manually. If done manually, vigorously flick the contents of the plate into a sink, flood the plate with water, and empty the water with a flick of the wrist. Repeat 6 times.
   h. Invert the plate over a laboratory towel to remove the excess water.
   i. As in the previous order of addition, add 80 µL (2 drops) of substrate, and add 40 µL (1 drop) of chromogen from kit.
      (1). If less than 3 or 4 well strips are used, substrate and chromogen may be added separately.
      (2). If more, freshly mix a 2:1 volume of the substrate and chromogen, respectively, and add 120 µL of the mixture to each well.
      NOTE: Use care to prepare sufficient reagent mixture to add to the contents of the wells plus a small reservoir so as not to interrupt the addition of the reagents. Any interruption in the addition of reagents may affect the color development relative to the standard curve. Discard the excess.
   j. Mix, cover with clean parafilm, and incubate the plate as before for 30 minutes.
   k. After incubation, add 40 µL (1 drop) of the stop solution (from the kit) to each well, and mix thoroughly until all of the blue has converted to yellow.
      NOTE: Plate must be read within 30 min after adding stop solution.

CALIBRATION AND QUALITY CONTROL:

7. Check the overall performance of the microplate reader according to the manufacturer’s instructions.
8. Prepare a calibration curve, using three standards: 100, 500, 2500 ng/L, each time the assay is run.
   To prepare the working standards in the appropriate matrix:
   a. Serially dilute an aliquot of the alachlor stock solution in a volume of matrix similar to the sample matrix yielding a 50,000 ng/L. If the methanol concentration of the samples is less than 1%, water may be used. However, the higher the methanol concentration of the samples, the more desirable it is to use the same % of methanol standards as in the samples.
   b. Using the 50,000 ng/L solution, prepare the working standards 100 ng/L, 500 ng/L and 25000 ng/L, in the matrix solvent.
9. Run controls, 1 for every three rows of the assay to determine possible drift.
10. Zero the reader against air or deionized water before reading the assay.
11. Determine the concentration of alachlor using the data reduction capabilities of the microtiter plate reader, or appropriate software. Use a semi-log or 4 parameter logit-log curve fit to the standard curve. Make the appropriate dilution corrections.
12. If data reduction capabilities are not available, perform the calculations as follows:
a. Average the optical density (OD) for the negative control.
b. Average the OD for each of the standards in the curve.
c. Determine the percent binding (%B) of the standards by:
   \[ \%B_s = \frac{\text{standard average}}{\text{negative control average}} \times 100 \]
d. Determine the %B of the samples by:
   \[ \%B_s = \frac{\text{sample average}}{\text{negative control average}} \times 100 \]
e. Prepare a calibration graph, %B vs. log concentration (ng/L) of alachlor.

13. Follow the EnviroGard alachlor assay guidelines to ensure accurate results [4].
   a. The coefficient of variation for optical density (OD) of each standard in the curve is 15%.
   b. The percent binding (%B) for each standard in the curve should also fall within the following ranges.
      - 100 µg/mL: 64-86%
      - 500 µg/mL: 33-55%
      - 2500 µg/mL: 11-21%
   NOTE: See the certification sheets of particular lots of kits provided with the assay.

MEASUREMENT:

14. Set wavelength on microtiter plate reader to 450 nm, and reference wavelength to 600 or 650 nm.
15. Zero the microtiter plate reader against air, or 200 µL of water in a blank well. Within 30 minutes of
adding the stop solution, read the plates at 450 nm.

CALCULATIONS:

16. Record the actual solution volumes to which the samples, \( V_s \) (L), and blanks, \( V_b \) (L), were diluted.
17. From the calibration graph, determine the concentration of the sample solutions of the samples,
\( C_s \) (ng/L), and media blanks, \( C_b \) (ng/L).
   NOTE: Blanks generally fall below the range of the calibration graph and are not included in the
calculations. Variable readings for the blanks indicate a problem with the assay.
18. Using the solution volume of the samples, \( V_s \) (L), calculate the concentration, \( C \) (mg/m³), of alachlor
in the volume of air sampled, \( V_{air} \) (L):
   \[ C = \frac{C_s V_s \cdot 10^{-3}}{V_{air}}, \text{mg/m}^3 \]
   NOTE: µg/L = mg/m³

EVALUATION OF METHOD:

This method was evaluated with fortified SDB-xc extraction disks that had clean humidified air passed
through them. The volume of air to be sampled was based on air levels found in a NIOSH field survey of
herbicide applicators [5], and a 1:100 dilution factor in the assay. If sampling is done for longer periods of
time, further dilution of the samples may be necessary. The method was evaluated over the range of 0.24
µg to 120 µg per sample. The sample precision was not statistically poolable over the entire range of the
levels studied. However, they were poolable at the 12-, 24-, and 48-µg range. The overall precision for
these samples was 0.0783, with an accuracy estimate of ±17.4%. The precision, bias, and accuracy at
each level studied also were determined (See Table 1). The average recovery of all levels was 100%.
Samples are stable for at least 7 days at ambient temperature and 30 days refrigerated. Breakthrough was
not evident at a capacity of 144 µg.
REFERENCES:


METHOD WRITTEN BY: Deborah Sammons and Eugene Kennedy, Ph.D., NIOSH/DPSE

TABLE 1. METHOD SUMMARY

<table>
<thead>
<tr>
<th>Arbitrary Exposure Limit (1 mg/m³)</th>
<th>Test Average (µg/sample)</th>
<th>Independent Average (µg/sample)</th>
<th>Precision (sampling and analysis)</th>
<th>Bias [(test/indep) -1]</th>
<th>Calculated Accuracy (± %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01x</td>
<td>0.259</td>
<td>0.24</td>
<td>0.109</td>
<td>0.080</td>
<td>27.7</td>
</tr>
<tr>
<td>0.1x</td>
<td>2.17</td>
<td>2.4</td>
<td>0.170</td>
<td>-0.094</td>
<td>35.3</td>
</tr>
<tr>
<td>0.5x</td>
<td>12.7</td>
<td>12.0</td>
<td>0.081</td>
<td>0.059</td>
<td>23.0</td>
</tr>
<tr>
<td>1x</td>
<td>23.9</td>
<td>24.0</td>
<td>0.063</td>
<td>-0.003</td>
<td>13.2</td>
</tr>
<tr>
<td>2x</td>
<td>48.9</td>
<td>48.0</td>
<td>0.090</td>
<td>0.019</td>
<td>18.4</td>
</tr>
<tr>
<td>5x</td>
<td>128.4</td>
<td>120</td>
<td>0.122</td>
<td>0.070</td>
<td>29.1</td>
</tr>
</tbody>
</table>

a Theoretical Exposure Concentrations were based on the NIOSH RELs of similarly classified pesticides.

b Precision is not statistically poolable.