Degradation dynamics of an oxime carbamate insecticide (methomyl) in aqueous medium of varying pH under laboratory simulated condition

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**ABSTRACT:** Laboratory degradation studies were performed in water at pH 4.0, 7.0 and 9.2 using methomyl (Lannate 12.5 L) formulation at the rates of 1.0 (T\textsubscript{1}) and 2.0 (T\textsubscript{2}) \mu gmL\textsuperscript{-1}. Water samples collected on 0 (2 h), 3, 7, 15, 30, 45, 60 and 90 days after treatments were processed for residue analysis of methomyl by HPLC equipped with Photo Diode Array Detector. In 60 days, dissipation was 84-87\% at pH 4.0, 71-77\% at pH 7.0, and 91-93\% at pH 9.2 in both treatments showing very little effect of pH on dissipation. The half-life periods observed were 20.76 and 22.13 days at pH 4.0, 27.87 and 28.67 days at pH 7.0 and 15.84 and 16.54 days at pH 9.2 at T\textsubscript{1} and T\textsubscript{2} doses respectively.

**Keywords:** methomyl; pH; dissipation; kinetics; insecticide

**Introduction**

Methomyl S-methyl N-[(methylcarbamoyl)oxy]-thioacetimidate [C\textsubscript{5}H\textsubscript{10}N\textsubscript{2}O\textsubscript{2}S] is an insecticide of oxime carbamate group (Fig. 1). The compound was introduced in 1966 as a broad spectrum insecticide. It is mainly used to control the larvae of the diamondback moth, *Plutella xylostella* (L) and its two major parasitoids, *Cotesia plutellae* (Kurdjumov) and *Oomyzus sokolowskii* (Kurdjumov). It is also used as an acaricide to control ticks and
spiders. It is used for foliar treatment of vegetable, fruit and field crops, cotton, commercial ornamentals, and in and around poultry houses and dairies. It is also used as fly bait. It is effective in two ways: (a) as a "contact insecticide," because it kills target insects upon direct contact, and (b) as a "systemic insecticide" because of its capability to cause overall "systemic" poisoning in target insects, after it is absorbed and transported throughout the pests that feed on treated plants. It is capable of being absorbed by plants without being "phytotoxic" or harmful, to the plant [1-3].

![Chemical structure of methomyl.](image)

Methomyl has low persistence in the soil environment and possesses the risk of groundwater contamination because of its high solubility in water [4]. Additionally, it is moderately to highly toxic to fish and highly toxic to aquatic invertebrates [4-6]. Therefore, persistence study of methomyl in soil-solution and pure water system has earned importance in respect of its possible food chain contamination. pH is a prime factor controlling dissipation, transformation and fate of pesticides in soil-water system. The present dissipation study of methomyl in water, has therefore, been carried out at different pH levels. Methomyl residue at different days interval was determined by reversed-phase high-performance liquid chromatography with fluorescence detection after postcolumn derivatization. The separation of carbamates is performed on a C8 column with water-methanol as mobile phase [7, 8].

**Material and Methods**

**Chemicals and reagents**

The certified reference standards of methomyl were purchased from Dr. Ehrenstorfer GmbH, Germany, and were of >99% purity. All the solvents were of HPLC grade or equivalent quality. Methomyl formulation (Lannate 12.5 L) was procured from local market. Sodium sulphate was washed repeatedly with distilled acetone and activated at 110 °C for 2 h before use. Stock solution (100 µg mL⁻¹) was prepared in methanol and working solution was prepared by diluting it.

**Preparation of matrices and application of chemical**

The pH of water was adjusted using buffer. Buffer capsules of pH 4.0, 7.0 and 9.2 from E. Merck were used for the purpose of preparing buffer solution. One capsule was required for 100 mL of distilled water to maintain the above mentioned pH. In a series of
Winchester bottles (10 L capacity) 6 L distilled water was kept and sixty capsules were added to each of the bottle. The bottles were then left in room temperature for overnight to homogenize the buffer solutions. For carrying out laboratory experiment, water (6 L) of each pH triplicate was spiked at the rate of 1.0 (T₁) and 2.0 (T₂) μg of a.i. per mL with methomyl formulation and stored in Winchester bottles from October to December, 2009 under room temperature (15-39.5 °C). Untreated control was also carried out simultaneously. Samples were drawn periodically on 0 (2 h), 3, 7, 15, 30, 45, 60, and 90 days after treatments and analyzed for methomyl residues.

**Extraction and clean-up of residues**

Representative 200 mL water sample was taken in 1 L separating funnel and 5-10 g sodium chloride was added to it. It was extracted thrice (100, 50, 50 mL) with dichloromethane by liquid-liquid partitioning. Organic phases were combined, passed through over sodium sulphate and concentrated on a rotary vacuum evaporator under reduced pressure at 40 °C followed by a gas manifold evaporator till near dryness. Final solution was made to 1 mL in methanol and subjected to HPLC analysis. No clearing was required as no interference peaks were observed during analysis.

**Estimation of residues**

Final analysis of methomyl in water samples were done by HPLC (Shimadzu Model No. SPD M 10 A) equipped with Photo Diode Array Detector. The C-18 reverse phase column (15 cm x 4.6 mm i.d.) along with guard column was used. The mixture of acetonitrile and water (4:6 v/v) was used as mobile phase for the detection of methomyl residue. The other parameters like flow rate, wave length (λ_max), retention time were 1 mL/min, 240 nm and 3.55±0.2 min, respectively (Fig. 2).

![Figure 2. HPLC chromatogram of methomyl in substrate.](image)

**Confirmation of the residues by LC-MS/MS**
The substrates were extracted using the above stated method and finally subjected to analysis in LC-MS (Quattro Micro system) coupled with Waters 2695 separation module. Detection as well as confirmation was carried out by Quattro Micro (Tandem mass spectrometry detector) fitted with an electro spray ionization source (ESI). The MS parameters were optimized in direct infusion mode using Mass Lynx Auto tune software program. The Ion Mode, Inter Channel Delay (s), Inter Scan Time (s), Span (Da), Dwell time (s), Cone (V), Collision Energy (eV), Molecular Ion (m/z) and Daughter Ion (m/z) are MRM, ES+, 0.010, 0.010, 0.2, 0.1, 17.00, 7.00, 163.00 and 87.60, respectively (Fig. 3).

Column: 100 x 2.1 mm; Symmetry ® C₁₈, 5 µ (RPC)

Mobile phase: Methanol/water (90:10, v/v) + 5 mM ammonium acetate

Flow rate: 0.3 mL/min.

Retention time: 3.39 ± 0.05 min.

![Figure 3. LC-MS/MS MRM transition for methomyl.](image)

**Calculation of residues**

The residue content was calculated by using the formula

\[
\text{Residue (in } \mu\text{g}^{-1}) = \frac{A_1 \times C \times V_1}{A_2 \times W \times V_2} \times R_f
\]

Where,

\(A_1\) = Area of methomyl from sample, in chromatogram.

\(A_2\) = Area of methomyl from standard, in chromatogram.

\(V_1\) = Total volume of sample (in mL).
C = Concentration of analytical standard in ppm (µg/ml) \times µL injected.

W = Weight of the final working sample drawn from laboratory sample obtained from raw sample after quartering technique (in g).

V_2 = Injected volume of sample (in µL).

R_f = Recovery factor.

Linearity was evaluated by linear regression analysis.

The residue data were subjected to regression analysis and the fit of the data to first order kinetics (C_t = C_0e^{-kt}) was confirmed by testing the statistical significance of correlation coefficient. The half-life values were calculated from dissipation constant calculated from regression analysis.

The calibration curves for the compounds were obtained by plotting the peak area against the concentration of the corresponding calibration standards. The limit of detection (LOD) of the test compounds was determined by considering a signal-to-noise ratio of 3 with reference to the background noise obtained for the blank sample, whereas the limits of quantification (LOQ) were determined by considering a signal-to-noise ratio of 10.

**Results and Discussion**

**Recovery Study and Method Validation**

To work out the extraction efficiency of methods employed for methomyl from aqueous phase of varying pH were spiked in triplicate at three different levels (i.e. at the rate of 0.5, 1.0 and 5.0 µg mL\(^{-1}\)) with the above mentioned insecticide. Average recoveries of methomyl from water varied from 86.0-89.6% at pH 4.0, 84.0-88.8% at pH 7.0 and 86.6-92.4% at pH 9.2 (Table 1). The linearity of the calibration curve was the range 0.05–2.00 µg mL\(^{-1}\) for methomyl with a correlation coefficient (R\(^2\)) of the calibration curve of >0.99. For matrix calibration, the R\(^2\) was also >0.99 for methomyl. The LOD and LOQ of the compound were 0.02 and 0.05 µg mL\(^{-1}\), respectively. The coefficients of variation regarding the repeatability and intermediate precision for methomyl at 0.5 µg mL\(^{-1}\) were 2.5% and 6.5%, respectively.

**Effect of pH**

As evident from the data in Table 2, at pH 4.0, initial residues of 0.96 µg mL\(^{-1}\) in treatment T\(_1\) dissipated to 0.86 µg mL\(^{-1}\) in 3 days, 0.45 and 0.15 µg mL\(^{-1}\) in 30 and 60 days, respectively. Corresponding dissipation were 10.07, 53.47 and 84.03%, respectively. In treatment T\(_2\), initial residues of 1.96 µg mL\(^{-1}\) dissipated to 1.72, 0.83 and 0.26 µg mL\(^{-1}\) in 3, 30 and 60 days after application with corresponding dissipation of
At pH 7.0, initial residues of 0.97 μgL⁻¹ in T₁ treatment, dissipated to 0.87, 0.56 and 0.28 μgL⁻¹ in 3, 30 and 60 days after treatment with corresponding dissipation of 10.07, 42.42 and 71.41% respectively, whereas in T₂ initial residues of 1.98 μgL⁻¹ dissipated to 1.80, 0.92 and 0.45 μgL⁻¹ in 3, 30 and 60 days after treatment showing...
dissipation of 9.26, 53.54 and 77.27%, respectively (Table 3).

Table 3. Dissipation of methomyl residues in water at pH 7.0

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>1.0 μg mL⁻¹</th>
<th>2.0 μg mL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R₁ R₂ R₃</td>
<td>Mean (± SD)</td>
</tr>
<tr>
<td>0</td>
<td>0.98 0.96 0.97</td>
<td>0.97 (±0.010)</td>
</tr>
<tr>
<td>3</td>
<td>0.88 0.85 0.89</td>
<td>0.87 (±0.021)</td>
</tr>
<tr>
<td>7</td>
<td>0.76 0.80 0.74</td>
<td>0.76 (±0.031)</td>
</tr>
<tr>
<td>15</td>
<td>0.71 0.68 0.66</td>
<td>0.69 (±0.026)</td>
</tr>
<tr>
<td>30</td>
<td>0.54 0.59 0.55</td>
<td>0.56 (±0.026)</td>
</tr>
<tr>
<td>45</td>
<td>0.43 0.36 0.38</td>
<td>0.39 (±0.037)</td>
</tr>
<tr>
<td>60</td>
<td>0.22 0.29 0.33</td>
<td>0.28 (±0.055)</td>
</tr>
<tr>
<td>90</td>
<td>0.09 0.12 0.09</td>
<td>0.10 (±0.017)</td>
</tr>
</tbody>
</table>

At pH 9.2, from treatment T₁ initial residues of 0.97 μg mL⁻¹ dissipated to 0.69, 0.52 and 0.17 μg mL⁻¹ in 3, 15 and 45 days after treatment with corresponding dissipation of 28.77, 46.09 and 82.12%, respectively. In T₂ residues of 1.96 μg mL⁻¹ on 0 (2 h) day dissipated to 1.56, 1.14 and 0.30 in 3, 15 and 45 days after treatment and corresponding dissipation was 20.43, 42.69 and 84.52%, respectively (Table 4). The Regression equation, Correlation Co-efficient and half-life for the dissipation of methomyl in water at different pH are presented at Table 5.

It is clear that methomyl residues in water dissipated more than 80% in 60 days at pH levels of 4.0, 7.0 and 9.2 in both treatments under laboratory conditions under temperature ranging from 15 to 39.5 °C. The degradation was slow during first 3 days followed by relatively faster degradation from 3rd day onward to 45 days after which it became slow (figures 4-6).

Almost identical degradation ranging from 71.41 to 93.03% at all the three pH levels during 60 days study indicate that there was no significant effect of pH on degradation, although dissipation was a little faster at pH 9.2 throughout the studies (Fig. 6). Half-life values at pH levels of 4.0, 7.0 and 9.2 varied from 22.35 to 21.76, 27.87 to 28.67 and 15.84 to 16.54 days, respectively. At all pH levels degradation was observed to be faster in T₁ than T₂. The slow dissipation at higher rate could attribute to inhibition of microbial activity.
Figure 4. Dissipation of methomyl at pH 4.0 (ppm = μg mL⁻¹)

Table 4. Dissipation of methomyl residues in water at pH 9.2

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>1.0 μg mL⁻¹</th>
<th>2.0 μg mL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R₁</td>
<td>R₂</td>
</tr>
<tr>
<td>0</td>
<td>0.98</td>
<td>0.96</td>
</tr>
<tr>
<td>3</td>
<td>0.70</td>
<td>0.67</td>
</tr>
<tr>
<td>7</td>
<td>0.60</td>
<td>0.63</td>
</tr>
<tr>
<td>15</td>
<td>0.54</td>
<td>0.52</td>
</tr>
<tr>
<td>30</td>
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<td>0.38</td>
</tr>
<tr>
<td>45</td>
<td>0.19</td>
<td>0.16</td>
</tr>
<tr>
<td>60</td>
<td>0.09</td>
<td>0.10</td>
</tr>
<tr>
<td>90</td>
<td>BDL</td>
<td>BDL</td>
</tr>
</tbody>
</table>

BDL=Below Detectable Limit

Table 5. Regression equation, Correlation Co-efficient and half-life for the dissipation of methomyl in water at different pH

<table>
<thead>
<tr>
<th>pH</th>
<th>Treatments (μg mL⁻¹)</th>
<th>Regression Equation</th>
<th>Correlation co-efficient</th>
<th>Half-life (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>1.0</td>
<td>y = 2.991-0.013x</td>
<td>0.986</td>
<td>22.35</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>y = 3.308-0.013x</td>
<td>0.983</td>
<td>21.65</td>
</tr>
<tr>
<td>7.0</td>
<td>1.0</td>
<td>y = 2.997-0.010x</td>
<td>0.993</td>
<td>27.87</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>y = 3.286-0.010x</td>
<td>0.976</td>
<td>28.67</td>
</tr>
<tr>
<td>9.2</td>
<td>1.0</td>
<td>y = 2.942-0.015x</td>
<td>0.979</td>
<td>15.84</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>y = 3.291-0.018x</td>
<td>0.973</td>
<td>16.54</td>
</tr>
</tbody>
</table>
Figure 5. Dissipation of methomyl at pH 7.0 (ppm = µg/mL⁻¹)

Figure 6. Dissipation of methomyl at pH 9.2 (ppm = µg/mL⁻¹)

Similar observations have been reported for prchloraz [9] and bentiocarb [10] in water where dissipation was independent of pH and was subject to photodegradation following pseudo first order kinetics. Dissipation of butachlor residues has also been reported [11] following pseudo first order kinetics in river waters. Whereas in another studies decomposition of trifluralin and metribuzin in water as pH and dose dependent, resulted in faster dissipation under alkaline conditions and at low dose of application [12,
Slightly faster dissipation of methomyl at alkaline pH has been observed in our studies also. Considering rapid dissipation of Methomyl at the tested doses in water system, its much faster degradation can be expected under field condition.

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References and Notes