Bull. Environ. Contam. Toxicol. (2006) 77:516–520

DOI: 10.1007/s00128-006-1094-8

Bulletin of Environmental Contamination and Toxicology

Methomyl Residue on Chinese Cabbage Grown under Greenhouse Conditions

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Received: 3 May 2006/Accepted: 28 August 2006

Pesticides are widely used throughout the world to prevent crop losses before and after harvesting. The indiscriminate use of these chemicals can result in their bioaccumulation in food and subsequent bioconcentration through the food chain. N-methylcarbamate (NMC) insecticides are widely used to protect crops because it has been proven that they are highly toxic in insects but generally low in toxicity in warm-blooded species. Carbamates are also much less persistent than organochlorine pesticides and produce fewer toxic byproducts. Because carbamates inhibit acetylcholinesterase, however, they are considered toxic for the environment and for human beings (Hartley and Kidd 1983; Worthing 1983). These insecticides include methomyl a broad-spectrum insecticide (Hayes and Laws 1990) that is marketed commercially in Korea and used for foliar treatment of vegetables, fruits and field crops (Tomlin 2000). Methomyl is effective in 2 ways: (1) as a contact and systemic insecticide; and (2) for leaving a short-term residue on plants (McEwen and Stephenson 1979).

Brassica campestris (Chinese cabbage) has long been consumed as a staple food by Koreans in various forms of fresh, salted, or fermented as kimchi. To fulfill the off-season demand for this crop, it has become a common practice to cultivate *B. campestris* under greenhouse conditions. Because the pattern of dissipation for pesticide residue within or on the crops varies considerably under greenhouse conditions compared to open-air conditions, we studied changes in the amount of methomyl residue on Chinese cabbage when it is applied by folia spraying under greenhouse conditions.

MATERIALS AND METHODS

A standard methomyl (*S*-methyl [*EZ*]-*N*-[methylcarbamoyloxy] thioacetimidate) was provided by the National Agricultural Products Quality Management Services. Organic solvents (acetonitrile and dichloromethane) used for extraction were of pesticide grade and; when used for other purposes, they were of analytical grade (Baker, NJ, USA). Sodium sulfate, which was used for pesticide analysis, was of pesticide grade.

This study was carried out in a greenhouse at the College of Agriculture and Life Science at Chonnam National University in Gwangju, Korea, where one plot was

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used to grow controls and 2 plots, measuring 12.8 m x 3.5 m each, were used as test plots; the experiments in all 3 plots were replicated 3 times. No insecticide sprays were applied in the test plots before or during these experiments.

The commercial formulation of methomyl (Methoran® 24.1% EC, SM-BT, Seoul, Korea) was applied to cabbage at the recommended rate (20 ml/20 L; (T_1), at twice the recommended rate (40 ml/20 L water; (T_2), or not at all (controls; (T_3) using a mechanical sprayer. The 2 active treatments were carried out between April 16 and May 25, 2005.

Five cabbages (weight: approx. 0.4 kg each) were collected at random from each plot beginning 2 hours after methomyl application and each day for the following 10 days. All samples were chopped immediately after harvesting, mixed with other samples harvested from the same plot, packed separately in plastic bags, labeled, and stored at –20°C until analyzed.

A 20-g sample of cabbage was weighed and transferred into a 250 ml homogenizer cup with 100 ml of acetonitrile. The mixture was macerated at 10,000 rpm for 5 min in a high-speed homogenizer (WiseMix™ HG15A, Daihan Scientific, Seoul, Korea). The homogenate was filtered through Whatman filter paper (No. 6) and Celite 545 resting on a porcelain Büchner funnel. The filtrate was then transferred into a 250 ml sampling bottle and evaporated until 20 ml of the solution was left. The residue solution was passed through a 500 ml separatory funnel, diluted with 100 ml of a saturated aqueous sodium chloride solution, and then partitioned twice with 70 ml of dichloromethane. After vigorous shaking for 3 min, the solvent phases were combined, dehydrated with 35 g of anhydrous sodium sulfate, and evaporated to dryness on a vacuum rotary evaporator (Büchi Rotavapor R-114, Germany) at 40°C (Büchi Waterbath B-480, Germany). The extract was recovered in 2 ml of methanol/water (7/3, v/v), sonicated for 3 min and filtrated through a syringe membrane filter.

We used a high-performance liquid chromatography (HPLC) system (Termo Spectra, Calif, USA) equipped with an autosampler (AS3500) containing a 100-µl-sample loop and a programmable fluorescence detector (FL3000) operated at an excitation wavelength of 330 nm and using emission wavelengths of 430 nm. The mobile phase was delivered by a P4000 quaternary pump coupled to a PCX 5100 post column reaction system (Pickering Laboratories, Mountain View, Calif, USA). The HPLC column was maintained at 40°C. The post-column reaction unit consisted of 2 reagent pumps (the NaOH solution and the o-phthaldehyde [OPA] solution were constantly pumped at a flow rate of 0.3 ml/min during the entire cycle sequence) and 2 reaction coils. The first reaction coil was heated to 100°C for NaOH hydrolysis, and the second one was kept at ambient temperature for OPA derivatization. The analytical column selected for analysis was a 5µm Capcell Pak C_{18} (4.6 mm ID x 250 mm; Shiseido Co. Ltd., Tokyo, Japan). The methanol/water mobile phase gradient for methomyl began with an initial composition of 50% MeOH, which increased to 80% over 10 min and then to

100% over 2 min. Before each injection, the HPLC system had to be stabilized for 8 min in the methanol/water mobile phase (50:50; v/v). The injection volume was 10 μl and the flow-rate was 1 ml/min. Methomyl was quantified by comparing the chromatographic peak areas in sample extracts with the corresponding peak areas for standard solutions containing known quantities of the respective substances.

Samples of untreated cabbage were fortified with the appropriate amount of the standard solutions to reach concentrations of 0.1 and 0.5 ppm. The samples were allowed to settle for 30 min prior to extraction. Afterward, they were processed according to the extraction procedure described above. Three replicates for each concentration were analyzed.

RESULTS AND DISCUSSION

This method was validated at 2 different concentrations corresponding to 20% of the maximum residue level (MRL) and at 100% of the MRL established by Korean Food and Drug Administration (KFDA). As shown in Table 1, the rates of recovery using this method were 99.2% and 93.1% at 0.1 and 0.5 ppm, respectively, with relative standard deviations (RSDs) of less than 5%. These low RSDs indicate good repeatability of the extraction. Our method meets the requirements of the European Union guidelines (1999), indicating that the method used can be considered accurate and precise when data are 70 to 110% accurate, with relative standard deviations not higher than 20%.

Table 1. Recovery of methomyl from cabbage.

Spiking Level	Recovery rate (%)			
(ppm)	1	2	3	M*± RSD
0.1	95.4	98.6	103.6	99.2 ± 4.1
0.5	93.5	92.2	93.5	93.1 ± 0.7

* Mean of 3 replicate studies.

The methomyl residues remaining on these cabbage plants after treatments are reported in Table 2. No residue was found on untreated (control) cabbages. The original methomyl residue levels were 6.5 and 14.7 ppm above the MRL on day 0 after treatment with the recommended methomyl level and twice the recommended level, respectively. The pesticide deposits were proportional to the amount of methomyl applied. When the concentration of methomyl on day 0 was compared in cabbages that were sprayed at the recommended rate with the concentration on those that were sprayed at twice that rate, we observed a direct correlation between the 2 treatments. More than 95% of the residue dissipated within 6 days from cabbages treated at the recommended rate compared with those treated at twice that rate, from which it took 7 days for more than 95% of the residue to dissipate. After 10 days, the residue was below the MRL established by the KFDA. It has been reported that less than 3% of the methomyl remained for one week after cabbage plants undergo foliar spraying with the insecticide

(US National Library of Medicine 1995). It is evident from the data in Table 2 that methomyl gradually dissipated incrementally with time. In Korea, this insecticide is registered for use on many crops, with an MRL of 0.05 to 5.0 mg/kg (Korea Food and Drug Administration 2005) and a pre-harvest interval of 7 days (Korea Crop Protection Association 2005)

Table 2. Change in residual methomyl levels on Chinese cabbages when applied with folia spraying.

Days after application	Treatment	Residue, ppm (M*±SD)	Dissipation rate %	Half life (d)
0	T_1 20 ml/20 L	6.5 ± 1.37	-	1.7
1		4.1 ± 0.37	37.1	
2		2.2 ± 0.30	66.3	
3		1.0 ± 0.04	84.4	
4		0.7 ± 0.07	89.2	
5		0.5 ± 0.12	92.5	
6		0.3 ± 0.00	96.2	
7		0.2 ± 0.01	96.6	
8		0.2 ± 0.01	96.6	
9		0.2 ± 0.04	97.5	
10		0.1 ± 0.00	98.3	
0	T_2 40 ml/20 L	14.7 ± 0.36	-	1.8
1		10.7 ± 1.00	27.0	
2		8.9 ± 0.20	39.7	
3		3.7 ± 0.73	74.6	
4		2.3 ± 0.10	84.1	
5		2.3 ± 0.09	84.4	
6		1.3 ± 0.13	91.3	
7		0.6 ± 0.02	95.7	
8		0.5 ± 0.03	96.3	
9		0.5 ± 0.02	96.6	
10		0.5 ± 0.02	96.9	

* Mean of 3 studies.

Many factors contribute to pesticide deposit and residue dissipation rates. Under similar environmental conditions, the characteristics of the crop (e.g., morphology, cuticle characteristics, stage of growth at treatment, growth rate) and pesticide application method (e.g., formulation, rate, water volume, pressure, and nozzle type) are most important (Ebeling 1963; Gunther et al 1977). The disappearance of methomyl was fitted to first-order kinetics irrespective of any type of treatment, with correlation coefficients (r^2) >0.95. The biological half-life ($t_{1/2}$) was calculated using the formula $t_{1/2} = \ln 2/k$, where the constant k is the slope of the linear regression. Field data showed that the methomyl residue on cabbage decreased with a half-life ($t_{1/2}$) of 1.7 and 1.8 days when applied at the recommended rate and twice the recommended rate, respectively. Kidd and James

(1991) and Hartley and Kidd (1983) reported a similar finding for methomyl when it was applied to leaves, with a 3- to- 7-day half-life. Our finding was supported by Cabras et al. (1988), who stated that foliar application, but not soil treatment, resulted in residue concentrations on lettuce that varied with the growth pattern of the lettuce. We cannot draw any conclusion from this experiment regarding the distribution of residue among different parts of the cabbage, because the entire cabbage was used for residue analysis.

In this study, we observed that methomyl dissipates gradually with increments of time. Its half-life was measured in days, and its use is not expected to cause any toxicological problems when the treated cabbage is consumed. Furthermore, allowing 10 days from application to harvest was adequate for keeping the methomyl residue below the MRL.

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