Emamectin benzoate residue analysis in ground and terrestrial field water in Lahore suburban area

Sheraz Ahmed¹, Fahim A. Qureshi¹, Amjad Islam¹,²* and Ahmed Adnan¹

¹Department of Chemistry, Government College University, Katchery Road, Lahore-54000, Pakistan.
²Industrial Chemicals Division, Guides Corporation, Davis Road, Lahore-54000, Pakistan.

Accepted 5 October, 2011

High performance liquid chromatography with diode array detector was used to determine the emamectin residue in ground water in Lahore suburban area. The samples were extracted with chloroform through solvent extraction by using a separating funnel. The chemicals used were chloroform, methanol (HPLC grade) and acetonitrile (HPLC grade). Mobile phase used was acetonitrile (98%) and methanol (2%). 10 samples were analyzed and emamectin benzoate was detected in 5 samples. Emamectin benzoate residue was detected in terrestrial and ground water. It was present in trace amount. The concentration range varies from 0.04 to 1.4 ppm of terrestrial field water. With respect to toxicity, it is class II pesticide by W.H.O. Presence of pesticides in large amounts can create a lot of diseases in human body.

Key words: New York codes rules and regulations (NYCRR), reference dose (RfD), United States environmental protection agency (USEPA).

INTRODUCTION

Emamectin benzoate belongs to the avermectin group and is administered to the fish through feed. It is a non-systemic insecticide which penetrates leaf tissue by transmural movement. It paralyses the Lepidoptera, which stops feeding within hours of ingestion and die. Emamectin benzoate is effective against several stages of the salmon louse, copepodite L, lepido and Lepidoptera. Ingestion of high dosage and is not disruptive to beneficial arthropods during integrated pest management. Emamectin benzoate is a very effective agricultural insecticide that is applied to various crops in a low concentration (Yoshii et al., 2000). It is used as an acaricides or parasites for animals and plants.

Emamectin benzoate is the 4′-deoxy-4′-epi-methylamino benzoate salt of avermectin B₁ (abamectin), which is similar structurally to natural fermentation products of Streptomyces avermitilis. Emamectin is a mixture of approximately 90% emamectin B₁a and approximately 10% emamectin B₁b. Emamectin benzoate is being developed as a newer broad spectrum insecticide for vegetables. The mechanism of action involves stimulation of high affinity gamma-amino butyric acid (GABA) receptors and a consequent increase in membrane chloride ion permeability. Animal studies point out a wide margin of safety because mammalian species are much less sensitive due to lower GABA receptor affinities and relative impermeability of the blood brain barrier (Sung et al., 2009).

It is a white to off-white powder. Its density is 1.20 (23°C), vapour pressure is 3 × 10⁻⁸ mm Hg (torr) and solubility in water is 0.024 g/L (pH 7, 25°C). The metabolites of emamectin are 8, 9-Z isomer (defined as 8, 9-Z), 4′-epi-(N-formyl-N-methyl) amino-4′-deoxy-avermectin B₁ (methyl formyl amino form, defined as MFA) and 4′-epi-amino-4′-deoxy-avermectin B₁ (amino form, defined as Amino) (Yoshii et al., 2004). Its melting point is 141 to 146°C. It is non-corrosive, posing a slight fire hazard if exposed to flame. It is soluble in acetone and methanol but hardly soluble in hexane. The half life of emamectin in water was found to vary between 0.7 days during summer conditions to 35.4 days during winter conditions. The difference is due to light sensitivity not temperature. Emamectin benzoate decomposes in a
kinetic phase with a half-life, which includes aerobic and anaerobic metabolism, of 174.2 days. The anaerobic half-life of emamectin benzoate is 427.4 days. Emamectin benzoate exhibits no mobility. It tightly bounds to soil and does not move readily in environment. Terrestrial field dissipation studies indicate a maximum half-life of 15 to 17 days. No quantifiable residues were found at 6 to 12 inches, and no detectable residues were found below 12 inches. Emamectin benzoate is highly toxic to marine/estuarine crustaceans. The leaching studies show that depending upon the type of soil, 0.1 to 0.82% of the applied material can be desorbed.

The acceptable daily intake (ADI) has been set at 1 µg/kg body weight by WHO/FAO. But the United States Environmental Protection Agency (USEPA) calculated an oral reference dose (RfD) for emamectin benzoate of 0.00025 mg/kg/day based on the no-observed-effect level (NOEL) from the sub chronic neurotoxicity in mice (0.075 mg/kg/day) and an uncertainty factor of 300. Environment Conservation Department in New York does not register this product as a “restricted use pesticide”. Emamectin benzoate is “not likely” to be a human carcinogen.

In plants, metabolism has been investigated in cabbage, lettuce, sweet corn and in other vegetables (Crouch et al., 1997; Crouch and Feely, 1995). Maximum emamectin benzoate residues established by USEPA in or on head and stem Brassica vegetables, celery and head lettuce each at 0.025 parts per million (ppm). The USEPA estimated that chronic dietary exposure to these residues would be no more than five percent of the population adjusted dose (PAD) for infants and children and no more than four percent of the reference dose (RFD) for adults. This chronic exposure analysis is based on the assumption that 25% of crops are treated and contain tolerance level residues.

Emamectin has neither antibacterial nor fungicidal properties. It is meant to kill salmon lice, which is a crustacean. It is the second class pesticide by WHO/FAO. Abamectin emamectin benzoate is an upgrade to improve the product, its activity is much higher than the avermectin and such as the stomach poisoning toxicity is avermectin 2146 times. In normal cases, the effect of emamectin benzoate was significantly better than abamectin, emamectin benzoate control diamondback moth should be better than the effect of avermectin (Yoshii et al., 2001) (Figure 1).

Several methods have been developed for the determination of emamectin residues in soil, crops and water (Hicks et al., 1997; Chukwudebe et al., 1997). Liquid chromatographic method using a UV detector was reported for the determination of emamectin residues in water (Blackwell et al., 2004). This work is different because it involves a diode array detector. High performance liquid chromatography is more favorable than conventional liquid chromatography for pesticide residue analysis. In Pakistan, emamectin is in use for the last four to six years and there is not much work reported dealing with residue analysis.

**MATERIALS AND METHODS**

Organic solvents (residue analysis grade) for dissolving and extracting were methanol (HPLC), chloroform and acetonitrile (HPLC grade) purchased from Merck (Germany). Standard material (95%) was obtained from Shanghai Taizhao Chemicals (China). Purity of the standard after recrystallization was more than 95%.
Stock standard solutions were prepared by accurately weighing 100 mg of standard emamectin benzoate and dissolved in acetonitrile and methanol (98:2), after which the volume is made to 100 ml. Working standard solutions of 1, 5, 10 and 20 µg were prepared by dilution with acetonitrile and methanol (98:2). For each standard solution took 0.25, 1.25, 2.5 and 5 ml of stock standard solution and 25 ml solvent, acetonitrile and methanol (98:2) for each working standard. Stock standard solution and working standard solutions were stored under refrigeration (4°C).

High-performance liquid chromatography (HPLC) is one of the premier analytical techniques widely used in analytical laboratories. High-performance liquid chromatography or high pressure liquid chromatography (HPLC) is a form of column chromatography used frequently in biochemistry and analytical chemistry to separate, identify, and quantify compounds. HPLC utilizes a column that holds chromatographic packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) used.

A high performance liquid chromatograph (HPLC), Agilent 1100, equipped with diode array detector (DAD) was used for the analysis of residues of emamectin benzoate. An HPLC column, Thermo scientific (4.6 mm ID; 250 mm length; 5 µm particle size; pore size 120 Å, stainless steel) was used for emamectin residue analysis. Mobile phase was a combination of acetonitrile (98%) and methanol (2%). Injection volume was 100 µl. The solvent was pumped at a flow rate of 1.2 ml/min with gradient elution. Solvents used for samples preparation were methanol and acetonitrile. Wave length of light was 246 nm. Analysis was carried out at room temperature (25°C).

### Sampling

10 samples of ground water and terrestrial field water (5 terrestrial and 5 ground water samples) were collected from different villages near Lahore. These water samples were collected from Kasoor (Ganda Sing border), Changa Manga, Patooki (Hunjaran Kalan), village Vinya and Raiwind. These samples were analyzed after 24 h being stored at 4°C until analyses. These samples were collected from December 2008 to February 2009 at temperature 12°C.

### Extraction

All samples (1000 ml of water for each sample) were extracted one by one from chloroform through solvent extraction using a separating funnel and were filtered through 0.4 µm filter paper one by one separately. Extracted samples were then allowed in the beakers to dry at room temperature after covering the beakers with aluminium foil with holes, after evaporation of chloroform from all samples, the residues were deposited at the bottom of the beakers. The residue from each beaker was dissolved in 25 ml solvent (98 ml acetonitrile : 2 ml methanol) and then was filtered once again through 0.4 µm filter paper one by one separately for making sample solution and analyzed by HPLC.

### RESULTS AND DISCUSSION

10 samples of ground water and terrestrial field water (5 terrestrial and 5 ground water samples) were analyzed for pesticide residue. Emamectin was identified in these samples from its retention time by comparing it with authentic standard. Comparison of these samples is shown in Table 1. Concentrations of these samples are shown in Table 2. Maximum residue limit (MRL) value of emamectin is 0.5 ppm by WHO/FAO in ground water. Terrestrial toxicity effect concentration from technical report of environment agency, Rio house (England) is 570 to 1318 (mg kg⁻¹).

Emamectin is a mixture of emamectin B₁a and emamectin B₁b. Emamectin B₁a is a major component and emamectin B₁b is the minor component. Each component has its own peak. Two peaks were obtained...
in the samples also. The concentration range varies from 0.04 to 1.4 ppm in which emamectin benzoate B$_{1a}$ concentration ranges from 0.42 to 1.4 ppm whereas emamectin benzoate B$_{1b}$ concentration ranges from 0.04 to 0.12 ppm.

Chromatogram of a water sample is shown in Figure 2. Figure 3 shows a calibration curve for emamectin B$_{1a}$ and it is a graph between concentration (on x-axis) and area (on y-axis). Figure 4 shows a calibration curve for emamectin B$_{1b}$.

At the time of sampling, the temperature was 12°C. With the temperature increase, the toxicity of avermectin against pests (Particularly virulent stomach poison) increases. However, emamectin benzoate and abamectin toxicity with rising temperature rate of increase is inconsistent.

Emamectin benzoate is poorly soluble in water. The hydrolysis half-life of emamectin benzoate is 19.5 weeks at pH 9 and stable at pH 5.2, 6.2, 7.2 and 8.2. At the time of sampling, pH of water samples varies from 6.2 to 7.8.
There are no chemical specific Federal or State drinking water standards for emamectin benzoate. Based on its chemical structure, emamectin benzoate falls under the 50 µg/L general New York State drinking water standard for "unspecified organic contaminants" (10 NYCRR Part 5, Public Water Systems), based on the USEPA Office of Pesticide Programs RfD of 0.00025 mg/kg/day and the Department of Environmental Conservation Water Quality Regulation procedures for deriving surface water and groundwater standards and guidance values from non-oncogenic effects (6 NYCRR - Part 702.5), an ambient water quality value for emamectin benzoate would be 1.8 µg/L.

Use of avermectin production levels are generally higher than that of emamectin benzoate (emamectin benzoate production, mostly 1 or 0.5%, while abamectin is generally 1.8 or 2%), the dilution factor are generally higher than the avermectin emamectin benzoate (avermectin 1000 to 1500 times, emamectin benzoate 2000 to 3000 times).

Due to its low application rate and the number of target crops limited crops, the labeled use of proclaim insecticide does not appear to pose significant risks to the workers or to the general public through dietary exposure.

Acknowledgements

The authors are grateful to Dr. Fahim Ashraf Qureshi and Dr. Ahmad Adnan from the Department of Chemistry, Government College (GC), University Lahore, Pakistan, for their critical review on this manuscript.

References


Hicks MB, Payne LD, Prabhu SV, Wehner TA (1997). Determination of...
emamectin benzoate in freshwater and seawater at pictogram-per-milliliter levels by liquid chromatography with fluorescence detection. J. AOAC Int., 80: 1098-1103.


