Residual dynamics of thiacloprid in medical herbs marjoram, thyme, and camomile in soil

YU Yun-long[1,*], WU Jia-lun[1], STAHLER Matthias[2,*], PESTEMER Wilfried[2]

1. Department of Plant Protection, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310029, China. E-mail: ylyu@zju.edu.cn
2. Institute for Ecotoxicology and Ecochemistry in Plant Protection, Federal Biological Research Centre for Agriculture and Forestry, Königin-Luise-Straße 19, D-14195 Berlin, Germany. E-mail: m.staehler@bba.de

Abstract

Thiacloprid is a new insecticide of the chloronicotinyl family. To assess its risk after application, residual characteristics of thiacloprid in marjoram, thyme, and camomile and in soil were studied under field conditions. The active ingredient was extracted from the plant material using a mixture of acetone-water. After filtration, the extract was concentrated to the aqueous phase, diluted with water, and portioned against ethyl acetate on a matrix solid phase dispersion column. Thiacloprid was extracted from soil using a mixture of methanol-water, filtered, and reextracted (clean up) with dichloromethane. The residues were quantified using HPLC-MS-MS. The methods were validated by recovery experiments. Thiacloprid residues in marjoram, thyme, and camomile and in soil persisted beyond 10, 14, 14, and 21 d but no residues were detected after 14, 21, 21, and 28 d, respectively. The data obtained in this study indicated that the biexponential model is more suitable than the first-order function to describe the decline of thiacloprid in fresh marjoram, fresh thyme, and dried camomile flowers with half-life ($t_{1/2}$) of 1.1, 0.7, and 1.2 d, respectively. However, both the first-order function and biexponential model were found to be applicable for dissipation of thiacloprid in soil with almost the same $t_{1/2}$ values of 3.5 and 3.6 d. The results indicated that thiacloprid dissipates rapidly and does not accumulate in the tested herbs and in soil.

Key words: thiacloprid; marjoram (*Majorana hortensis*); thyme (*Thymus vilgaris*); camomile (*Matricaria chamomilla*); residue

Introduction

Thiacloprid, (Z)-3-(6-chloro-3-pyridylmethyl)-1,3-thiazolidin-2-ylidenecyanamide, is the second member of the Bayer's chloronicotinyl insecticide (CNI) family (Jeschke *et al.*, 2001). The structure is shown in Fig.1. It has activity not only against sucking insects such as aphids, whiteflies, and some jassids but is also active against weevils, leafminers, and *Cydia pomonella* in apples and various species of beetles (e.g. *Leptinotarsa decemlineata*, *Antonomus pomorum*, *Lissorhoptrus oryzophilus*, *Lema oryzae*) and shows good plant compatibility in all relevant crops (Elbert *et al.*, 2000; Ciglar and Barić, 2002). On the basis of its high insecticidal activity with a favorable ecological profile and safety to bees, it is particularly useful in horticulture as well as in modern crop protection systems. The use of thiacloprid is expected to increase because it has been registered for use in many crop plants (Dhivahar and Dhandapani, 2003; Gualco *et al.*, 2003; Walunj and Pawar, 2004; Sannino *et al.*, 2005).

However, very little information is available in previously published reports on the behavior of thiacloprid in plants and soils (Oliver *et al.*, 2005). Bikramjit and Gajbhiye (2004) examined the behavior of thiacloprid in soil under field conditions and found that the dissipation of the residues followed first-order kinetics with half-life of 3.2–3.3 d. In the study of Krohn (2001), the half-life in soil under field conditions were calculated to be 9–27 d in northern Europe and 10–16 d in southern Europe. Premalatha *et al*. (2003) examined residual levels of thiacloprid in cottonseed, lint, and oil. Klein (2001) investigated the metabolism of thiacloprid in plants. Nevertheless, no reports on dissipation kinetics of thiacloprid in plants could be found.

To assess the safety of its application on medicinal herbs for control of insect pests, a field test was conducted to examine thiacloprid residues in marjoram, thyme, and camomile flowers, as a cooperative program between China and Germany. The objectives of this study were to investigate the residual dynamics of thiacloprid in the medicinal herbs marjoram, thyme, and camomile, as well as in soil and to evaluate the safety of its application on such kinds of plants.

1 Materials and methods

1.1 Chemicals

The formulation CALYPSO® (480 g a.i./L) from Bayer Crop Science, Germany, was used for plant treatments.
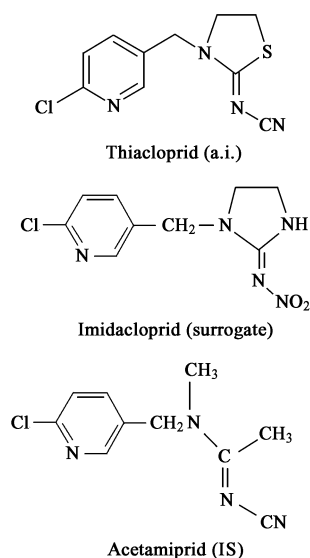
Fig. 1 Structural formulae of test substance, the surrogate, and the internal standard (IS) substance.

The standard active ingredient (thiacloprid), surrogate (imidacloprid), and the internal standard (acetamiprid) together with certificates were kindly supplied by Dr. Ehrenstorfer, GmbH, Germany. Acetonitrile (RIEDEL-DEHAËN, Germany) and ethyl acetate (VDR, Germany) were HPLC-grade solvents, whereas acetone (FLUKA, SIGMA-ALDRICH) was a residue analysis grade solvent.

1.2 Field trials and sampling

Trials with three herbs were carried out on plot 18 of the field station of the Federal Biological Research Centre for Agriculture and Forestry (BBA) in Berlin, Germany, from early July to the end of August 2005. The soil in the experimental station was characterized as follows: 77.1% sand, 16.1% silt, 6.8% clay, 2.1% organic matter, pH 6.1.

Meteorological data were continuously recorded throughout the experiments by an automatic weather station of BBA. The climatic data in proximity of the treatment period and the daily average of the climatic data during the entire experiment are shown in Fig.3c.

The dissipation experiment was conducted in 24-m^2 plots in the field. Sowing rates of the varieties used were 2.5 kg seeds/hm^2 for camomile and 8.0 kg seeds/hm^2 for marjoram and thyme. When camomile was in the flowering phase and marjoram and thyme were approximately 15 cm in height, the formulated insecticide (480 g a.i./L) was sprayed at a dose of 0.12 L in 400 L water/hm^2 using HEGE 29 sprayer on 25 July, 2005. This treatment was repeated 10 d after the first application. All treatments were replicated thrice. Epigeal parts of marjoram and thyme plants and camomile flowers, and soil were sampled at random from the plots 2 h, and 1, 2, 3, 5, 7, 11, and 14 d after the last application. After sampling was complete, the collected samples were immediately transported to the laboratory, where they were chopped, thoroughly mixed, and divided into three subsamples each for marjoram, thyme and camomile. A total of 25 g (fresh weight) of marjoram or thyme was weighed and placed in glass

jars and stored at –20°C until analysis. The sample of camomile flowers was dried in an oven at 60°C for 24 h, then 5 g of dried camomile flowers was weighed and placed in a glass jar and stored under similar conditions as were marjoram and thyme.

Soil was passed through a 2-mm sieve, and soil moisture was determined immediately using a METTLER balance-system PM 4600 with heater device LP 10. Fifty grams of soil was weighed and placed in a glass jar and stored at –20°C until extraction of thiacloprid.

1.3 Extraction of thiacloprid from the herbs

For extraction of thiacloprid residues from the herbs, the procedures described by Schöning and Placke (2001) were used with modification. Surrogate (2.5 μg per 25 g sample) and acetone-water (150 ml, 3:1 in volume) were added to the sample jar and then homogenized at a speed of 10000 r/min for 3 min. The mixture was decanted and filtered through a 7-cm Buchner funnel with 5 g of Celite 545, the filter cake was washed twice, successively with 70 ml of acetone-water (3:1) and 30 ml of acetone. The extracts were collected in a 250-ml measuring cylinder, and acetone was added to make the volume up to 250 ml. A total of 50 ml of the mixture was transferred into a flask and concentrated on a rotary evaporator (BÜCHI, Germany) under conditions of water bath temperature of 30°C and pressure of 0.025 Pa to remove the organic solvent. The mixture that was remaining in the flask was then transferred onto an EXTRELUT NT 20 column (VARIAN, USA) and eluted with 60 ml of ethyl acetate, 15 min after the transfer. The eluant was collected and rotary evaporated to about 1 ml and then to dryness under a nitrogen stream. The internal standard acetamiprid was added to the residue and the volume was made up to 1 ml with acetonitrile and was ready for analysis using HPLC-MS-MS. Recovery of the overall method was always checked for each sample by simultaneous addition of surrogate imidacloprid at concentration of 0.1 mg/kg. The standard series of concentration with matrix (matrix standard) was set to be 10, 25, 50, 100, 250, 500, 750, and 1000 ng/ml for quantitative determination.

1.4 Extraction of thiacloprid from the soil

Distilled water was added to the glass jar to make up the soil moisture up to 10%, and 90 ml of methanol was added. After shaking at 230 r/min for 1 h, the mixture was filtered and collected in a 250-ml separatory funnel containing 50 ml of distilled water and 2 ml of saturated sodium chloride and extracted thrice with 30 ml of dichloromethane. All extracts were passed through anhydrous sodium sulfate, collected in a flask, and then subjected to the procedures described above for the herbs.

1.5 Determination of thiacloprid by HPLC-MS-MS

All analyses of thiacloprid were carried out on an API 2000 (Applied Biosystems, USA) system equipped with a Turbo Ion Spray Source (ESI). Separation was carried out on XTerra MS C18 (WATERS, USA). The elution solvent

consisted of acetonitrile and acetic acid, its gradient for separation of thiacloprid is listed in Table 1. Positive ESI-MS/MS was carried out using a spray voltage of 26 eV. The target compound thiacloprid as well as surrogate imidacloprid were determined.

1.6 Recovery efficiency studies

Known quantities of thiacloprid and surrogate imidacloprid in acetone were added to untreated samples of marjoram, thyme, and camomile and to soil at different fortification levels. The active ingredient thiacloprid and surrogate imidacloprid were extracted and analyzed as described previously.

1.7 Data analysis

Two models were adopted to interpret the relationship between residues and time. First-order model, $C_t = C_0 e^{-kt}$, where C_t (mg/kg) is the residue after time t, C_0 (mg/kg) is initial residue, and k is dissipation rate constant (d^{-1}). It is widely used to describe the fate of pesticides in soil and plants (Beulke and Brown, 2001). Compared with monophasic first-order kinetics, biexponential model consists of two exponential terms, $C_t = ae^{k_1 t} + be^{k_2 t}$, where C_t (mg/kg) is the residue after time t, a (mg/kg) and b (mg/kg) are constants, and k_1 (d^{-1}) and k_2 (d^{-1}) are dissipation rate constants of the first and the second component, respectively (Gustafson and Holden, 1990).

2 Results and discussion

2.1 Recovery study

The average recoveries of thiacloprid fortified in fresh marjoram, thyme, and dried camomile flowers and in soil are shown in Table 2. In most cases, the recoveries of thiacloprid were >70% with coefficients of variation (CV) not exceeding 20%. The detection limits are 0.01 mg/kg for thyme and marjoram and 0.05 mg/kg for camomile flowers. Fig.2 shows the chromatograms of blank and thiacloprid-fortified samples. These data are generally considered to be satisfactory for residue determinations.
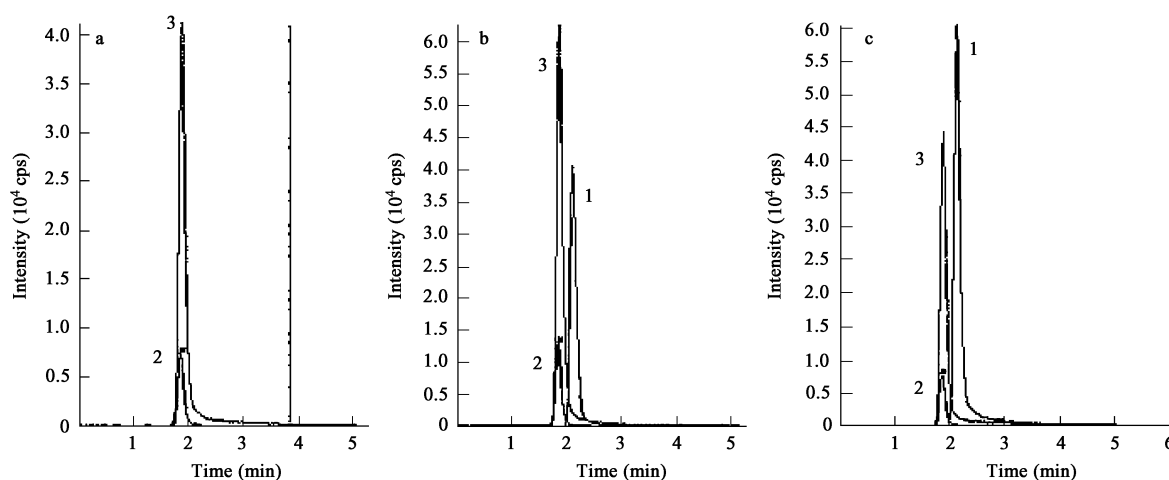

Fig. 2 Chromatograms of blank (a), of a standard (b) and a marjoram sample 1 d after application (c) each with thiacloprid (1), imidacloprid (2), and acetamiprid (3).

Table 1 Gradient of solvents used in this study

Step	Total time (min)	Flow rate (μl/min)	A (%) Acetonitrile + 0.1% acetic acid	B (%) Acetonitrile + 0.1% acetic acid
0	0.0	400.00	50.0	50.0
1	3.0	400.00	50.0	50.0
2	3.1	400.00	100.0	0.0
3	6.0	400.00	100.0	0.0
4	6.1	400.00	50.0	50.0
5	10.0	400.00	50.0	50.0

Table 2 Recoveries of thiacloprid fortified in fresh marjoram, fresh thyme, and dried camomile flowers

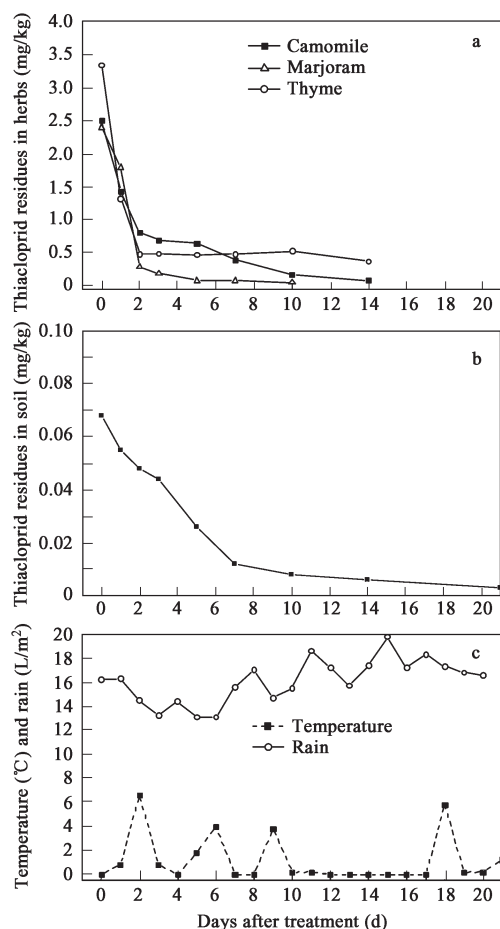| Fortification level (mg/kg)	Recovery (CV, %)			
	Fresh marjoram	Fresh thyme	Dried camomile flowers	Soil
1	80 (8.5)	80 (4.1)	88 (10.9)	92 (2.4)
0.5	84 (11.0)	78 (10.6)	71 (4.6)	96 (1.9)
0.1	90 (11.5)	94 (9.6)	87 (14.6)	83 (19.8)
0.05	92 (19.5)	94 (4.0)	93 (8.3)	69 (2.3)
0.01	nd	100 (19.5)	nd	75 (11.0)

nd: Not detectable.

Fig. 3 Dissipation of thiacloprid in three herbs (a) and soil (b), average daily temperature and daily rainfall (c) during the experimental period.

2.2 Residual dynamics of thiacloprid in marjoram, thyme, and camomile

The data on residual dynamics obtained for thiacloprid in marjoram, thyme, and camomile are shown in Fig.3a. The decline of thiacloprid in these herbs was more rapid in the first 2 d than in the later period. This may be attributable to evaporation of thiacloprid from plant surfaces and to eluting by rainfall. It rained 0.2, 0.8, and 6.6 mm on the first 3 d after treatment (Fig.3c). This might have accelerated the decline of thiacloprid on herbs, leading to the rapid disappearance of thiacloprid.

To evaluate the decline of thiacloprid in these herbs, residual data should be subjected to statistical analysis. A linear regression can be obtained after an appropriate transformation of the residual and/or time values. Levels

of residue can generally be interpreted using a first-order model, which allows for the linearization of data by plotting the logarithms of the residues ($\ln C$) versus time. However, this type of interpretation is not always suitable because pesticides often dissipate more quickly at first and then much more slowly during the following period of time than is shown by the function of the first-order model. In this case, thiacloprid disappeared rapidly from all three plants within the first 2 d. Two days later, however, thiacloprid disappeared more slowly. Therefore, the first-order model and the biexponential model are usually used together to study the dissipation of the residues in plants before harvest and to determine the statistical parameters that describe such behavior. The statistical data from the first-order model and the biexponential model for disappearance of thiacloprid in the herbs are summarized in Table 3. It could be concluded that the biexponential model is more suitable to describe the disappearance behaviors of thiacloprid on fresh marjoram, fresh thyme, and dried camomile flowers with higher values of determination coefficients r^2. The half-life of thiacloprid on marjoram, thyme, and camomile was calculated to be 1.1, 0.7, and 1.2 d, respectively, using the biexponential model.

Fig.3a indicates that the amount of the insecticide thiacloprid deposited on marjoram, thyme, and camomile depends on the plant species. Two hours after treatment, the concentrations of thiacloprid on marjoram and camomile were found to be 2.5 and 2.4 mg/kg respectively, which were much lower than that on thyme (3.3 mg/kg). Jayakrishnan and Gopal (2005) found that the initial concentration of thiacloprid on eggplant fruits was 0.87 mg/kg with normal dose (72 g a.i/hm^2) and 1.813 mg/kg with double dose (144 g a.i/hm^2) 15 d after application, 0.313 mg/kg and 0.678 mg/kg of thiacloprid residues persisted on the fruits for either dose, respectively. The $t_{1/2}$ values of thiacloprid under field conditions were in the range of 10.8–12.3 d. In this study, the initial concentrations of thiacloprid on marjoram, thyme, and camomile were measured to be 2.4, 3.3, and 2.5 mg/kg, respectively, at the recommended dose (58 g a.i./hm^2), which were higher than those observed on eggplant fruits.

The maximum residue limit (MRL) of thiacloprid in protected lettuce, herbs, and baby leaf brassica has been proposed to be 2.0 mg/kg (Eun *et al.*, 2005). According to the biexponential model obtained from this study, the thiacloprid concentration after application of the recommended dose (58 g a.i./hm^2) in marjoram, thyme, and camomile had reduced to 2.0 mg/kg within 0.3, 0.5, and 0.4

Table 3 Statistical data of thiacloprid dissipation in marjoram, thyme, and camomile and in soil

Model	Herbs	Equation	$t_{1/2}$ (d)	r^2
First-order model	Marjoram	$C_t = 2.5260e^{-0.6695t}$	1.0	0.928
	Thyme	$C_t = 2.9698e^{-1.2190t}$	0.6	0.965
	Camomile	$C_t = 2.3408e^{-0.3967t}$	1.8	0.933
	Soil	$C_t = 0.06913e^{-0.1965t}$	3.5	0.984
Biexponential model	Marjoram	$C_t = 3.4933e^{-0.5484t} - 0.9686e^{0.3200t}$	1.1	0.932
	Thyme	$C_t = 2.9226e^{-1.2852t} + 0.4198e^{0.0045t}$	0.7	0.993
	Camomile	$C_t = 1.4368e^{-1.1986t} + 1.0745e^{-0.1537t}$	1.2	0.989
	Soil	$C_t = 0.2312e^{-0.1623t} - 0.1623e^{-0.1499t}$	3.6	0.984

d, respectively. In addition, thiacloprid was not detectable 10, 14, and 14 d after application in marjoram, thyme, and camomile (Fig.3). This indicates that thiacloprid will not accumulate in the tested medical herbs after being applied as crop protection chemical. It can therefore be deduced that the application of thiacloprid on marjoram, thyme, and camomile is of no concern on the herb quality.

2.3 Residual dynamics of thiacloprid in soil

Compared with the dissipation curves of thiacloprid in marjoram, thyme, and camomile, the decreasing trend of thiacloprid in soil was observed to be different (Fig.3b). The statistical data of thiacloprid dissipation in soil are shown in Table 3. The first-order and biexponential models were both found to be sufficient to simulate dissipation of thiacloprid with almost the same determination coefficients of 0.98 and 0.98; the half-life of thiacloprid in soil was calculated to be 3.5 and 3.6 d, respectively. This shows good agreement with the previous results reported by Bikramjit and Gajbhiye (2004). Bikramjit and Gajbhiye (2004) found that the dissipation of thiacloprid in soil followed first-order kinetics with half-life of 3.2–3.3 d. However, Krohn (2001) indicated that the half-life of thiacloprid in soil measured under field conditions ranged from 9 to 27 d in northern Europe and from 10 to 16 d in southern Europe. Generally, dissipation of pesticides in soil depends on soil types and environmental conditions. In this study, thiacloprid dissipation may also differ under different environmental conditions. The amount of thiacloprid residues in soil was not detectable 21 d after treatment. Overall, rapid dissipation of thiacloprid in soil would be expected from the data of this and previous studies. Moreover, it has been reported that thiacloprid is strongly adsorbed and that it is slightly mobile in soil (Krohn, 2001; Oliver *et al.*, 2005); hence, it has no potential for leaching into groundwater. It could therefore be concluded that thiacloprid is compatible with environmental quality.

3 Conclusions

The data reported in this study suggested that the decline of thiacloprid in marjoram, thyme, and camomile could be well described by the biexponential model with half-life ($t_{1/2}$) of 1.1, 0.7, and 1.2 d, respectively. Both first-order function and biexponential model are suitable to characterize the dissipation of thiacloprid in soil. The results of this study indicated that thiacloprid dissipates rapidly and does not accumulate in marjoram, thyme, and camomile and in soil. On the basis of the results of this study and its high activity against sucking insects, weevils, leafminers, Cydia pomonella, and various species of beetles (Elbert *et al.*, 2000; Ciglar and Barić, 2002), the new insecticide thiacloprid can be safely used for the control of the pests on marjoram, thyme, and camomile.

References

Beulke S, Brown C D, 2001. Evaluation of methods to derive pesticide degradation parameters for regulatory modeling[J]. Biol Fertil Soils, 33: 558–564.

Bikramjit S, Gajbhiye V T, 2004. Persistence and leaching of thiacloprid in soil under field and/or laboratory conditions[J]. Pestic Res J, 16(1): 62–65.

Ciglar B I, Barić B, 2002. Monitoring the appearance and possibility for prevention of the apple sawfly in croatia[J]. J Pestic Sci, 75: 41–45.

Dhivahar P, Dhandapani N, 2003. Bioefficacy of the new molecule thiacloprid (Calypso 240 SC) against rice yellow stem borer, Scirpophaga incertulas (Walker)[J]. Indian J Plant Protect, 31(1): 144–145.

Elbert A, Erdelen C, Khnhold J *et al.*, 2000. Thiacloprid, a novel neonicotinoid insecticide for foliar application[C]. The BCPC Conference: Pests and diseases, Vol.1 Proceedings of an international conference held at the Brighton Hilto, 21–26.

Eun H, Arao T, Hokakubo S *et al.*, 2005. Food crop grouping for setting maximum residue limits (MRLs) and facilitating availability of pesticides for minor crop uses–Cucurbit vegetables group[J]. J Pestic Sci, 30: 145–152.

Gualco A, Cantoni A, Gollo M *et al.*, 2003. Thiacloprid (CalypsoReg) a novel neonicotinoid for pest control on pome fruits, stone fruits, vegetables, flowers and ornamentals[J]. Informatore Fitopatologico, 53(4): 39–44.

Gustafson D I, Holden L R, 1990. Nonlinear pesticide dissipation in soil–a new model based on spatial variability[J]. Environ Sci Technol, 24:1032–1038.

Jayakrishnan S M, Gopal M, 2005. Comparative persistence of chlorpyrifos and thiacloprid on eggplant fruits and managing fruit and shoot borer, Leucinodes orbonalis Guen[C]. 229th American Chemical Society Meeting. San Diego, CA. March 16, 2005.

Jeschke P, Moriya K, Lantzsch R *et al.*, 2001. Thiacloprid (Bay YRC 2894) –A new member of the chloronicotinyl insecticide (CNI) family[J]. Pflanzenschutz Nachrichten Bayer, 54(2): 147–160.

Klein O, 2001. Behaviour of thiacloprid (YRC 2894) in plants and animals[J]. Pflanzenschutz Nachrichten Bayer, 54(2): 209–240.

Krohn J, 2001. Behaviour of thiacloprid in the environment[J]. Pflanzenschutz Nachrichten Bayer, 54: 281–290.

Oliver D P, Kookana R S, Quintana B, 2005. Sorption of pesticides in tropical and temperate soils from Australia and the Philippines[J]. J Agric Food Chem, 53: 6420–6425.

Premalatha R, Kuttalam S, Jayakumar R *et al.*, 2003. Residues of thiacloprid in cotton seed, lint and oil[J]. Pestic Res J, 15(1): 55–57.

Sannino L, Piro F, Contiero M, 2005. Field performance of thiacloprid against the tobacco flea beetle Epitrix hirtipennis (Melsheimer)[J]. Informatore–Fitopatologico, 55(3): 36–38.

Schöning R, Placke F J, 2001. Residue analytical method for the determination of residues of YRC 2894 in/on plant materials by HPLC with electrospray ionization and MS/MS–detection[J]. Pflanzenschutz Nachrichten Bayer, 54: 261–280.

Walunj A R, Pawar S A, 2004. An evaluation of thiacloprid against pests of chilli[J]. Tests Agrochemicals Cultivars, 25: 6–7.