Environ Monit Assess (2016) 188: 528
DOI 10.1007/s10661-016-5536-4

Chronic and acute risk assessment of human exposed to novaluron-bifenthrin mixture in cabbage

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Received: 29 February 2016 / Accepted: 9 August 2016 / Published online: 22 August 2016

Abstract Based on the dissipation and residual level in cabbage determined by gas chromatography coupled with an electron capture detector (GC-ECD), chronic and acute risk assessments of the novaluron and bifenthrin were investigated. At different spiked levels, mean recoveries were between 81 and 108 % with relative standard deviations (RSDs) from 1.1 to 6.8 %. The limit of quantification (LOQ) was 0.01 mg kg^{-1}, and good linearity with correlation coefficient (>0.9997) were obtained. The half-lives of novaluron and bifenthrin in cabbage were in the range of 3.2~10 days. Based on the consumption data in China, the risk quotients (RQs) of novaluron and bifenthrin were all below 100 %. The chronic and acute risk of novaluron in cabbage was relatively low, while bifenthrin exerts higher acute risk to humans than chronic risk. The obtained results indicated that the use of novaluron-bifenthrin mixture does not seem to pose any chronic or acute risk to humans even if cabbages are consumed at high application dosages and short preharvest interval (PHI).

Keywords Risk assessment · Novaluron · Bifenthrin · Cabbage

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Abbreviations

GC-ECD	Gas chromatography with electron capture detector
RSD	Relative standard deviation
LOQ	Limit of quantification
RQ	Risk quotient
PHI	Preharvest interval
FAO	Food and Agriculture Organization
MRL	Maximum residue limit
ADI	Acceptable daily intake
SC	Suspension concentrate
SPE	Solid-phase extraction
ICAMA	Institute for the Control of Agrochemicals
NEDI	National estimated daily intake
NESTI	National estimated short-term intake
ARfD	Acute reference dose
STMR	Supervised trials median residue
LP	Large portion consumed
HR	Highest residue
CA	Concentration addition
IA	Independent action

Introduction

Novaluron [(±)-1-[3-chloro-4-(1,1,2-trifluoro-2-rifluorometh-oxyethoxy)-phenyl]-3-(2,6-difluoro-benzoyl)urea], developed by Makhteshim-Agan Industries Ltd., is a new insect growth regulator (Cutler and

Scott-Dupree 2007). It is a potent suppressor of important lepidopteran and coleopteran pests and can provide control of several homopteran and dipteran pests (Portilla et al. 2014; Trostanetsky et al. 2015; Keefer et al. 2015; Kim et al. 2014). Novaluron is registered as an insecticide for crops including apples, potatoes, cotton, and brassicas (FAO 2003). The compound is considered eco-friendly or green pest-controlling agent (Malhat et al. 2014).

Bifenthrin [(2-methyl-1,1-biphenyl-3-yl)-methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcycloprpanecarboxylate] is a widely used insecticide both in agriculture and in urban areas. It is one of the nonsystemic pyrethroids with a broad spectrum of insecticidal and acaricidal activity (Tewary et al. 2005) and is highly toxic to aquatic organisms (Parry et al. 2015). Several recent studies of bifenthrin have demonstrated effects on cytotoxicity, neurobehavioral toxicology, and estrogenic potential (Zhang et al. 2015; Dar et al. 2015; Cao et al. 2014; Crago and Schlenk 2015).

A risk assessment for pesticides is usually defined as the characterization of the potential adverse health effects of human exposure to hazard of pesticides. The methods for estimation exposure are integral to the risk assessment process used for setting of legally enforceable limits. For assuring the safety of public health, regulatory agencies define maximum residue limits (MRLs) for pesticides (EC Regulation 2005; FAMIC 2000; EPA 2015). A common health based guidance value for long-term daily exposure is the acceptable daily intake (ADI): the estimated amount of a substance in food or drinking water, expressed on a body weight basis, which can be consumed every day for a lifetime by humans without presenting an appreciable risk to their health (MacLachlan and Meller 2012). The MRL of novaluron for group of brassica is set at 0.7 mg kg^{-1}, while it is 0.2 mg kg^{-1} for bifenthrin in cabbage, established by China (GB 2763-2014).

With regular and repeated pesticide application, pests may quickly develop resistance. Mixtures of pesticides, which show broader spectrums and higher insecticidal activities, have played an important role in extending pesticide lifetimes (Barros et al. 1999). However, traditional risk assessment of toxic chemicals routinely focuses on effects assessment of single chemical evaluation, which may underestimate the risk associated with toxic action of mixtures (Chen et al. 2014a, b).

In this paper, the residues of novaluron and bifenthrin in cabbage were determined by GC-ECD. The aims of this study are as follows: (1) to establish a GC-ECD method for simultaneous analysis of novaluron and bifenthrin in cabbage, (2) to evaluate the dissipation kinetics and terminal residues of novaluron and bifenthrin in cabbage, and (3) to assess the chronic and acute risk of this pesticide to humans based on field trial data.

Materials and methods

Chemicals and reagents

Analytical standard of novaluron (purity >98.7 %) was obtained from Sigma (USA). Analytical standard of bifenthrin (purity >99.5 %) was obtained from Dr. Enrenstorfer GmbH (Germany). A formulation of a suspension concentrate (SC) containing 5 % novaluron and 5 % bifenthrin was used. Analytical grade of acetonitrile, acetone, sodium chloride, and petroleum ether was purchased from Beijing Chemical Reagent Co., Ltd. (Beijing, China). Petroleum ether was distilled before use and fractioned at 60–70 °C. The florisil solid-phase extraction (SPE) column (1 g, 6 mL) was purchased from ANPEL Laboratory Technologies, Inc. (Shanghai, China).

Field trials and sample collection

Field trials including dissipation study and terminal residue study were conducted at Beijing, Anhui province, and Hunan province from 2013 to 2014. The trial was conducted according to “Standard Operating Procedures on Pesticide Registration Residue Field Trials” (ICAMA 2007). The area of each plot was 15 m^2 and was separated from each other by a buffer area. Each treatment consisted of three replicate plots and a control plot. A plot with no application history of novaluron and bifenthrin was selected. During the course of experiment, applications of similarly structured pesticide were forbidden.

To investigate the dissipation of novaluron and bifenthrin, cabbage crop were sprayed with SC at dosage of 187.5 g a.i. ha^{-1}. Representative cabbage samples were collected at 2 h and 1, 2, 3, 5, 7, 10, 14, 21, and 30 days after application. For the terminal residue trial, the SC was applied at two dosages of 37.5 and 56.25 g a.i. ha^{-1}. Each dosage level was designed to spray two and three times with interval of 7 days. Representative cabbage samples were collected 7, 14, and 21 days after last spraying. All samples were homogenized and stored at −20 °C for further analysis.

Sample preparation

The homogenized cabbage samples of 10 g were weighed into a 50-mL Teflon centrifuge tube, and then, 20 mL of acetonitrile was added. The tube was shaken vigorously by hand for 1 min and then treated to ultrasonic extraction for 15 min. After addition of 7 g sodium chloride, the samples were shaken vigorously by hand for 1 min and centrifuged for 5 min at 3000 rpm. Thereafter, a portion of supernatant (10 mL) was evaporated to near dryness using a vacuum rotary evaporator at 35 °C.

For the cleanup, the florisil SPE cartridge was conditioned with 5 mL of petroleum ether. The concentrated extracts were dissolved in 20 mL acetone/petroleum ether (1:9, *v*/*v*) and transferred to the cartridge. The eluent was collected and concentrated to dryness on a vacuum rotary evaporator at 35 °C. The residues were redissolved in 2.5 mL acetone/petroleum ether (1:9, *v*/*v*) for GC-ECD analysis.

GC-ECD analysis

Novaluron and bifenthrin were determined using an Agilent 7890A GC-ECD. Separation was carried out on a HP-5 (30 m × 0.32 mm × 0.25 μm) capillary column. The injector was operated at 270 °C with an injection volume of 1 μL. The oven temperature was programmed to ramp from 100 °C (held for 5 min), was raised to 150 °C at 10 °C min^{-1}, and then raised to 260 °C at 30 °C min^{-1} for 5 min. Nitrogen was used as the carrier gas at a flow rate of 2 mL min^{-1}. The detector was maintained at 300 °C. The retention time of novaluron and bifenthrin were 7.4 and 15.5 min, respectively.

Dissipation kinetics

The dissipation dynamics and half-lives of novaluron and bifenthrin in cabbage were calculated using the first-order kinetics equations (Eq. 1):

$$C_t = C_0 e^{-kt} \quad (1)$$

where C_t represents the concentration of the pesticide residue at the time of t, C_0 represents the initial deposits after application, and k is the degradation rate constant (day^{-1}). The half-life ($t_{1/2}$) is defined as the time required for the pesticide residue level to fall to half of the initial residue level after application and was calculated from the k value for each experiment, $t_{1/2} = \ln 2/k$.

Risk assessment

The risk of pesticides in food is assessed from the amount of ingested pesticides as follows (Eqs. 2~5):

$$\mathrm{NEDI} = \mathrm{STMR} \times \mathrm{F/bw} \quad (2)$$

$$\mathrm{RQ_c} = \mathrm{NEDI/ADI} \quad (3)$$

$$\mathrm{NESTI} = \mathrm{HR} \times \mathrm{LP/bw} \quad (4)$$

$$\mathrm{RQ_a} = \mathrm{NESTI/ARfD} \quad (5)$$

where NEDI is national estimated daily intake, ADI is acceptable daily intake, NESTI is national estimated short-term intake, ARfD is acute reference dose (all in μg kg^{-1} bw day^{-1}); STMR is supervised trials median residue (mg kg^{-1}); F is consumption of food agro-product (g day^{-1}); bw is body weight (kg); RQ_c is chronic risk quotient; LP is the large portion consumed (g day^{-1}); HR is highest residue (mg kg^{-1}); and RQ_a is acute risk quotient.

RQ_c ≤100 % (or RQ_a ≤100 %) indicates that the chronic risk (or acute risk) of a pesticide to humans is acceptable, while RQ_c >100 % (or RQ_a >100 %) represents an unacceptable chronic risk (or acute risk) and a higher RQ_c (or RQ_a) indicates greater risk (Sun et al. 2016).

Results and discussion

Method validation

For the preparation of calibration curves, mixture standard of novaluron and bifenthrin was diluted either with pure solvent (acetone/petroleum ether (1:9, *v*/*v*)) or blank matrix extracts in series at 0.01, 0.05, 0.1, 0.25, 0.5, 1, and 1.5 mg L^{-1}. Good linearity and correlation coefficients (>0.9997) between concentrations and peak areas were obtained.

A series of blank samples fortified with novaluron and bifenthrin mixture at levels of 0.01, 0.2, 0.7, 1, and

Table 1 Average recoveries and RSDs of novaluron and bifenthrin in cabbage ($n = 5$)

Pesticide	Spiked level (mg/kg)	Average recovery (%)	RSD (%)
Novaluron	0.01	91	6.8
	0.2	87	5.1
	0.7	98	4.7
	1	81	4.6
	2	84	3.6
Bifenthrin	0.01	100	6.8
	0.2	94	6.6
	0.7	105	1.7
	1	108	4.6
	2	101	1.1

2 mg kg^{-1} were prepared for method validation. Five replicates were taken for recovery experiment. The fortified recoveries were ranged from 81 to 108 % with RSDs of 1.1~6.8 %. The LOQ was 0.01 mg kg^{-1}, for both novaluron and bifenthrin in cabbage. The average recoveries and RSDs are shown in Table 1. The results confirmed that the developed method is suitable for determination of novaluron and bifenthrin in cabbage. Figure 1 shows the GC-ECD chromatograms of novaluron and bifenthrin.

The dissipation of novaluron and bifenthrin in cabbage

Dissipation rate is one of the most important parameters in predicting the fate of pesticides in the crop. The half life ($t_{1/2}$), dissipation regressive equation, and correlation coefficient (r) are summarized in Table 2. The initial concentrations of novaluron in cabbage were in range of 0.6~4.5 mg kg^{-1} with half-lives of 3.5~10 days. The initial concentrations of bifenthrin in cabbage were in range of 0.6~4.7 mg kg^{-1} with half-lives of 3.2~9.6 days.

The dissipation study is an important evaluation and would be helpful for the proper and safe use of pesticide. A gradual and continuous decrease of pesticide residues in cabbage was observed. For both of novaluron and bifenthrin, the longest half-life was obtained in Beijing, while the shortest one was obtained in Anhui. Dissipation rate may be influenced by many factors, including the properties of the pesticide, the plant species, the climate (light, temperature, humidity), growth rate, and

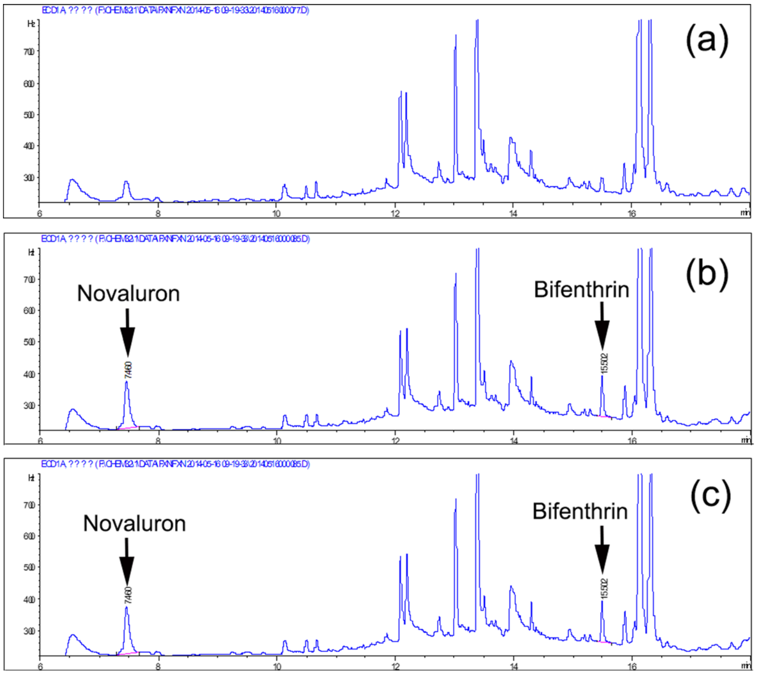

Fig. 1 GC-ECD chromatograms of novaluron and bifenthrin: **a** blank cabbage sample, **b** matrix-matched standard (0.02 mg L^{-1}), and **c** cabbage spiked with novaluron and bifenthrin (0.01 mg kg^{-1})

Table 2 Dissipation regressive equation, correlation coefficient (r), and half-lives ($t_{1/2}$) of novaluron and bifenthrin in cabbage

Pesticide	Location	2013			2014		
		Equation	r	$t_{1/2}$	Equation	r	$t_{1/2}$
Novaluron	Beijing	$C = 0.9e^{-0.06T}$	−0.972	10	$C = 1.1e^{-0.09T}$	−0.970	7.4
	Anhui	$C = 4.5e^{-0.17T}$	−0.973	4.1	$C = 3.1e^{-0.20T}$	−0.975	3.5
	Hunan	$C = 2.2e^{-0.13T}$	−0.830	5.3	$C = 0.6e^{-0.13T}$	−0.875	5.3
Bifenthrin	Beijing	$C = 1.1e^{-0.07T}$	−0.986	9.6	$C = 1.2e^{-0.10T}$	−0.977	6.9
	Anhui	$C = 4.7e^{-0.17T}$	−0.947	4.1	$C = 3.2e^{-0.22T}$	−0.973	3.2
	Hunan	$C = 2.6e^{-0.14T}$	−0.838	5.0	$C = 0.6e^{-0.15T}$	−0.900	4.6

so on (Arias-Estevez et al. 2006). In this experiment, the temperature and humidity of Anhui and Hunan were higher than Beijing, which may make a longer half-life in Beijing. However, further study is necessary to reveal the relevance between influencing factors and dissipation rate. There is a obvious difference between initial residue of three sites, which may be result of different size of cabbage when applied pesticide. Common variety of cabbage in the locality was selected in field trial. The size of cabbage when applied pesticide may be different among three sites, which make different amount of pesticide adhere to cabbage. Calculated by first-order kinetics equation, the half-lives were not influenced by initial residue. As a result of equal proportions in the mixed formulation (5 % for both novaluron and bifenthrin), the initial residue levels of novaluron and bifenthrin were similar. In previous studies, the half-lives of novaluron in chilli, brinjal, and tomato were 2.08, 1.80~1.95, and 1.80~2.08 days, respectively (Malhat et al. 2014; Das et al. 2007). Furthermore, the half-lives of bifenthrin in tea, tomato, and wheat were 0.52~1.32, 1.83~2.32, and 2.4~10.5 days (Tewary et al. 2005; Chauhan et al. 2012; You et al. 2013). The different crop varieties and weather in field trials may lead to different rate of dissipation.

The terminal residues of novaluron and bifenthrin in cabbage

The cabbage was sampled three times during the harvest period. When the SC was sprayed at low dosage (37.5 g a.i. ha^{-1}) over two and three times application, the terminal residues of novaluron and bifenthrin in cabbage were 0.01~0.18 mg kg^{-1}; when it was sprayed at high dosage (56.25 g a.i. ha^{-1}) over two and three times application, the terminal residues were 0.01~0.23 mg kg^{-1}.

Exposure risk assessment

The average consumption and the large portion consumption of cabbage in China are reported at 185.4 and 209.4 g day^{-1}, respectively (Jin 2008). The mean adult body weight was 60 kg (FAO 2009). The MRL and ADI of novaluron were set at 0.7 mg kg^{-1} and 10 μg kg^{-1} bw day^{-1} (GB 2763-2014). The 2005 JMPR concluded that the short-term intake of novaluron residues is unlikely to present a public health concern; thus, the ARfD of novaluron was unnecessary (ARS 2010a, b). According to the results of terminal residue trials, the STMR of novaluron was 0.01 mg kg^{-1}. The RQ_c of novaluron has been obtained. The MRL, ADI, and ARfD of bifenthrin were set at 0.2 mg kg^{-1}, 10 μg kg^{-1}

Table 3 The risk assessment of novaluron and bifenthrin in cabbage

Pesticide	STMR (mg kg^{-1})	HR (mg kg^{-1})	NEDI (μg kg^{-1} bw day^{-1})	NESTI (μg kg^{-1} bw day^{-1})	RQ_c (%)	RQ_a (%)
Novaluron	0.01	–	0.03	–	0.3	–
Bifenthrin	0.01	0.20	0.03	0.7	0.3	7.0

bw day^{-1}, and 10 μg kg^{-1} bw day^{-1} (GB 2763-2014; ARS 2010a, b). According to the results of terminal residue trials, the STMR and HR of bifenthrin were 0.01 and 0.20 mg kg^{-1}, respectively. The RQ_c of both novaluron and bifenthrin were 0.3 %, while the RQ_a of bifenthrin was 7.0 %. The risk assessment of novaluron and bifenthrin is summarized in Table 3.

The results of risk assessment showed that the RQ_c and RQ_a were all below 100 %, which indicate that the residues of novaluron and bifenthrin in cabbage were relatively safe to humans even at high dosage and shortest PHI. The lager value of RQ_a (7.0 %) than RQ_c (0.3 %) of bifenthrin indicates that bifenthrin exerts higher acute risk to humans than chronic risk. Nevertheless, it is a remarkable fact that the risk assessment in present study is on single pesticide. For a predictive assessment of the aquatic toxicity of chemical mixtures two competing concepts are available: concentration addition (CA) and independent action (IA) (Faust et al. 2003). CA was introduced by Loeve and Muischnek (1926). This model is based on a dilution principle and was designed for chemicals with a similar mechanism of action and has proven effective in several settings (Hermens et al. 1984; Konemann 1981). The overall effective concentration for CA model can be calculated by adding up all the effective concentrations of all compounds. IA was first applied to biological data by Bliss (1939). IA is designed for mixtures of chemicals that have distinct mechanisms of action, and its usefulness has been confirmed in several settings (Faust et al. 2003; Backhaus et al. 2004). On the one hand, Deneer assesses the usefulness of CA based on literature data from 1972 to 1998. Deviations from CA were observed in only 8 % of all mixtures which are rare, and those that do occur are mostly limited in extent and tend to cancel each other out (Deneer 2000). On the other hand, as for IA model, Hass discovered that combinations of similarly acting antiandrogens are able to produce developmental effects in male offspring of rats (Hass et al. 2007). Chen found that the combination-index method could accurately predict the combined toxicity (Chen et al. 2014a, b; Chen et al. 2014a; Chen et al. 2015; Wang et al. 2015). Previous study also indicated that one single chemical does not always drive the effect of a non-potency adjusted mixture (Hadrup et al. 2013).

The health risk of novaluron and bifenthrin based on the toxicity predicted by CA is relatively low. The use of novaluron-bifenthrin mixture does not seem to pose any chronic or acute risk to humans in China even if cabbages are consumed at high dosages and short PHIs. The results could provide guidance for the reasonable use of pesticides and serve as a reference for establishing MRLs. However, to obtain the actual risk assessment of combinations of novaluron and bifenthrin, further toxicology studies to judge whether CA or IA is appropriate are required.

Acknowledgments This study was supported by the National Science Foundation of China (item nos. 21177155 and 31401773).

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