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Research Article

Dissipation, Residue Analysis, and Dietary Risk Assessment of Chlorantraniliprole and Indoxacarb in Rice

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Abstract: A residue analytical method for the determination of chlorantraniliprole and indoxacarb in brown rice, rice husk, and rice straw was validated using a revised QuEChERS method coupled with high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), and field trials were conducted in 12 regions to study the dissipation and final residues of the two pesticides in rice crop. In the method validation, chlorantraniliprole and indoxacarb had average recoveries ranging from 79% to 110%, with relative standard deviations (RSD) of 1.2% to 12.2%. The dissipation experiments showed that the half-lives of chlorantraniliprole in brown rice and rice husk were 12.4-29.8 and 4.6-11.2 d, respectively, whereas the dissipation half-lives of indoxacarb were 10.3-20.4 and 4.4-8.1 d, respectively. As for the final residues in the 12 regions, rice samples were collected on the 28 and 35 d after the last application. The maximum residues of chlorantraniliprole and indoxacarb in rice grain were 0.38 mg/kg and 0.080 mg/kg, respectively, and in brown rice, the maximum residues of chlorantraniliprole and indoxacarb were 0.028 mg/kg and 0.040 mg/kg, respectively. These values were all below the relevant Maximum Residue Limits (MRLs) in China. Using the final residue results and the food consumption data, the long-term dietary risk assessment indicated that the residues of these two pesticides in rice would not pose unacceptable dietary risk to the general population.

***Keywords*:** chlorantraniliprole, indoxacarb, rice, residue, dissipation dynamics, dietary risk assessment

1. Introduction

Chlorantraniliprole is an anthranilic diamide insecticide, which can bind specifically to ryanodine receptors (RyR) in insect muscles, therefore allowing the uncontrolled release of calcium from internal stores. This response mechanism can impair the muscle contraction regulation and ultimately leads to the die of the target organism [1]. Chlorantraniliprole is widely used due to its low toxicity toward non-target organisms, including animals, fish, etc. [2]. Indoxacarb is an effective insecticide for controlling target insects, and the mechanism of action was that it blocks the sodium channels in the nerve cells of pests, causing the loss of function of the nerve cells and eventually the death of the target pests [3].

Rice is one of the staple food crops for the vast majority of the world's population, and China is the world's largest rice producer as well as the rice-consuming country. With the increase in rice planting areas, the problem of rice pests and diseases has become more serious [4]. Chemical control is a common and effective means of controlling crop pests and diseases. The high control efficiency of chlorantraniliprole and indoxacarb makes them ideal for controlling

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rice pests [5-6]. For the safety use of chlorantraniliprole and indoxacarb on rice, different countries have established Maximum Residue Levels (MRLs) (Table 1). The MRLs for these two pesticides were found to be 0.5 mg/kg for chlorantraniliprole and 0.1 mg/kg for indoxacarb in both unhusked rice and brown rice in China. However, despite increased production and use, the residue levels in rice grain and straw after field application of chlorantraniliprole and indoxacarb and the dietary risk assessment have not been reported.

Table 1. The MRLs of chlorantraniliprole and indoxacarb in rice in different countries

Compound	Crop type	China	CAC	USA	EU
chlorantraniliprole	Unhusked rice	0.5	-	-	-
	Brown rice	0.5	-	-	-
	rice	-	0.4	0.15	0.4
indoxacarb	Unhusked rice	0.1	-	-	-
	Brown rice	0.1	-	-	-
	rice	-	-	-	0.01

As for the residue analysis of chlorantraniliprole, HPLC and LC with tandem mass spectrometry (LC-MS/MS) have been mentioned as some residue analysis methods of chlorantraniliprole in different foodstuffs like grapes [7] and tomato [8]. The residues of chlorantraniliprole in rice have also been studied, in which acetonitrile extraction was performed on rice samples, followed by a glass column cleaning procedure and HPLC-UV analysis [9]; however, the sample preparation process was tedious, complex, and solvent-intensive. In paddy ecosystems (soil, straw, paddy water, brown rice), the residues of chlorantraniliprole and degradation dynamics were investigated using LC-MS/MS, and four kinds of sorbents were used for cleanup of the matrices [10].

Residue analysis of indoxacarb was mainly focused on vegetables [8], fruits [11] , milk and egg [12], and rice [13]. The QuEChERS method was employed to determine indoxacarb in rice-related matrices by LC-MS/MS, in which the rice samples were cleaned up by N-propyl ethylene diamine (PSA) and Octadecylsilyl, ODS (C18), while the soil samples were not purified [13]. Besides, the degradation and residues of indoxacarb enantiomers were determined in rice using chiral liquid chromatography-tandem high-resolution mass spectrometry [14].

As the compound pesticide formulation (the mixed formulation contains two or more pesticides) is being extensively used on rice, the reports on the mixed formulation of chlorantraniliprole and indoxacarb in rice are mainly focused on efficacy determination [15-17], such as the control effect and dosage. However, the residue and dissipation dynamics after the application of the compound formulation of chlorantraniliprole and indoxacarb have not been reported. Moreover, the hazard quotient (HQ) of chlorantraniliprole and indoxacarb has not been reported either.

The goals of this study were to develop a practical, quick, and effective residue analysis method for determining both chlorantraniliprole and indoxacarb in rice matrices (brown rice, husks, and straw), as well as to look into the residue condition and dissipation dynamics of the two pesticides. Since rice is a daily consuming foodstuff, the potential dietary intake risks for general consumers were assessed using the field trial residue results, food consumption, and toxicological data.

2. Materials and methods

2.1 *Chemicals and reagents*

Acetonitrile of chromatographic grade were obtained from Fisher Chemicals; a Haide deionized water system in Shandong, China, was used to obtain ultrapure water. Beijing Chemical Reagent Company provided formic acid, magnesium sulfate ($MgSO_4$), and sodium chloride (NaCl). Tianjin Bona Agela Technology Co. Ltd. provided PSA. The pesticide standards of chlorantraniliprole (98.3%) and indoxacarb (97.8%) were bought from Shanghai Pesticide Research Institute Co., Ltd. The mixed formulation of 15% chlorantraniliprole-indoxacarb (5% + 10%) suspension was provided by Anhui Meilan Agricultural Development Co. Ltd. for field trials.

The standard stock solutions of chlorantraniliprole (1,000 mg/L) and indoxacarb (1,000 mg/L) were prepared separately in acetonitrile. The standard mixtures of serial concentrations were prepared from the stock solution by dilution with acetonitrile.

The instrument utilized was the LCMS-8045 with an Athena C18-WP column (3.0 μm, 2.1 mm, and 50 mm). Approximately 85:15 (v/v) of acetonitrile and 0.1% formic acid water were used as the mobile phase. The flow rate was 0.3 mL/min, the injection volume was 1 μL, and the column temperature remained at 40 °C. The MS/MS parameters are shown in Table 2 and were obtained using positive electrospray. The drying gas flow rate was 10.0 L/min, and the required temperature setting for the ion source was 300 °C.

Table 2. The MS/MS transitions and optimized parameters

Compound	Precursor ion	Product ion	Q1 Pre Bias (V)	Collision Energy (eV)	Q3 Pre Bias (V)
chlorantraniliprole	483.7	452.80*	-10.0	-14.0	-27.0
		285.85	-13.0	-16.0	-30.0
Indoxacarb	527.9	203.0*	-22.0	-41.0	-19.0
		150.0	-24.0	-26.0	-13.0

* Represents the quantitative ion.

2.2 *Field trials*

Field trials were carried out in 2020 in 12 main rice-growing Provinces (Cities) in China, which included Zhaodong City of Heilongjiang Province, Hanzhong City of Shaanxi Province, Xinyang City of Henan Province, Jinhua City of Zhejiang Province, Huaibei City of Anhui Province, Xiantao City of Hubei Province, Changsha City of Hunan Province, Jiujiang City of Jiangxi Province, Gaoan City of Jiangxi Province, Nanning City of Guangxi Province, Kunming City of Yunnan Province, and Zhaoqing City of Guangdong Province. Table 3 listed the geometric locations, soil types, and typical climate conditions of the 12 field trial locations. The final residues and dissipation of the two target pesticides were studied simultaneously in the field experiments. According to the "Guideline for the Assessment of Pesticide Residues in Crops" in China, the field experiments conformed to good agricultural practices. The final residues in three matrices (brown rice, rice husk and straw) were studied at all the 12 locations, and for the dissipation study, only four of these locations (the Provinces of Anhui, Hunan, Guangxi, and Guangdong) were selected for the dissipation dynamic in only brown rice and rice husk matrices.

The field application scheme for the two pesticides is shown in Table 4. In the 12 experimental regions, the treatment areas were each 100 m^2, in which 15% chlorantraniliprole-indoxacarb suspension was sprayed on the rice crops during the occurrence of rice young larvae. In order to study the state of residual in the worst case of the two pesticides, the dose and application times were established as the maximum case, which was 56.25 g a.i./ha and sprayed twice. In the dissipation experiment, the rice grain (contains the brown rice and rice husk) samples (at least 2 kg) were collected randomly at 2 h, 7 d, 14 d, 28 d, and 35 d after application. For the terminal residue experiment, both the rice grain and rice straw samples (at least 2 kg each) were collected randomly at 28 and 35 d after spraying. The collected rice straw was smashed and the rice grains were threshed, and then the resulting brown rice and rice husk samples were packed separately to avoid cross-contamination, and the experimental samples were placed at -18 °C.

Table 3. The geometric locations, soil type, and typical climate involved in the field trial locations

Regions	Site	Geometric Location	Soil type	Typical Climate
Heilongjiang	Heshan Village, Dongfa Township, Zhaodong City,	125°98' N, 46°07' E	loamy soil	Temperate monsoon climate
Shaanxi	Xujiaxiang Village, Xixiang County, Hanzhong City	32°98' N, 107°83' E	loamy soil	Subtropical monsoon climate
Henan	Dai Zhai Village, Cao Huanglin Town, Xinyang City	32°18' N, 114°81' E	yellow-brown loam	Temperate monsoon climate
Zhejiang	Sumeng Township Agricultural Academy Base, Jinhua City	29°08' N, 119°65' E	red and yellow loam	Subtropical monsoon climate
Anhui	Gaoyue Town, Huaibei City	33°95' N, 116°80' E	sandy loam	Subtropical monsoon climate
Hubei	Liuhe Village, Changtankou Town, Xiantao City	30°37' N, 113°45' E	rice soil	Subtropical monsoon climate
Hunan	Chunhua Town, Changsha City	28°31' N, 113°29' E	clay	Subtropical monsoon climate
Jiujiang	Duchang County, Jiujiang City, Jiangxi Province	29°27' N, 116°18' E	clay	Subtropical monsoon climate
Gaoan	Ashibu Town, Gaoan City, Jiangxi Province	28°28' N, 115°26' E	clay	Subtropical monsoon climate
Guangxi	Xixiangtang District, Nanning City	22.83°' N, 108°30' E	red loam	Subtropical monsoon climate
Yunnan	Huangpo Village, Fumin County, Kunming City	25°22' N, 102°50' E	red loam	Subtropical monsoon climate
Guangdong	Dawan Town, Gaoyao District, Zhaoqing City	23°03' N, 112°45' E	sandy loam	Subtropical monsoon climate

Table 4. Field application scheme of the formulation

Pesticide commercial formulations	Recommend dose (g a.i./ha)[b]	PHI (d)	Application times	Intervals for dissipation residues (d)	Intervals for terminal residues (d)
15% chlorantraniliprole-indoxacarb suspension [a]	56.25	28	2	2 h, 7, 14, 28, 35	28, 35

[a] 15% chlorantraniliprole-indoxacarb (5% + 10%) suspension
[b] 'g a.i./ha' indicates gram of active ingredients per hectare

2.3 *Sample preparation*

The samples of mashed brown rice (5.0 g), rice husks (2.0 g), and rice straw (2.0 g) were weighed and put into 50 mL centrifuge tubes. Following the addition of 5 mL water with 0.1% formic acid and 10 mL of acetonitrile, the tubes were oscillated for 5 min. The mixture was then vortexed for 1 min after 3 g of NaCl was added. After that, the tubes were centrifuged at 3,800 rpm for 5 min, and 1 mL of the supernatant was taken for further dispersive solid-phase extraction (d-SPE) cleanup. For the cleanup, 50 mg of PSA and 150 mg of $MgSO_4$ were accurately weighed into a 2 mL centrifuge tube, and then the extracted supernatant of brown rice, rice husks, and rice straw were added into the tube for purification. The tubes were vortexed for 1 min and centrifuged at 10,000 rpm for 1 min at high speed. After centrifugation, the supernatant (acetonitrile phase) was filtered through an organic membrane with 0.22 μm pore size for

HPLC-MS/MS analysis.

2.4 *Dietary risk assessment*

Dietary risk assessment was conducted using terminal residue data and local dietary consumption information according to the international common method [18] and the guideline of World Health Organization [19]. Neither chlorantraniliprole nor indoxacarb has an acute reference dose (ARfD) for short-term dietary intake risk. To calculate the risk of long-term dietary exposure, the Hazard Quotient (HQ), defined as the ratio of the National Estimated Daily Intake (NEDI) to the Acceptable Daily Intake (ADI), was used.

HQ = [NEDI/(ADI × BW)] × 100%, in which BW stands for "body weight," and 63 kg was selected as the default for the general population in China; ADI (Acceptable Daily Intake) is the amount of a substance that can be ingested daily by humans over a lifetime without producing a detectable health hazard; NEDI is the National Estimated Daily Intake (mg/kg bw);

NEDI = Σ(*STMR* × *F*), and F stands for the average daily food (rice) intake of Chinese citizens in urban and rural areas; STMR stands for Supervised Trials Median Residue (mg/kg);

When HQ < 100%, consumers are considered to be at an acceptable dietary risk.

3. Results and discussion

3.1 *Validation of analytical method*

QuEChERS (quick, easy, cheap, effective, rugged and safe) approach [20] has been used to prepare samples for the measurement of pesticide residues in a variety of matrices due to its benefits including requiring less solvent, employing less lab equipment, and attaining high sample throughput. In this study, QuEChERS was used and revised for the simultaneous measurement of chlorantraniliprole and indoxacarb in rice matrices. The method was then assessed for fortified recoveries, relative standard deviation (RSDs), limit of quantification (LOQ), linearity, and matrix effects in accordance with the method validation standard.

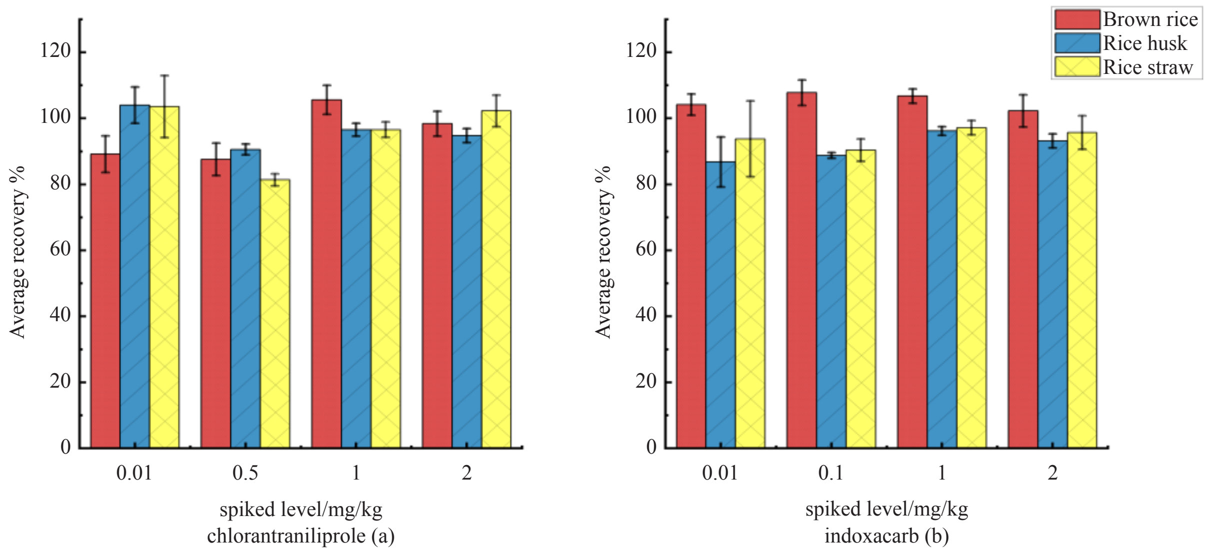

Figure 1. Average recoveries of chlorantraniliprole (a) and indoxacarb (b)

The blank brown rice, rice husk, and rice straw samples were spiked with the standard solutions of chlorantraniliprole and indoxacarb at 4 spiking levels, which were 0.01, 0.5, 1.0, and 2.0 mg/kg and 0.01, 0.1, 1.0, and 2.0 mg/kg, respectively. The spiking recoveries and RSDs of the five replicates were used to assess the accuracy and precision of the method. Recoveries between 70% and 120% with RSD of less than 20% were considered qualified for the method.

The results (Figure 1) showed that the average recoveries were 81%-106% and 87%-108% for chlorantraniliprole and indoxacarb, respectively, and the precision of the method, in terms of RSDs, was 2.2%-9.3% and 1.2%-12.2%. The LOQ was 0.01 mg/kg for both analytes in these three rice matrices. The results showed that the method was reliable and sensitive for the determination of chloroantraniliprole and indoxacarb in rice matrices.

The standard solutions of chlorantraniliprole and indoxacarb were diluted with the matrix blank extracts to prepare the matrix-matched calibration solutions at 0.005, 0.05, 0.1, 0.25, 0.5, and 1.0 mg/L for brown rice and at 0.002, 0.02, 0.1, 0.25, 0.5, and 1.0 mg/L for rice husk and rice straw. The linearity parameters listed in Table 5 showed a good linear correlation ($R^2 > 0.99$) between the peak area and the mass concentration of chlorantraniliprole and indoxacarb in the range of 0.005-1.0 mg/L for brown rice and between 0.002-1.0 mg/L for rice husk and straw.

Table 5. The linear equations and determination coefficients (R^2) of the matrix-matched or solvent-only calibration curves of two pesticides in rice matrices

Compound	Matrices	Linear equations and R^2	ME(%)
Chlorantraniliprole	ACN	y = 5,031,611.3894x + 10,638.2172 R^2 = 0.9993	-
	Brown rice	y = 5,129,367.8799x + 22,828.6981 R^2 = 0.9999	1.9%
	Rice husk	y = 3,923,872.7871x + 47,968.8571 R^2 = 0.9997	-22.0%
	Rice straw	y = 4,808,468.5399x + 153,728.3156 R^2 = 0.9991	-4.4%
Indoxacarb	ACN	y = 3,011,055.0343x + 2,568.1933 R^2 = 0.9999	-
	Brown rice	y = 2,992,992.6859x - 551.1778 R^2 = 0.9998	-0.6%
	Rice husk	y = 2,679,245.9044x - 2,654.5555 R^2 = 0.9998	-11.0%
	Rice straw	y = 3,093,350.2788x - 6,603.4537 R^2 = 0.9998	2.7%

Matrix Effect refers to the enhancement or suppression of the response signal caused by the co-eluted matrix components. The following equation was used to calculate the matrix effect (ME) [21]:

$$ME = \left(\frac{Slope\ of\ \ calibration\ curve\ in\ matrix\ extracts}{Slope\ of\ \ calibration\ curve\ in\ pure\ solvent} - 1 \right) \times 100\%$$

When ME is in -20%-20%, it shows weak matrix effect; when ME > 20% or ME < -20%, it means matrix enhancement or matrix suppression effect. In this study, both chlorantraniliprole and indoxacarb showed weak matrix effects in most of the three rice matrices. Among them, the matrix effects of chlorantraniliprole in brown rice, rice husk, and rice straw were 1.9%, -22.0%, and -4.4%, respectively, while the matrix effects of indoxacarb were -0.6%, -11.0%, and 2.7%, respectively. Specific data sources are presented in Table 5. Despite of the weak effect, the matrix-matched calibration curves were used for precise quantification.

3.2 *Residue dissipation study*

The residue dynamic study in brown rice and rice husk was carried out with the goal of estimating the residue dissipation and half-lives of the pesticides during the crop cultivation. The residual dissipation dynamics of the two pesticides in the two matrices (brown rice and rice husk) are shown in Figure 2 and Figure 3, with detailed data in Table 6. When the residue was lower than the LOQ (< 0.01 mg/kg), 0.01 mg/kg was used in the calculation.

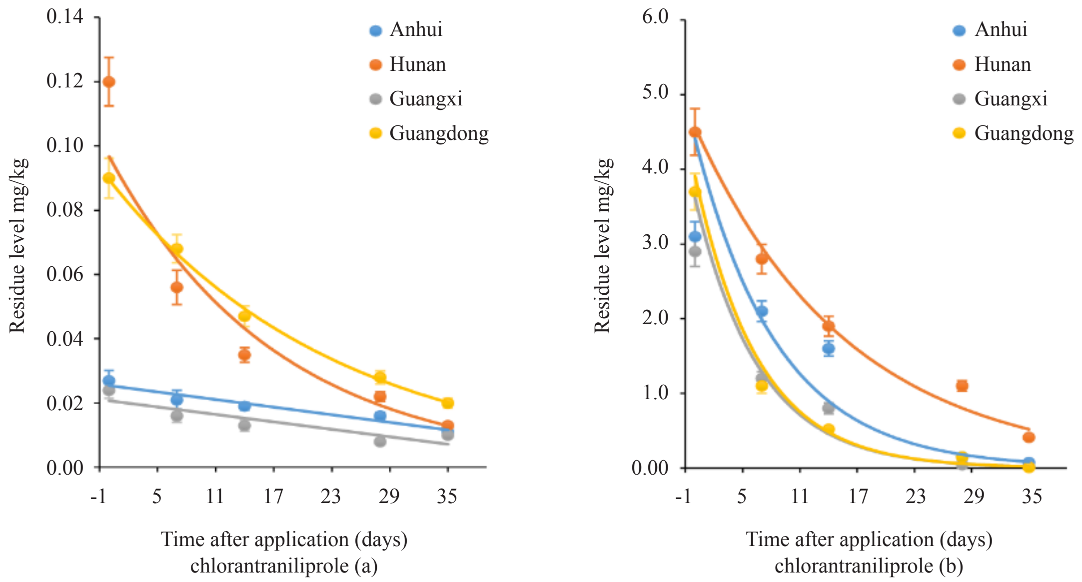

Figure 2. Dissipation of chlorantraniliprole in brown rice (a) and rice husk (b) at four field trial locations

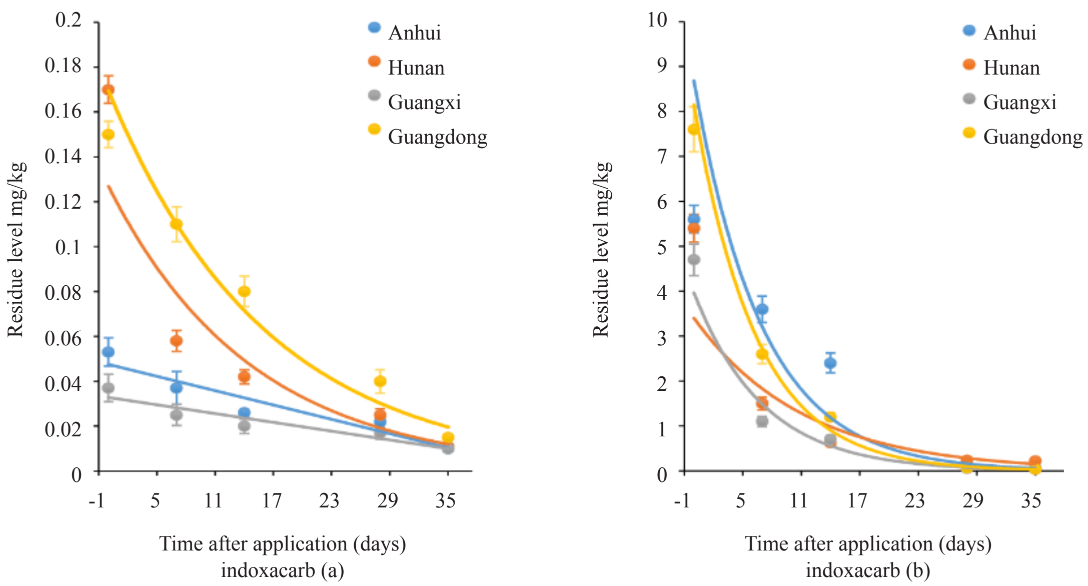

Figure 3. Dissipation of indoxacarb in brown rice (a) and rice husk (b) at four field trial locations

The residues of chlorantraniliprole and indoxacarb in both brown rice and rice husk samples decreased gradually with time, and the dissipation rates were mostly higher than 90% in 28 or 35 d after application. Although brown rice was wrapped in rice husks during its growth process, very low levels of pesticide residues were still detected in brown rice samples. Most likely it was due to the high level of pesticide residues in the rice husks penetrated into the brown rice when harvested on the day of application or when the rice grains were shelled, resulting in the brown rice being stuck with the pesticides.

Table 6. Dissipation of chlorantraniliprole and indoxacarb in rice at four field trial locations

Compound	Matrix	Dissipation	Field trial locations			
			Anhui	Hunan	Guangxi	Guangdong
chlorantraniliprole	Brown rice	Equation	$y = -0.0004x + 0.0254$	$y = 0.0912e^{-0.056x}$	$y = -0.0004x + 0.0207$	$y = 0.0896e^{-0.043x}$
		R^2	$R^2 = 0.9356$	$R^2 = 0.9037$	$R^2 = 0.7983$	$R^2 = 0.9975$
		$T_{1/2}$/d	29.8	12.4	21.8	16.1
	Rice husk	Equation	$y = 4.4818e^{-0.114x}$	$y = 4.5662e^{-0.062x}$	$y = 3.6184e^{-0.148x}$	$y = 3.9118e^{-0.15x}$
		R^2	$R^2 = 0.8562$	$R^2 = 0.9895$	$R^2 = 0.9755$	$R^2 = 0.9947$
		$T_{1/2}$/d	6.1	11.2	4.7	4.6
indoxacarb	Brown rice	Equation	$y = -0.0011x + 0.0475$	$y = 0.1269e^{-0.067x}$	$y = -0.0007x + 0.0328$	$y = 0.1695e^{-0.062x}$
		R^2	$R^2 = 0.9038$	$R^2 = 0.8966$	$R^2 = 0.8829$	$R^2 = 0.9751$
		$T_{1/2}$/d	19.1	10.3	20.4	11.2
	Rice husk	Equation	$y = 9.1413e^{-0.143x}$	$y = 2.8817e^{-0.086x}$	$y = 3.9651e^{-0.14x}$	$y = 8.1516e^{-0.157x}$
		R^2	$R^2 = 0.7905$	$R^2 = 0.8995$	$R^2 = 0.9766$	$R^2 = 0.9976$
		$T_{1/2}$/d	4.8	8.1	5.0	4.4

The initial residues of chlorantraniliprole at 2 h after application in brown rice varied at the four experimental sites, which were higher (0.09-0.12 mg/kg) in Hunan and Guangdong while lower in Anhui and Guangxi (0.024-0.027 mg/kg). The initial residues of indoxacarb had a similar pattern, with residue levels higher in Hunan and Guangdong (0.15-0.17 mg/kg) and lower in Anhui and Guangxi (0.037-0.053 mg/kg). This phenomenon might due to the higher humidity and temperature in Hunan and Guangdong Provinces, which caused the higher penetration and translocation of the pesticides from rice husk and straw into the brown rice.

Due to the very low pesticide residue levels, the dissipation pattern of the pesticides in brown rice (Figure 2a and Figure 3a) in Anhui and Guangxi did not follow an exponential decay law as expected. Instead, it mostly decreased proportionally with time and fitted with the zero-order kinetic equation with R^2 between 0.7983~0.9356, and the half-lives of chlorantraniliprole and indoxacarb were 21.8-29.8 d and 19.1-20.4 d, respectively. On the contrary, the dissipation pattern in Hunan and Guangdong Provinces followed an exponential decay law as usual, and fitted with the first-order kinetic equation with the coefficient of determination (R^2) of 0.8966-0.9975. The half-lives were relatively shorter, which were 12.4-16.1 d and 10.3-11.2 d for the two pesticides, respectively.

Unlike in brown rice, the initial residues of chlorantraniliprole in rice husk were relatively high and did not vary much at the four test sites, and the residues ranged from 2.9 to 4.5 mg/kg. Accordingly, the dissipation pattern in rice husks at all the four sites (Figure 2b and Figure 3b) followed the exponential decay law, and fitted with the first-order kinetic equation with the coefficient of determination (R^2) of 0.7905-0.9976. The half-lives did not vary much either, ranging from 4.6 to 11.2 d. Similarly, the initial residues of indoxacarb in rice husk were relatively consistent among the four sites, ranging from 5.4 to 7.6 mg/kg. Correspondingly, the half-lives did not vary much, ranging from 4.4 to 8.1 d. Overall, the residues in rice husk were much higher than in brown rice; however, in rice husk, the half-life was shorter. Pesticides dissipated more slowly in brown rice than in rice husk due to the fact that rice husks were directly exposed to the air and easily dissipated with the light and rain.

The literature [10] showed that chlorantraniliprole dissipated in water, soil, and rice straw following the first-order kinetic equation, and the half-lives were 0.85, 16.0, and 3.5 d, respectively. The other study was reported showing that the half-lives of chlorantraniliprole in paddy water, soil, and straw were 3.1-5.0 d, 6.6-9.0 d, and 8.9-9.9 d, respectively [9]. However, no study has been done on the dissipation of chlorantraniliprole in brown rice and rice husk. Combined with the half-lives in brown rice (12.4-29.8 d) and rice husk (4.6-11.2 d) of this study, it can be roughly analyzed that chlorantraniliprole dissipated fast in rice husk, straw, and paddy water. Brown rice has the longest half-lives, but the residue levels were relatively very low.

The dissipation study on indoxacarb in rice [14] found that the half-lives of R-indoxacarb and S-indoxacarb were 4.20-4.33 and 3.45-3.57 d in rice straw. In this study, the half-lives of indoxacarb in rice husk and brown rice were 4.4-8.1 and 10.3-20.4 d, respectively, which inferred that indoxacarb dissipated faster in rice straw than in husk and brown rice.

3.3 *Results of terminal residue*

According to the pesticide efficacy field trial conducted by the formulation provider, the estimated pre-harvest interval (PHI) of the tested formulation was 28-35 d. Therefore, the residues on 28 and 35 d after application of the two pesticides in brown rice, rice husk and rice straw at 12 locations were listed as the terminal residues (Table 8). Table 7 summarizes the STMR (supervised trial median residue) and HR (highest residue) at 28 and 35 d using the terminal residue data for each matrix. The residue of the two pesticides in unhusked rice was calculated by following equation: Residue in unhusked rice = 77.6% × residue in brown rice + 22.4% × residue in rice husk, in which, 77.6% and 22.4% are the average weight percentage of brown rice and rice husk in the unhusked rice samples. When the residue was lower than the LOQ (< 0.01 mg/kg), 0.01 mg/kg was used in the calculation.

Table 7. Terminal residue results of chlorantraniliprole and indoxacarb

Compound	Sampling interval (d)	Brown rice		Rice husk		Rice straw		Unhusked rice	
		STMR (mg/kg)	HR (mg/kg)	STMR (mg/kg)	HR (mg/kg)	STMR (mg/kg)	HR (mg/kg)	STMR (mg/kg)	HR (mg/kg)
chlorantraniliprole	28	< 0.01	0.028	0.15	1.9	0.082	0.42	0.044	0.38
	35	< 0.01	0.02	0.035	0.67	0.057	0.27	0.017	0.14
indoxacarb	28	< 0.01	0.04	0.12	0.36	0.14	0.68	0.04	0.08
	35	< 0.01	0.031	0.038	0.22	0.098	0.47	0.023	0.058

On 28-35 d after application, the terminal residues of chlorantraniliprole were < 0.01-0.028 mg/kg in brown rice, < 0.01-1.9 mg/kg in rice husk, < 0.01-0.42 mg/kg in rice straw, and < 0.01-0.38 mg/kg in unhusked rice, respectively. The terminal residues of indoxacarb were < 0.01-0.04 mg/kg in brown rice, < 0.01-0.36 mg/kg in rice husk, < 0.01-0.68 mg/kg in rice straw, and < 0.01-0.08 mg/kg in unhusked rice, respectively. The wide distribution of the residues in rice husk and straw may be due to the occasionally uneven spray droplets, which is inevitable in agricultural operations. All the terminal residues of chlorantraniliprole and indoxacarb in brown rice and unhusked rice were below the corresponding

MRLs in China (0.5 mg/kg and 0.1 mg/kg, respectively), therefore, the PHI was recommended to be 28 d to ensure that the harvested brown rice is safe for the consumption. The MRL of indoxacarb in rice in the EU is much lower (0.01 mg/kg), and the big difference among MRLs may be a concern in the international trade between the different countries.

Table 8. Terminal residue of chlorantraniliprole and indoxacarb in brown rice, rice husk, rice straw and unhusked rice

Regions	Preharvest interval (days)	Residues of chlorantraniliprole (mg/kg)				Residues of indoxacarb (mg/kg)			
		Brown rice	Rice husk	Rice straw	Unhusked Rice	Brown rice	Rice husk	Rice straw	Unhusked Rice
Heilongjiang	28	< 0.01	1.2	0.25	0.25	< 0.01	0.17	0.54	0.043
	35	< 0.01	0.30	0.25	0.068	< 0.01	0.19	0.37	0.046
Shanxi	28	0.022	0.13	0.070	0.044	0.024	0.13	0.15	0.045
	35	< 0.01	0.024	0.045	0.013	< 0.01	0.039	0.11	0.016
Henan	28	< 0.01	0.21	0.20	0.050	< 0.01	0.36	0.32	0.080
	35	< 0.01	0.037	0.17	0.015	< 0.01	0.029	0.24	0.014
Zhejiang	28	< 0.01	1.9	0.077	0.38	< 0.01	0.15	0.24	0.038
	35	< 0.01	0.67	0.068	0.14	< 0.01	0.082	0.20	0.024
Anhui	28	0.016	0.15	0.086	0.043	0.021	0.11	0.072	0.041
	35	0.011	0.075	0.033	0.025	< 0.01	0.059	0.062	0.026
Hubei	28	< 0.01	0.016	0.024	0.011	< 0.01	0.015	0.010	0.011
	35	< 0.01	0.024	0.021	0.013	< 0.01	0.021	< 0.01	0.012
Hunan	28	0.024	1.1	0.066	0.265	0.025	0.24	0.10	0.073
	35	0.013	0.41	0.029	0.102	0.011	0.22	0.085	0.058
Jiujiang	28	0.010	0.47	0.162	0.10	< 0.01	0.21	0.12	0.072
	35	< 0.01	0.18	0.085	0.044	< 0.01	0.036	0.064	0.032
Gaoan	28	< 0.01	0.012	0.16	0.010	0.036	0.046	0.68	0.017
	35	< 0.01	< 0.01	0.10	0.010	0.031	0.040	0.47	0.010
Guangxi	28	< 0.01	0.044	0.42	0.018	0.017	0.057	0.64	0.026
	35	< 0.01	0.021	0.27	0.012	< 0.01	0.037	0.38	0.047
Yunnan	28	< 0.01	0.028	0.033	0.014	< 0.01	0.026	0.037	0.013
	35	< 0.01	< 0.01	0.032	0.010	< 0.01	< 0.01	0.026	0.010
Guangdong	28	0.028	0.15	0.019	0.025	0.04	0.073	0.057	0.02
	35	0.02	< 0.01	< 0.01	0.018	0.015	0.039	0.059	0.021

The final residues of chlorantraniliprole and indoxacarb in rice straw were much higher than those in rice grain and brown rice, which were 0.01-0.42 mg/kg and 0.01-0.68 mg/kg, respectively. This phenomenon may be related to the conductive transportation of nutrients during plant growth that led to an accumulation of pesticides.

3.4 *Dietary risk assessment for general population*

Long-term dietary risk assessment was conducted in accordance with the WHO Guidelines for Estimating Dietary Intake of Pesticide Residues Based on the Terminal Residues of Field Experiments (1997) [19]. According to the Chinese Dietary Guidelines, in this study, food consumption (F) of rice and its products is 0.2399 kg/day. The evaluation of dietary intake takes into account the edible component of all the pesticide-registered crops in China. For those registered food crops for which the STMR was not available, the MRL value was used instead [18, 22]. The calculated hazard quotient (HQ) for each food crop was illustrated (Figure 4), and Table 11 listed the computed HQ findings. Specific data sources are presented in Table 9 and Table 10.

Table 9. Average body weight, rice consumption, NEDI and HQ of different age and gender groups in China

Groups	Weight[a] (kg)	Cereal consumption[b] (kg)	NEDI/BW (10^{-3} mg/kg bw)		HQ (%)[c]	
			chlorantraniliprole	indoxacarb	chlorantraniliprole	indoxacarb
2~7 male	19.825	0.2183	0.110	0.110	0.0055	1.10
2~7 female	18.9	0.2183	0.116	0.116	0.0058	1.16
8~12 male	35.97	0.336	0.093	0.093	0.0047	0.93
8~12 female	34.0	0.336	0.099	0.099	0.005	0.99
13~19 male	59.9	0.4618	0.077	0.077	0.0039	0.77
13~19 female	51.5	0.3688	0.072	0.072	0.0036	0.72
20~50 male	72.87	0.4757	0.065	0.065	0.0033	0.65
20~50 female	58.22	0.3869	0.066	0.066	0.0033	0.66
51~65 male	70.53	0.461	0.065	0.065	0.0033	0.65
51~65 female	60.6	0.3918	0.065	0.065	0.0033	0.65
> 65 male	66.8	0.3988	0.060	0.060	0.003	0.60
> 65 female	58.87	0.3263	0.110	0.110	0.0055	1.10

[a] The data of body weight and cereal consumption are from the Fifth China total diet study
[b] Cereals and cereals products include rice, wheat, corn, and their products
[c] HQ = [(NEDI/BW)/ADI] × 100%, the ADI of chlorantraniliprole and indoxacarb were 2.0 and 0.01 mg/kg bw

Table 10. Long-term dietary risk assessment of chlorantraniliprole in rice

Variety of food	Consumption (kg)	NEDI [a] (mg)	Daily Allowable Intake (mg)	HQ [b] (%) (Hazard quotient)	Proportion of HQ for each food group [c] (%)
Rice and its products	0.2399	0.002399	ADI*63	0.0019	0.03%
Noodles and their products	0.1385	0.00277		0.0022	0.04%
Other Cereals	0.0233	0.000466		0.0004	0.01%
Potatoes	0.0495	0.00099		0.0008	0.01%
Dried beans and their products	0.016	0.0008		0.0006	0.01%
Dark colored vegetables	0.0915	3.66		2.9048	46.57%
Light colored vegetables	0.1837	3.674		2.9159	46.75%
Fruits	0.0457	0.0914		0.0725	1.16%
Nuts	0.0039	0.000078		0.0001	0.001%
Vegetable oil	0.0327	0.0654		0.0519	0.83%
Sugar, starch	0.0044	0.0022		0.0002	0.003%
Soya sauce	0.009	0.36		0.2857	4.58%
Total	0.8381	7.8605	126	6.24	100%

[a] NEDI = $\Sigma(STMR \times F)$, STMR of chlorantraniliprole is 0.01 mg/kg
[b] HQ = [NEDI/(ADI × BW)] × 100%
[c] Risk percentage of rice intake = STMR of brown rice × F (rice)/(ADI × BW)

For chlorantraniliprole, the NEDI for the general population was 7.8605 mg, and the HQ was 6.24%, showing that the use of chlorantraniliprole has no unacceptable long-term risk for the general population in China. In addition, the use of chlorantraniliprole on rice only accounted for 0.03% of the potential risks. However, for indoxacarb, the calculated NEDI was 2.3526 mg, which caused the high HQ of 373.42%. The risk source in Figure 3 showed that the high HQ was mainly from the light-colored vegetables (leaf lettuce). This is because the high MRL of 10 mg/kg was used for the risk calculation of light-colored vegetables since their STMR is not available. The use of MRL instead of STMR overestimated the potential dietary intake risks of indoxacarb. Fortunately, the risk percentage of rice intake was only 0.1%, which indicated that the use of indoxacarb in rice crops would not have unacceptable long-term dietary risks for the general population.

It's crucial to evaluate the risk of food consumption in accordance with the physiological and lifestyle traits of various populations, since people of different ages and genders may have varied dietary patterns. According to various ages and genders, the Chinese population was divided into 12 groups for this study. The specific division basis and age are shown in Figure 5. The Research Report on China's Total Diet included information on rice consumption by age and gender groups (Table 12). As shown in Figure 5, the calculated group HQ (%) of chlorantraniliprole and indoxacarb was 0.003%-0.006% and 0.60%-1.16%, respectively, which were all much lower than 1. Among the different age groups, children were at the relatively highest risk of pesticide exposure, with HQ (%) ranging from 0.0047-0.006% and 0.93%-1.16%, respectively, mainly due to their lower body weight than adults. With increasing age, the chronic dietary risk indicates a general declining trend because of the relative increase of the bodyweight, which caused an increasing allowance daily intake. An exception was that women over 65 years were at significantly increased risk. This may be related to their relatively lower body weight. Besides that, the exposure risk of indoxacarb was relatively greater than that of chlorantraniliprole due to their significantly different ADIs.

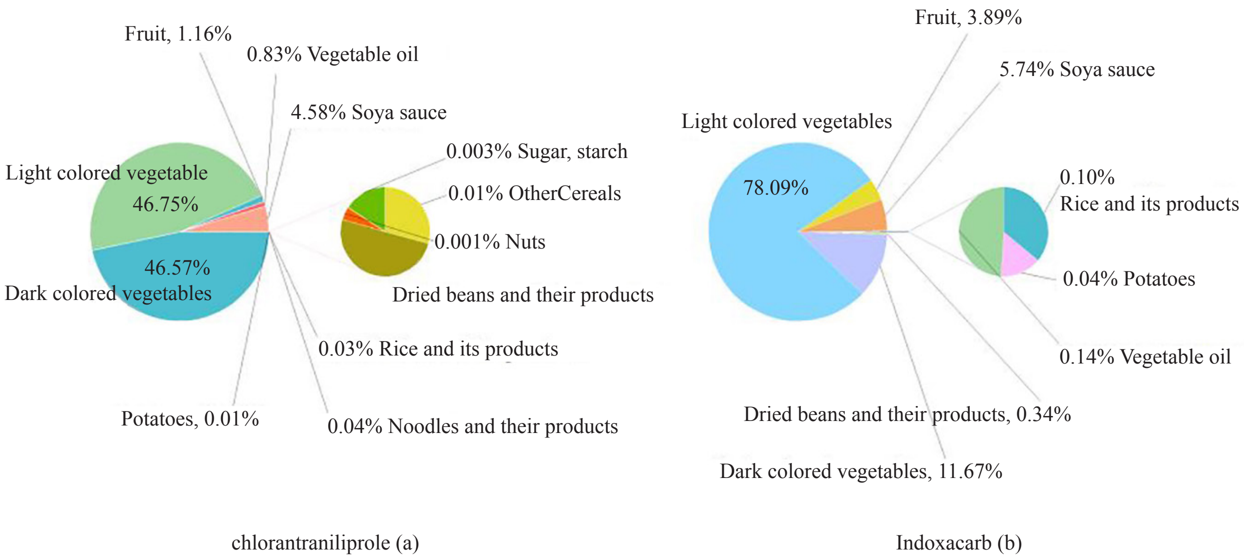

Figure 4. Contribution percentage of several food categories to the chlorantraniliprole (a) and indoxacarb (b) long-term risk quotient.

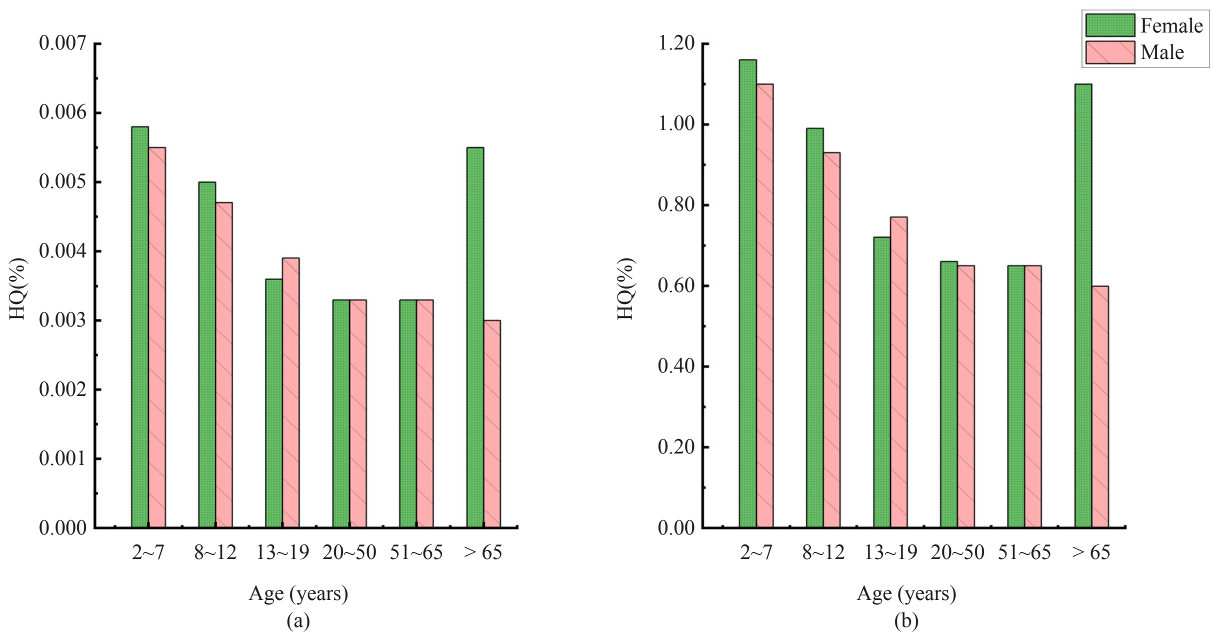

Figure 5. Chronic dietary intake risk to different population groups of chlorantraniliprole (a) and indoxacarb (b) in rice

Table 11. Assessment of the long-term dietary risks of chlorantraniliprole and indoxacarb

Compound	Assessment of the long-term dietary risks			
	ADI (mg/kg bw)	NEDI[a] (mg)	HQ[b] (%)	Risk percentage of rice intake[c] (%)
chlorantraniliprole	2.0	7.8605	6.24	0.03
indoxacarb	0.01	2.3526	373.42	0.10

[a] NEDI = Σ(*STMR* × *F*), in which NEDI is for National Estimated Daily Intake (mg/kg bw); STMR stands for Supervised Trials Median Residue (mg/kg); and F stands for the average daily food intake of Chinese citizens in urban and rural areas.
[b] HQ = [NEDI/(ADI × BW)] × 100%, in which BW stands for "body weight," and 63 kg was selected as the default for the Chinese governments' general population[23].
[c] Risk percentage of rice intake = STMR of brown rice × F (rice)/(ADI × BW)

Table 12. Long-term dietary risk assessment of indoxacarb in rice

Variety of food	Consumption (kg)	NEDI[a] (mg)	Daily Allowable Intake (mg)	HQ[b] (%) (Hazard quotient)	Proportion of HQ for each food group[c] (%)
Rice and its products	0.2399	0.002399	ADI*63	0.38	0.10%
Potatoes	0.0495	0.00099		0.16	0.04%
Dried beans and their products	0.016	0.0008		1.27	0.34%
Dark colored vegetables	0.0915	0.2745		43.57	11.67%
Light colored vegetables	0.1837	1.837		291.59	78.09%
Fruits	0.0457	0.0914		14.51	3.89%
Vegetable oil	0.0327	0.00327		0.52	0.14%
Soya sauce	0.009	0.135		21.43	5.74%
Total	0.668	2.35256	0.63	373.42	100%

[a] NEDI = Σ(*STMR* × *F*), STMR of indoxacarb is 0.01 mg/kg
[b] HQ = [NEDI/(ADI × BW)] × 100%
[c] Risk percentage of rice intake = STMR of brown rice × F (rice)/(ADI ×BW)

4. Conclusions

In this study, a method was developed for the simultaneous determination of chlorantraniliprole and indoxacarb residues in brown rice, rice husk, and rice straw using QuEChERS and HPLC-MS/MS. The experimental data indicated that the residual degradation half-lives in brown rice and rice husk were 12.4-29.8 d and 4.6-11.2 d, respectively, for chlorantraniliprole and were 10.3-20.4 d and 4.4-8.1 d for indoxacarb. The results of the terminal residue experiment showed that the maximum residues of chlorantraniliprole and indoxacarb in brown rice and unhusked rice were all lower than the MRLs for chlorantraniliprole and indoxacarb 28 d after the application. Children had the highest exposure risk to the two pesticides, according to an analysis of the hazards associated with chronic food consumption in various gender and age groups. Based on the results of the field trials, the long-term dietary risk assessment for chlorantraniliprole and indoxacarb in rice revealed no unacceptable risks to the health of general population in China.

The findings offer evidence to support the safe, rational, and scientific use of chlorantraniliprole and indoxacarb on rice.

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Conflict of interest

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