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Fate characteristics and risk identification of thifluzamide in buckwheat across China: Analytical method development, occurrence, and health assessment

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ABSTRACT

Elaborating on the fate tendency of thifluzamide (thiazole-amide fungicide) in buckwheat based on nationwide application is vital for grain security and human health based on nationwide application. A rapid and sensitive analytical method was developed to trace thifluzamide in buckwheat matrices using an ultrahigh-performance liquid chromatography-tandem triple quadrupole mass spectrometer (UHPLC-MS/MS), with a retention time of 2.90 min and limit of quantitation (LOQ) of 0.001 mg/kg. Thifluzamide could be stably stored for 84 d in buckwheat matrices under −20 °C under dark condition. The occurrence, dissipation and terminal magnitudes of thifluzamide were reflected by the primary deposition of 0.02–0.55 mg/kg, half-lives of 12–14 d, and highest residues of 0.41 mg/kg. The long-term risks (*ADI%*) of thifluzamide were 37.268 %–131.658 % in registered crops, and the risks for the rural population were significantly higher than those of the urban population. The unacceptable dietary risks of thifluzamide should be continuously emphasized for children aged 2–7 with an *ADI %* values of 100.750 %–131.658 %. A probabilistic model was further introduced to evaluate the risk discrepancy of thifluzamide in buckwheat, showing the risks in Tartary buckwheat (*Fagopyrum tararicum Gaerth*) were 1.5–75.4 times than that in sweet buckwheat (*Fagopyrum esculentum Moench*). Despite the low risks for dietary buckwheat, the high-potential health hazards of thifluzamide should be pay more attention given the increasing applications and cumulative effects.

1. Introduction

Buckwheat appears as an annual herb and is also known as triangle wheat, flower wheat, and polygonum buckwheat, containing two cultivated varieties of sweet buckwheat (*Fagopyrum esculentum Moench*) and Tartary buckwheat (*Fagopyrum tararicum Gaerth*). Buckwheat is regarded as the only false grain crop of the Polygonum family that has high nutritional and medical values. Buckwheat production will reach approximately 4 million tons worldwide in 2021 (Zou et al., 2023). China was the world's second largest buckwheat producer and exporter, with cultivated areas of 0.3–0.5 million hm^2 and outputs of 0.3–0.5 million tons in 2021, and the planting ratio of *Fagopyrum esculentum Moench* and *Fagopyrum tararicum Gaerth* was approximately 2:1 (Ren et al., 2022). Previous study showed that buckwheat was rich in various nutrients and contained effective ingredients including flavonoids, polyphenols and active peptides. It also has a low glycemic index, and can enhance human immunity, with antitumor, antioxidation and other effects (Huda et al., 2021). However, buckwheat has suffered many serious diseases as the gradual expansion of its planting, and a total of 19 diseases in buckwheat have been reported on the global, including 14 fungal diseases (such as sheath blight), 5 other diseases, 2 bacterial diseases, 3 viral diseases and 3 nematodes as well as 5 other diseases (Qi et al., 2020). Thifluzamide has shown extraordinary control effects on many fungal diseases, such as *Puccinia, Ustilago, Rhizoctonia, Corticiaceae* and *Tilletlac-eae*, especially for sheath blight on rice and wheat. Thifluzamide was active against standing blight and striated blight caused

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by *basidiomycete fungi* in buckwheat by inhibiting succinic dehydrogenase in the tricarboxylic acid cycle (Chen et al., 2015).

Thifluzamide, 2′,6′-dibromo-2-methyl-4′-trifluoromethoxy-4-trifluoromethyl-1,3-thiazole-5-carboxanilide, appears as a new systemic broad-spectrum thiazole-amide fungicide with great systemic characteristics and long-term efficacy (Lewis et al., 2016) developed by Monsanto Company (MO, USA). The melting point of thifluzamide was 178 °C, and its solubility in water was 1.6 mg/L. The partition coefficient and its vapor pressure of the analyte were measured as 4.1 and 1.01×10^{-06} mPa at 20 °C, respectively (Lewis et al., 2016). The crops that registered in China for thifluzamide were buckwheat, potato, peanut, sorghum, rice, and Dendrobium officinale etc. However, thifluzamide is classified as a grade III toxicity and have adverse effects on human health (Lewis et al., 2016). Zebrafish also suffered from the damage of thifluzamide by inhibiting its succinate dehydrogenase (SDH) (Yang et al., 2020) and affecting its lipid metabolism (Yang et al., 2018). Thifluzamide has potential harm to earthworms at the concentration of 10.0 mg/kg (Yao et al., 2020). In addition, the alpha diversity and relative abundance of fungi as well as the community structure of fungal communities in soil can be reduced by thifluzamide exposure. The residual dose of thifluzamide in soil being greater than 10 mg/kg, would cause certain soil ecological risks (Yao et al., 2022).

The reported studies related to thifluzamide have focused on its analytical methods, risk assessment, ecological toxicity, etc. The analysis methods of thifluzamide mainly include gas chromatography (Kumar et al., 2018) and liquid chromatography (Han et al., 2022) have been documented for thifluzamide. However, there is a lack of thifluzamide in buckwheat matirces. Thifluzamide dissipated quickly in maize plants including maize and cowpea with half-lives of 0.2–2.4 d, but it degraded slowly in soil with half-lives of 4.6–15.9 d (Ma et al., 2018; Han et al., 2022). The half-lives of thifluzamide in rice crops and the corresponding cultivation environment were obviously different (Wei et al., 2015). Studies have shown that the risk quotient of thifluzamide in corn was 0.03 % (Ma et al., 2018), and the risk magnitude in peanut seeds from different provinces of China were 0.00019 %–0.00046 % (Cao et al., 2020). However, the risk quotient of thifluzamide in banana was 117.8 % (Zhou et al., 2020), which indicated unacceptable health risks for the general population were found in banana with a risk quotient of 117.8 % (Zhou et al., 2020). In addition, there are currently studies that combine thifluzamide with nanoparticles to improve its availability in plants (Zhao et al., 2022). The combination of pesticide and nanotechnology has become a research hotspot. New nanomaterials with photocatalytic ability and antibacterial activity have great potential (Yousefi et al., 2019). With the progress of the times, consumers pay increasing attention to food security. Ensuring food security not only improves the health level and quality of life of consumers but is also an important guarantee for promoting social stability and harmony. Remarkably, the maximum concentration limit standard of thifluzamide in buckwheat products has not been established worldwide; therefore, it was necessary to study the risk assessment and behavior of thifluzamide in buckwheat.

In present study, a sensitive ultrahigh-performance liquid chromatography-tandem triple quadrupole mass spectrometry (UHPLC-MS/MS) method was first developed to trace thifluzamide in buckwheat. providing a methodological basis for follow-up investigations of storage properties, occurrence, dissipation, and terminal concentration. Compared with HPLC (high-performance liquid chromatography) and UPLC (ultra-performance liquid chromatography), UHPLC (ultrahigh-performance liquid chromatography) possesses the advantages of more precision and speed, less use of solvent, and increased affordability (Nahar et al., 2020). In addition, this study clarified the storage stability of thifluzamide in buckwheat matrix was clarified to confirm the effectiveness of the determination data from the field samples and discussed the influencing factors. Moreover, field experiments were carried out in the main buckwheat-producing areas to explore the concentration, occurrence, dissipation, terminal magnitudes and influencing factors of thifluzamide and contributing factors. Besides, the long-term risks of thifluzamide were assessed, and the exposure risks among consumers groups with different ages, regions and genders, based on two mathematical models of deterministic model and probabilistic model, were used for risk assessment. The deterministic model was simple and feasible, but it could not reflect the individual differences among different populations. The probabilistic model could calculate individual dietary exposure and determine the occurrence probability of dietary risks, representing a more accurate distribution of dietary exposure. The study provided technical support for the accurate qualitative and quantitative analysis of thifluzamide in buckwheat and a theoretical basis for the development of its maximum residues limits, which was also conducive to the rational use of thifluzamide in actual agricultural production and provided a scientific basis for human health risk management.

2. Materials and methods

2.1. Sample collection and storage preparation

Representative *Fagopyrum esculentum Moench* and *Fagopyrum tararicum Gaerth* samples were randomly collected from each trial site, excluding the edges and ends of the plots, as far as preferably healthy and disease-free samples. At least 1 kg of *Fagopyrum esculentum Moench* and *Fagopyrum tararicum Gaerth* samples of at least 1 kg with normal growth and no disease were collected from different directions and parts of plots and put into the sample container. *Fagopyrum esculentum Moench* and *Fagopyrum tararicum Gaerth* samples were collected at increasing intervals at 0 d (2 h), 7 d, 21 d, 28 d and 35 d for the later pharmacokinetic dissipation study. Samples of mature buckwheat were collected at preharvest intervals (PHIs) of 21 and 28 d after the last application in accordance with FAO/WHO recommendations for the terminal level study of thifluzamide. All samples were divided into two parts weighing 150 g, followed by being classified, packaged, labeled, and stored in a –20 °C low temperature freezer. The storage stability samples of *Fagopyrum esculentum Moench* and *Fagopyrum tararicum Gaerth* were prepared according to the demands of NY/T 3094 "Guidelines for Testing storage Stability of Pesticide Residues in Plant-derived Agricultural Products". The blank sample was added to 50 μL of 100 mg/L working solution and kept in the dark at –20 °C, and the samples were sampled at 0, 28, 42, 56 and 84 d to evaluate the degradation rate of thifluzamide during storage period. Detailed pesticide application, sample handling, and instrumental analysis methods would be provided in the supplementary materials.

2.2. Sample extraction and purification

Representative homogenized samples (5 ± 0.05 g) of buckwheat were weighed into 50 mL polytetrafluoroethylene centrifuge tubes followed by adding 10 mL ultrapure water to the buckwheat sample and swirling for 1 min, and 10 mL acetonitrile was subsequently added, swirled for 5 min, and oscillated for 5 min by a CK-2000 high-throughput tissue grinder (TH Morgan, Beijing, China). Next, 6 g of NaCl was added, and the mixture was repeatedly vortexed for 5 min, followed by centrifugation for 5 min with a Frontier FC 5706 centrifuge (OHAUS, Nänikon, Switzerland) at 6000 r/min. Finally, 1 mL of the supernatant was transferred into a single-use centrifuge tube. The resulting supernatant was withdrawn and reconstituted by the initial mobile phase, and filtered through a 0.22 μm nylon syringe filter into an autosampler vial for UHPLC-MS/MS injection.

2.3. Instrumentations

Ultra-performance liquid chromatography-triple quadrupole tandem mass spectrometry (Waters ACQUITY UPLC H-Class/Xevo TQD) equipped with an ACQUITY UPLC HSS T3 column (50 mm × 2.1 mm, 1.8 μm) was employed to detect the thifluzamide in buckwheat. Gradient elution was initialized by 10/90 vol of MeCN (solvent A) and 0.05 % FA in water (solvent B) for 1 min and then increased from 10 % to 90 % of solvent A over 2.5 min, held at 90 % of solvent A for 0.1 min, and then equilibrated by 10 % of solvent A for 1.5 min, providing a total analysis time of 5 min. The column temperature, flow rate and the injection volume were set at 30 °C, 0.35 mL/min, and 5 μL, respectively. To obtain better sensitivity, the positive mode ESI of the electrospray ionization source (ESI) of positive mode was selected. The ion source temperature was 150 °C, the capillary voltage was 3.8 kV, the solvent removal temperature was 500 °C, the cone hole gas flow rate was 50 L/h, and the solvent removal gas flow rate was 1000 L/h. The multiple reaction monitoring (MRM) transitions *m/z* 526.81/147.96, *m/z* 526.81/506.81 and *m/z* 526.81/486.84 were selected for confirmation and quantification when the collision energies were set as 38, 8 and 30 V, respectively. The cone voltage was set at 38 V. The dwell time and the retention time of thifluzamide were 0.108 s and 2.90 min, respectively. Masslynx NT v.4.1 SCN 940 was applied for instrument control and data acquisition and processing.

2.4. Statistical analysis and mathematical calculations

Statistical analysis was performed to determine whether there were significant differences in the thifluzamide contents among different regions and sexes. Differences in datasets that were normally distributed were assessed by one-way ANOVA with Duncan's post hoc test by SPSS 22.0 (IBM, Beijing, China). Statistical significance (p) was set at <0.05 (2-tailed). The posterior distribution of each thifluzamide concentration was assessed with Monte Carlo simulations to reduce uncertainty in the intake evaluations. The theoretical models and calculations are detailed in the supplementary material.

2.5. Quality assurance/quality control

The specificity, linearity, matrix effect (ME), limit of detection (LOD), limit of quantitation (LOQ), repeatability, reproducibility and stability were positively validated based on the analytical quality control criteria of SANTE/11312/2021 (SANTE, 2021). The specificity of the developed method was verified by the analysis of a buckwheat blank in the same plot, with no interference detected at the retention time of thifluzamide (2.90 min). By measuring the calibration curves obtained from the solvent MeCN and the matrix extract, with reference to the correlation coefficient (r) of 0.9999, a satisfactory linearity of 0.001–1.5 mg/kg was observed. Buckwheat samples with high concentrations of target compounds were uniformly diluted in a linear range, and a satisfactory linearity of 0.001–1.5 mg/kg was observed with reference to the correlation coefficient (r) of 0.9999. By measuring the calibration curves obtained from the solvent MeCN and the matrix extract, significant matrix suppression effects were observed for thifluzamide (–14 %) in buckwheat. Consequently, the external matrix-matched standard was selected for the accurate quantitation of thifluzamide to reduce the matrix effect and obtain the true detection data in the sample. The limit of detection (LOD) of thifluzamide was 7.39×10^{-6} mg/kg, which was determined at a threefold signal-to-noise (peak-to-peak) ratio of three under the above conditions. The limit of quantification (LOQ) in the buckwheat matrix was 0.001 mg/kg, which was the lowest concentrations with satisfactory recoveries and the relative standard deviation (RSD), which was evaluated as 0.001 mg/kg. A recovery experiment was performed by spiking a standard working solution of thifluzamide at low (LOQ), medium (10-fold LOQ) and high levels (100-fold LOQ) to cover the maximum concentrations of the samples in five replicates. The recovery was satisfactory (92–109 %), and the RSD was less than 10 %. The stability of thifluzamide was also assessed weekly in both the working solution and the matter-matched solution during the experiment, and no significant differences were found in the concentration of thifluzamide in the study, with p greater than 0.05 with 95 % probability. Typical UHPLC—MS/MS (MRM) chromatograms of thifluzamide in 0.001 mg/L standard solution are shown in Fig. S1.

3. Results and discussion

3.1. Storage stability of thifluzamide in Fagopyrum esculentum Moench and Fagopyrum tararicum Gaerth

The concentrations of thifluzamide in sweet buckwheat (*Fagopyrum esculentum Moench*) and Tartary buckwheat (*Fagopyrum tararicum Gaerth*) remained stable in the range of 1.00–1.06 mg/kg, with degradation rates of <30 %. The results indicated that thifluzamide could be stably stored during dark experiments at –20 °C. The quality control (QC) recoveries of thifluzamide in *Fagopyrum esculentum Moench* and *Fagopyrum tararicum Gaerth* were between 97 % and 110 % during 84 d of storage. The factors affecting the storage stability of thifluzamide may be pH, storage temperature, physicochemical properties of pesticide and pretreatment conditions of matrices (Tiryaki et al., 2008; Bian et al., 2020; Kawar et al., 1973; Mastovská and Lehotay, 2004). First, the storage stability of thifluzamide might be attributed to the pH value, and thifluzamide could be stored stably in a matrix with a pH of 5–9. The pH of buckwheat (7.7) was higher than that of cowpea (4.6). Thus, the degradation rate of thifluzamide in buckwheat (–12 %–4 %) was lower than that in cowpea (2.1 %–19.1 %) for 12 weeks under stored at –20 °C for 12 weeks(Han et al., 2022). During the storage period, thifluzamide suffered different degrees of loss due to its own properties and environmental impacts, which directly affected the reliability of experimental data, affects the establishment of pesticide registration and pesticide maximum residue limits, and ultimately leads to food safety problems. Therefore, it was necessary to study the storage stability of thifluzamide in the samples to be analyzed. Thifluzamide was stably stored in buckwheat under frozen conditions, which might result from the low-temperature frozen storage slowing the degradation of thifluzamide by decreasing the respiratory intensity of buckwheat crops. In addition, the storage stability of thifluzamide may also be related to its physicochemical properties of agrochemicals(Bian et al., 2022) and the corresponding enzyme activities in buckwheat (Yang et al., 2019). The storage stability of thifluzamide was affected by multiple factors under different storage conditions, and the mechanism should be further revealed by further investigations.

3.2. Occurrence and degradation of thifluzamide during Fagopyrum esculentum Moench cultivation

The initial concentrations of thifluzamide in sweet buckwheat (*Fagopyrum esculentum Moench*) were 0.55 mg/kg and 0.02 mg/kg in Trial #1 (112.78°E, 40.62°N) and Trial #2, respectively, and the dissipation rates were 88.9 % and 87.9 % after 35 d. Moreover, the pharmacokinetic degradation of thifluzamide conformed to pseudo-first-order kinetics well, with determination coefficients (r) of 0.9673 and 0.8919. The resolution dynamic curve of Trial #1 was $C = 0.654e^{-0.058\ t}$, and the matrix standard curve was used for quantitative determination, with a $T_{1/2}$ of 12 d. The curve of Trial #2 was $C = 0.0162e^{-0.049\ t}$, with a $T_{1/2}$ of 14 d. The results showed that the initial deposition amount of thifluzamide in Trial #1 was 23 times than that in Trial #2. The temperature in Trial #2 (2–14 °C) was slightly higher than that in Trial #1 (–4–11 °C), and the precipitation was 4 times that in Trial #1. High temperature may promote the pharmacokinetic degradation of pesticides in plants(Farha et al., 2016). With the increase of atmospheric temperature, the number of soil microorganisms increased, and some studies showed that aerobic microorganisms could promote the degradation of thifluzamide in soil(Gupta and Gajbhiye, 2004). Furthermore, compared with Trial #1, the soil pH value in Trial #2 was lower (6.9), and the OM % was higher (3.1 %). Low pH may promote the hydrolysis of thifluzamide in soil water (Gangwar and Rafiquee, 2007), and organic matter is a fundamental driver of thifluzamide biodegradation in soil

(Hussain et al., 2013). The original deposit of thifluzamide in buckwheat would be also affected by crop variety, plant height, plant density, application machinery, weather conditions and cultivation facilities (Wang et al., 2019).

Moreover, the pharmacokinetic degradation of thifluzamide conformed to pseudo-first-order kinetics well, with determination coefficients (r) of 0.9673 and 0.8919. The resolution dynamic curve of thifluzamide was $C = 0.654e^{-0.058\ t}$ in Trial #1, with a half-life ($T_{1/2}$) of 12 d. The curve was $C = 0.0162e^{-0.049\ t}$ in Trial #2, with a $T_{1/2}$ of 14 d. The degradation half-lives ($T_{1/2}$) of thifluzamide were significantly different among different crops, with $T_{1/2}$ values of 1.7–4 d in cowpea (Han et al., 2022) and 9.1–11.6 d in peanut(Cao et al., 2020). The longest $T_{1/2}$ of thifluzamide was observed in soil (4.56–15.84 d), whereas the shortest $T_{1/2}$ was observed in corn (0.19–0.22 d) (Ma et al., 2018).

3.3. Terminal levels and MRL comparison of thifluzamide in *Fagopyrum esculentum Moench* and *Fagopyrum tararicum Gaerth*

As shown in Table S1, the terminal levels of thifluzamide in the buckwheat crops were explored in 24 trial plots spanning four climatic zones from northern to southern China. The final concentrations of thifluzamide were 0.0048–0.41 mg/kg, when the highest levels appeared in Trial #4 (0.27–0.41 mg/kg), and the lowest appeared in Trial #2 (0.0048–0.0054 mg/kg). The concentrations of thifluzamide in *Fagopyrum tararicum Gaerth* in Trial #4 were 1.5–75.4 times than those in *Fagopyrum esculentum Moench* in Trial #2. First, *Fagopyrum tararicum Gaerth* leaves were wide triangular, and their surface area was larger than that of *Fagopyrum esculentum Moench* leaves, which was more likely to cause pesticide accumulation. This was the main reason for the highest concentration in Trial #4. In addition, the high temperature of Trial #4 decreased the photolysis rate of thifluzamide on *Fagopyrum tararicum Gaerth* leaves (Xi, 2021). The lower terminal level of thifluzamide in *Fagopyrum esculentum Moench* was caused by high rainfall in Trial #2, which increased the amounts of leaching and runoff of the agrichemicals (Fang et al., 2006). The increase of rainfall not only increases the scour to leaves, but also may accelerate the leaching of thifluzamide in soil and further reduce the content of thifluzamide absorbed by buckwheat crops in soil(Farha et al., 2016). In addition, the initial concentrations amount of Trial #2 was also low. The crop variety planted in Trial # 1–3 was *Fagopyrum esculentum Moench*. Similarly, compared with Trials #1–3, Trial #1 had low rainfall, the rainfall at Trial # 2 and 3 was 2.8–4.2 times that of Trial # 1, so the terminal level of Trial # 1 was much higher than Trials #2 and 3(Farha et al., 2016).

According to China GB2763-2021(2022), the MRL of thifluzamide in rice was 7 mg/kg, and in wheat, it is 0.5 mg/kg for wheat. Japan regulated thifluzamide in rice at 1 mg/kg (2012). Food safety is an important guarantee for people's life safety and health. As an important link of food safety, food inspection plays a vital role. The establishment of a maximum residue limit standard is an important technical means to strengthen the risk management of pesticide residues, which is closely related to the potential health risks of human beings. At present, the maximum residue limit standard of thifluzamide in buckwheat products has not been established in the world. This experiment provided a theoretical basis and scientific reference for the formulation of MRL of thifluzamide in buckwheat, and the MRL value was recommended to be 1 mg/kg. Therefore, it is suggested to speed up formulating a MRL of thifluzamide in buckwheat straw to avoid the exposure risks.

3.4. Long-term risk analyses of thifluzamide

Total chronic dietary risks of thifluzamide were elaborated regarding *Fagopyrum esculentum Moench* and *Fagopyrum tararicum Gaerth* crops and all registered crops in China by a deterministic model, and the risk quotients in *Fagopyrum esculentum Moench* and *Fagopyrum tararicum Gaerth* crops were further calculated by a probabilistic model. The involved populations were divided by age, sex and region. Based on deterministic model estimates. The long-term risks (*%ADIs*) of thifluzamide range from 37.2 % to 131.6 %, as shown in Fig. 1. The peak value was greater than 100 %, indicating that consumers would suffer from unacceptable long-term health risks. The risk quotients for rural population were higher than that for urban population. By comparing the dietary consumption parameters of the urban population and rural population, the rural population has lower body weight and higher dietary consumption(Jin, 2008). A decline in *%ADI* was obtained with age, where children aged 2–3 suffered the highest exposure risks of up to 131.658 %, with the exception of the rural male group, rural males aged 4–6 suffered the maximal risks. This is due to the higher unit intake of buckwheat for rural male population aged 4–6(Jin, 2008). It is worth noting that >100 % of the risk quotients were found in children aged 2–7, and the risk of thifluzamide in children should be focused on in subsequent studies. Besides, the dietary risk of thifluzamide in rice accounted for 59.2 % of the total chronic risk, ranking first, and the risk factor for peanut crop accounted for the lowest (0.044 %). This may due to do with how the different preferences of population for different crops changes with the population(Li, 2017).

A deterministic model was used to estimate the risk quotient of thifluzamide in buckwheat at 0.18 %–0.78 % of the total chronic risk, and a probabilistic model was used to further assess potential thifluzamide exposure (Fig. 2). The dietary exposure assessment of pesticide concentrations gradually changed from qualitative to quantitative, from the certainty assessment of population to the assessment of individual distribution, from point assessment to more comprehensive probability assessment with the continuous refinement of assessment requirements, and put forward requirements on the description of variability and uncertainty of assessment results(EPA 2005; EFSA, 2012). The probabilistic model is a common method to calculate the incidence, variability and uncertainty of dietary risks and is the main tool for pesticide exposure assessment in European and American countries(EPA 2005; EFSA, 2012). As shown in Table 1**,** with the 50th percentiles to 99.9th, the *%ADI* values of thifluzamide increased from 0.315 % to 2.638 % with the 50th to 99.9th percentiles, which was far lower than 100 % and indicated that the long-term exposure of thifluzamide from buckwheat were within an acceptable range. Statistical gaps in chronic exposure between regions and between age groups of relevant populations were clarified ($p < 0.05$). Significantly, the long-term risks of thifluzamide for the rural group by buckwheat consumption were higher than those for the urban group ($p < 0.05$). The *%ADI* of the deterministic model was between 0.316 %–0.653 % and 0.161 %–0.635 %, while the *%ADI* of the probabilistic model was between 1.206 %–2.638 % and 0.315 %–2.563 %. The risks quotients although deterministic model was simple required with relatively less data, and the underestimation of actual dietary exposure of the high consumers were possible to appear when buckwheat was not consumed by a large proportion of the population. Comparatively, probabilistic distributional analysis can offer more information on the variability in dietary exposure of thifluzamide via the

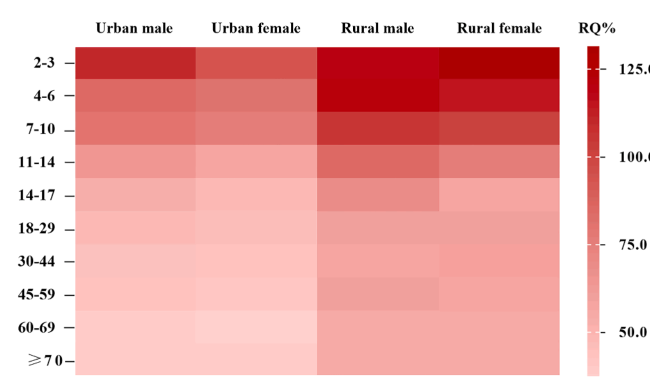

Fig. 1. Long-term risks in Crop registration for various populations.

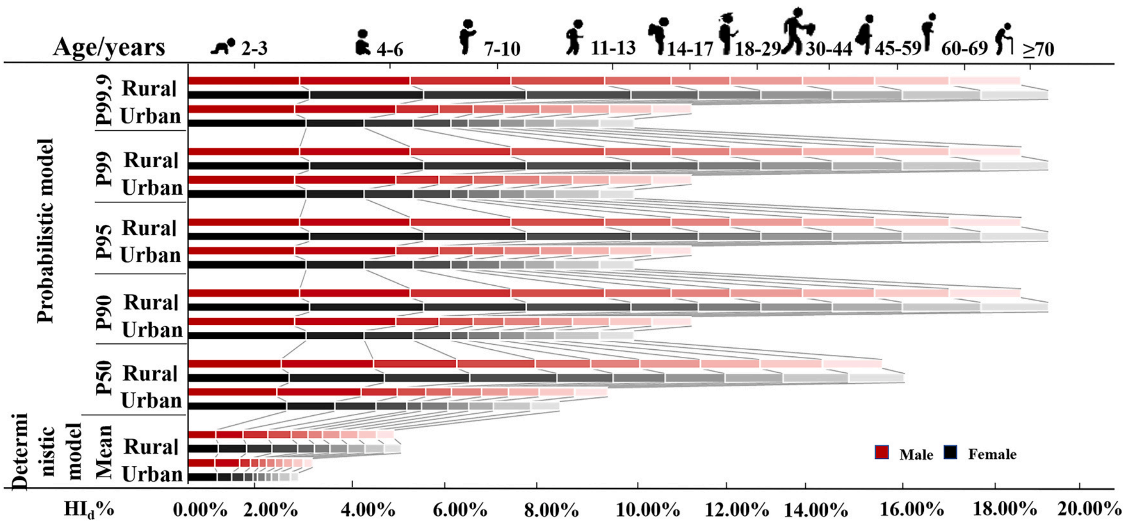

Fig. 2. Long-term risks by deterministic and probabilistic assessment.

Table 1
Long-term risk estimation for dietary thifluzamide exposure in buckwheat for population groups between deterministic and probabilistic models.

Population		Deterministic model		Probabilistic model									
Sex	Age	Urban	rural	urban					rural				
		mean	mean	P50	P90	P95	P99	P99.9	P50	P90	P95	P99	P99.9
male	2–3	0.635%	0.653%	2.136%	2.563%	2.563%	2.563%	2.563%	2.198%	2.638%	2.638%	2.638%	2.638%
	4–6	0.312%	0.613%	1.050%	1.260%	1.260%	1.260%	1.260%	2.064%	2.477%	2.477%	2.477%	2.477%
	7–10	0.264%	0.551%	0.888%	1.066%	1.066%	1.066%	1.066%	1.854%	2.224%	2.225%	2.225%	2.225%
	11–13	0.201%	0.564%	0.677%	0.813%	0.813%	0.813%	0.813%	1.897%	2.277%	2.277%	2.277%	2.277%
	14–17	0.094%	0.362%	0.315%	0.378%	0.378%	0.378%	0.378%	1.219%	1.463%	1.463%	1.463%	1.463%
	18–29	0.170%	0.336%	0.573%	0.688%	0.688%	0.688%	0.688%	1.132%	1.358%	1.358%	1.358%	1.358%
	30–44	0.133%	0.385%	0.449%	0.539%	0.539%	0.539%	0.539%	1.296%	1.555%	1.556%	1.556%	1.556%
	45–59	0.161%	0.375%	0.541%	0.649%	0.650%	0.650%	0.650%	1.263%	1.516%	1.516%	1.516%	1.516%
	60–69	0.241%	0.424%	0.810%	0.972%	0.972%	0.972%	0.972%	1.426%	1.712%	1.712%	1.712%	1.712%
	≥ 70	0.189%	0.362%	0.636%	0.763%	0.763%	0.763%	0.763%	1.218%	1.462%	1.462%	1.462%	1.462%
female	2–3	0.572%	0.599%	1.926%	2.311%	2.312%	2.312%	2.312%	2.017%	2.421%	2.421%	2.421%	2.421%
	4–6	0.543%	0.595%	1.828%	2.194%	2.195%	2.195%	2.195%	2.003%	2.403%	2.404%	2.404%	2.404%
	7–10	0.233%	0.540%	0.784%	0.941%	0.941%	0.941%	0.941%	1.818%	2.181%	2.182%	2.182%	2.182%
	11–13	0.184%	0.505%	0.619%	0.743%	0.743%	0.743%	0.743%	1.699%	2.039%	2.039%	2.039%	2.039%
	14–17	0.166%	0.358%	0.560%	0.672%	0.672%	0.672%	0.672%	1.206%	1.448%	1.448%	1.448%	1.448%
	18–29	0.194%	0.316%	0.654%	0.785%	0.785%	0.785%	0.785%	1.064%	1.277%	1.277%	1.277%	1.277%
	30–44	0.174%	0.391%	0.585%	0.702%	0.703%	0.703%	0.703%	1.315%	1.578%	1.579%	1.579%	1.579%
	45–59	0.198%	0.385%	0.668%	0.801%	0.801%	0.801%	0.801%	1.295%	1.554%	1.554%	1.554%	1.554%
	60–69	0.226%	0.405%	0.761%	0.914%	0.914%	0.914%	0.914%	1.361%	1.634%	1.634%	1.634%	1.634%
	≥ 70	0.217%	0.386%	0.730%	0.876%	0.876%	0.876%	0.876%	1.298%	1.558%	1.558%	1.558%	1.558%

consumption of buckwheat. The risks quotient showed a decreasing trend with increasing age, and the greatest long-term risks were 2.638 % in children aged 2–3. This was because the children group intaked more per unit of body weight than the other groups. The risk quotient for urban population dropped to its lowest at age 14–17, while the risk quotient for rural population dropped to its lowest at age 18–29. In addition, there no statistically significant difference in the potential exposure risks between male and female populations ($p > 0.05$), with risk quotients of). This is due to the fact that male and female eat the same diet. The 0.315 %–2.638 % for males and 0.560 %–2.421 %, respectively. Moreover, the risk quotient in Trial #4 (0.300 %–2.096 %) was much higher than that in the other three sites (0.005 %–0.757 %), which was caused by the high final concentrations in Trial #4. The crop variety of test site 4 is *Fagopyrum tararicum Gaerth*, and the concentration of Trial #4 in the terminal levels is much higher than that of other Trials. Food safety is related to the national economy and people's livelihood. Pesticide residue is a sensitive and concerning topic in food safety. The results of long-term dietary exposure risk assessment can provide a theoretical basis for the formulation of pesticide residue limit standards and a scientific reference for the formulation of regulations. They contribute to the improvement of the population's dietary structure, and human health is closely related. It is of great significance for agricultural production management, agricultural product quality safety and human health evaluation.

4. Conclusions

In summary, an effective and sensitive UHPLC—ESI—MS/MS method was established to trace thifluzamide in buckwheat with an LOQ of 0.001 mg/kg, which could be a suitable alternative to trace thifluzamide in relevant matrices. Thifluzamide can be stably stored in buckwheat for 84 d from field collection to laboratory detection, ensuring data reliability and validity during sample storage. The primary deposition, $T_{1/2}$, STMR and HR were 0.02–0.55 mg/kg, 12–14 d, 0.11 and 0.41 mg/kg, respectively, which were conducive to promoting the MRLs establishment of thifluzamide in buckwheat and the corresponding exposure evaluation for consumer populations. A joint assessment of buckwheat and other registered crops showed that thifluzamide had high potential chronic risks for the consumer population (ADI%, 37.268 %–131.658 %). The long-term risks values were decreased with increasing age, and children aged 2–6 suffered from the worst exposure. The chronic risks for rural residents were significantly higher than those for urban residents ($p < 0.05$), and there was no significant difference between genders ($p > 0.05$). The risk quotient of *Fagopyrum tararicum Gaerth* was 1.5–75.4 times that of *Fagopyrum esculentum Moench* by using a probabilistic model to reduce the uncertainty and variability of risk assessment. This study provides scientific references for the food safety of thifluzamide in buckwheat and dietary optimization for consumer populations.

Ethics approval

Not applicable.

Consent to participate

All authors involved in this paper have confirmed their participation in the experimental study and the preparation of the paper.

Consent to publish

All authors approved the paper for publication.

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CRediT authorship contribution statement

Zhao Lilin: Methodology, Project administration. **Chen Zenglong:** Writing – review & editing, Funding acquisition. **Liu fengjiao:** Data curation, Methodology. **Zhang hongxia:** Investigation, Methodology. **Ren Xin:** Data curation, Formal analysis. **Chen Li:** Formal analysis, Writing – review & editing. **Xu zhanbo:** Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.115833.

References

Bian, Y., Wang, Y., Liu, F., Li, X., Wang, B., 2020. The stability of four organophosphorus insecticides in stored cucumber samples is affected by additives. Food Chem. 331, 127352 https://doi.org/10.1016/j.foodchem.2020.127352.

Bian, Y., Feng, Y., Zhang, A., Qi, X., Pan, J., Han, J., Ma, X., Liang, L., 2022. Residue distribution and risk assessment of bifenazate and its metabolite in garlic plant. Food Chem. 379, 132013 https://doi.org/10.1016/j.foodchem.2021.132013.

Cao, J., Wu, X., Xu, J., Dong, F., Liu, X., Zheng, Y., 2020. Residual levels and dietary risk assessment of thifluzamide in peanut. J. Int. J. Environ. Anal. Chem. 102, 3461–3470. https://doi.org/10.1080/03067319.2020.1770242.

Chen, WeiTao, Li, MingHui, Li, WenXi, 2015. Dissipation and residue level of thifluzamide in rice field ecosystem. J. J. Chem. 1–8. https://doi.org/10.1155/2015/848252.

EFSA, 2012. Guidance on the Use of Probabilistic Methodology for Modelling Dietary Exposure to Pesticide Residues. Available online: 〈https://efsa.onlinelibrary.wiley.com/DOI/epdf/10.2903/j.efsa.2012.283〉 (accessed on 2 April 2023).

Fang, H., Yu, Y.L., Wang, X., Shan, M., Wu, X.M., Yu, J.Q., 2006. Dissipation of chlorpyrifos in pakchoi-vegetated soil in a greenhouse. J. Environ. Sci. (China) 18 (4), 760–764.

Farha, W., Abd El-Aty, A.M., Rahman, M.M., Shin, H.C., Shim, J.H., 2016. An overview on common aspects influencing the dissipation pattern of pesticides: a review. Environ. Monit. Assess. 188 (12), 693. https://doi.org/10.1007/s10661-016-5709-1.

Gangwar, S.K., Rafiquee, M.Z.A., 2007. Kinetics of the acid hydrolysis of isoproturon in the absence and presence of sodium lauryl sulfate micelles. Colloid Polym. Sci. 285, 587–592. https://doi.org/10.1007/s00396-006-1596-2.

Gupta, S., Gajbhiye, V.T., 2004. Adsorption-desorption, persistence and leaching behavior of thifluzamide in alluvial soil. Chemosphere 57 (6), 471–480. https://doi.org/10.1016/j.chemosphere.2004.06.033.

Han, S., Bi, Y., Han, L., Song, S., Ye, Z., Qin, F., Lv, X., 2022. Residue behavior and risk assessment of pyraclostrobin and thifluzamide in cowpea. Bull. Environ. Contam. Toxicol. 108 (4), 786–790. https://doi.org/10.1007/s00128-021-03421-2.

Huda, M.N., Lu, S., Jahan, T., Ding, M., Jha, R., Zhang, K., Zhang, W., Georgiev, M.I., Park, S.U., Zhou, M., 2021. Treasure from garden: bioactive compounds of buckwheat. Food Chem. 335, 127653 https://doi.org/10.1016/j.foodchem.2020.127653.

Hussain, S., Devers-Lamrani, M., Spor, A., Rouard, N., Porcherot, M., Beguet, J., Martin-Laurent, F., 2013. Mapping field spatial distribution patterns of isoproturon-mineralizing activity over a three-year winter wheat/rape seed/barley rotation. Chemosphere 90, 2499–2511. https://doi.org/10.1016/j.chemosphere.2012.10.080.

Jin, S., 2008. Survey Report on the Nutrition and Health of Chinese Residents: Data Seton the Status of Nutrition and Health in 2002. People's Hygiene Press,, Beijing.

Kawar, N.S., de, Batista G.C., Gunther, F.A., 1973. Pesticide stability in cold-stored plant parts, soils, and dairy products, and in cold-stored extractives solutions. Residue Rev. 48 (0), 45–77. https://doi.org/10.1007/978-1-4615-8498-8_2.

Kumar, P., Ahlawat, S., Chauhan, R., Kumar, A., Singh, R., Kumar, A., 2018. In vitro and field efficacy of fungicides against sheath blight of rice and post-harvest fungicide residue in soil, husk, and brown rice using gas chromatography-tandem mass spectrometry. Environ. Monit. Assess. 190 (9), 503 https://doi.org/10.1007/s10661-018-6897-7.

Lewis, K.A., Tzilivakis, J., Warner, D., Green, A., 2016. An international database for pesticide risk assessments and management. Hum. Ecol. Risk Assess.: Int. J. 22 (4), 1050–1064. https://doi.org/10.1080/10807039.2015.1133242.

Li, C., Liu, R., Li, L., Li, W., He, Y., Yuan, L., 2017. Dissipation behavior and risk assessment of butralin in soybean and soil under field conditions. Environ. Monit. Assess. 189 (9), 476 https://doi.org/10.1007/s10661-017-6185-y.

Ma, C., Liu, Z., Qi, Y., Wang, S., Cao, X., Wang, J., She, Y., Shao, Y., Shen, J., Zhang, C., Jiang, Z., 2018. Residue behavior and risk assessment of thifluzamide in the maize field ecosystem. Environ. Sci. Pollut. Res. Int. 25 (21), 21195–21204. https://doi.org/10.1007/s11356-018-2211-z.

Mastovská, K., Lehotay, S.J., 2004. Evaluation of common organic solvents for gas chromatographic analysis and stability of multiclass pesticide residues. J. Chromatogr. A 1040 (2), 259–272. https://doi.org/10.1016/j.chroma.2004.04.017.

Nahar, L., Onder, A., Sarker, S.D., 2020. A review on the recent advances in HPLC, UHPLC and UPLC analyses of naturally occurring cannabinoids (2010-2019). Phytochem. Anal.: PCA 31 (4), 413–457. https://doi.org/10.1002/pca.2906.

Qi, YangJu, Chen, Zhenjiang, Li, Zhenxia, Liu, Hui, Wang, Li.Hua, Li, Chunjie, 2020. Research progress on buckwheat diseases. J. Pratacult. Sci. 01, 75–86. https://doi.org/10.11829/j.issn.1001-0629.2019-0143.

Ren, Changzhong, Shan, Fang, Wang, Min, Li, Yunlong, 2022. Buckwheat nutritional and functional research and product development. J. Chin. J. Grain Oil 11, 261–269. https://doi.org/10.20048/j.carolcarroll nki. Issn 1003-0174.000001.

Tiryaki, O., Baysoyu, D., Seçer, E., Aydin, G., 2008. Testing the stability of pesticides during sample processing for the chlorpyrifos and malathion residue analysis in cucumber, including matrix effects. Bull. Environ. Contam. Toxicol. 80 (1), 38–43. https://doi.org/10.1007/s00128-007-9308-2.

Wang, W., Teng, P., Liu, F., Fan, T., Peng, Q., Wang, Z., Hou, T., 2019. Residue analysis and risk assessment of oxathiapiprolin and its metabolites in cucumbers under field conditions. J. Agric. Food Chem. 67 (46), 12904–12910. https://doi.org/10.1021/acs.jafc.9b03961.

Wei, L.N., Wu, P., Wang, F.R., 2015. Dissipation and degradation dynamics of thifluzamide in rice field. J. Water Air Soil Pollut. 226, 130. https://doi.org/10.1155/2015/848252.

Xi, N., Li, Y., Chen, J., Yang, Y., Duan, J., Xia, X., 2021. Elevated temperatures decrease the photodegradation rate of pyrethroid insecticides on spinach leaves: implications for the effect of climate warming. Environ. Sci. Technol. 55 (2), 1167–1177. https://doi.org/10.1021/acs.est.0c06959.

Yang, L., Song, X., Zhou, X., Zhou, Y., Zhou, Y., Gong, D., Luo, H., Deng, Y., Yang, D., Chen, L., 2019. Residual behavior and risk assessment of butralin in peanut fields. Environ. Monit. Assess. 192 (1), 62 https://doi.org/10.1007/s10661-019-8013-z.

Yang, Y., Dong, F., Liu, X., Xu, J., Wu, X., Qi, S., Liu, W., Zheng, Y., 2018. Thifluzamide affects lipid metabolism in zebrafish (Danio reio). Sci. Total Environ. 633, 1227–1236. https://doi.org/10.1016/j.scitotenv.2018.03.302.

Yang, Y., Dong, F., Liu, X., Xu, J., Wu, X., Zheng, Y., 2020. Thifluzamide induces the toxic effects on zebrafish (Danio rerio) via inhibition of succinate dehydrogenase (SDH). Environ. Pollut. (Barking, Essex: 1987) 265 (Pt B), 115031. https://doi.org/10.1016/j.envpol.2020.115031.

Yao, X., Zhang, F., Qiao, Z., Yu, H., Sun, S., Li, X., Zhang, J., Jiang, X., 2020. Toxicity of thifluzamide in earthworm (Eisenia fetida). Ecotoxicol. Environ. Saf. 188, 109880 https://doi.org/10.1016/j.ecoenv.2019.109880.

Yao, X., Liu, Y., Liu, X., Qiao, Z., Sun, S., Li, X., Wang, J., Zhang, F., Jiang, X., 2022. Effects of thifluzamide on soil fungal microbial ecology. J. Hazard. Mater. 431, 128626 https://doi.org/10.1016/j.jhazmat.2022.128626.

Yousefi, S.R., Amiri, O., Salavati-Niasari, M., 2019. Control sonochemical parameter to prepare pure ZnFeO nanostructures and study their photocatalytic activity. Ultrason. Sonochem. 58, 104619 https://doi.org/10.1016/j.ultsonch.2019.1046190.352.654.

Zhao, P., Wang, C., Zhang, S., Zheng, L., Li, F., Cao, C., Cao, L., Huang, Q., 2022. Fungicide-loaded mesoporous silica nanoparticles promote rice seedling growth by regulating amino acid metabolic pathways. J. Hazard. Mater. 425, 127892 https://doi.org/10.1016/j.jhazmat.2021.127892.

Zhou, Min, He, XiuFen, Dong, CunZhu, 2020. Residue digestion and dietary risk assessment of diethyl ether and thifluzamide on banana. J. J. Trop. Crops 41 (3), 596–602. https://doi.org/10.3969/j.issn.1000-2561.2020.03.024.

Zou, L., Wu, D., Ren, G., Hu, Y., Peng, L., Zhao, J., Garcia-Perez, P., Carpena, M., Prieto, M.A., Cao, H., Cheng, K.W., Wang, M., Simal-Gandara, J., John, O.D., Rengasamy, K.R.R., Zhao, G., Xiao, J., 2023. Bioactive compounds, health benefits, and industrial applications of Tartary buckwheat (Fagopyrum tataricum). Crit. Rev. Food Sci. Nutr. 63 (5), 657–673. https://doi.org/10.1080/10408398.2021.1952161.