RESIDUE AND DIETARY RISK ASSESSMENT OF CHLORMEQUAT CHLORIDE IN LIAONING PEANUTS (*ARACHIS HYPOGAEA* L.)

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Abstract. A detection method for chlormequat chloride (CC) in peanuts was established using ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). The residual levels of CC in peanuts and its effects on peanut growth and yield were evaluated. The dietary risk of CC residues in peanuts was assessed according to the guidelines of the Pesticide Residue Joint Meeting (JMPR). The results indicate that the half-life of CC in peanut plants is 1.2 days. Assuming that the degradation of CC is 90%, it takes 7.4 days. The dietary exposure levels of CC residues in peanuts for various populations in China are 0.51-1.40 μg/(kg bw·d), with no dietary risk. The recommended dosage for peanut application is 75 g ai/ha, sprayed once, the dietary intake risk is 0.0046-0.052. The application dose of 225 g ai/ha of CC was sprayed twice, and the residual amount of CC in peanut kernels was 0.2 mg/kg higher than the maximum residue limit (MRL), indicating a residual risk, but the dietary intake risk was at an acceptable level. This study provides an important basis for ensuring the safety of peanut consumption and formulating relevant regulatory policies.
Keywords: *plant growth regulator, maximum residue limit, overgrowth, dietary exposure, high performance liquid chromatography tandem mass spectrometry*

Introduction

Peanuts (*Arachis hypogaea* L.) are rich in various beneficial nutrients to human health, such as protein, fat, carbohydrates, vitamins, minerals, and phospholipids. They are an important source of high-quality plant oils and proteins (Gama and Adhikari, 2019). Peanut is resistant to barren drought, has strong adaptability and biological nitrogen fixation ability, and can play a role in fertilizing soil fertility (Hou et al., 2024), improving soil quality (Qin et al., 2023) and optimizing ecology (Sun, 2015), and has become one of the important crop varieties in the adjustment of planting structure.

Peanut overgrowth is a common issue in cultivation, characterized by leaf hypertrophy, field shading, increased distance between fruit needles and the ground, delayed needling, insufficient nutrition for reproductive growth, and significant impacts on flower production (Si et al., 2009). Chlormequat chloride (CC) is a widely used plant growth inhibition regulator. Currently, 50% CC is registered and commercially available. It can inhibit the synthesis of gibberellin, prevent plant physiological barren growth, promote reproductive growth, deepen leaf color, shorten plant internode, and enhance plant stress resistance and increase production (Chen et al., 2016). According to a report by the Joint Meeting of Pesticide Residues (JMPR) in 2000, the pesticide has been registered in 33 crops, including 23 food crops (barley, cereals, etc.). 5 kinds of vegetables (eggplant, tomato, etc.) and 5 kinds of fruits (grapes, pears, sugarcane, etc.) (Zhang et al., 2008). In China, it is only registered in cotton, tomato, corn and wheat,

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but because it can increase crop yield and increase efficiency, there are many literatures and pesticide manufacturers recommend it to be used on grain and oil crops, especially peanuts. However, excessive and irrational use of the CC can lead to abnormal plant growth, and there is also a teratogenic risk for children and pregnant women (Nisse et al., 2015). CC remains in the soil, and the decay period is as long as 2 years, causing crops to fail to grow normally, resulting in loss of agricultural output and quality (Huang et al., 2017; Xiagedeer et al., 2016).

With the extensive use of plant growth regulators in agricultural production, more and more attention has been paid to their rational application and residue monitoring. China is the largest exporter of peanuts, mainly exporting to countries such as Europe, Southeast Asia, and Japan (Liao, 2020). There are significant regional differences in the formulation of CC residue regulations. According to the 2014 UK Pesticide Use Survey, the estimated area treated with CC ranked fifth among all pesticide sprayed arable land, with the highest usage of 2389 tons (Garthwaite et al., 2014). In addition, the annual production of CCC in China exceeds 10000 tons (Li et al., 2012). Therefore, people are increasingly concerned about the residues of CCC in food, which is reflected in the maximum residue limits (MRL) set by many regulatory agencies. At present, the maximum residue limit (MRL) in peanuts in China is 0.2 mg/kg (Ministry of Agriculture and Rural Affairs of the People's Republic of China, 2021), while the European Commission (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/mrls) and the Japan Food Chemical Research Foundation (http://db.ffcr.or.jp/front) prescribe the MRL of peanuts to be 0.01 mg/kg. This difference has led to strict restrictions on China's peanut exports, causing huge losses to the peanut export industry.

With the increasing public attention to health, there is also a growing concern about food safety residues and dietary risks. CC is toxic to the bone development of male rats, affecting the development of chondrocytes, osteoblasts, and osteoclasts through growth hormone (GH) and insulin-like growth factor 1 (IGF-1) (Huang et al., 2017; Jia et al., 2018). Excessive spraying of CC can cause plants to stop growing, even shrink, and easily cause environmental pollution, seriously affecting the normal growth of next season's crops (Lin et al., 2024). As a chemical pesticide, there have been reports on the application and yield quality impact of CC in peanuts (Li et al., 2015; Zhong et al., 2013; Meng, 2007). This study conducted a standardized residue test for the application of CC in peanuts and established a UPLC-MS/MS detection method for CC in peanuts. The degradation dynamics of CC in peanut plants and the final residue in peanut kernels were studied using this method, and the dietary intake risk assessment of CC residue in peanut kernels was conducted. This study recommends the safe concentration and spraying frequency of CC in peanuts, providing important basis for ensuring peanut consumption safety and formulating relevant regulatory policies.

Materials and methods

Instruments and reagents

Ultra-performance liquid chromatography tandem mass spectrometer (Waters UPLC Exo TQ, USA); Zhongjia High Speed Centrifuge HC-3514, Anhui Zhongke Zhongjia Technology Co., Ltd; Dinghaoyuan RS-1 Vortex Mixer, Beijing Dinghaoyuan Technology Co., Ltd; Paynt Ultra Pure Water Machine, Beijing Xiangshunyuan Technology Co., Ltd; 0.22 μ m needle filter, 50 mL polypropylene plastic centrifuge

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tube, Xinkang Medical Equipment Co., Ltd; Chromatography column Waters HSS T3 C18 (2.1 mm × 100 mm, 1.8 μ m).

Chlormequat chloride standard [100 μ g/mL, Environmental Quality Supervision, Inspection and Testing Center of the Ministry of Agriculture (Tianjin)]; Methanol, acetonitrile, and formic acid require chromatographic purity grade (Beijing Dima Technology Co., Ltd.); Purified water (Hangzhou Wahaha Group); The field experiment tested a 50% CC agent (Sichuan Runer Technology Co., Ltd.).

Field experiment design

The field experiment was conducted following the ‘Standard Operating Procedures for Pesticide Registration Residue Field Experiments’ and ‘Guidelines for Pesticide Registration Experiments’ (NY/T 788-2004).

In 2023, the experiment was conducted in Kangping County, Shenyang City, Liaoning Province. The commercially available 50% CC was prepared in the following concentrations: low concentration (D, 50%CC diluted 5000 times, 45 g ai/ha), medium concentration (M, 50%CC diluted 3000 times, 75 g ai/ha), high concentration (G, 50%CC diluted 1000 times, 225 g ai/ha), and a blank control. Each treatment concentration was applied once and twice, and each treatment method was repeated three times, with a treatment area of 30 m^2. The method of foliar spraying was used to apply the CC in the period of peanut flowering and injection. During the harvest period, no less than 2.0 kg of peanut shell samples were taken from each plot by random five-point method. After drying, the samples were ground and mixed well. 200 g powder was obtained by quartering method and stored at -20°C for analysis.

Establishment of chlormequat chloride detection method

Sample preparation

Weigh the ground peanut seed powder sample 5.0 g, add 3 mL water and 10 mL acetonitrile into a 50 mL centrifuge tube, and shake for 30 min. Add the reagent kit (NaOAc 1.5 g, Mg SO_4 6 g), cover with a lid and mix quickly. Centrifuge at 10000 r/min for 5 min, remove the supernatant into a glass bottle (1.5 mL) and pass it through a 0.22-μm filter membrane for detection and analysis on the machine.

Chromatographic conditions

Mobile phase A: methanol; B: 0.1% formic acid aqueous solution; The gradient elution program (Lan et al., 2017) is: 0-2.5 min, 5% A; 2.5~8.0 min, 5%A~90%A; 8.0~10.0 min, 90%A; 10.0~10.5 min, 90%A~5%A; 10.5~12.0 min, 5% A. Flow rate: 200 μ L/min; Column temperature: 30°C; Injection volume: 1 μL.

Dietary intake risk assessment

According to the maximum residue limit (MRL) of the dwarfing hormone residue test in this experiment, evaluate the national daily intake (NEDI) of dwarfing hormone according to *Equation 1*, and calculate the dwarfing hormone risk quotient (RQ) according to *Equation 2* (Li et al., 2024, 2020; Bu et al., 2022).

$$\text{National estimated daily intake: NEDI} = \sum [\text{STMRi (STMR Pi)} \times \text{Fi}] \quad \text{(Eq.1)}$$

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$$\text{Risk merchant: } RQ = NEDI/(ADI \times b.w.) \quad \text{(Eq.2)}$$

In the formula: STMRi (supervised trials median residue, STMR) is the median value of the standardized residue test (mg/kg); STMR-Pi is the median value (mg/kg) of the standardized residual test after processing factor correction; Fi is the intake of the general population (kg); ADI is the daily allowable intake (mg/kg b.w.), and b.w. is the average body weight per capita of Chinese residents.

When $RQ \leq 1$, the impact of dietary intake risk on the health of the general population is at an acceptable risk level, and the smaller the risk quotient value, the lower the risk; When $RQ > 1$, it indicates an unacceptable risk, and the higher the value, the greater the risk.

Results and analysis

Reliability of testing methods

In the concentration range of 0.01~1 mg/L, the linear relationship was good, and the quantification was accurate in this concentration range, and the correlation coefficient $r = 0.9998$. Add CC at concentrations of 0.01 mg/kg, 0.02 mg/kg, and 0.05 mg/kg for spiked recovery experiments. Repeat 5 times for each concentration level to calculate the average recovery rate and relative standard deviation (RSD). Under the three different addition levels (*Table 1*), the average recovery rate of CC in peanuts is 92.4%~105.2%, with RSD ranging from 2.07%~8.82%. The addition recovery rate and relative standard deviation meet the requirements of the “Guidelines for Pesticide Residue Testing in Crops”, and the method is reliable.

Table 1. *Average recovery rate of CC addition in peanuts (n = 5)*

Sample	Add level (mg/kg)	Recovery rate (%)						RSD (%)
		1	2	3	4	5	Average	
Peanut fruit	0.01	112	104	116	98	96	105.2	8.67
	0.02	91	98	96	88	93	93.2	3.96
	0.05	95	98	97	94	93	95.4	2.07
Peanut plants	0.01	114	108	106	92	97	103.4	8.82
	0.02	88	94	92	92	96	92.4	2.97
	0.05	94	99	92	96	98	95.8	2.86

Degradation dynamics of CC in peanut plants

The degradation kinetics of CC in peanut plants followed a first-order kinetic model, with the degradation curve described by $y=11.025e^{-0.066x}$ (R^2=0.9908), as shown in *Figure 1*. By calculation, the half-life of CC in peanut plants is 1.2 days. When CC is dissolved by 90%, it takes 7.4 days.

Final residues of CC in peanuts and straw

Spray different concentrations of CC on peanuts once or twice, and sample and measure the final residual values of CC in peanuts after the last application at harvest. The residue of peanut CC is between 0.025 and 0.24 mg/kg, with a median STMR of

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0.041 mg/kg (*Table 2*). The final residue level of CC after spraying 225 g ai/ha twice was 0.24 mg/kg, exceeding the Chinese national standard of 0.2 mg/kg, indicating a residual risk.

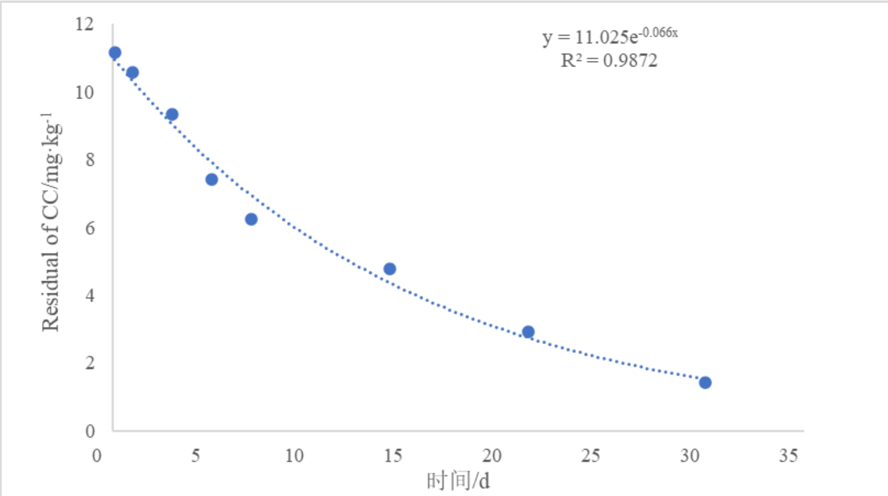

Figure 1. *Degradation dynamics of CC in peanut plants*

Table 2. *Final residual levels of CC in peanuts*

Number	Application dosage (g ai/ha)	Application times	Average detection value of CC (mg/kg)
1	45	1	0.025
2	45	2	0.032
3	75	1	0.062
4	75	2	0.074
5	225	1	0.14
6	225	2	0.24
7	CK	–	＜0.01

Take the average of three measurements

Effect of CC on the growth trend of peanuts

The growth of peanut was significantly affected by the application of CC to peanut (*Table 3*). From the perspective of growth status, the peanut plants could be significantly dwarfed by spraying different concentrations of CC 1~2 times during the acupuncture period of peanut flowering, making the plants robust. The plant height of each treatment was lower than that of the control (CK), with significant differences. Spraying CC also increased peanut yield, with a concentration of 75 g ai/ha, spraying twice resulted in the greatest increase in yield, with an average plot yield of 17.54 kg, which was significantly different from the CK.

Dietary risk assessment

This study selected four groups of people, including children aged 2-6 years old, standard individuals (adult males engaged in light physical labor), urban and rural

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residents, as the evaluation objects. The median value of the standardized residue test was 0.24 mg/kg of CC in peanuts determined in this experiment. The weight information of each group required for evaluation was sourced from the "Chinese Resident Nutrition and Health Status Survey Report III - 2002 Resident Physical Fitness and Nutritional Status" (Shen et al., 2024), and the consumption of peanuts was calculated according to the intake of high consumption groups in the same age group (Ministry of Health of the People's Republic of China, 2004). The ADI value of CC is 0.05 mg/kg·bw, and the short-term dietary intake and risk quotient are calculated according to formulas (1) and (2). From the results in *Table 4*, it can be seen that there are differences in the intake of CC residues in peanuts among different populations. Children (2-6 years old) have a higher exposure to CC than other populations, but the value is very low, and the risk quotient is far below 100%. The dietary risk of urban population is lower than that of rural population. In 2000, FAO/WHO joint meeting reported that the international estimate of short-term intake (IESTI) of CC in pears exceeded its acute reference dose (ARfD, 0.05 mg/kg bw), with values of 240% for the general population and especially 700% for children up to six years of age (FAO, 2000). Researchers analyzed CC in one hundred 24 h urine samples collected from non-occupationally exposed individuals in the general population, and the result showed that CC was detectable in all samples with a mean level of 5.6 ng/mL urine (Lindh et al., 2011). Our research results indicate that the dietary risk of CC in peanuts is extremely low.

Table 3. *Effects of CC on peanut growth trend*

Number	Application dosage (g ai/ha)	Application times	Plant height (cm)	Number of branches (pcs)	Number of fruit needles (pcs)	Aboveground dry matter accumulation (g)	Yield (kg)
1	45	1	51.7ab	6.41c	12.18d	7.42bc	15.12c
2	45	2	52.2ab	6.85c	13.84c	7.48bc	16.14b
3	75	1	47.4b	7.68b	15.00b	7.54ab	16.32b
4	75	2	48.6b	7.75b	15.58b	7.62ab	17.54a
5	225	1	44.3c	8.01a	16.54a	7.86a	17.12a
6	225	2	46.2c	8.25a	16.63a	7.88a	17.48a
7	CK	–	54.3a	6.25d	11.75e	5.52c	14.63d

Plant height, number of branches, number of fruit needles, aboveground dry matter accumulation, and yield are all average values. The letters indicate differences at the $P < 0.05$ level

Table 4. *Short term dietary intake and acute risk assessment of CC residues in peanuts for various populations*

Group	Weight (kg)	Peanut consumption ($g \cdot d^{-1}$)	Intake F ($kg \cdot d^{-1}$)	National estimated daily intake [mg/(kg bw.)]	Risk quotient RQ (%)
Children aged 2-6 years old	15.18	24.9	0.00164	3.94×10^{-4}	0.052
Standard individuals	63.00	35.7	0.00057	1.37×10^{-4}	0.0043
Urban people	66.57	33.4	0.00050	1.20×10^{-4}	0.0036
Rural people	61.13	35.8	0.00059	1.42×10^{-4}	0.0046

Conclusion

Chlormequat chloride (CC) is a widely used plant growth regulator in agricultural production. This study established a UPLC-MS/MS method for analyzing CC residues in peanuts, demonstrating high sensitivity, good reproducibility, and accuracy and

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precision that meet the requirements of pesticide residue analysis and detection. The results of the digestion dynamics and final residue test indicate that the digestion dynamics of CC in peanuts follow the first-order kinetic equation, with a residual half-life of 1.2 days. The median residue of CC in peanuts during the harvest period is 0.041 mg/kg, indicating a low residual amount.

Applying CC on peanuts can inhibit excessive plant growth and increase yield. Spraying at a concentration of 75 g ai/ha twice can achieve a yield of 17.54 kg in the plot, which is 2.91 kg higher than the control with significant differences. The recommended dosage for peanut application is 75 g ai/ha, sprayed once, the dietary intake risk is 0.0046-0.052. The application dose of 225 g ai/ha of CC can increase yield too, but the residual amount of CC in peanut kernels was 0.2 mg/kg higher than the maximum residue limit (MRL), indicating a residual risk, but the dietary intake risk was at an acceptable level. From the perspective of economic benefits and health, applying CC at 75 g ai/ha, the recommended dosage once can increase yield and reduce residue levels. At the same time, green prevention and control of peanut overgrowth can also be achieved through field cultivation and management.

Discussion

The residual levels of CC vary among different peanut plant tissues, likely due to differences in their physiological structures and functions. Leaves have a larger surface area and participate in active physiological processes such as gas exchange, which may result in different absorption and metabolic rates of CC compared to tissues such as roots. At the cellular level, differences in cell wall permeability and the distribution and activity of intracellular transport proteins can affect the accumulation and residue of CC in different tissues.

In terms of environmental conditions, temperature has a significant impact on the residue of CC. Higher temperatures may accelerate CC degradation, as increased temperature enhances chemical reactivity and alters the molecular structure of CC. However, environments with high humidity may affect the adhesion and infiltration of CC on plant surfaces. High humidity may dilute the concentration of CC or promote its migration and transformation within plant tissues. The soil type is also related to it. Different soil textures, acidity, organic matter content, etc. can alter the adsorption and desorption balance of CC, thereby affecting the absorption and residual levels of CC by peanut plants.

Regarding the impact on future pesticide regulations, the results of this study suggest that more precise guidelines should be established for the use of CC in peanut cultivation. For example, setting different dosages and safety intervals based on differences in environmental conditions in different regions. Considering the potential impact of residual CC on health and safety, long-term low-dose intake of CC may interfere with the human endocrine system, affect hormone balance, and lead to a series of health problems such as developmental abnormalities and reproductive dysfunction. Spraying methods will also affect the residue. Dissipation and potential risk of tristyrylphenol ethoxylates (TSPEOs) in two common application modes were studied: spraying and root irrigation. The concentration of total TSPEOs in peanut plants was significantly higher when sprayed (435–37,693 μg/kg) than in root irrigation (24–1602 μg/kg) (Wang et al., 2024).

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Studies have shown that peeling, cleaning, and boiling peanuts can remove pesticide residues, but frying, stir frying, and baking can increase the residual levels of some pesticides; During the oil extraction process, the residual levels of 13 pesticides in peanut oil increased, and the residual levels of 25 pesticides in peanut meal increased (Cui et al., 2024). For consumers, in order to reduce the residual intake of CC, before eating peanuts, the method of soaking in clear water for many times can be used to remove part of CC remaining on the surface of peanuts by the dissolution of water. When cooking, high-temperature frying or boiling can also promote the decomposition of CC to a certain extent, reduce its residue and ensure food safety.

Author contributions. 1. Qiujun Lin: Methodology, Software, Investigation, Formal Analysis, Writing-Original Draft. 2. Xianxin Wu: Data Curation, Investigation, Writing - Original Draft. 3. Chunjing Guo: Visualization, Investigation, Writing-Review & Editing. 4. Tianshu Peng: Visualization, Investigation, Formal Analysis. 5. Lina Li: Visualization, Investigation, Formal Analysis. 6. Jianzhong Wang (Corresponding Author): Funding Acquisition, Resources, Supervision, Writing - Review & Editing.

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