# Residue and Risk Assessment of Kresoxim-methyl in Apple Under Field Conditions

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**Abstract:** A rapid and effective method was developed for determination of kresoxim-methyl residue in apple. Field trials were designed to evaluate the dissipation and residue of kresoxim-methyl in apple at Hebei (China), Shandong (China) and Anhui (China). The dietary exposure and risk assessment were also conducted by using the calculation of risk quotient (RQ). The samples were extracted by Acetone and ethyl acetate, cleaned up by Florisil solid phase extraction column and detected by gas chromatograph with DB-1701 capillary column and electron capture detector. The results showed good average recoveries of 80%-99% with relative standard deviations of 0.9%-1.3% at the spiked level of 0.02, 0.20 and 2.0 mg /kg. The limit of detection (LOD) was $1.0 \times 10^{-12}$ g and the limit of quantification (LOQ) was 0.02 mg /kg. The results were found that the half lives of kresoxim-methly in apple of Hebei (China), Shandong (China) and Anhui (China) were 6.09, 12.6 and 10.0 d respectively, and the terminal residue was below MRL (0.2 mg/kg) 14 days after application, the RQ of kresoxim-methyl were far below 1. It predicted that it was safe for human being when kresoxim-methyl was used to control Apple tree diseases.

**Keywords:** Kresoxim-methyl, Apple, Residue, Risk Assessment

## 1 Introduction

Kresoxim-methyl is a kind of methacrylic acid ester antiseptic, it acts as a mitochondrial respiratory inhibitor. By transferring the electrons between the cytochromes, it inhibits the respiration of mitochondrial and destroys the energy synthesis in order to act as a bactericide. It is able to prevent from invading of the pathogenic spore and it can effectively inhibit the germination of the spore and the development of the leaves of mycelium. It is widely used on early blight of tomato, powdery mildew of strawberry and cucumber, venturia pirina, ring rot of apple and Powdery mildew spot and leaf spot etc [1-4]. According to European Food Safety Authority, 2010 [5], the ADI (Acceptable Daily Intake) value of kresoxim-methyl is 0.4 mg /kg bw. China's Nation Standard GB 2763-2014 "National food safety standard — Maximum residue limits for pesticides in food" has stipulated that the MRL (Maximum Residue Limit) value of an apple should be 0.2 mg/kg.

Currently, the method of calibration of kresoxim-methyl pesticide including separation and quantification using high performance liquid chromatography (HPLC) with UV-detector [6-7] or using gas chromatography with flame ionization detector (FID) [8]. Residual quantity analysis method is commonly used on growing plants of rice, wheat, cucumber, strawberry and apple etc., the residues analysis of kresoxim-methyl in grain harvestry, fruits and soil as well as the study of the degradation pattern [9-15]. Ge etc. [9-11] have used gas chromatography electron capture detector (ECD) to detect and analyze the residue of kresoxim-methyl in cucumber and soil, and they studied the pattern of degradation dynamics of kresoxim-methyl on cucumber. Liu Congyun [12] and his team have adopted the LC - MS method to detect the kresoxim-methyl residue of cucumber and at the same time completed the dietary risk assessment of kresoxim-methyl in cucumber. Yu Xueli [13-15] and her team have used the gas chromatography electron capture detector (ECD) to detect the residue of kresoxim-methyl in wheat, rice and soil.

In this research, the extract of apple sample was purified with florisil SPE column. A method to detect the residue of

kresoxim-methyl in apple was developed by using gas chromatography capillary column to separate and using electron capture detector (ECD) to detect. The method was used to study the degradation dynamics and the final residue of 40% kresoxim-methyl deflocculant of apple fields in different areas in China, at the same time, calculated the dietary exposure of pesticide and perform the risk assessment of the detection result of sample residues.

# 2 Materials and Method

## 2.1. Agentia and Apparatus

Standard substance of kresoxim-methyl (99.0%) provided by the National pesticide products quality supervision and Inspection Center, china; 40% kresoxim-methyl SC provided by Jiangxi Zhengbang biological chemical Limited by Share Ltd (China); Sodium chloride, anhydrous sodium sulfate (analytical reagent AR) provided by Tianjin concord technology co., Ltd (China); Ethyl acetate, acetone, hexane (chromatographic pure) provided by Fisher U.S.; SPE-Florisil column (1 g, 6 mL) provided by Supelco (USA).

Gas chromatograph (Shimadzu GC 2010 plus, Shimadzu, Japan), with electron capture detector and chromatography workstation; homogenizer (T25, IKA German); solid phase extraction apparatus (Beijing KangLin Technology Co., Ltd., China); nitrogen blowing instrument (DCY-24S Qingdao Haike instrument Co., Ltd.)

## 2.2. Field Testing

The experiment was carried out in Shijiazhuang, Hebei province, China; Yantai, Shandong province, China; Suzhou, Anhui province, China. 40% kresoxim-methyl SC was used for testing.

### 2.2.1. Digestion Dynamic Test

To investigate the dissipation of kresoxim-methyl in plant, apples were sprayed with kresoxim-methyl formulation (40% SC) of the dosage of 300 mg a.i. /kg. Three areas were set to repeat experiment and put 2 trees in each area. Areas are equipped with isolation belt, and compared areas were also set. The pesticide was sprayed when apples in the trees had a diameter of 3 cm and perform sample collection 1 h, and 1, 3, 5, 7, 14, 21, 30, 45 days after spraying. Afterwards, The content of kresoxim-methyl in the apple samples was determined using the method developed earlier.

### 2.2.2. Final Residue Test

Low dosage (200 mg a.i. /kg) and high dosage (300 mg a.i. /kg) of the pesticide was applied 3 and 4 times respectively in an interval of 7 days. Three areas were set to repeat experiment and 2 trees in each area. In addition, A blank region was set for comparison. The pesticide was sprayed when apples in the trees had a diameter of 3 cm and perform sample collection 14, 21 and 28 days after spraying. The content of kresoxim-methyl was Determined.

## 2.3. Preparation of the Sample

### 2.3.1. Extraction

25.0 g of sample, exact 40 ml of acetone and 60 mL of ethyl acetate was broke up with a homogenate machine, after that operate ultrasonic oscillation for 5 mins. Put them aside until the layers are completely separated. Transfer the upper layer into a 100 mL stopper measuring cylinder which contains 5 g of sodium chloride, continue shaking it for 1 min and put it aside for another 10 min. When the layers were completely separated, collect 50 ml of the upper layer liquid, transfer it through anhydrous sodium sulfate and wash it using a small amount of acetone. Perform vacuum steam at 45 degrees Celsius to nearly dry and add 3 ml of n-hexane and carry on to nearly dry. Dissolve the residue into 2 ml of n-hexane and purify it with florisil column.

### 2.3.2. Purification

Wash the florisil column with 5 mL of acetone / hexane (20:80, v/v) and 5 mL of hexane, filter the washing liquid and when the liquid level is lower than the surface of the infilling, fill the column with the sample liquid and dispose it. After that, use 10 mL of acetone / hexane (20:80, v/v) to wash the beaker in two times and fill the column with the sample liquid. Collect the elution liquid and blow it with nitrogen at 40 degrees Celsius to nearly dry and ethyl acetate was used to make it up to 5 ml.

## 2.4. Chromatography Condition

Chromatography column: DB-1701 (30 m×0.25 mm×0.25 μm, Agilent Technologies); column temperature: keep it at 80°C for 1 min and increase the temperature to 260°C with a speed of 20°C /min, and keep this temperature for 5.5 min; Inlet temperature: 250°C, perform shunt injection with a split ratio of 10; Detector temperature: 300°C; Carrier gas: N2 (99.999%); Flux: 2.0 mL/min; Tail blowing: 30 mL/min; Sample size: 1.0 μL.

Under the above chromatography condition, retention time: kresoxim-methyl 11.4 min.

## 2.5. Risk Assessment

Use equation (1) and (2) to calculate Dietary exposure of pesticide and perform risk assessment [16]

$$EED \ (mg /kg \ bw) = CRL \ (mg /kg) \times FI \ (kg) \div bw \ (kg) \quad (1)$$

$$RQ = EED \ (mg /kg \ bw) \div ADI \ (mg /kg \ bw) \quad (2)$$

Where:
In equation (1): EED is estimated exposure dose
CRL is calculated residue level
FI is Food intake (refers to table 1)
In equation (2): bw is Body weight
RQ is Risk quotient
ADI is acceptable daily intake
When the risk quotient (RQ)>1, a high risk which is not acceptable exists, the higher the RQ the more the risk; when RQ<1, the risk detected is acceptable, the lower the RQ, the

lesser the risk [16].

**Table 1.** *Body weight and fruit intake of different crowds [17].*

| Crowd | Body weight/kg | Fruit intake/[g/(person·d)] |
|---|---|---|
| Age 2-4 boy | 14. 1 | 43. 7 |
| Age 2-4 girl | 13. 4 | 44. 4 |
| Male age 18-30 | 60. 5 | 41. 8 |
| Female age 18-30 | 52. 6 | 52. 9 |
| Male age 60-70 | 61. 3 | 33. 8 |
| Female age 60-70 | 54. 3 | 34. 8 |

# 3. Results and Analysis

## 3.1. Linearity Range and Detection Limit

Acetic ether was used as the solvent to prepare the standard solution of kresoxim-methyl of 100 μg/mL, and the solution was diluted into 0.01, 0.05, 0.1, 1.0, 2.0, 5.0 μg/mL series of standard solutions. Under the above instrument condition, the series of standard solutions were detected and graphs were constructed of the peaks on standard solution concentration (μg/mL) while drawing standard working curves for kresoxim-methyl. The linear equation of kresoxim-methyl is $Y=258536X+25866$, the correlation coefficient $r=0.9992$ where $Y$ is the peak area, $X$ is the standard concentration of solution (μg/mL). The limit of detection of kresoxim-methyl when calculated using 3 times of noise-signal ratio is $1.0\times10^{-12}$ g.

## 3.2. Accuracy and Precision of the Method

0.02, 0.20 and 2.0 mg /kg of standard substance of kresoxim-methyl were add into blank apple sample separately, and recovery test was repeated for five times for each concentration using the constructed method. The result (table 2) has shown that when the addition was between 0.02 and 2.0 mg/kg, the average percent recovery ranged from 80% to 90%, and the relative standard deviation (RSD) ranged from 0.9% to 1.3%. The limit of quantitation (LOQ) was 0.02 mg/kg when using the lowest addition level method.

**Table 2.** *The average recoveries and RSD of kresoxim-methyl in apple (n=5).*

| Spiked level/mg/kg | recovery/% | | | | | | RSD/% |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Average | |
| 0.02 | 99 | 100 | 101 | 98 | 99 | 99 | 1.0 |
| 0.20 | 90 | 92 | 90 | 92 | 89 | 91 | 1.3 |
| 2.0 | 80 | 80 | 79 | 81 | 79 | 80 | 0.9 |

## 3.3. Degradation Dynamics

**Table 3.** *Dynamic residue of kresoxim-methyl in apples from Hebei, Shandong and Anhui.*

| Time after spraying/d | Residue level detected/(mg/kg) | | |
|---|---|---|---|
| | Hebei (china) | Shandong (china) | Anhui (china) |
| 0 | 0.648 | 0.437 | 0.528 |
| 1 | 0.392 | 0.257 | 0.468 |
| 3 | 0.316 | 0.230 | 0.289 |
| 5 | 0.184 | 0.203 | 0.286 |
| 7 | 0.034 | 0.154 | 0.262 |
| 14 | 0.029 | 0.072 | 0.161 |
| 21 | 0.025 | 0.060 | 0.075 |
| 30 | 0.020 | 0.042 | 0.065 |
| 45 | <0.020 | 0.032 | 0.020 |

Refers to table 3 for the dynamic residue of kresoxim-methyl in apples from Hebei (china), Shandong (china) and Anhui (china). The origin adhesion quantities of kresoxim-methyl in apples from the three areas are 0.648, 0.438 and 0.528 mg/kg respectively. The resolution equation are $C_t = 0.30e^{-0.115t}$, $C_t =0.26e^{-0.0552t}$ and $C_t=0.43e^{-0.0691t}$ respectively, the correlation coefficient are -0.8537, -0.9343 and -0.9875 respectively, the half-lifes are 6.09 d, 12.6 d and 10.0 d respectively.

## 3.4. The Terminal Residues and Risk Assessment

Upon using 200 mg/kg, the maximum recommended dose of pesticide and 300 mg/kg, 1.5 times of the recommendation, the residues detected 14, 21 and 28 days after the final application of kresoxim-methyl in apples from different areas was 0.062~0.391 mg/kg, 0.025~0.283 mg/kg and 0.02~0.139 mg/kg respectively. Refers to table 4 for the average residues results in three different areas.

**Table 4.** *The terminal residues of kresoxim-methyl in apple.*

| Dosage a.i / (mg /kg) | The number of spraying | Average residue/(mg/kg) | | |
|---|---|---|---|---|
| | | Hebei (china) | Shandong (china) | Anhui (china) |
| 200 | 3 | 0.194 | 0.242 | 0.115 |
| | 4 | 0.224 | 0.167 | 0.136 |
| 300 | 3 | 0.258 | 0.191 | 0.172 |
| | 4 | 0.266 | 0.197 | 0.182 |

Refers to table 5 for results of the dietary risk assessment of kresoxim-methyl in apple using the maximum terminal average residual.

***Table 5.*** *The dietary risk assessment of kresoxim-methyl in apple.*

| crowd | Estimated exposure dose/[mg/(kg bw·d)] | | | RQ | | |
|---|---|---|---|---|---|---|
| | Hebei (china) | Shandong (china) | Anhui (china) | Hebei (china) | Shandong (china) | Anhui (china) |
| Age 2-4 boy | 0.0008 | 0.0008 | 0.0006 | 0.0021 | 0.0019 | 0.0014 |
| Age 2-4 girl | 0.0009 | 0.0008 | 0.0006 | 0.0022 | 0.0020 | 0.0015 |
| Male age 18-30 | 0.0002 | 0.0002 | 0.0001 | 0.0005 | 0.0004 | 0.0003 |
| Female age 18-30 | 0.0003 | 0.0002 | 0.0002 | 0.0007 | 0.0006 | 0.0005 |
| Male age 60-70 | 0.0001 | 0.0001 | 0.0001 | 0.0004 | 0.0003 | 0.0003 |
| Female age 60-70 | 0.0002 | 0.0002 | 0.0001 | 0.0004 | 0.0004 | 0.0003 |

## 4. Conclusion

A rapid and effective method was developed by using GC-ECD for determination of kresoxim-methyl in apple. The kresoxim-methyl dissipation rates and terminal residues in apple fields were also studied to evaluate consumer safety. The results show that the half-lives in apple were 6.09 d (Shijiazhuang, China), 12.6 d (Shandong, China) and 10.0 d (Anhui, China) respectively under the field condition. It can be demonstrated that after 14 days of using the appropriate amount dose set by experiment, the residual risk quotient values of kresoxim-methyl in apple are much lower than 1: thus the dietary risk of customers caused by residual in apple is extremely low if the diseases of apple are prevented and cured by experimental dose of 40% kresoxim-methyl deflocculant. Therefore, it is safe for human body when recommended amount of pesticide is used in China.

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