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Carboxin and its major metabolites residues in peanuts: Levels, dietary intake and chronic intake risk assessment

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ABSTRACT

We developed an ultra-performance liquid chromatography-tandem mass spectral method to determine the fungicide carboxin and its metabolites, oxycarboxin and carboxin sulfoxide in peanut samples. The method was used to detect the concentration of the analytes in the samples from fields and markets. The total residue quantities in peanut kernels were used to evaluate the chronic dietary risk of total carboxin upon peanut consumption. The estimated dietary intake of carboxin from peanuts whose seeds had been treated with carboxin at the recommended dose was between 0.020% and 0.344% of acceptable daily intake and the risk was found to be negligible. The chronic dietary risk assessment from markets and commercial field samples for various groups of humans indicated that the group with the greatest degree of exposure was 45 to 75-year-old women who lived in rural areas. However, their acceptable daily intake percentage was 0.006%, meaning that their health risk was extremely small.

1. Introduction

The peanut is an important commercial crop (Wan, Zhang, & Sun, 2005), containing kernels rich in oil, fat, and protein, which makes it a good nutritional and medicinal source. The peanut shell and straw byproducts are also rich in nutrients and might find medicinal use (Ferreyra, Pachepsky, Collino, & Acock, 2000; Hashem, Abdel-Halim, El-Tahlawy, & Hebeish, 2009) or as livestock and poultry roughage. However, fungus-related diseases reduce peanut, pod, and fodder yields and decrease the quality of peanut oil (Mondal & Badigannavar, 2015). These sporadically occurring fungus-related diseases can reduce the economic value of a peanut harvest by 30% or more (Subrahmanyam & Mcdonald, 1987). Carboxin, (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide), also commercialized as Vitavax, is a systemic anilide fungicide introduced in 1969 by Uniroyal Chemical Co. and approved for use in the EU subject to member state approval. It efficiently prevents and cures Basidiomycetes-induced diseases, including smut, rust, rot and blight, in peanut seeds and seedlings (Wallnöfer, Königer, Safe, & Hutzinger, 1971; Akgul, Ozgonen, & Erkilic, 2011; Rakholiya, 2015). However, extensive use of carboxin in agricultural applications increases the possibility that it will be consumed by humans (Bozdogan & Yarpuz-Bozdogan, 2015).

Carboxin is oxidized in the environment and in plants by abiotic and biotic reactions (Balasubramanya & Patil, 1980a, 1980b) to carboxin sulfoxide and sometimes in small amounts to oxycarboxin (Chin, Stone, & Smith, 1970) (Fig. S1). Both carboxin sulfoxide and oxycarboxin have been reported as metabolites in peanut seeds and peanut cell suspension (Larson & Lamoureux, 1984); these two oxides have bactericidal activity (Isidori et al., 2012). Carboxin sulfoxide is readily formed, is more persistent in the environment, and is more mobile than carboxin (Dellagreca, Iesce, Cermola, Rubino, & Isidori, 2004; USEPA, 2004). Oxycarboxin also acts as a fungicide and is effective against rust in cereals and vegetables (Dębska, Gnusowski, & Zygmunt, 1979). Oxycarboxin in plants is more stable and persists for a longer period of time than carboxin does (Hustert, Moza, & Kettrup, 1999). Because carboxin sulfoxide and oxycarboxin are the main metabolites of carboxin and have a toxicity very similar to that of carboxin (EFSA, 2010), the definition for risk assessment and monitoring of residual carboxin includes carboxin, carboxin sulfoxide, and oxycarboxin, the sum of the three compounds being expressed as total carboxin (EFSA, 2010). To

Abbreviations: LC-MS/MS, Liquid chromatography-tandem mass spectrometry; QuEChERS, Quick, Easy, Cheap, Effective, Rugged, and Safe; ADI, acceptable daily intake; MRL, maximum residue level; MeCN, acetonitrile; PSA, Primary secondary amine

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ensure the safety of peanut consumption by humans, quantification of residual total carboxin on peanut matrices and evaluation of the risks posed by residual total carboxin are important.

Prior to this report and to the best of our knowledge, a sensitive analytical method for total carboxin had not been reported. A few analytical methods have been developed to determine carboxin levels (Bozdogan et al., 2015) and carboxin plus oxycarboxin levels. Farrow and co-workers reported a gas chromatographic method to determine carboxin levels in grain (Farrow, Hoodless, & Hopkinson, 1975). Tafuri and co-workers described a gas chromatographic method that uses a nitrogen-selective detector to determine carboxin and oxycarboxin levels in grain (Tafuri, Patumi, Businelli, & Marucchini, 1978). Ma and co-workers developed a QuEChERS and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method to determine carboxin levels in wheat (Ma et al., 2016). However, no investigation has attempted to quantify residual total carboxin levels in peanuts and related straw samples. There is a need for a rapid and sensitive analytical method to identify and quantify residual total carboxin in peanut kernels, shells, and related straw samples, to ensure food and environmental safety.

Although monitoring of a compound provides knowledge of its residual level in a matrix, in terms of food safety, other information is needed (Łozowicka, Kaczyński, Jankowska, Rutkowska, & Hrynko, 2012). To evaluate if a pesticide possesses a chronic dietary intake risk to humans, an estimation of its daily intake by humans should be made, and then this estimation should be compared with toxicological endpoint value such as the acceptable daily intake (ADI) (Chen et al., 2012). The ADI of carboxin is 0.008 mg kg^{-1} bw d^{-1} established by EFSA, CAC and China, and it is 0.1 mg kg^{-1} bw d^{-1} for US EPA. Risk assessment methods for pesticide residues reported in the literature have mostly been based on deterministic assessments (point estimates), as such methods are simple and feasible. However, evaluation of the results does not reflect individual differences among different populations (Duan, Guan, Li, Li, & Luo, 2016). Recently, probabilistic methods have often been used to make food safety risk assessments. They can better quantify the variability of the results (Caldas, Boon, & Tressou, 2006). When evaluating the risk, data from supervised field trials may reflect whether a potential hazard to humans exists when the maximum allowed dose of a pesticide is applied. However, that quantity will generally overestimate the typical intake because samples obtained from field where usage of pesticides was at maximum approved dose and timing under the Good Agricultural Practices are very unlikely to occur in practice (Chen et al., 2012). Therefore, assessment of samples obtained at markets may more accurately reflect the chronic dietary risk to humans, meaning that it is necessary to evaluate the chronic dietary intake of samples from fields and markets using a probabilistic assessment.

As noted above, the present work aimed to develop a rapid and effective analytical method to determine residual total carboxin in peanut kernels, shells and related straw samples, then exploring the residue behavior of carboxin and its major metabolites in peanuts from supervised field trials and market-basket survey. Further chronic dietary risk was evaluated for Chinese cohorts consuming peanuts containing residual carboxin and its metabolites, to understand the health risk. The European Union, America, and Korea have set a maximum residue level (MRL) for carboxin in peanuts of 0.02 mg kg^{-1}; however, China currently does not have an MRL value for total carboxin in peanuts. This work provided the scientific evidence needed to allow the Chinese government to establish an MRL and recommend a proper dose of carboxin for peanut seeds from the perspective of dietary risk assessment.

2. Materials and methods

2.1. Chemicals and reagents

Carboxin (98.2% purity) was purchased from Qinchengyixin Technology Co., Ltd. (Beijing, China); oxycarboxin (99.0% purity) and carboxin sulfoxide (99.8% purity) were purchased from Alta Scientific Co., Ltd. (Tianjin, China). Chromatographic-grade acetonitrile (MeCN) and formic acid (98%) were purchased from Sigma-Aldrich (Steinheim, Germany). Analytical grade anhydrous magnesium sulfate ($MgSO_4$) (98%), sodium chloride (NaCl) (99.5%), MeCN, and aqueous ammonia (25%) were purchased from Beijing Chemical Company (Beijing, China). Ultrapure water was prepared using a Milli-Q system (Millipore, Bedford, MA). Primary secondary amine (PSA, 40 μm) and Florisil sorbents (40 μm) were provided by Agela Technologies Inc. (Tianjin, China).

2.2. Sample collection

Peanut kernels were obtained from the field trial described below, and from supermarkets, wholesale markets, farmer's markets, and peanut plant fields cultivated for commercial distribution.

2.2.1. Field trial study

Supervised field trials were conducted during 2015 and 2016 in Shandong (118.83E, 36.71 N), Henan (112.93E, 35.08 N), and Anhui (117.57E, 32.87 N) provinces according to instructions in the *Standard Operating Procedures on Pesticide Registration Residue Field Trials* issued by the Institute of the Control of Agrochemicals, Ministry and Agriculture, People's Republic of China. Peanut seeds were coated with a solution of carboxin·thiram 400 g/L Suspension Concentrate provided by Nanjing MacDermid Chemical Co., Ltd. at a dose of 120 g active ingredient/100 kg seed (the recommended dosage) or 180 g active ingredient/100 kg seed (1.5 times recommended dosage) before sowing. Each experimental field had three replicate plots, each 30 m^2 in area and isolated by irrigation channels. At harvest time, samples of peanut kernels, shells, and straw (each at least 1 kg in mass) were randomly collected from each plot. Samples were immediately sent to the laboratory and stored at −20 °C until used. Then the samples were analyzed to get the final residue data.

2.2.2. Sample collection from commercial fields and markets

A total of 200 peanut samples were collected during 2016 from commercial peanut plant production areas in the major provinces and municipalities of China, namely Guangxi, Jiangxi, Anhui, Jiangsu, Shandong, Shanxi, Beijing, and Liaoning. Some of the samples were collected during the harvest season from the fields, and others were collected from supermarkets, wholesale markets, and farmer's markets in the major provinces and municipalities in China. For these samples, their pesticide backgrounds were not known. Sample sizes were at least 1 kg. Samples were packed into zip-lock bags, then immediately delivered to the laboratory, and stored at −20 °C until used.

2.3. Analytical procedures

2.3.1. Sample extraction and cleanup

Peanut samples were separated into kernels and shells, and then the kernels, shells, and straw were comminuted in a blender. The extraction procedure applied was based on the QuEChERS method. The procedure was as follows: Samples (5 g of kernels, 5 g of straw and 2 g of shells) were individually added into 50-mL Teflon centrifuge tubes, and then 5 mL of ultrapure water and 10 mL of MeCN/1% (*v/v*) aqueous ammonia were added. The tubes were shaken vigorously for 10 min after which 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added, and then the samples were shaken for 5 min. Next, samples were centrifuged at 2811*g* for 5 min. Each supernatant was filtered through a 0.22-μm

nylon syringe filter with 100 μL of each sample then being individually transferred into a brown sampler vial and diluted with 900 μL of MeCN for UPLC-MS/MS.

2.3.2. UPLC-MS/MS conditions

All analyses were performed using a Waters Acquity UPLC system coupled to a XEVO TQ-S tandem quadrupole mass spectrometer equipped with an electrospray ionization source (Waters Corp., Milford, MA). Good chromatographic separation and retention behavior were achieved using an ACQUITY UPLC HSS T3 column (2.1 mm × 100 mm, 1.8-μm particle size; Waters Corp.). The mobile phase consisted of acetonitrile and water. Elution was performed in gradient mode (0–1.5 min, 10–90% acetonitrile; 1.5–3.0 min, 90% acetonitrile; 3–3.1 min, 90–10% acetonitrile; 3.1–5.0 min, 10% acetonitrile). The flow rate was 300 μL/min and the injection volume was 3 μL.

The mass spectrometer was operated in positive ionization and multiple reaction-monitoring modes. Typical conditions were: capillary voltage +3.5 kV; source temperature 150 °C; desolvation temperature 300 °C; nitrogen served as the cone and desolvation gases at rates of 150 and 800 L h^{-1}, respectively. All parameters were optimized to obtain the maximum sensitivity and resolution (Table S1). MassLynx software (version 4.1) was used to collect and analyze the data.

2.4. Dietary intake and chronic intake risk assessment

To assess human consumer risk resulting from exposure to low levels of total residual carboxin *via* peanut consumption, we assessed the risk of consuming carboxin and its metabolites *via* peanut kernels from fields for which seeds had been treated with the recommended dose of carboxin or treated with 1.5 times recommended dose. Risk assessments were also made for the samples from commercial fields and markets, for which pesticide usage was not known.

Probabilistic approach (Duan et al., 2016) was used to assess the dietary exposure. The estimated daily intake of carboxin depends on the carboxin concentration in the peanut kernels and the amount of peanuts consumed. The national estimated daily intake (NEDI) was determined using the following equation (Li et al., 2016): NEDI = LR × F/bw, where LR is the amount of residual total carboxin (mean or percentage of the pesticide residual level, mg kg^{-1}), F is the average peanut consumption (g/person/day), bw is the average body weight (kg). The values for F and bw (Table S2) were obtained from the 2002 Chinese National Nutrition and Health Survey. Demographic information concerning the participants, including their age and sex, were obtained from the survey and included in the risk analysis. Because food consumption and body mass differ for Chinese living in rural and urban areas, the surveyed population was subdivided as rural and urban residents.

The percentage ADI (%ADI), which is a measure of the chronic exposure risk, was used to evaluate the risk of chronic dietary intake and was expressed as: %ADI = (NEDI/ADI) × 100%. The risk probability is positively correlated with the %ADI value, and the greater the %ADI value is, the greater the chronic exposure is. When the %ADI value is < 100%, the risk is considered acceptable and will not constitute a long-term health threat; conversely, when the %ADI value is > 100%, the risk is considered unacceptable.

We calculated the risk associated with dietary exposure to total carboxin using Monte Carlo and bootstrap construction methods (Duan et al., 2016). Latin hypercube sampling was performed multiple times (n) for the bootstrap method (Dai et al., 2016). First, the sum of the carboxin, carboxin sulfoxide, and oxycarboxin concentrations for the field and market samples were measured, and samples that were found to contain no residual fungicide (< LOQ) were assigned a value of LOQ/2 (Tsoutsi, Konstantinou, & Hela, 2008). An optimum fitting distribution was obtained using all the data and determined to be appropriate according to the Chi-Squared, Anderson-Darling, and Kolmogorov-Smirnov tests. Statistics for individual samples, including the mean values and percentiles (P50, P90, P97.5, P99.9), were calculated and the confidence intervals for all statistical data were recorded. The number of iterations (n) and the number of simulations in the simulation procedures were 10,000 and 100, respectively, which resulted in 10^6 (10,000 × 100) simulations being performed to guarantee the reliability of the results (Huang et al., 2015). Sampling and fitting were performed using the commercially available software package @Risk (Version 5.5, for Excel, Professional Edition).

3. Results and discussion

3.1. UPLC-MS/MS optimization parameters

Multiple reaction monitoring mode was used for MS/MS to identify and quantify carboxin, carboxin sulfoxide and oxycarboxin and the spectral parameters were optimized using samples of the standards (100 μg L^{-1}) that were directly injected into the spectrometer. UPLC-ESI-MS/MS spectra for the three compounds were acquired by direct injection at different cone voltages. Protonated molecules served as the precursor ions for MS/MS; the precursor ions were fragmented at different collision voltages, and the selected reaction monitoring was adjusted to obtain the highest degree of sensitivity. Two intense fragment ions were observed in the product-ion spectrum of each analyte and were chosen as the quantitative and qualitative product ions for each compound. The molecular weights, the precursor ions, cone voltages, and corresponding collision voltages are listed in Table S1. Satisfactory chromatographic separation and peak shapes were achieved for the three compounds with the ACQUITY UPLC HSS T3 column. The retention times were 1.66 min for carboxin sulfoxide, 1.88 min for oxycarboxin, and 2.19 min for carboxin under those conditions (Fig. S2).

3.2. Optimization of extraction and purification procedures

Based on the QuECHERS method, the selection of an extraction solvent and the choice of a clean-up procedure are critical for the simultaneous analysis of residual concentrations of multiple pesticides in different matrices. Typically, MeCN is the first solvent considered because it typically has a better extraction efficiency and extracts less matrix components than do other solvents (Zhao et al., 2014). We first examined the extracting efficiency of MeCN for each of the three matrices spiked with the three analytes each at a concentration of 100 μg kg^{-1}. Unfortunately, the recoveries for carboxin and its two metabolites were unsatisfactory, the recoveries of carboxin were ≤59.0%. For the peanut shell, the recoveries of carboxin sulfoxide were higher than 120%. For the peanut straw, the recoveries of the two metabolites were less than 70% (Fig. S3A). The addition of acid (Du et al., 2017) or base (Barreto, Ribeiro, Hoff, & Dalla Costa, 2016) into an extraction solvent has been shown to increase the extraction of various analytes. Therefore, MeCN/2% (v/v) formic acid and MeCN/2% (v/v) ammonia were tested for their extraction abilities. When MeCN/2% (v/v) formic acid was used, the recovery of carboxin from each of the matrices was < 70%, and the recovery of carboxin sulfoxide from peanut kernels and shells was > 140% (Fig. S3A). Conversely, for MeCN/2% (v/v) ammonia the recovery of each target compound from each matrix was satisfactory (73.5–117.1%; Fig. S3A).

Next, MeCN solutions containing 1% (v/v) or 1.5% (v/v) ammonia were tested as extraction solvent. As shown for peanut shells in Fig. S3B, for example, recoveries (75.7–117.1%) were always acceptable for MeCN containing 1, 1.5, or 2% (v/v) ammonia. The recoveries of carboxin and carboxin sulfoxide improved as the concentration of ammonia increased, although for oxycarboxin there is no clear correlation. Taking the results for the three compounds into consideration, we selected MeCN/1% (v/v) ammonia in water as the extraction solvent.

To efficiently clean up the MeCN/1% (v/v) ammonia solution used for extraction, a QuEChERS procedure incorporating a dispersive solid-phase extraction was first considered. The clean-up abilities of two

Table 1
Recoveries (n = 15; %), and RSD_r and RSD_R percentages for the target compounds from different matrices at four spiked concentrations.

Matrix	Spiked level (μg kg^{-1})	Carboxin			Oxycarboxin			Carboxin sulfoxide		
		Recovery	RSD_r	RSD_R	Recovery	RSD_r	RSD_R	Recovery	RSD_r	RSD_R
Peanut kernel	1	101.0	4.5	5.1	91.0	6.5	7.7	100.3	5.6	5.9
	10	112.3	3.8	4.7	98.3	5.1	8.9	106.5	3.3	6.9
	100	84.5	3.1	3.7	83.2	3.0	3.7	85.2	3.2	3.6
	500	115.4	2.6	4.8	114.5	2.5	3.1	110.5	3.0	4.9
Peanut shell	1	80.3	8.2	9.6	72.8	2.4	3.5	97.8	8.4	12.1
	10	80.7	3.1	6.9	71.7	1.9	2.7	89.6	6.7	8.8
	100	84.4	17.9	16.9	90.7	7.5	9.4	118.3	6.7	9.9
	500	82.9	5.6	7.5	76.8	1.9	10.3	101.0	7.4	9.6
Peanut straw	1	77.4	6.7	9.4	80.5	5.6	7.0	101.4	4.8	5.6
	10	77.8	10.2	12.5	74.9	4.4	5.1	91.2	10.1	9.5
	100	96.4	6.9	6.7	90.8	4.4	4.6	105.8	3.0	6.4
	500	78.1	5.5	8.9	73.7	2.3	3.3	94.2	3.2	6.2

common sorbents, PSA (50 mg) and Florisil (50 mg) (Zan, & Chantara, 2007), were tested using the different matrices spiked with each of the analytes at 100 μg kg^{-1}. Because of the high sensitivity of the Xevo TQ-S mass spectrometer detector, after each QuEChERS procedure, the treated samples were each diluted 10-fold with MeCN before UPLC-MS/MS. To test the effects of the sorbents, the extracts were also diluted 10-fold with pure MeCN without the use of sorbent. Concerning the recoveries of the analytes, we observed no significant differences between the two sorbents and the samples diluted 10-fold with MeCN, except in the case of carboxin sulfoxide from peanut shells for which the recovery was 120% greater when the purification procedure included PSA or Florisil. The recovery and RSD values were acceptable when the extracts from all matrices were diluted 10-fold with pure MeCN and in the absence of a sorbent. Consequently, treatment of the samples did not require a clean-up step, which saved time and cost. By diluting a sample before analysis, it reduces the injection volume of the samples and the matrix effect. In summary, MeCN/1% (*v/v*) ammonia served as the extraction solvent, and the extracts were diluted 10-fold with MeCN instead of subjecting them to a clean-up procedure.

3.3. Analytical method validation

3.3.1. Linearity and LOQ

The optimized method was used to determine the concentration of carboxin and its two metabolites in the matrices. Blank samples (peanut kernels, shells and straw) were extracted by the optimized extraction method. Matrix-matched standard solutions were obtained at 1, 5, 10, 50, 100 and 500 μg L^{-1} by adding blank sample extracts to each serially diluted standard solution. A six-point calibration curve for each matrix and each analyte was plotted for linear regression analysis with the concentration of each analyte between 1 μg L^{-1} and 500 μg L^{-1}. The linear regression equations including slope, intercept, and the determination coefficients (R^2) are given in Table S3. Satisfactorily linear determination coefficients ($R^2 \geq 0.9972$) were obtained for the curves of each analyte in each matrix, and the LOQ was 1 μg kg^{-1} for all matrices.

3.3.2. Matrix effects

Overcoming matrix effects is a major challenge when developing a mass spectral procedure that can quantify a target compound(s) because co-eluting contaminants may enhance or suppress the analyte response (Payá et al., 2007), which is also dependent on the instrument, the analyte and its concentration, and the sample pre-treatment procedure (Famiglini, Palma, Pierini, Trufelli, & Cappiello, 2008). Therefore, in the present study, the effect of matrix on the MS/MS detector using the proposed method was investigated in the three matrices. The matrix effect could be calculated as follows:

matrix effect (ME%)= 100 × (slope of calibration curves in matrix − slope of calibration curves in solvent) /slope of calibration curves in solvent.

The effects that the matrices had on the detection intensities of the three analytes ranged from −68.97% to −1.97% (Table S3). Therefore, it was important that we employed matrix-matched calibration standards to accurately quantify the analytes in the different samples.

3.3.3. Precision and trueness

The precision and trueness of our method were evaluated using the data obtained for the recovery assays. Blank samples, prepared in quintuplicate, were spiked at one of four concentrations (1, 10, 100, and 500 μg kg^{-1}). The precision of the method was evaluated as repeatability (RSD_r) and reproducibility (RSD_R) values. The RSD_r values were taken as the standard deviation of the recovery percentages from spiked samples analyzed on the same day. The RSD_R values were taken as the standard deviation of the spiked samples analyzed on three distinct days. The mean recovery values were between 71.7% and 118.3% with both types of RSD values ≤17.9% for all samples (Table 1). These values are considered to be satisfactory according to the Document SNATE/11945/2015 guidelines (mean recovery between 70 and 120%, and RSD ≤20%) and indicated that the developed method could be used to determine the levels of carboxin and its two metabolites in peanut kernels, shells and straw.

3.4. Residual levels in peanut samples

3.4.1. Analysis of samples from carboxin-treated seeds

Peanut seeds were treated with carboxin·thiram at a dose of 120 g active ingredient/100 kg (the recommended dosage) and 180 g active ingredient/100 kg (1.5 times recommended dose) before sowing. The amounts of carboxin and its metabolites on the peanuts grown from these seeds were assayed after harvesting, as were samples of the straw in which the seeds had been grown (Table 2). To assess the risk associated with peanut consumption, the total carboxin concentration was calculated (Table 2). The total carboxin residual values in the straw were relatively large, with the largest value being 346 μg kg^{-1}; conversely, the residual values were much smaller for shells (9.69–24.3 μg kg^{-1}), and the residual levels for most kernel samples were less than the LOQ. The occurrence and detected carboxin sulfoxide concentrations were greater than those for carboxin and oxycarboxin.

The residual values for straw when 1.5 times recommended dose was applied to the seeds were greater than when the recommended dose was applied to the seeds. For peanut kernels and shells, the residual values when the recommended dose was applied were greater than when the larger dose was applied, which may be because the absorption of carboxin in the kernels and shells were disproportionate

Table 2
Carboxin and its metabolites residues in harvested peanuts samples (μg kg^{-1}, $n = 3$).

Compounds	Location	Shandong				Henan				Anhui			
	dosage	Low dosage		High dosage		Low dosage		High dosage		Low dosage		High dosage	
	Year	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Carboxin	Kernel	ND	3.56 ± 0.02	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Shell	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Straw	4.00 ± 0.03	4.00 ± 0.04	4.03 ± 0.07	3.98 ± 0.02	4.50 ± 0.06	3.97 ± 0.01	4.74 ± 0.25	3.97 ± 0.01	4.09 ± 0.05	4.00 ± 0.01	4.17 ± 0.05	4.04 ± 0.07
Oxycarboxin	Kernel	ND	5.75 ± 0.31	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Shell	12.3 ± 0.76	12.8 ± 2.43	11. ± 0.15	11.4 ± 0.60	11.9 ± 0.18	11.0 ± 0.12	12.2 ± 0.41	11.0 ± 0.11	11.1 ± 0.15	11.4 ± 0.0.16	11.2 ± 0.07	13.4 ± 0.36
	Straw	26.2 ± 1.59	24.1 ± 5.60	48.1 ± 2.47	18.9 ± 1.53	19.3 ± 0.19	23.6 ± 7.38	21.9 ± 0.67	17.8 ± 0.22	17.1 ± 0.04	18.5 ± 0.13	17.3 ± 0.13	37.2 ± 0.01
Carboxin sulfoxide	Kernel	3.79 ± 1.53	8.70 ± 0.71	2.87 ± 1.12	ND	3.01 ± 0.31	ND	2.71 ± 0.77	ND	ND	ND	ND	2.09 ± 0.14
	Shell	3.87 ± 2.51	14.0 ± 1.74	1.82 ± 0.36	1.91 ± 0.82	4.17 ± 0.94	ND	3.13 ± 0.99	ND	ND	1.56 ± 0.09	1.45 ± 0.08	4.38 ± 0.42
	Straw	88.3 ± 13.6	126 ± 31.7	320 ± 86.8	25.7 ± 8.01	3.00 ± 0.93	1.01 ± 0.38	20.7 ± 3.44	3.07 ± 0.40	ND	4.19 ± 0.12	ND	6.07 ± 0.33
Sum of carboxin	Kernel	3.55 ± 1.43	16.8 ± 7.29	2.69 ± 1.05	ND	2.82 ± 0.29	ND	2.54 ± 0.72	ND	ND	ND	ND	1.96 ± 0.13
	Shell	14.5 ± 2.48	24.3 ± 3.13	12.1 ± 0.41	11.8 ± 3.16	14.4 ± 0.81	9.70 ± 0.18	13.69 ± 0.62	9.69 ± 0.26	9.79 ± 0.13	11.5 ± 0.08	11.2 ± 0.13	15.0 ± 0.71
	Straw	110 ± 12.9	144 ± 28.15	346 ± 81.7	44.7 ± 7.55	24.3 ± 0.78	25.7 ± 4.45	43.38 ± 3.42	22.5 ± 0.56	19.1 ± 2.34	24.2 ± 2.22	19.4 ± 2.42	42.5 ± 2.14

ND, not detected.

in comparison with straw.

3.4.2. Residual carboxin analytes in peanut kernels collected from commercial fields and markets

Carboxin and its metabolites were detected in 8.5% of the 200 peanut kernel samples, and the presence of carboxin sulfoxide was significantly greater compared with those of oxycarboxin and carboxin. The greatest concentration of the total residual carboxin was 34.4 μg kg^{-1} found in one field sample collected from Shandong province. The detection frequency was 26.19% for samples from Shangdong, 21.05% for samples from Beijing, 2.50% for samples from Anhui, and 7.69% for samples from Guangxi. The detection rates and the detected concentrations for the samples from Shandong were the largest found, but still considerably less than the MRL value established by the European Union.

3.5. Chronic intake risk assessment

A probabilistic evaluation model (He et al., 2015) was built to estimate the health risks for a Chinese cohort, using the Monte Carlo simulation software package @Risk to fit the distribution of total carboxin in peanut kernels. The total residual carboxin data were processed for distribution fitting; the optimum fitting distribution was selected. The results showed that the data fit from the carboxin-treated seed experiment conformed to an Invgauss distribution and that the data for the peanuts obtained from markets and fields conformed to a Loglogistic distribution. Mean values and percentiles (P50, P90, P97.5, P99.9 in this work) were obtained from the distribution.

The chronic exposure risks for the Chinese cohorts sorted by age, place of residence, and sex are listed in Table S2. The P50 values represent the median exposure of total carboxin by the cohort, and values at P90, P97.5, P99.9 represent greater degrees of exposure. The ADI% values for all groups were < 100% and increased with increasing levels of exposure. At P99.9, those with the greatest degree of exposure were women between the ages of 45 and 75 who lived in rural communities; however, their ADI% values were only 0.3436% and 0.0057% for kernel samples from the carboxin-treated seed experiments and the commercial market and field samples, respectively. The dietary exposure levels of carboxin for all groups were far below the ADI values. Therefore, consumption of peanuts containing residual total carboxin does not present a potential health risk.

Table 3 provides a comparison between the dietary exposure to carboxin according to the data for peanuts grown from carboxin-treated seeds and those obtained from commercial markets and fields. The hypothetical risk of chronic exposure had the market and field samples been consumed was much smaller than that for the peanuts grown from the treated seeds, however, it is unlikely the hypothetical risk would be as great as that determined for the carboxin-treated seed samples because probably the carboxin dose would be smaller than that used in our experiments. The results for the commercial market and field samples had a remarkably reduced chronic intake risk assessment compared with those from the samples obtained from carboxin-treated seeds, which indicated that pesticides were employed correctly and that the chronic dietary intake risk of carboxin *via* peanut consumption would have been very small. The probabilistic assessment results demonstrated that the total carboxin concentration in peanut kernels does not pose a health risk and that application of carboxin to peanut seeds in China at the recommended dose is safe for consumption of the peanuts harvested from the related plants.

4. Conclusions

We developed a method employing a modified QuEChERS sample preparation procedure to simultaneously quantify carboxin and its two major metabolites in peanut kernels, shells, and related straw samples. The method is simple, fast, and reliable, and the recoveries and LOQs

Table 3
Chronic dietary-intake risk assessment of carboxin according to peanut consumption for cohorts according to age, environmental and sex.

Sample types	gender	age (years)	rural				urban			
			P50	P90	P97.5	P99.9	P50	P90	P97.5	P99.9
Samples from the markets and commercial fields	male	2–6	1.1085×10^{-4}	1.5767×10^{-4}	1.9942×10^{-4}	3.3165×10^{-4}	6.3925×10^{-4}	9.0925×10^{-4}	1.1500×10^{-3}	1.9125×10^{-3}
		6–18	4.3042×10^{-4}	6.1221×10^{-4}	7.7431×10^{-4}	1.2877×10^{-3}	2.2145×10^{-4}	3.1498×10^{-4}	3.9838×10^{-4}	6.6253×10^{-4}
		18–45	3.2910×10^{-4}	4.6810×10^{-4}	5.9204×10^{-4}	9.8459×10^{-4}	3.5040×10^{-4}	4.9840×10^{-4}	6.3037×10^{-4}	1.0483×10^{-3}
		45–75	4.1276×10^{-4}	5.8710×10^{-4}	7.4255×10^{-4}	1.2349×10^{-3}	3.7434×10^{-4}	5.3244×10^{-4}	6.7342×10^{-4}	1.1199×10^{-3}
		≥75	3.0122×10^{-4}	4.2844×10^{-4}	5.4188×10^{-4}	9.0118×10^{-4}	3.2470×10^{-4}	4.6184×10^{-4}	5.8413×10^{-4}	9.7143×10^{-4}
	female	2–6	3.0260×10^{-4}	4.3041×10^{-4}	5.4438×10^{-4}	9.0533×10^{-4}	4.1510×10^{-4}	5.9042×10^{-4}	7.4675×10^{-4}	1.2419×10^{-3}
		6–18	2.4123×10^{-4}	3.4311×10^{-4}	4.3396×10^{-4}	7.2170×10^{-4}	3.8702×10^{-4}	5.5049×10^{-4}	6.9624×10^{-4}	1.1579×10^{-3}
		18–45	2.9733×10^{-4}	4.2291×10^{-4}	5.3488×10^{-4}	8.8953×10^{-4}	3.4184×10^{-4}	4.8623×10^{-4}	6.1497×10^{-4}	1.0227×10^{-3}
		45–75	1.8975×10^{-3}	2.6989×10^{-3}	3.4135×10^{-3}	5.6769×10^{-3}	2.9861×10^{-4}	4.2474×10^{-4}	5.3720×10^{-4}	8.9338×10^{-4}
		≥75	1.9255×10^{-4}	2.7387×10^{-4}	3.4639×10^{-4}	5.7605×10^{-4}	2.0511×10^{-4}	2.9174×10^{-4}	3.6898×10^{-4}	6.1364×10^{-4}
Samples grown from carboxin-treated seeds	male	2–6	2.6662×10^{-4}	2.0376×10^{-3}	5.6575×10^{-3}	2.0072×10^{-2}	1.5375×10^{-3}	1.1750×10^{-2}	3.2625×10^{-2}	0.1158
		6–18	1.0352×10^{-3}	7.9115×10^{-3}	2.1967×10^{-2}	7.7936×10^{-2}	5.3262×10^{-4}	4.0704×10^{-3}	1.1302×10^{-2}	4.0098×10^{-2}
		18–45	7.9154×10^{-4}	6.0491×10^{-3}	1.6796×10^{-2}	5.9590×10^{-2}	8.4278×10^{-4}	6.4407×10^{-3}	1.7883×10^{-2}	6.3448×10^{-2}
		45–75	9.9276×10^{-4}	7.5869×10^{-3}	2.1066×10^{-2}	7.4739×10^{-2}	9.0034×10^{-4}	6.8806×10^{-3}	1.9105×10^{-2}	6.7782×10^{-2}
		≥75	7.2448×10^{-4}	5.5367×10^{-3}	1.5373×10^{-2}	5.4541×10^{-2}	7.8095×10^{-4}	5.9683×10^{-3}	1.6571×10^{-2}	5.8794×10^{-2}
	female	2–6	7.2781×10^{-4}	5.5621×10^{-3}	1.5444×10^{-2}	5.4793×10^{-2}	9.9838×10^{-4}	7.6299×10^{-3}	2.1185×10^{-2}	7.5162×10^{-2}
		6–18	5.8019×10^{-4}	4.4340×10^{-3}	1.2311×10^{-2}	4.3680×10^{-2}	9.3085×10^{-4}	7.1138×10^{-3}	1.9752×10^{-2}	7.0078×10^{-2}
		18–45	7.1512×10^{-4}	5.4651×10^{-3}	1.5174×10^{-2}	5.3837×10^{-2}	8.2219×10^{-4}	6.2834×10^{-3}	1.7447×10^{-2}	6.1898×10^{-2}
		45–75	4.5638×10^{-3}	3.4877×10^{-2}	9.6841×10^{-2}	0.3436	7.1821×10^{-4}	5.4888×10^{-3}	1.5240×10^{-2}	5.4070×10^{-2}
		≥75	4.6310×10^{-4}	3.5392×10^{-3}	9.8268×10^{-3}	3.4864×10^{-2}	4.9332×10^{-4}	3.7700×10^{-3}	1.0468×10^{-2}	3.7139×10^{-2}

were satisfactory for the tested matrices. The total residue quantities in peanut kernels were used to evaluate the intake risk of total carboxin due to peanut consumption, and the results showed that the chronic risk exposure was always < 0.3436%, which indicated that, when used at the recommended dose to protect peanuts against fungal diseases, carboxin should not harm humans who consume peanuts from the treated seeds. The amounts of carboxin and its major metabolites in peanuts and its chronic exposure risk were found to be small for samples collected from commercial fields and markets. Therefore, the dietary intake of carboxin pesticide residues from peanut consumption for Chinese consumers does not pose a potential risk.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2018.09.087.

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