Multiple Pesticide Residues in Bananas Sold in Tangshan City and Dietary Risk Assessment

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Abstract [**Objectives**] This study was conducted to investigate the pesticide residue status of bananas in Tangshan area and the dietary intake risk of consumers. [**Methods**] High-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was applied to detect 70 pesticide residues in 191 batches of banana samples extracted from Tangshan area, and chronic and acute risk assessments were conducted. [**Results**] Among the 191 batches of bananas, 172 batches of samples were detected to contain agrochemicals, with a detection rate of 90.05%. Seventeen agrochemicals were detected, accounting for 24.29% of the total agrochemicals tested. There was a phenomenon of multiple pesticide residues in a single sample, and high detection rates were found in carbendazim (49.21%), prochloraz (41.88%), pyraclostrobin (34.03%) and imidacloprid (30.37%). According to GB 2763-2021 *National Food Safety Standards—Maximum Residue Limits for Pesticides in Food*, the over-standard rate of imidacloprid was 17.8%; and the chronic and acute dietary risks of the 17 pesticide residues were all less than 100%. [**Conclusions**] The pesticide residues in bananas consumed by consumers on a daily basis do not pose unacceptable dietary risks.

Key words Bananas; Pesticide residues; Dietary intake; Risk assessment
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Bananas (*Musa nana* Lour.) are fragrant, nutritious and have certain medicinal value[1]. They can be harvested all year round, and are one of the fruits with the largest consumption and trade volume in the world[2-3]. China is a big country in banana production and consumption in the world[4]. As an important cash crop in China's tropical areas, Banana is mainly distributed in Guangdong, Guangxi, Yunnan, Hainan, Fujian, Taiwan Province and other provinces (regions)[5]. Pests and diseases that affect banana growth and development mainly include leaf spot, scab, anthracnose, root-knot nematode disease, *etc*. Chemical control plays an active role in controlling and preventing banana pests and diseases. However, due to the growing scale of banana industry, the occurrence base of pests and diseases keeps accumulating, and some growers frequently abuse pesticides in pursuit of yield, which increases the risk of pesticide residues exceeding the standard to a certain extent, thus posing a threat to personal health[6].

In recent years, there have been many reports on the behavior and dietary risk assessment of pesticide residues in vegetables and fruits in various fields. Liu *et al.* [7] and Tang *et al.* [8] respectively assessed the dietary intake risk of pesticide residues in kiwifruit and celery, but there have been few reports on the chronic and acute dietary intake risk assessment of pesticide residues in bananas[9-11]. In this study, the data on pesticide residues in bananas in Tangshan City from 2020 to 2021 were summarized and analyzed, and the chronic and acute dietary intake risks were evaluated, aiming to clarify the pesticide residue pollution situation and dietary risk index of bananas in Tangshan City and provide basic data for supervision of pesticide residues in bananas and reference for establishing residue limit standards for metalaxyl and dimethomorph in bananas in China.

Materials and Methods

Sample collection and preparation

Banana samples were collected and prepared in accordance with NY/T789-2004 *Guideline on Sampling for Pesticide Residue Analysis*. From 2020 to 2021, a total of 191 batches of banana samples were randomly sampled from wholesale markets, traditional markets, supermarkets and other sampling points in various counties and districts of Tangshan City. After a single banana sample was mixed evenly, a random sample of 2 kg was taken. Next, small pieces or segments were cut from different parts and treated. Each obtained sample was chopped and thoroughly mixed. A portion obtained by the quartering method or all of a sample was homogenized using a tissue crusher, and then added into a labeled polyethylene sample bottle, which was stored at – 18 ℃ for testing.

Instruments and reagents

LCMS-8040 liquid chromatography-tandem mass spectrometer (equipped with ESI source, Shimadzu Company of Japan); SiO-6512 full-automatic pretreatment equipment (Beijing Ability Tech

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Co., Ltd.), TTL-DCLL nitrogen blowing instrument (Beijing Tongtailian Technology Development Co., Ltd.); JJ-200 electronic balance (Changshu Shuangjie Instrument Manufacturing Plant); SZ13022-NY 0.22 μm organic microporous membrane (Tianjin Linghang Experimental Equipment Co., Ltd.).

Seventy kinds of pesticide reference materials such as imidacloprid, prochloraz and pyraclostrobin (1 000 μg/ml, Institute of Environmental Protection, Ministry of Agriculture); methanol and acetonitrile (chromatographic grade, purity > 99.9%, Dikma Technologies); formic acid (chromatographic grade, purity > 88.0%, Xiya Chemical Technology (Shangdong) Co., Ltd.); ammonium acetate (chromatographic grade, purity > 99.9%, Tianjin Guangfu Fine Chemical Research Institute); extraction package, R104 zirconia beads and V03 double-layer extraction purification tube (Beijing Ability Tech Co., Ltd.); test water (Guangzhou Watson's Food & Beverage Co., Ltd.).

Sample pretreatment

The QuEChERS method was used for sample pre-treatment. First, a 10 g of sample was weighed (accurate to 0.01 g) and added with 10 ml of acetonitrile, 4 ml of water, zirconia beads and a material bag for extraction and purification. The supernatant was directly determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and sieved through 0.22 μm organic microfiltration membrane.

Detection items, detection methods and judgment basis

There were 70 kinds of agrochemicals in four categories (Table 1), including 47 kinds of pesticides such as acetamiprid and imidacloprid, 19 kinds of fungicides such as acetamiprid and imidacloprid, three acaricides such as carbendazim and metalaxyl, and one herbicide, pendimethalin.

According to GB/T 20769-2008 *Determination of 450 pesticides and related chemicals residues in fruits and vegetables—LC-MS-MS method*[12], 70 pesticide residues in bananas were determined.

Whether the pesticides exceeded limits and the registration information of pesticides were determined according to GB 2763-2021 *National Food Safety Standards—Maximum Residue Limits for Pesticides in Food*[13] and China Pesticide Information Network[14], and pesticides without limits could not be judged. Qualified rate (including those undetected and detected but not exceeding the maximum residue limits) and unqualified rate were analyzed, and the calculation formulas are as follows:

Sample detection rate (%) = Number of samples detected/Total number of samples × 100%

Qualified rate (%) = Number of qualified sample batches/Total number of sample batches × 100%

Over-standard rate (%) = Number of unqualified sample batches/Total number of sample batches × 100%

Table 1 Categories and names of agrochemicals tested

Agrochemical	Name
Pesticide	Cyromazine, methamidophos, acephate, omethoate, aldicarb sulfoxide, aldicarb sulfone, clothianidin, chlordimeform, methomyl, dipterex, thiamethoxam, carbofuran-3-hydroxy, dimethoate, imidacloprid, aldicarb, dichlorvos, acetamiprid, thiacloprid, carbofuran, carbaryl, pirimicarb, isocarbophos, phorat-sulfoxide, phorat-sulfone, isazofos, cadusafos, tebufenozide, isofenphos-methyl, chlorobenzuron, triazophos, sulfotep, buprofezin, methidathion, malathion, phorate, diazinon, phoxim, profenofos, emamectin benzoate, fenthion, chlorfluazuron, chlorpyrifos, avermectin, fipronil desulfinyl, fipronil, fipronil-sulfide, fipronil-sulfone
Fungicide	Propamocarb, propamocarb hydrochloride, carbendazim, probenazole, triadimenol, diethofencarb, myclobutanil, pyrimethanil, tebuconazole, metalaxyl, zoxamide, flusilazole, fenbuconazole, propiconazole, trifloxystrobin, dimethomorph, prochloraz, ifenoconazole, pyraclostrobin
Acaricide	Bifenazate, propargite, pyridaben
Herbicide	Pendimethalin

Dietary risk assessment

Chronic dietary risk The chronic dietary risk assessment was calculated by $\%ADI$, and the formula is as follows:

$$\%ADI=\frac{(STMR\times P-ADI)}{bw}\times 100$$

In the formula, $STMR$ is the average level of pesticide residues in samples (mg/kg); P is the daily fruit consumption of adult residents (kg/d)[15], calculated at 0.11 kg/d; ADI is the acceptable daily intake (mg/kg), according to GB2763-2021 *National Food Safety Standard—Maximum Residue Limits of Pesticides in Food*; and bw is the average body weight (kg), calculated as 53.23 kg[16]. The higher the $\%ADI$ value, the higher the risk. When $\%ADI > 100\%$, it indicates that the risk is unacceptable. Conversely, when $\%ADI < 100\%$, it indicates that the risk is acceptable.

Acute dietary risk The acute dietary risk assessment was calculated using $\%ARfD$, and the formula is as follows:

$$ESTI=\frac{[U\times HR\times V+(LP-U)\times HR]}{bw}$$

$$\%ARfD=\frac{ESTI}{ARfD}\times 100$$

In the formulas, $ESTI$ is the short-term intake value, and U is the single fruit weight (kg) of the edible portion of bananas, which is 0.767 3 kg[16]; HR is the highest residual value of pesticides detected in the sample (mg/kg), taking the 97.5 percentile value of the pesticide residual value; V is the variation factor, generally 3; LP is a large meal (kg), and an adult large meal value is 0.850 3 kg[16]; and $ARfD$ is the acute reference quantity (mg/kg). The higher the $\%ARfD$ value, the higher the risk. When $\%ARfD > 100\%$, the risk is unacceptable. Otherwise, the risk is acceptable.

Data analysis

Microsoft Excel 2019 was used to collect data and make statistical analysis.

The four seasons of spring, summer, autumn and winter were represented by I, II, III and IV, respectively. In specific, the four seasons are from March to May, June to August, September to November, and December to February, respectively[17].

Results and Analysis

Detection and analysis of pesticide residues in bananas

Among the 191 batches of bananas, 172 batches of samples were detected to contain agrochemicals, with a detection rate of 90.05%. Among them, 36 batches were unqualified, with an unqualified rate of 18.85%.

Of the 70 agrochemicals tested, 17 agrochemicals were detected (Table 2), accounting for 24.29% of the total agrochemicals tested, including nine fungicides and eight pesticides. Among them, four agrochemicals showed detection rates higher than 30%, and they were, from high to low, respectively, carbendazim, prochloraz, pyraclostrobin and imidacloprid, of which the detection rates were 49.21%, 41.88%, 34.03% and 30.37%, respectively. The detection rates of seven agrochemicals ranged from 1.05% to 17.8%, namely thiamethoxam, metalaxyl, thiabendazole, acetamiprid, tebuconazole, fenbuconazole and clothianidin, of which the detection rates were 7.33%, 3.66%, 2.62%, 2.09% and 1.05% respectively. The detection rates of five agrochemicals were 0.52%, namely dimethomorph, fipronil, fipronil sulfone and omethoate. Eight kinds of agrochemicals were found to exceed the standards, including six kinds of pesticides, namely imidacloprid, thiamethoxam, clothianidin, fipronil, fipronil sulfone, omethoate, and three kinds of fungicides, namely carbendazim, difenoconazole and chlorpyrifos. Among them, imidacloprid exceeded its standard most frequently, with 34 times exceeding the standard, and the exceeding rate was as high as 17.8%, which is basically consistent with the previous research report that imidacloprid exceeded the limit for up to 28 times[18]. Ma *et al.*[19] also showed that the detection rates of prochloraz, pyraclostrobin and imidacloprid were 42.86%, 40.82% and 34.69%, respectively.

According to the results of GB 2763-2021, metalaxyl and dimethomorph were not set with maximum residue limits for bananas[13].

Table 2 Residues of 17 agrochemicals in bananas

Agrochemical name	Agrochemical category	Detection times//times	Detection rate//%	Residual level mg/kg	Mean mg/kg	Number of times exceeding the standard//times	Over-standard rate//%	Maximum residue limit//mg/kg
Carbendazol	Fungicide	94	49.21	0.006 – 3.780	0.148	1	0.52	2
Prochloraz	Fungicide	80	41.88	0.007 – 0.661	0.083	0	0	5
Pyraclostrobin	Fungicide	65	34.03	0.006 – 0.884	0.11	0	0	1
Imidacloprid	Pesticide	58	30.37	0.010 – 0.315	0.084	34	17.8	0.05
Difenoconazole	Fungicide	34	17.8	0.007 – 1.385	0.139	1	0.52	1
Thiamethoxam	Pesticide	14	7.33	0.006 – 0.371	0.053	6	3.14	0.02
metalaxyl	Fungicide	7	3.66	0.007 – 0.055	0.02	–	–	–
Probenazole	Fungicide	5	2.62	0.030 – 0.244	0.165	0	0	5
Acetamiprid	Pesticide	5	2.62	0.015 – 0.082	0.035	0	0	3
Tebuconazole	Fungicide	4	2.09	0.015 – 0.200	0.065	0	0	3
Fenbuconazole	Fungicide	2	1.05	0.016 – 0.025	0.021	0	0	0.05
Clothianidin	Pesticide	2	1.05	0.010 – 0.033	0.022	1	0.52	0.02
Dimethomorph	Fungicide	1	0.52	0.019	0.019	–	–	–
Fipronil	Pesticide	1	0.52	0.013	0.013	1	0.52	0.005
Fipronil sulfone	Pesticide	1	0.52	0.006	0.006	1	0.52	0.005
Omethoate	Pesticide	1	0.52	0.041	0.041	1	0.52	0.02
Chlorpyrifos	Pesticide	1	0.52	0.023	0.023	0	0	2

The maximum residue limit value " – " stands for no maximum residue limit value in the standard.

Pesticide residues in bananas in different quarters

In 2020, most batches were detected in quarter II, and 80 batches were sampled, showing a detection rate of 91.25% and a maximum over-standard rate of 16.25%. In quarter I, 26 batches were sampled, and the detection rate and over-standard rate were 92.31% and 15.38%, respectively. Eighteen batches were sampled in quarter III, and the detection rate and qualified rate were 66.67% and 100%, respectively. In 2021, most batches were detected in quarter III, and 32 batches were sampled, showing a detection rate of 96.88% and an over-standard rate of 37.50%. Seven batches were sampled in quarter I, showing a detection rate of 85.71% and lowest over-standard rate of 14.29%. Twenty eight batches were sampled in quarter II, and the detection rate and over-standard rate were 92.86% and 21.43%, respectively. During quarter IV of 2020 – 2021, no sampling inspection was conducted (Table 3).

In 2020, the highest detection rate was found on carbendazim in quarter I, carbendazim and prochloraz in quarter II, and carbendazim, pyraclostrobin and prochloraz in quarter III. In 2021, carbendazim and difenoconazole were found to have higher detection rates in quarter I, and the agrochemicals with higher detection rates in quarter II and quarter III were highly consistent with the previous year (Fig. 1 – Fig. 6).

After investigation and study, it was found that difenoconazole can be mixed with carbendazim for control of sigatoka, but they have little mobility and degraded slowly in soil. As a result, it is easy for them to pollute the soil environment, and to cause liver disease and chromosome aberration when they are enriched in the body[20]. Prochloraz, as a low-toxic preservative, can be mixed with fungicides and is a common agrochemical in banana storage and transportation. Imidacloprid can effectively control *Pentalonia nigronervos*, but it is degradation-resistant and cause residue easily. Therefore, carbendazim, prochloraz, imidacloprid and difenoconazole are widely used by fruit farmers to control *Fusarium* wilt, leaf spot, anthracnose and *P. nigronervos* in bananas, which is consistent with the test results.

Table 3 Detection rate and over-standard rate in different quarters

	Quarter	Detection batch//Times	Not detected (qualified)	Detected but not exceeding the standard (qualified)	Detected and exceeding standard (unqualified)	Detection rate//%	Qualified rate//%	Over-standard rate//%
2020	I	26	2	20	4	92.31	84.62	15.38
	II	80	7	60	13	91.25	83.75	16.25
	III	18	6	12	0	66.67	100.00	0.00
	IV	0	0	0	0	0.00	0.00	0.00
2021	I	7	1	5	1	85.71	85.71	14.29
	II	28	2	20	6	92.86	78.57	21.43
	III	32	1	19	12	96.88	62.50	37.50
	IV	0	0	0	0	0.00	0.00	0.00

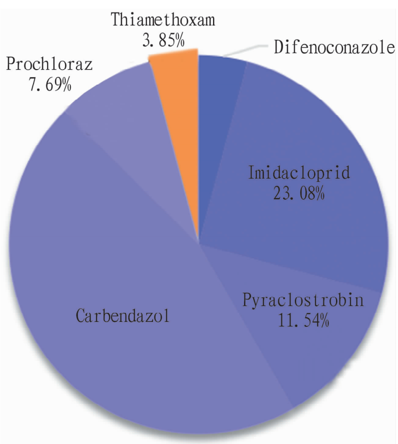

Fig. 1 Quarter I in 2020

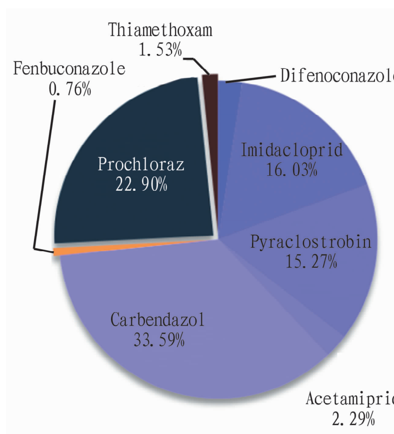

Fig. 2 Quarter II in 2020

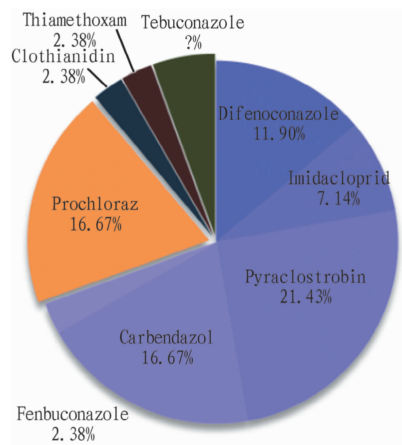

Fig. 3 Quarter III in 2020

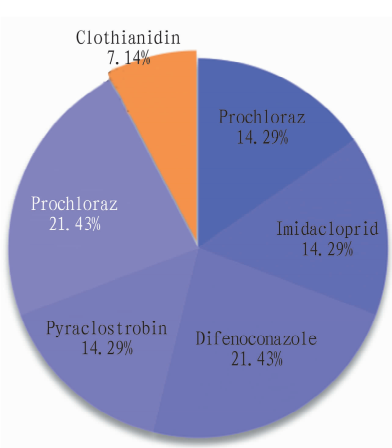

Fig. 4 Quarter I in 2021

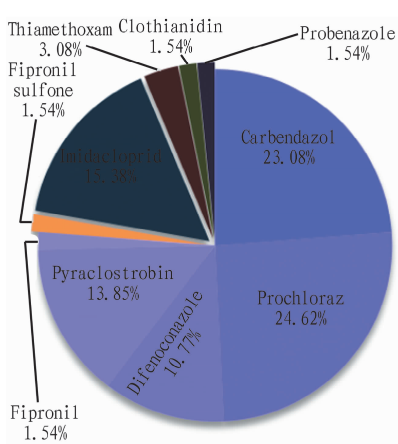

Fig. 5 Quarter II in 2021

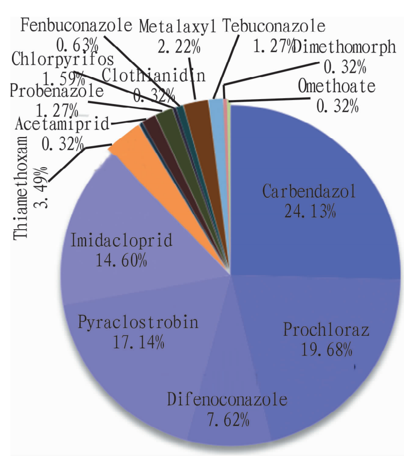

Fig. 6 Quarter III in 2021

No analysis was made in quarter IV due to no sampling inspection.

Assessment of chronic dietary risk

The risk of chronic dietary intake (% ADI) of the 17 agrochemicals in bananas ranged from 0.020% to 28.242%, far below 100%, and the highest risk was omethoate with 28.242%, followed by fipronil with 13.432%. The %ADI values of other agrochemicals such as prochloraz, carbendazim and difenoconazole were all in the range of 0.020% – 6.200%. The %ADI values of 17 agrochemicals were much lower than 100%, so the chronic dietary risk of agrochemical residues in bananas was acceptable.

Assessment of acute dietary risk

The risk of acute dietary intake (%ARfD) ranged from 0.14% to 53.03%. In specific, the highest %ARfD was found on pyrazolin, at 53.03%, and those with %ARfD values in the range of 10.58% – 19.41% included fipronil, prochloraz and difenoconazole, the %ARfD values of which were 19.41%, 17.20% and 10.85%, respectively. There were eight kinds with %ARfD ranging from 1.03% to 9.18%, namely, omethoate, fipronil sulfone, carbendazim, acetamiprid, imidacloprid, thiabendazole and chlorpyrifos, the %ARfD values of which were 9.18%, 8.96%, 5.20%, 3.46%, 2.89%, 1.27%, 1.09% and 1.03%, respectively. The %ARfD values of other four kinds, tebuconazole, fenbuconazole, clothianidin and dimethomorph, were less than 1%. From the above analysis, it could be seen that the %ARfD values of the 17 agrochemicals in bananas were far below 100%, so the risk of acute dietary intake of bananas was acceptable.

Table 4 Assessment of chronic dietary risk

Agrochemical name	ADI mg/kg bw	Average residual level//mg/kg	%ADI//%
Omethoate	0.000 3	0.041	28.242
Fipronil	0.000 2	0.013	13.432
Fipronil sulfone	0.000 2	0.006	6.200
Difenoconazole	0.01	0.139	2.872
Prochloraz	0.01	0.083	1.715
Carbendazol	0.03	0.148	1.019
Pyraclostrobin	0.03	0.11	0.758
Chlorpyrifos	0.01	0.023	0.475
Tebuconazole	0.03	0.065	0.448
Probenazole	0.1	0.165	0.341
Imidacloprid	0.06	0.084	0.289
Fenbuconazole	0.03	0.021	0.145
Thiamethoxam	0.08	0.053	0.137
Acetamiprid	0.07	0.035	0.103
Metalaxyl	0.08	0.02	0.052
Clothianidin	0.1	0.022	0.045
Dimethomorph	0.2	0.019	0.020

Table 5 Assessment of acute dietary risk

Agrochemical	Maximum residue//mg/kg	97.5 percentile value//mg/kg	ARfD//mg/kg	ESTI//mg/kg	%ARfD//%
Metalaxyl	0.055	0.050 4	–	0.002 3	–
Pyraclostrobin	0.884	0.591 8	0.05	0.026 5	53.03
Fipronil	0.013	0.013 0	0.003	0.000 58	19.41
Prochloraz	0.661	0.383 9	0.1	0.017 2	17.20
Difenoconazole	1.385	0.726 6	0.3	0.032 6	10.85
Omethoate	0.041	0.041 0	0.02	0.001 8	9.18
Fipronil sulfone	0.006	0.006 0	0.003	0.000 27	8.96
Carbendazol	3.78	0.580 7	0.5	0.026 0	5.20
Acetamiprid	0.082	0.077 2	0.1	0.003 5	3.46
Imidacloprid	0.315	0.258 3	0.4	0.011 6	2.89
Thiamethoxam	0.371	0.282 9	1	0.012 7	1.27
Probenazole	0.244	0.242 8	1	0.010 9	1.09
Chlorpyrifos	0.023	0.023 0	0.1	0.001 0	1.03
Tebuconazole	0.200	0.187 2	0.9	0.008 4	0.93
Fenbuconazole	0.025	0.024 8	0.2	0.001 1	0.56
Clothianidin	0.033	0.032 4	1	0.001 5	0.15
Dimethomorph	0.019	0.019 0	0.6	0.000 85	0.14

The ARfD value " – " indicates that there is no acute reference dose yet or no need for it.

Conclusions and Discussion

In this study, 70 kinds of pesticide residues in 191 batches of banana samples from Tangshan area were investigated. The results showed that the detection rate of samples was 71.20%, and the over-standard rate was 18.85%. Seventeen agrochemicals were detected, including carbendazim, prochloraz, pyraclostrobin, imidacloprid, difenoconazole, thiamethoxam, metalaxyl, thiabendazole, acetamiprid, tebuconazole, fenbuconazole, clothianidin, dimethomorph, fipronil, fipronil sulfone, omethoate and chlorpyrifos. The residue levels of eight agrochemicals, such as fipronil and imidacloprid, exceeded the maximum residue limits (MRLs) specified in the standard. Among them, carbendazim were detected in 94 batches, with a detection rate of 49.21%, and imidacloprid exceeded the standard in 34 batches, with a detection rate of 17.8%. Both metalaxyl and dimethomorph were detected, but GB2763-2021 has not yet established a maximum residue limit for them. This study can provide a reference for establishing residue limit standards for metalaxyl and dimethomorph in bananas in China[13]. In summary, the phenomenon of pesticide residues in bananas is more prominent.

In this study, chronic and acute dietary risk assessments were conducted on 191 batches of banana samples collected. The assessment results showed that the %ADI values of 17 agrochemicals, represented by carbendazim and imidacloprid, were between 0.020% and 28.242%, much lower than 100%, indicating an acceptable risk. The % ARfD values were between 0.14% and 53.03%, below 100%, indicating an acceptable risk.

In order to improve the efficacy of agrochemicals, fruit farmers often use combinations of multiple agrochemicals to control diseases and pests in bananas, but the residual toxicity of mixed agrochemicals is not yet clear. Because of limited data, the toxicity of mixed pesticide residues was not evaluated in this study, and further analysis will be made after the data are completed.

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