

## Residues and risk exposure assessment of 6-benzylaminopurine for bean sprouts in Shandong, China

YING JIANG – QING SUN – PEIRUI XIAO – YING HAO – TIANLIANG ZHANG – ZUNHUA CHU

### Summary

6-Benzylaminopurine (6-BAP) is an important ingredient in production of “toxic bean sprouts”. In order to assess health risk from 6-BAP residue in bean sprouts on a large sample size and wide sample range among large survey population, 6-BAP was detected in 149 out of 1 494 bean sprouts samples collected in 2016–2020 in Shandong Province of China. High performance liquid chromatography-tandem mass spectrometry was used for analysis. Those 10.0 % samples contained 6-BAP residue above the maximum residue limit (MRL) and the average level was 0.007 mg·kg<sup>-1</sup>. Hazard quotient (HQ) of 6-BAP was 0.0 % for the general population and 0.2 % for consumers. The daily exposure of 6-BAP was accounted for 0.0 % of the the acceptable daily intake (ADI) and the estimated daily intake (EDI) of all subjects surveyed was below ADI. From the perspective of public health, the occurrence of 6-BAP residue in bean sprouts could not pose a serious health risk problem. Strict regulations on the management of 6-BAP residue in bean sprouts and human health risk assessment should be recommended.

### Keywords

risk exposure assessment; plant growth regulator; acceptable daily intake; chronic intake risk assessment; public health

Plant growth regulators (PGR), also known as plant exogenous hormones, are a class of pesticides which regulate the growth and development of plants. They are widely used in modern agricultural production [1]. PGR can increase crop yield, improve quality, enhance resistance, extend shelf life as well as preserve freshness and quality [2, 3]. However, PGR have certain toxicity and residue characteristics. Abuse, misuse or irrational use of PGR may lead to yield reduction, quality decline, excessive residue content or dietary risk increase and other issues [4–7].

6-Benzylaminopurine (6-BAP), a multifunctional PGR classified as a plant hormone, is an important ingredient in the production of “toxic bean sprouts” [8]. 6-BAP is the first synthetic cytokinin, which can promote plant cell growth, inhibit decomposition of chlorophyll, nucleic acids and pro-

teins in plant leaves, increase the content of amino acids and delay leaf senescence [9–11]. It is extensively used in agriculture and horticulture [12], for example in cultivation of maize [13], watermelon [14] and *Diospyros oleifera* [15], which are economically important crops in China. In the production process of bean sprouts, 6-BAP can promote shortening of the growth cycle and improve their yield, quality as well as flavour [16]. However, 6-BAP irritates mucous membranes and skin, or cause nausea and vomiting [17], even be toxic to humans when ingested over a long period, which is characterized by damage to the reproductive and nervous systems, leading to precocious puberty, and cancer [18]. 6-BAP is estrogenic with neuroendocrine disrupting properties [19], eliciting toxicity to the endothelial system of human umbilical vein endothelial cells (HUVEC) [8].

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The maximum reference limit (*MRL*) and the acceptable daily intake (*ADI*) for 6-BAP are different in many countries and organizations. China has limited the extent of 6-BAP use and banned it in the production of bean sprouts in 2015 [20]. *ADI* for 6-BAP established by the Joint FAO/WHO Expert Committee on Pesticide Residues (JMPR) is 0.05 mg·kg<sup>-1</sup> body weight (bw) [21], with a value 0.02 mg·kg<sup>-1</sup> bw set by Australia and Canada [22] and 0.01 mg·kg<sup>-1</sup> bw by European Union [23]. No need for an acute reference dose (*ARfD*) was recognized by European Union [23], from which the evaluation benchmark for this study was derived.

In recent years, there has been an increase surge in the use of PGR in China, which has become a country with their highest use. However, no systematic risk assessment of exposure to PGR in bean sprouts has been conducted, especially regarding 6-BAP. Therefore, based on the consumption among residents and 6-BAP residue for bean sprouts in Shandong Province, this study was conducted to assess the risk exposure of 6-BAP in bean sprouts, to provide a scientific basis for the revision of the 6-BAP residue limit standards and to take preventive actions to minimize human health risk.

## MATERIALS AND METHODS

### Apparatus and reagents

High-performance liquid chromatography (HPLC) grade acetonitrile, formic acid, methanol and ammonium acetate (analytical-reagent grade) were obtained from Tedia (Fairfield, Ohio, USA). Magnesium sulfate, anhydrous, sodium acetate, anhydrous and sodium chloride (all analytical reagent grade, Tedia) were heated at 600 °C for 4 h in the muffle furnace and cooled down in a desiccator. Primary and secondary amine (PSA) adsorbent, C18 adsorbent and graphitized carbon black (GCB) adsorbent were purchased from Agilent Technologies (Santa Clara, California, USA). 6-BAP standard, concentration 100 µg·ml<sup>-1</sup> and purity 99.4 % (w/w), was purchased from Dr. Ehrentorfer (Ausberg, Germany). 6-BAP standard stock solution in various concentrations was prepared in methanol and stored in dark below 4 °C, which was reconstituted every two weeks. A volume of 1.0 ml of the 6-BAP standard was transferred into a 100 ml volumetric flask and diluted with methanol for 6-BAP mixed standard stock solutions (1000 µg·l<sup>-1</sup>), which were stored at -20 °C for a maximum of 30 days. Volumes of 1 µl, 2 µl, 5 µl, 10 µl, 20 µl, 50 µl, 100 µl of

the mixed standard stock solution were removed using a micro sampler, and diluted to 1 ml with methanol for working standard mixed solutions (1.00 µg·l<sup>-1</sup>, 2.00 µg·l<sup>-1</sup>, 5.00 µg·l<sup>-1</sup>, 10.00 µg·l<sup>-1</sup>, 20.00 µg·l<sup>-1</sup>, 50.00 µg·l<sup>-1</sup>, 100.00 µg·l<sup>-1</sup>). These were freshly prepared for immediate use.

### Sample collection

A total of 1 494 bean sprout samples, including 817 mung bean sprouts and 677 soybean sprouts, were randomly selected from the farmland and vegetable wholesale markets, supermarkets and free market in 108 counties (districts and cities) in all 16 prefecture-level cities of Shandong province in 2016–2020. Each bean sprout sample was of at least 500 g. Samples were stored wrapped in aluminium foil at 4 ± 1 °C and analysed within 48 h.

### Extraction and clean-up

The samples were chopped and homogenized by cryogenic milling. Then, 10.0 g of the homogenized sample or a blank sample of 10.0 g were accurately weighed and placed into a 50 ml centrifuge tube followed by adding 1.0 g sodium chloride and 1.0 g anhydrous sodium acetate, then mixed with 10 ml acetonitrile containing 0.2% (v/v) formic acid. The mixture was vortex-mixed for 1 min and then added 4 g anhydrous magnesium sulfate, vortex-mixed for 2 min and centrifuged at 1 790 ×g for 5 min. A volume of 1 ml of the acetonitrile extract was transferred into a 1.5 ml microtube with 50 mg of C18 adsorbent, 50 mg PSA adsorbent, 10 mg GCB adsorbent and 150 mg anhydrous magnesium sulfate. The mixture was vortex-mixed for 2 min and centrifuged at 11 190 ×g for 5 min. The supernatant was withdrawn and analysed by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS).

### HPLC-MS/MS analysis

AB Sciex QTRAP 4500 HPLC-MS/MS (Sciex, Framingham, Massachusetts, USA) online combined system was employed, with injection volume of 2 µl and flow rate of 0.20 ml·min<sup>-1</sup>. An Agilent Zorbax Eclipse Plus C18 column (100 mm × 2.1 mm, particle size 3.5 µm; Agilent Technologies) was used at 40 °C. The mobile phase A was methanol and mobile phase B was 0.1% (v/v) formic acid in water. Elution involved initial step from 0.0 min to 2.0 min with 10 % A and 90 % B, then from 2.0 min to 6.0 min with 10 % A and 90 % B, from 6.0 min to 8.0 min with 90 % A and 10 % B, from 8.0 min to 10.0 min with 10 % A and 90 % B. The mass spectrometer operated in electrospray ionisation (ESI) mode with ionspray voltage (*IS*) set at

+5 500 V/−4 500 V. Data were acquired by multi-response monitoring (MRM). The mass detection method was selected-ion monitoring (SIM) at −29 eV and −45 eV. Full-scan modes were between 50  $m/z$  and 300  $m/z$ . The atomiser evaporation temperature was set at 500 °C, with 30 units for curtain air (CUR), 50 units for nebulizer gas (ion source gas 1, GS1) and auxiliary heated gas (ion source gas 2, GS2). The retention time ( $t_R$ ) from the sample injection to the appearance of the peak apex for the chromatogram was 10 min, and declustering potential ( $DP$ ) was at −97 V, while qualitative analysis and quantitative analysis were set at 224.0/105.9  $m/z$  and 224.0/132.9  $m/z$ , respectively.

### Quality control

The residues were qualitatively determined by retention time and quantified by an external standard calibration curve method. A procedural blank was analysed for every batch of five samples to avoid any interference or contamination. Good linear correlation was achieved owing to the correlation coefficient for the regression equation of 0.983. Relative standard deviations of the method were from 7.2 % to 8.4 % and the average recovery rate was from 91.6 % to 106.0 %, indicating acceptable repeatability of the method. The limit of detection ( $LOD$ ) was 0.003 mg·kg<sup>−1</sup>, determined as 3 times the signal-to-noise ratio. The limit of quantification ( $LOQ$ ) was 0.009 mg·kg<sup>−1</sup>, calculated based on a signal-to-noise ratio of 10.

### Characteristics of study participants

As shown in Tab. 1, 47.9 % of 4 063 participants aged 1 years and over in this study were male and 82.9 % were adults. The average age was 41.50 years, with 62.69 kg for the average weight.

### Health risk assessment

The long-term or chronic consumer health risk was evaluated based on estimated daily intake ( $EDI$ ) and  $ADI$  (Eq. 1 and Eq. 2).

$$EDI = \sum_{i=1}^n \frac{F_i \times C_i}{1000 \times BW_i} \quad (1)$$

$$HQ = \frac{EDI}{ADI} \quad (2)$$

where  $EDI$  of 6-BAP residue is expressed in milligrams per kilogram of body weight per day,  $F_i$  is the  $i$ -th average bean sprouts consumption per person (in grams per day),  $C_i$  is the 6-BAP residue level for the  $i$ -th bean sprouts (in milligrams per kilogram),  $BW_i$  is the  $i$ -th body weight (in kilograms),  $HQ$  is the chronic intake risk assessment

**Tab. 1.** Characteristics of the study subjects.

Age (years)	$n$	Gender		Weight [kg]	
		Male	Female	Male	Female
1–5	210	105	105	18.75	18.53
6–11	307	149	158	35.47	32.28
12–17	178	87	91	56.75	52.97
18–44	1 389	646	743	76.15	62.47
45–59	1 219	597	622	73.86	64.02
≥ 60	760	361	399	71.49	63.47
Total	4 063	1 945	2 118	67.5	58.27

$n$  – number of participants.

and  $ADI$  is the acceptable daily intake (in milligrams per kilogram of body weight per day).

The data on bean sprouts consumption and body weight were based on Shandong Resident Food Consumption Survey Program in 2017–2020, an annual province-wide survey focusing on representative samples of households by geographic location, population distribution and economic development level in Shandong. The program was designed to provide data on the food and water consumption on three non-consecutive days, together with physical condition at individual levels in Shandong province.

Exposure probability distribution for 6-BAP was calculated by @RISK software (Palisade, Carson, California, USA), a quantitative risk analysis software, to construct the data model with Monte Carlo simulations. The results were obtained by Latin hypercube sampling method with 10 000 random samples from the data for distribution functions of bean sprouts consumption, 6-BAP residue in bean sprouts and the weight data for the investigated population in Shandong province.

## RESULTS

### 6-BAP residue in bean sprouts

Tab. 2 summarizes the content ranges of 6-BAP in bean sprouts. In 1 494 bean sprouts samples, 149 samples of 6-BAP were detected, with 42 for mung bean sprouts and 107 for soybean sprouts. All 149 (10.0 %) samples contained 6-BAP residue above  $MRL$  and the detection rate in soybean sprouts (15.8 %) was 3 times higher than in mung bean sprouts (5.1 %). The average residue content was 0.007 mg·kg<sup>−1</sup>, while the highest residue content was 1.070 mg·kg<sup>−1</sup> in mung bean sprouts.

**Tab. 2.** Content of 6-benzylaminopurine in bean sprouts.

Bean sprouts	Positive samples	Detection rate [%]	Content [mg·kg <sup>-1</sup> ]						
			Mean	Median	P <sub>95</sub>	P <sub>97.5</sub>	P <sub>99</sub>	P <sub>99.9</sub>	Maximum
Mung bean sprouts	42	5.1	0.005	nd	0.010	0.020	0.035	0.386	1.070
Soybean sprouts	107	15.8	0.009	nd	0.034	0.077	0.155	0.366	0.385
Total	149	10.0	0.007	nd	0.022	0.042	0.106	0.386	1.070

nd – not detected, which meant that 6-benzylaminopurine (6-BAP) residue content was below limit of detection.  
P<sub>95</sub> – 95th percentile for content of 6-BAP, P<sub>97.5</sub> – 97.5th percentile for content of 6-BAP, P<sub>99</sub> – 99th percentile for content of 6-BAP, P<sub>99.9</sub> – 99.9th percentile for content of 6-BAP.

**Tab. 3.** Consumption of bean sprouts by general and consumer population.

	n	Consumption [g·d <sup>-1</sup> ]								
		Mean	Median	Minimum	P <sub>90</sub>	P <sub>95</sub>	P <sub>97.5</sub>	P <sub>99</sub>	P <sub>99.9</sub>	Maximum
<b>General population</b>										
Mung bean sprouts	4 063	3.57	0.00	0.00	0.00	33.33	55.00	83.33	150.00	200.00
Soybean sprouts	4 063	3.14	0.00	0.00	0.00	21.67	50.00	83.33	133.33	166.67
Total	4 063	6.71	0.00	0.00	25.00	53.33	83.33	100.00	170.00	200.00
<b>Consumers</b>										
Mung bean sprouts	284	51.06	40.00	6.67	100.00	116.67	136.67	171.67	200.00	200.00
Soybean sprouts	253	50.43	40.00	3.33	100.00	110.00	133.33	150.00	166.67	166.67
Total	517	52.73	45.00	3.33	100.00	117.33	137.33	166.67	200.00	200.00

n – number of consumers, P<sub>90</sub> – 90th percentile for consumption of bean sprouts, P<sub>95</sub> – 95th percentile for consumption of bean sprouts, P<sub>97.5</sub> – 97.5th percentile for consumption of bean sprouts, P<sub>99</sub> – 99th percentile for consumption of bean sprouts, P<sub>99.9</sub> – 99.9th percentile for consumption of bean sprouts.

**Tab. 4.** Estimated daily intake of 6-benzylaminopurine in bean sprouts among the general and consumer population.

	EDI per body weight [10 <sup>-6</sup> mg·kg <sup>-1</sup> ]							HQ
	Mean	Median	P <sub>90</sub>	P <sub>95</sub>	P <sub>97.5</sub>	P <sub>99</sub>	Maximum	
<b>General population</b>								
1–5 years	1.543	0.000	8.202	11.667	13.889	16.204	20.741	0.00
6–11 years	1.082	0.000	4.188	7.656	11.667	17.677	31.551	0.00
12–17 years	0.597	0.000	0.000	5.426	7.000	14.359	17.107	0.00
18–44 years	0.816	0.000	3.241	6.282	9.333	11.647	21.212	0.00
45–59 years	0.747	0.000	2.593	6.222	8.944	11.733	26.667	0.00
≥ 60 years	0.676	0.000	1.558	5.786	8.617	12.444	21.875	0.00
Male	0.773	0.000	2.842	6.222	9.167	11.667	31.551	0.00
Female	0.858	0.000	3.111	6.751	10.078	14.933	26.667	0.00
Total	0.817	0.000	2.917	6.462	9.516	13.333	31.551	0.00
<b>Consumers</b>								
1–5 years	31.360	33.939	45.161	56.000	62.222	62.222	62.222	0.00
6–11 years	23.190	18.421	46.667	54.756	57.613	94.652	94.652	0.00
12–17 years	19.930	16.473	43.077	51.320	51.320	51.320	51.320	0.00
18–44 years	16.840	13.965	32.375	37.692	42.000	48.938	63.636	0.00
45–59 years	19.230	17.500	32.200	45.016	49.412	60.677	80.000	0.00
≥ 60 years	18.570	15.556	35.654	43.750	55.462	65.625	65.625	0.00
Male	17.760	15.634	33.939	38.889	46.667	55.462	94.652	0.00
Female	20.730	17.449	41.067	48.904	60.345	63.636	80.000	0.00
Total	19.270	16.279	35.654	45.016	54.756	62.222	94.652	0.00

EDI – estimated daily intake, P<sub>90</sub> – 90th percentile for estimated daily intake of 6-benzylaminopurine (6-BAP), P<sub>95</sub> – 95th percentile for estimated daily intake of 6-BAP, P<sub>97.5</sub> – 97.5th percentile for estimated daily intake of 6-BAP, P<sub>99</sub> – 99th percentile for estimated daily intake of 6-BAP, HQ – hazard quotient.

### Consumption

Data on consumption of bean sprouts by the general and consumer population are shown in Tab. 3. Among the general population, the average consumption of bean sprouts was  $6.71 \text{ g}\cdot\text{d}^{-1}$ , of which  $3.57 \text{ g}\cdot\text{d}^{-1}$  was for mung bean sprouts and  $3.14 \text{ g}\cdot\text{d}^{-1}$  for soybean sprouts. The number of consumers for bean sprouts was 517 in Shandong Province, including 184 for mung bean sprouts, 253 for soybean sprouts, with 20 consumers eating two sprouts. Among the consumer population, the average consumption of bean sprouts was  $52.73 \text{ g}\cdot\text{d}^{-1}$ , of which green sprouts represented  $51.06 \text{ g}\cdot\text{d}^{-1}$  and soybean sprouts represented  $50.43 \text{ g}\cdot\text{d}^{-1}$ . The median consumption of bean sprouts was  $45.00 \text{ g}\cdot\text{d}^{-1}$ , with  $200.00 \text{ g}\cdot\text{d}^{-1}$  and  $166.67 \text{ g}\cdot\text{d}^{-1}$  for the highest consumption of green bean sprouts and soybean sprouts, respectively.

### Estimated daily intake

As shown in Tab. 4, the average *EDI* of 6-BAP in bean sprouts among the general population was  $0.817 \times 10^{-6} \text{ mg}\cdot\text{kg}^{-1} \text{ bw}$ , all accounting for 0.0 % of *ADI*. The average *EDI* in females was higher than in males, while the highest *EDI* was the opposite. Among different population groups, the average *EDI* ranked first in 1–5 years ( $1.543 \times 10^{-6} \text{ mg}\cdot\text{kg}^{-1} \text{ bw}$ ), followed by 6–11 years ( $1.082 \times 10^{-6} \text{ mg}\cdot\text{kg}^{-1} \text{ bw}$ ) and 18–44 years ( $0.816 \times 10^{-6} \text{ mg}\cdot\text{kg}^{-1} \text{ bw}$ ). The highest *EDI* from males aged 6–11 years was  $31.551 \times 10^{-6} \text{ mg}\cdot\text{kg}^{-1} \text{ bw}$ , 0.3 % of *ADI*. *EDI* of 0 participant exceeded *ADI*, same for mung bean sprouts and soybean sprouts.

The average *EDI* of 6-BAP in bean sprouts among the consumer population was

$19.270 \times 10^{-6} \text{ mg}\cdot\text{kg}^{-1} \text{ bw}$ , representing 0.2 % of *ADI*, ranged from  $16.840 \times 10^{-6} \text{ mg}\cdot\text{kg}^{-1} \text{ bw}$  and 0.2 % for 18–44 years, to  $31.360 \times 10^{-6} \text{ mg}\cdot\text{kg}^{-1} \text{ bw}$  and 0.3 % for 1–5 years. The average and median *EDI* in females were higher than in males, with the opposite for the highest *EDI*, same for mung bean sprouts and soybean sprouts. The top 3 rankings for the average *EDI* were 1–5 years, 6–11 years and 12–17 years. The highest *EDI* was from  $51.320 \times 10^{-6} \text{ mg}\cdot\text{kg}^{-1} \text{ bw}$  for males aged 12–17 years  $94.652 \times 10^{-6} \text{ mg}\cdot\text{kg}^{-1} \text{ bw}$  and 0.9 % for males aged 6–11 years in bean sprouts. *EDI* of all participants was below *ADI* in all sprouts.

Strict enforcement of the 6-BAP in the bean sprouts (that is, removal of data for 6-BAP exceeding the *MRL*) could reduce the *EDI* to  $0.000 \text{ mg}\cdot\text{kg}^{-1} \text{ bw}$  and to 0.0 % of the *ADI* for the general and consumer population, the reduction of which were 100.0 %.

### Assessment of exposure probability

As shown in Fig. 1, the average daily exposure of 6-BAP in bean sprouts among residents of Shandong province was  $3.957 \times 10^{-7} \text{ mg}\cdot\text{kg}^{-1} \text{ bw}$ , which accounted for 0.0 % of *ADI*. 6-BAP exposure interval values at 95% probability was  $(0.000\text{--}1.370) \times 10^{-6} \text{ mg}\cdot\text{kg}^{-1} \text{ bw}$  and *HQ* 0.00, while the maximum value was  $26.440 \times 10^{-6} \text{ mg}\cdot\text{kg}^{-1} \text{ bw}$  and 0.3 % of *ADI*, with all subjects surveyed below *ADI*.

## DISCUSSION

This study was a specialized research on 6-BAP in bean sprouts with the aim of providing data on

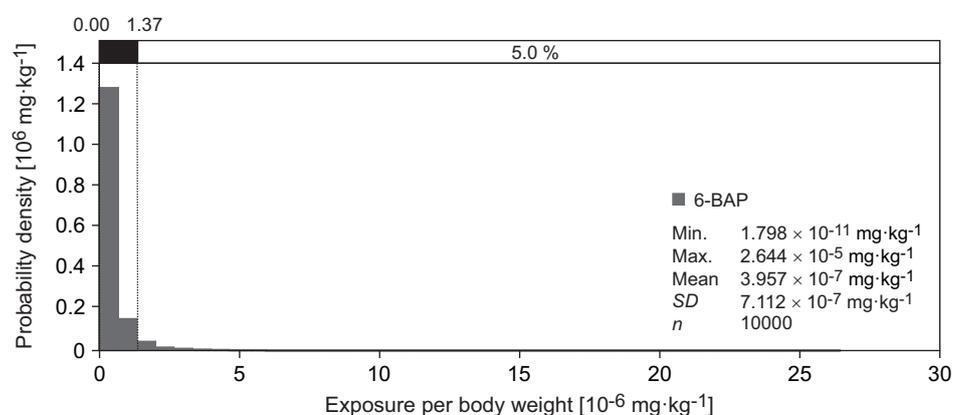


Fig. 1. Assessment of exposure probability of 6-benzylaminopurine from bean sprouts.

6-BAP – 6-benzylaminopurine, *SD* – standard deviation, *n* – number of samples.

6-BAP exposure interval at 95% probability was  $(0.000\text{--}1.370) \times 10^{-6} \text{ mg}\cdot\text{kg}^{-1}$  per body weight in modelling 10 000 random samples.

6-BAP residues on a large sample size and wide sample range. It aimed to assess the exposure to 6-BAP among a large survey population, with the general and consumer population. The detection rate of bean sprouts in our study was 10.0 %, with 5.1 % for mung bean sprout and 15.8 % for soybean sprouts, all above *MRL* in accordance with the limiting criteria. The average residue for 6-BAP in bean sprouts was 0.007 mg·kg<sup>-1</sup>, and the highest residue was 1.070 mg·kg<sup>-1</sup> in mung bean sprouts, with 0.385 mg·kg<sup>-1</sup> for soybean sprouts. The average consumption of bean sprouts was 6.71 g·d<sup>-1</sup> among the general population, while that was 52.73 g·d<sup>-1</sup> for consumers. *EDI* in both was 0.817 × 10<sup>-6</sup> mg·kg<sup>-1</sup> bw and 19.270 × 10<sup>-6</sup> mg·kg<sup>-1</sup> bw, accounting for 0.0 % and 0.2 % of *ADI*, respectively. The top 2 rankings in both was 1–5 years and 6–11 years.

The 6-BAP exposure values ranged from 1.798 × 10<sup>-11</sup> mg·kg<sup>-1</sup> bw to 2.644 × 10<sup>-5</sup> mg·kg<sup>-1</sup> bw, and 6-BAP exposure values at 95% probability were (0.000–1.370) × 10<sup>-6</sup> mg·kg<sup>-1</sup> bw, calculated by @Risk software in Monte Carlo simulations with statistical modelling. The assessment of exposure probability was 3.957 × 10<sup>-7</sup> mg·kg<sup>-1</sup> bw for average value and 26.440 × 10<sup>-6</sup> mg·kg<sup>-1</sup> bw for highest value. However, the highest *EDI* for bean sprouts was 31.551 × 10<sup>-6</sup> mg·kg<sup>-1</sup> bw for general subjects and 94.652 × 10<sup>-6</sup> mg·kg<sup>-1</sup> bw for consumer subjects, both in males aged 6–11 years. The average *EDI* in females were higher than in males, with the opposite for the highest *EDI*, below 1.0 % of *ADI*. The risk exposure assessment showed that the potential risk of exposure and the health risk to humans from 6-BAP in bean sprouts was minimal.

Previously, a study by GONG et al. [24] showed that the detection rate in mung bean sprouts was 2.1 %, with 0.044 mg·kg<sup>-1</sup> for the highest residue, even no residue was in soybean sprouts. In our study, all the detection results were higher than those in the studies mentioned above. *EDI* among the survey population in that study was 8.32 × 10<sup>-6</sup> mg·kg<sup>-1</sup> bw in the general and consumer population. However, the highest *EDI* was 5.96 × 10<sup>-6</sup> mg·kg<sup>-1</sup> bw for the group aged 4–10 years, which was below but similar to this study. Another study conducted in Shanghai city found no residues in table grapes and strawberries [25]. The same negative result was found for peppers, tomatoes, cucumbers and eggplants in Zhejiang province and Jiangsu province [26]. In those published studies, the data for 6-BAP residue and risk exposure assessment were higher than in this study. What attracted our attention was that 6-BAP was detected in bean sprouts in

2016–2020, because 6-BAP for bean sprouts was banned by law in 2015. In China, bean sprouts are the very common vegetable, which can be produced and sold by an ordinary person without 6-BAP usage knowledge. Moreover, the regulatory authorities for the production and sale of bean sprouts are varied in different regions. As a result, the government should strengthen the regulation, and popularize the concept of food safety to the bean sprout producers, especially to 6-BAP users.

To assess the health risk from 6-BAP residue in bean sprouts, our study adopted a cautious approach and exaggerated the risks by conservative guidelines. If 6-BAP residue was not detected in bean sprouts, 1/2 *LOD* was substituted for the value [27], and it may lead to the uncertainty in this study [28]. Although 6-BAP residue was detected in accordance with the standards for the detection of PGR residues in bean sprouts, *LOD* and *LOQ* were set at 0.003 mg·kg<sup>-1</sup> and 0.009 mg·kg<sup>-1</sup>, respectively, due to differences in testing personnel and technical operations, to provide consistency with other studies for comparison of test results. In addition, bean sprouts may cause combined exposure to multiple compounds active by a single pathway or various pathways [29], and uncertainty in the quantitative evaluation of the risk may exist since only 6-BAP residue in bean sprouts was monitored and no joint exposure risk assessment was considered. Given the small consumption of bean sprouts and the lag in consumption data can also lead to assessment uncertainty, the uncertainty and variability were characterized by probability distribution based on Monte Carlo simulations. Unlike *ADI*, the chronic 6-BAP residue intake through bean sprouts was lower than the critical level. From the perspective of public health, the occurrence of 6-BAP residue in bean sprouts could not pose a serious health risk problem.

This study demonstrated that potential risk of exposure to 6-BAP in bean sprouts could be under control in all ages. However, toddlers and children are vulnerable groups owing to lower body weight and relatively high level exposure to 6-BAP residue. This signifies that toddlers and children are at higher risk of 6-BAP residues exposure than the general population and therefore compliance with food safety requirements should be emphasized as important as nutrition [30]. Besides, the management, control and knowledge of 6-BAP usage should be improved. Also, 6-BAP residue detection and human health risk assessment need to be strengthened regularly in potentially contaminated production processes. A more comprehensive evaluation of multiple exposure to various

PGR compounds through bean sprouts regarding specific population such children should be recommended.

## CONCLUSIONS

6-BAP was detected in 149 out of 1494 (10.0 %) bean sprouts samples, all exceeding the *MRL*. The average residue content was 0.007 mg·kg<sup>-1</sup>. The risk exposure assessment and the health risk to humans from 6-BAP in bean sprouts was found to be minimal. The top *EDI* for general and consumer population was in 1–11 years old. Based on our results, the government should strengthen the regulation of the use of 6-BAP in bean sprouts. Also, food safety concepts and awareness of 6-BAP should be popularized to users. Besides, 6-BAP residue detection and human health risk assessment need to be strengthened regularly.

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## REFERENCES

- Zhang, X. – Peng, T. – Li, X. – Zhang, W. – Miao, Q. – Li, C.: Analysis on the residue and safety of plant growth regulators. *Shipin anquan zhiliang jiance xuebao – Journal of Food Safety and Quality*, *10*, 2019, pp. 614–619. DOI: 10.3969/j.issn.2095-0381.2019.03.011. In Chinese.
- McMillan, T. – Tidemann, B. D. – Odonovan, J. T. – Izydorczyk, M. S.: Effects of plant growth regulator application on the malting quality of barley. *Journal of the Science of Food and Agriculture*, *100*, 2020, pp. 2082–2089. DOI: 10.1002/jsfa.10231.
- Narayanwamy, P. – Anawal, V. V. – Ekabote, S. D.: Effects of plant growth regulators on flowering, fruit set and yield of ‘Bhagwa’ of pomegranate (*Punica granatum* L.). *Acta Horticulturae*, *1254*, 2019, pp. 129–134. DOI: 10.17660/ActaHortic.2019.1254.20.
- Xu, A.: Research advance in the toxicity and residue of plant growth regulator in vegetables in China. *Zhongguo shucai – China Vegetables*, *1*, 2009, pp. 1–6. ISSN: 1000-6346. In Chinese.
- Gao, R. – Chen, L. – Zheng, M. – Zhang, W.: The approach of human health risk assessment from pesticides. *Nongyaoxue xuebao – Chinese Journal of Pesticide Science*, *6*, 2004, pp. 8–14. DOI: 10.3321/j.issn:1008-7303.2004.03.002. In Chinese.
- Hasan, F. – Ansari, M. S.: Ecotoxicological hazards of herbicides on biological attributes of *Zygotramma bicolorata* Pallister (Coleoptera: Chrysomelidae). *Chemosphere*, *154*, 2016, pp. 398–407. DOI: 10.1016/j.chemosphere.2016.03.137.
- Troudi, A. – Amara, I. B. – Soudani, N. – Bouaziz, H. – Boudawara, T. – Zeghal, N.: Oxidative stress induced by gibberellic acid in bone of suckling rats. *Ecotoxicology and Environmental Safety*, *74*, 2011, pp. 643–649. DOI: 10.1016/j.ecoenv.2010.10.010.
- Gong, G. – Kam, H. – Bai, Y. – Cheang, W. S. – Wu, S. – Cheng, X. – Giesy, J. P. – Lee, S. M.: 6-benzylaminopurine causes endothelial dysfunctions to human umbilical vein endothelial cells and exacerbates atorvastatin-induced cerebral hemorrhage in zebrafish. *Environmental Toxicology*, *39*, 2024, pp. 1258–1268. DOI: 10.1002/tox.24012.
- Liu, Y. – Zhou, N. – Luo, C. – Zhang, Q. – Sun, P. – Fu, J. – Li, S. – Li, Z.: Shoot organogenesis and regeneration from leaf seedlings of *Diospyros oleifera* Cheng. *Plants (Basel)*, *12*, 2023, article 3507. DOI: 10.3390/plants12193507.
- Zheng, C. – Zhu, Y. – Wang, C. – Guo, T.: Wheat grain yield increase in response to pre-anthesis foliar application of 6-benzylaminopurine is dependent on floret development. *PLoS One*, *11*, 2016, article e0156627. DOI: 10.1371/journal.pone.0156627.
- Zhang, L. – Shi, X. – Hou, H. – Lin, Q. – Zhu, S. – Wang, G.: 6-Benzyladenine treatment maintains storage quality of Chinese flowering cabbage by inhibiting chlorophyll degradation and enhancing antioxidant capacity. *Plants (Basel)*, *12*, 2023, article 334. DOI: 10.3390/plants12020334.
- Wang, H. – Wang, S. – Chen, H. – Li, H. – Nie, X. – Zhang, X. – Liu, B. – Du, H.: Determination of 6-benzylaminopurine with ordered mesoporous carbon modified electrode via electrodeposition. *Huaxue shiji – Chemical Reagents*, *41*, 2019, pp. 54–57. DOI: 10.13822/j.cnki.hxsj.2019006601. In Chinese.
- Yu, T. – Xin, Y. – Liu, P.: Effects of 6-benzyladenine (6-BA) on the filling process of maize grains placed at different ear positions under high planting density. *Plants (Basel)*, *12*, 2023, article 3590. DOI: 10.3390/plants12203590.
- Li, X. – Gao, M. – Guo, Y. – Zhang, Z. – Zhang, Z. – Chi, L. – Qu, Z. – Wang, L. – Huang, R.: 6-Benzyladenine alleviates NaCl stress in watermelon (*Citrullus lanatus*) seedlings by improving photosynthesis and upregulating antioxidant defences. *Functional Plant Biology*, *50*, 2023, pp. 230–241. DOI: 10.1071/FP22047.
- Fu, J. – Liu, H. – Hu, J. – Liang, Y. – Liang, J. – Wuyun, T. – Tan, X.: Five complete chloroplast genome sequences from *Diospyros*: genome organization and comparative analysis. *PLoS ONE*, *11*, 2017, article e0159566. DOI: 10.1371/journal.pone.0159566.
- Ding, J. Z. – Yin, T. – Yu, X. – Zhao, S. M. – Xiong, S. B. – Zhan, W.: Effects of exogenous gibberellins, 6-benzyladenine and minerals on the growth of hydroponic mung bean sprouts. *Plant Physiology Journal*, *47*, 2011, pp. 501–504. DOI: 10.13592/j.cnki.ppj.2011.05.011. In Chinese.
- Xie, H. – Zhou, M. – Zhao, H. – Wang, Y. – Jiang, W. – Zhao, S.: Determination of three exog-

- enous plant hormone residues in bean sprout by high performance liquid chromatography-quadrupole-time of flight mass spectrometry. *Chinese Journal of Chromatography*, 32, 2014, pp. 493–498. DOI: 10.3724/SP.J.1123.2013.12029. In Chinese.
18. Wang, W. X. – Wang, B. R. – Liu, Z. H. – Xia, X.: Developmental toxicity and alteration of gene expression in zebrafish embryo exposed to 6-benzylaminopurine. *Chemosphere*, 233, 2019, pp. 336–346. DOI: 10.1016/j.chemosphere.2019.05.261.
  19. Gong, G. – Kam, H. – Chen, H. – Chen, Y. – Cheang, W.S. – Giesy, J.P. – Zhou, Q. – Lee, S.M.: Role of endocrine disruption in toxicity of 6-benzylaminopurine (6-BA) to early-life stages of zebrafish. *Ecotoxicology and Environmental Safety*, 232, 2022, article 113287. DOI: 10.1016/j.ecoenv.2022.113287.
  20. Announcement on the prohibition of the use of 6-benzyladenine and other substances in the production of bean sprouts. In: State Administration for Market Regulation [online]. Beijing : State Administration for Market Regulation, 2015 [cited 2 August 2024]. <[https://www.samr.gov.cn/spjys/tzgg/art/2024/art\\_e05c46118a1e4fba8c4df587dc0b2123.html](https://www.samr.gov.cn/spjys/tzgg/art/2024/art_e05c46118a1e4fba8c4df587dc0b2123.html)> In Chinese.
  21. Song, W.: The *ADI* and *ARfD* of pesticides assessed by JMPR. *Nongyao kexue yu guanli – Pesticide Science and Administration*, 8, 2009, pp. 12–17. DOI: 10.3969/j.issn.1002-5480.2009.08.002. In Chinese.
  22. Zhang, Z. – Hu G. – Wang W. – Zheng W. – Wang, Q.: Health risk assessment of 6-benzylaminopurine residue in bean sprouts. *Nongyaoxue xuebao – Chinese Journal of Pesticide Science*, 18, 2016, pp. 77–85. DOI: 10.16801/j.issn.1008-7303.2016.0009. In Chinese.
  23. European Food Safety Authority: Conclusion on the peer review of the pesticide risk assessment of the active substance 6-benzyladenine. *EFSA Journal*, 8, 2010, article 1716. DOI: 10.2903/j.efsa.2010.1716.
  24. Gong, C. – Dong, F. – Wang, Z. – Xing, Y. – Sun, Y.: Risk evaluation of plant growth regulator exposure in commercially available bean sprouts based on Monte Carlo simulation. *Nongchanpin zhiliang yu anquan – Quality and Safety of Agro-products*, 16, 2018, pp. 30–33,42. DOI: 10.3969/j.issn.1674-8255.2018.06.006. In Chinese.
  25. Li, X. – Wu, H. – Zhao, X. – Chen, L. – Zhou, C. – Li, J. – He, X.: Plant growth regulator residues and dietary risk assessment of table grapes and strawberries in Shanghai. *Nongyaoxue xuebao – Chinese Journal of Pesticide Science*, 24, 2022, pp. 152–160. DOI: 10.16801/j.issn.1008-7303.2021.0162. In Chinese.
  26. Song, W. – Xu, H. – Wang, W. – Yang, G. – Shao, Y. – Liu, X. – Wang, Q.: Risk assessment of dietary intake on residue of plant growth regulators in vegetable: a case study in Jiangsu and Zhejiang. *Nongchanpin zhiliang yu anquan – Quality and Safety of Agro-products*, 15, 2017, pp. 9–14,20. DOI: 10.3969/j.issn.1674-8255.2017.01.002. In Chinese.
  27. Wang, X. – Wu, Y. – Chen, J.: Low level data processing for food contamination monitoring. *Zhonghua yufang yixue zazhi – Chinese Journal of Preventive Medicine*, 36, 2002, pp. 278–279. DOI: 10.3760/j:issn:0253-9624.2002.04.022. In Chinese.
  28. Sui, H. – Jia, X. – Liu, Z. – Li, F. – Yan, W.: Sources, selection principles and uncertainty analyses of data for dietary exposure assessment of chemicals in foods. *Weisheng yanjiu – Journal of Hygiene Research*, 46, 2011, pp. 791–794. DOI: 10.19813/j.cnki.weishengyanjiu.2011.06.035. In Chinese.
  29. Yang, G. – Chen, C. – Wang, Q. – Zhao, H. – Zhang, Z. – Cai, Z. – Qian, Y.: Risk assessment for combined exposure of multi-residue of pesticides. *Nongyaoxue xuebao – Chinese Journal of Pesticide Science*, 17, 2015, pp. 119–127. DOI: 10.3969/j.issn.1008-7303.2015.02.01. In Chinese.
  30. Jiang, Y. – Zhuang, M. – Xiao, P. – Wang, K. – Song, J. – Liu, H. – Zhao, J. – Chu, Z.: Pesticide residues and health risk assessment in radishes in Shandong. *Journal of Food Science*, 87, 2022, pp. 4751–4760. DOI: 10.1111/1750-3841.16088.

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