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Research Paper

Occurrence and Health Risk of Patulin and Pyrethroids in Fruit Juices Consumed in Bangkok, Thailand

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ABSTRACT

The mycotoxin patulin (PAT) is well known as a natural contaminant of apple- and other fruit-based products. Pesticides are a group of chemicals abundantly used in agriculture to maximize productivity by protecting crops from pests and weeds. Because of their harmful health effects, PAT and pesticides are strictly monitored. The current study was undertaken to investigate the significance of PAT and pyrethroid insecticide contamination in a variety of fruit juices in Bangkok. To do this, a total of 200 fruit juice samples, consisting of 40 samples each of apple, apricot, peach, pineapple, and grape juice, were collected from supermarkets in Bangkok, Thailand. PAT contamination in a variety of fruit juices was detected using validated liquid chromatography–tandem mass spectrometry, and pyrethroid insecticides (cypermethrin, cyfluthrin, and flumethrin) were analyzed using a gas chromatography equipped with micro-electron capture detector. The survey found that PAT concentrations were lower than the maximum residue limit established by European Union. The results of the present study suggest that the risk of exposure to harmful levels of PAT, cypermethrin, cyfluthrin, and flumethrin in fruit juices is very low in urban areas of Thailand.

Key words: Cyfluthrin; Cypermethrin; Flumethrin; Fruit juices; Liquid chromatography–electrospray ionization–tandem mass spectrometry; Patulin

Patulin (PAT, 4-hydroxy-4*H*-furo-[3,2-*c*]pyran-2(6*H*)-one) is a secondary metabolite produced by certain species of the *Aspergillus* and *Penicillium* genera, predominantly *Aspergillus clavatus, Penicillium expansum, Penicillium aspergillus, and Penicillium byssochlamys (3, 25)*. PAT is found mainly in apples and its products, and occasionally in other fruits such as pears, apricots, peaches, and grapes, and is produced in the rotten parts of these fruits *(7)*. The contamination of food products with PAT has been observed worldwide, especially in apple juice *(4–7, 20, 21, 27, 34, 35, 38, 39, 44–46)*. PAT induces acute adverse effects, including convulsions, dyspnea, pulmonary edema, and gastrointestinal tract distension *(42),* and chronic adverse effects, which involve genotoxic, immunotoxic, immunosuppressive, and teratogenic effects and protein synthesis inhibition *(1, 17, 26, 30, 40)*. With respect to its considerable toxicity, various guidelines, recommended maximum concentrations, and legislation limits have been established in many countries. The Codex Alimentarius Commission *(10)* and U.S. Food and Drug Administration *(37)* have recommended a maximum level of 50 μg L^{-1} for fruit juices and their products. In the case of fruit-based baby food, the European Union has strict legislation limiting the occurrence of PAT to concentration of <10 μg kg^{-1} *(14)*. Infants and small babies are more susceptible to the intake of various toxins because of their very specific dietary requirements, low body weight, higher metabolic rate, and low ability to detoxify hazardous contaminants and xenobiotics *(33)*.

Pesticides, including pyrethroid insecticides, have been widely used in the cultivation and postharvest storage of certain crops to control weeds, insect infestation, and plant diseases. The wide application of these pesticides provides benefits in increasing agricultural production, but the pesticides can become a risk to both animals and humans via the food chain *(2, 23, 24)*. The comprehensive control of these contaminants is not an easy task because currently there are over 1,000 active pesticide substances registered in the European Union. The World Health Organization *(41)* has reported that roughly 3 million pesticide poisonings occur annually and result in 220,000 deaths worldwide. According to the World Health Organization *(43),* food consumption consists, on average, of 30% of fruits and vegetables, and it is well known that fruits and vegetables

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are more contaminated by pesticides than are products of animal origin *(8)*. Governments and international organizations *(13)* regulate the use of pesticides by setting maximum residue levels in food.

Given the widespread consumption of fruit juices, including apple, apricot, peach, pineapple, and grape juice, it is very important to investigate the natural occurrence of these significant mycotoxin and pyrethroid insecticides. This study focused on investigating the degree of PAT, cypermethrin, cyfluthrin, and flumethrin contamination in a variety of fruit juices from Bangkok, Thailand, in relation to the guidelines for assuring food safety.

MATERIALS AND METHODS

Toxins and chemicals. PAT, cypermethrin, cyfluthrin, and flumethrin standards were purchased from Sigma Aldrich (St. Louis, MO). Other reagents and chemicals were of analytical grade. Purified water was produced using the Milli-Q water purification system (Millipore, Inc., Bedford, MA).

Sample collection. A total of 200 fruit juice samples consisting of 40 samples each of apple, apricot, peach, pineapple, and grape juice were collected from supermarkets in Bangkok, Thailand. All the samples selected in this survey were collected from 10 different supermarkets in Bangkok from January to April 2016. Most of the samples were of Thai origin, and the rest were from other countries. The apple juice samples were from Thailand (17), Singapore (6), Malaysia (1), Japan (1), Turkey (1), the United States (8), Australia (3), France (1), Ukraine (1), and Austria (1). The peach juice samples were from Thailand (25) and South Africa (15). The apricot juice samples were from Thailand (5), Turkey (9), and South Africa (26). The grape juice samples were from Thailand (33), Singapore (1), Turkey (2), the United States (3), and England (1). All the pineapple juice samples were from Thailand (40). Different brands were selected to have a representative sample of products sold in the Bangkok supermarkets. The samples included both pasteurized and fresh (refrigerated) fruit juices. The samples were stored at –20°C until analysis.

Sample preparation. The extraction and cleanup methods for PAT, cypermethrin, cyfluthrin, and flumethrin in fruit juice were performed as described previously *(18, 29, 38),* with slight modifications. Briefly, 5 mL of fruit juice was applied on a solid-phase cartridge (InertSep, PLS-3; size 200 mg/mL; GL Sciences Co., Tokyo, Japan) after the cartridge had been preconditioned with 10 mL of methanol (RCI Labscan, Bangkok, Thailand) followed by 10 mL of Milli-Q water using a vacuum manifold. The cartridge was washed with 5 mL of 1% sodium bicarbonate (Ajax Finechem Ltd., Auckland, New Zealand) solution followed by 5 mL of 0.1% formic acid (Fisher Scientific, Leicester, UK) solution. The cartridge was aspirated under vacuum until most of the water had been removed. Then, 5 mL of methanol was added at a low flow rate to elute the compounds that remained on the cartridge. The eluate was then evaporated to dryness under a nitrogen stream at 40°C on a heating block (Eyela, Tokyo Rikakikai Co., Tokyo, Japan). The residue was reconstituted with 1 mL of methanol-water solution (50/50 [v/v]). After being passed through a Minisart RC filter (pore size of 0.22 μm; Sartorius AG, Goettingen, Germany), the reconstituted residue was analyzed for PAT using liquid chromatography–tandem mass spectrometry (LC-MS/MS).

For the extraction of cypermethrin, cyfluthrin, and flumethrin from the fruit juice, 4 mL of the fruit juice sample was added to 5 mL of ethyl acetate with acetone (1:1 [v/v]) and submitted to extraction, by agitation (Vortex-Genie 2, Scientific Industries, Bohemia, NY), for 20 min. Then, the organic phase was separated by centrifugation at 2,500 × *g* for 10 min, and the supernatant was then collected. The residue was reextracted using 5 mL of ethyl acetate (RCI Labscan) and acetone (RCI Labscan) (1:1 [v/v]). The two portions collected were combined and cleaned up using the solid-phase extraction florisil cartridge; the solvent was then passed through a polytetrafluoroethylene filter (pore size of 0.22 μm; Sartorius AG). The elute was subsequently collected and dried under a nitrogen stream at 40°C. The reconstituted residue was redissolved in 2 mL of ethyl acetate and analyzed for cypermethrin, cyfluthrin, and flumethrin using a gas chromatograph equipped with micro-electron capture detector (GC-μECD; Agilent 7890B, Agilent Technologies, Waldbronn, Germany).

LC-(electrospray ionization)-MS/MS. The liquid chromatography analysis was performed using an Agilent 1260 Infinity (Agilent Technologies) consisting of a binary pump, a vacuum degasser, a column oven and an autosampler. The chromatographic separation was performed using a ZORBAX Eclipse Plus RRHD C18 column (2.1 by 50 mm, particle size 1.8 μm; Agilent Technologies). The column was maintained at 40°C. The mobile phase consisted of 5 mM ammonium acetate solution (mobile phase A) and methanol (mobile phase B). The gradient program of the mobile phase was as follows: 0 to 2.5 min, 100% mobile phase A; 2.5 to 3.5 min, from 100 to 5% mobile phase A; 3.5 to 6.0 min, 5% mobile phase A; 6.0 to 8.0 min, from 5 to 100% mobile phase A; and then reequilibration at 100% mobile phase A until 12 min. The mobile phase solution was filtered through a 0.22-μm membrane and ultrasonically degassed prior to application. The flow rate was 0.4 mL/min, and the injection volume was 5 μL. The temperature of the autosampler was set at 4°C.

A triple quadrupole mass spectrometer was used (6460 triple; Agilent Technologies) equipped with an electrospray ionization source operated in negative ion mode under the multiple reaction monitoring mode. The ionization source parameters were optimized as follows: capillary voltage, 3,500 V; gas temperature, 300°C; gas flow, 8 L/min; and nebulizer, 48 lb/in^2. Under these conditions, PAT formed $[M + CH_3COO]^-$ ions at *m/z* 153. The molecular ions and fragments employed for PAT were as follows: Q1: *m/z* 153 to 109 (quantifier), collision energy of 1 eV; and Q3: *m/z* 153 to 81 (qualifier), collision energy of 4 eV.

GC-μECD. Sample extracts were analyzed for cypermethrin, cyfluthrin, and flumethrin in fruit juices using GC-μECD. The separation was achieved using an HP-5 fused silica capillary column (30 m by 0.32 mm by 0.25 μm; Agilent J&W GC column, Agilent Technologies). Helium and nitrogen were used as the carrier and makeup gas at constant flow rates of 2 and 20 mL min^{-1}, respectively. The injector and detector temperatures were 260 and 315°C, respectively. The oven temperature was held at 150°C for 1 min and then programmed to 225°C at 12°C min^{-1} and to 300°C at 15°C min^{-1}. The total assay time was 25 min per sample injection.

Quantification and method validation. We performed the validation of the LC-MS/MS method for PAT to assess the efficiency of this analytical method by investigating the recovery, repeatability, linear working range, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and matrix effects in accordance with the guidelines of European Commis-

sion Decision 2002/657/EC *(11)*. The linearity of an analytical procedure is its ability (within a given range) to produce test results that are directly proportional to the concentration (amount) of analyte in the sample. We conducted linear regression analysis for the PAT standard under the optimized LC-MS/MS conditions. Recovery and precision (repeatability, expressed as relative standard deviation [as a percentage]) were determined within-day by analyzing seven replicates containing PAT at three different quality control levels: 2, 5, and 10 μg L^{-1}. The interday precisions were determined by analyzing the quality control samples on five different days (one batch per day). The calibration standard concentrations were prepared in three replicates by spiking the working standard solution into blank samples to yield final concentrations of 1, 2.5, 5, 10, 25, and 50 μg L^{-1}. The matrix effects were determined for the tested matrices by spiking blank fruit samples of apple, apricot, peach, pineapple, and grape juice. With respect to the validation method using the GC-μECD for cypermethrin, cyfluthrin, and flumethrin in fruit juices, the determinations of recovery, LOD, LOQ, accuracy, and precision were performed. The LOD and LOQ of the method were evaluated as the signal-to-noise value of 3:1 and 10:1, respectively.

Fungal isolation and identification. The dilution plate method was used for the juice samples. We added 1 mL of each sample to 9 mL of sterilized water in the test tube. We mixed the solution thoroughly; we then removed 1 mL (10%) of the volume with a sterile volumetric pipette and transferred this to a new test tube containing a quantity of solution equal to that originally present in the first test tube. The solution was mixed thoroughly again. This iterative 10-fold dilution was done three to four times. Then we spread 0.1 mL of each dilution onto potato dextrose agar (Merck, Darmstadt, Germany) in a petri dish. After the petri dishes were incubated at 28°C for 3 to 5 days, the fungal isolates were selected and transferred to slant potato dextrose agar tubes and kept as a pure cultures for identification.

The fungal isolates were cultured onto Czapek agar, Czapek yeast (autolysate) extract agar, and malt extract agar (Difco, Oxford, UK) with three replicates for each medium. The samples were incubated at 28°C for 7 and 14 days. Macroscopic features such as the conidial color, colony diameter, mycelia color, exudates, reverse color, soluble pigment, sclerotia, and cleistothecia of each sample were recorded. Microscopic features such as the morphology of the seriation, vesicles, conidia, stipes, cleistothecial wall, asci, and ascospores were examined under stereo (Olympus, Tokyo, Japan) and compound (Carl Zeiss, Jena, Germany) microscopes *(19)* and compared with features in identification keys and species descriptions *(28, 31, 32)*.

Dietary exposure risk of PAT, cypermethrin, cyfluthrin, and flumethrin. The estimated daily intake (EDI; in milligrams per kilogram of body weight per day) of each chemical residue including PAT, cypermethrin, cyfluthrin, and flumethrin was calculated by multiplying the mean concentration of the residue by the consumption rate (250 mL day^{-1}) and dividing the result by the body weight, assuming the average weight of a child to be 18.9 kg and the average weight of an adult to be 60 kg *(36)*. The EDIs of the chemical residues were calculated as in equation 1.

$$\mathrm{EDI} = \sum \mathrm{RL}_i \times \frac{F_i}{\mathrm{BW}} \quad (1)$$

where RL_i is the residue level of the fruit juice (mg kg^{-1}), F_i is the fruit juice consumption rate per day (250 mL day^{-1}), and BW is the body weight (kg).

The risk assessment was calculated by comparing the concentrations of the residues detected with the established acceptable daily intake *(16)*. In this study, the hazard quotient (HQ) was used to indicate the long-term risk assessment. HQ can be calculated as in equation 2 *(22)*.

$$\mathrm{HQ} = \frac{\mathrm{EDI}}{\mathrm{ADI}} \times 100\% \quad (2)$$

where ADI is the acceptable daily intake. The HQs are summed up to give the chronic hazard index (cHI). When cHI > 100, the juice should be considered a risk to consumers; when cHI < 100, the juice is an acceptable or low risk. The cHI can be calculated as in equation 3.

$$\mathrm{cHI} = \sum \mathrm{HQ} \quad (3)$$

RESULTS AND DISCUSSION

Method validation and quality assurance. Regarding PAT detection, the LC-MS/MS method used in this study showed good linearity range, intraday and interday precision, and accuracy for the quantification of PAT. The correlation coefficient (r^2) values of the PAT calibration curves were 0.9969. The intra- and interday precisions (relative standard deviations) of the PAT spiked samples ranged from 2.9 to 6.6% and from 4.9 to 8.3%, respectively. The average recoveries for PAT from apple, apricot, peach, pineapple, and grape juices were 84 to 90%. The LOD and LOQ values of PAT were 0.5 and 1.5 μg L^{-1}, respectively, for the apple, pineapple, and grape juices; they were 0.6 and 2 μg L^{-1}, respectively, for the apricot and peach juices. Thus, LC-MS/MS has been identified as a sensitive analytical technique for determining PAT concentrations in fruit juices. With respect to cypermethrinin, cyfluthrin, and flumethrin (the pyrethroid insecticides) in fruit juices, the LOD values were 2, 2, and 1 μg L^{-1}, respectively, and the LOQ values were 4, 6, and 5 μg L^{-1}, respectively. The recovery percentages were fortified at 0.5 μg L^{-1} for cypermethrin and at 0.25 μg L^{-1} for cyfluthrin and flumethrin at 98.2, 88, and 92.8%. The intra- and interday precisions (relative standard deviations) of the spiked samples ranged from 6.43 to 10.32% and from 6.29 to 9.2%, respectively.

Monitoring survey. The method described here was applied to a monitoring survey of PAT, cypermethrin, cyfluthrin, and flumethrin in apple, apricot, peach, pineapple, and grape juices. The summary of the PAT, cypermethrin, cyfluthrin, and flumethrin contamination found in the fruit juices tested is shown in Table 1. PAT contamination was quantifiable in 11% (22 of 200) of all the collected samples, in 20% (8 of 40) of apple juice samples at levels from 1.70 to 9.48 μg L^{-1}, in 10% (4 of 40) of apricot juice samples at levels from 2.03 to 6.27 μg L^{-1}, in 7.5% (3 of 40) of peach juice samples at levels from 3.74 to 5.63 μg L^{-1}, and in 17.5% (7 of 40) of grape juice samples at levels from 1.86 to 3.53 μg L^{-1}; however, its concentration was below the LOQ of the method in the pineapple juice samples. Although PAT was detected in apple, apricot, peach and grape juices, its concentrations

TABLE 1. *Patulin, cypermethrin, cyfluthrin, and flumethrin contamination in apple, apricot, peach, pineapple, and grape juices consumed in Bangkok, Thailand*[a]

Fruit juice	Patulin			Cypermethrin			Cyfluthrin			Flumethrin		
	Positive samples (%)	Mean ± SD (μg L^{-1})	Range (μg L^{-1})	Positive samples (%)	Mean ± SD (μg mL^{-1})	Range (μg mL^{-1})	Positive samples (%)	Mean ± SD (μg mL^{-1})	Range (μg mL^{-1})	Positive samples (%)	Mean ± SD (μg mL^{-1})	Range (μg mL^{-1})
Apple	20 (8/40)	4.71 ± 2.50	1.70–9.48	25 (10/40)	0.19 ± 0.09	0.08–0.36	2.5 (1/40)	0.04 ± 0.00	0.04–0.04	17.5 (7/40)	0.04 ± 0.01	0.02–0.05
Apricot	10 (4/40)	4.51 ± 1.83	2.03–6.27	30 (12/40)	0.83 ± 0.74	0.12–2.77	17.5 (7/40)	0.11 ± 0.15	0.02–0.45	22.5 (9/40)	0.06 ± 0.01	0.05–0.08
Peach	7.5 (3/40)	4.43 ± 1.04	3.74–5.63	57.5 (23/40)	0.91 ± 0.48	0.19–2.19	67.5 (27/40)	0.05 ± 0.04	0.01–0.16	47.5 (19/40)	0.05 ± 0.01	0.04–0.07
Pineapple[b]	0 (0/40)	<LOQ	<LOQ	25 (10/40)	0.74 ± 0.69	0.10–1.90	27.5 (11/40)	0.15 ± 0.11	0.04–0.46	25 (10/40)	0.08 ± 0.03	0.05–0.14
Grape	17.5 (7/40)	3.05 ± 0.57	1.86–3.53	22.5 (9/40)	0.17 ± 0.04	0.13–0.26	7.5 (3/40)	0.01 ± 0.001	0.012–0.013	5 (2/40)	0.05 ± 0.01	0.05–0.06

[a] Numbers in parentheses are the number of positive samples/total number of samples. Means are the means of quantifiable levels.
[b] <LOQ, less than limit of quantification.

were below the maximum legislative limits established by European Commission Regulation 1881/2006 *(14)*. PAT contamination was higher in apple juice than in the other fruit juices. The maximum permitted level of PAT in fruit juices and nectars, especially apple juice and apple juice ingredients in other beverages marketed in Europe is 50 μg L^{-1} *(12)*. The permitted threshold is lower for apple juices labeled and sold as intended for consumption by infants and young children (10 μg kg^{-1}). This study was conducted on a limited number of samples collected in Bangkok; the sample products are familiar to the Thai people and are widely consumed by a large population in Thailand. However, a larger sample size is needed to confirm these data in further studies.

The fungal cultures were preliminarily identified by their colony morphology and microscopic characteristics. The fungal isolates from the apple, apricot, peach, pineapple and grape juice samples belonged mainly to the *Aspergillus* and *Penicillium* genera (Table 2; Supplement 1); *Aspergillus fumigatus, Aspergillus flavus,* and *Aspergillus niger* were the predominant species found. Only certain species of *Aspergillus* and *Penicillium* produce PAT, however; therefore, further identification using other techniques such as PCR is required. In addition, all the fungus isolated came from fresh (refrigerated) fruit juices.

Three pyrethroid insecticides, cypermethrin, cyfluthrin, and flumethrin, were quantifiable in 32% (64 of 200), 24.5% (49 of 200), and 23.5% (47 of 200) of all the collected samples. The incidence of contamination was higher in the peach juice samples than in other fruit juices (Table 1). In the peach juice samples, the residue levels were quantifiable in 57.5% (23 of 40), 67.5% (27 of 40), and 47.5% (19 of 40) at levels ranging from 0.19 to 2.19 μg mL^{-1}, from 0.01 to 0.16 μg mL^{-1}, and from 0.04 to 0.07 μg mL^{-1}, respectively. The maximum allowable residue levels of cypermethrin and cyfluthrin in food are 1 and 0.1 mg mL^{-1} *(9, 15)*.

Health risks of PAT, cypermethrin, cyfluthrin, and flumethrin. We calculated the risk assessment of PAT, cypermethrin, cyfluthrin, and flumethrin exposure via the consumption of apple, apricot, peach, pineapple, and grape juices (Table 3). The value of cHI was calculated for children and adults. The result showed that the cHI values of all the chemical residues tested were <100 in the fruit juice samples (Table 3).

In summary, the results of this study suggest that the risk of harmful PAT, cypermethrin, cyfluthrin, and flumethrin exposure via the consumption of apple, apricot, peach, pineapple, and grape juices is very low in the urban areas of Thailand; however, further studies with a larger sample size are needed to confirm these data. The levels of all the chemical residues tested in this study were well below the legislated maximum residue level. Molds such as those belonging to the PAT-producing *Aspergillus and Penicillium* genera were also identified; testing the growth of these molds during the storage of the food samples is warranted.

TABLE 2. *Fungal species isolated and identified from apple, apricot, peach, pineapple, and grape juices*[a]

Fungal isolation	Apple juice		Apricot juice		Peach juice		Pineapple juice		Grape juice	
	Positive samples	Incidence (%)	Positive samples	Incidence (%)	Positive samples	Incidence (%)	Positive samples	Incidence (%)	Positive samples	Incidence (%)
Aspergillus candidus	NT	0	NT	0	1–40	2.50	NT	0	NT	0
A. flavus	1/40	2.50	NT	0	4/40	10	3/40	7.50	1/40	2.50
A. fumigatus	2/40	5	1/40	2.50	4/40	10	3/40	7	4/40	10
A. niger	2/40	5	2/40	5	2/40	5	5/40	12.50	2/40	5
A. terreus	NT	0	NT	0	1/40	2.50	NT	0	NT	0
Aspergillus spp.	2/40	5	NT	0	1/40	2.50	NT	0	NT	0
Emericella variecolor	NT	0	NT	0	NT	0	1/40	2.50	NT	0
Emericella spp.	NT	0	NT	0	NT	0	NT	0	1/40	2.50
Neosartorya spp.	NT	0	NT	0	NT	0	1/40	2.50	NT	0
Penicillium spp.	1/40	2.50	2/40	5	1/40	2.50	7/40	17.50	NT	0
Syncephalastrum spp.	NT	0	NT	0	NT	0	NT	0	1/40	2.50
Talaromyces spp.	NT	0	NT	0	NT	0	1/40	2.50	NT	0
Trichoderma spp.	NT	0	NT	0	NT	0	NT	0	NT	0

[a] Positive samples are presented as number of positive samples/total number of samples. NT, none detected.

TABLE 3. *Health risk assessment based on daily intake of patulin, cypermethrin, cyfluthrin, and flumethrin contamination in apple, apricot, peach, pineapple, and grape juices*[a]

	Children[b]									Adult[c]								
	Patulin		Cypermethrin		Cyfluthrin		Flumethrin			Patulin		Cypermethrin		Cyfluthrin		Flumethrin		
Fruit juice	EDI (μg kg^{-1})	HQ	EDI (μg kg^{-1})	HQ	EDI (μg kg^{-1})	HQ	EDI (μg kg^{-1})	HQ	cHI	EDI (μg kg^{-1})	HQ	EDI (μg kg^{-1})	HQ	ED (μg kg^{-1})	HQ	EDI (μg kg^{-1})	HQ	cHI
Apple	0.96	0.89	0.12	0.25	0.01	0.05	0.00	0.05	1.24	0.00	0.00	0.04	0.08	0.00	0.02	0.00	0.02	0.11
Apricot	0.00	0.01	0.55	1.10	0.03	0.14	0.00	0.07	1.32	0.00	0.00	0.14	0.35	0.01	0.04	0.00	0.02	0.42
Peach	0.00	0.01	0.60	1.20	0.01	0.07	0.00	0.07	1.35	0.00	0.00	0.17	0.38	0.00	0.02	0.00	0.02	0.42
Pineapple	—	—	0.49	0.98	0.04	0.19	0.00	0.10	1.28	—	—	0.15	0.31	0.01	0.06	0.00	0.03	0.40
Grape	0.00	0.00	0.11	0.23	0.00	0.02	0.00	0.07	0.32	0.00	0.00	0.04	0.07	0.00	0.01	0.00	0.02	0.10

[a] EDI, estimated daily intake; HQ, hazard quotient; cHI, chronic hazard index; —, not determined because the PAT level was <LOQ (see Table 1).
[b] Average body weight 18.9 kg.
[c] Average body weight 60 kg.

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SUPPLEMENTAL MATERIAL

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