Risk Assessment Studies
Report No. 56

Chemical Hazard Evaluation

Pyrrolizidine Alkaloids in Food

January 2017
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EXECUTIVE SUMMARY

This study aims to determine the total sum of 1,2-unsaturated pyrrolizidine alkaloids (PAs) in selected food items, to estimate the dietary exposure to PAs of the Hong Kong adult population and to assess the associated health risks.

2. PAs are a group of secondary compounds that are produced by plants all over the world. To date, over 660 PAs and their corresponding N-oxide derivatives have been identified from more than 6 000 plant species. PAs are the most widely distributed natural toxins and cases of human toxicity caused by the use of toxic plant species as herbal teas or traditional medicines and the consumption of grain or grain products (flour or bread) contaminated with PA-containing seeds have been reported. Overseas studies showed that humans are also exposed to PAs through honey, tea, milk, eggs and offal; however, cases of human poisoning resulting from exposure through these sources have not been reported.

3. PAs are esters composed of a necine base and one or more necic acids. The necine base can either be saturated or contain a double bond (i.e. unsaturated) in the 1,2 position. Toxic PAs are those which contain unsaturated necine bases whereas the ones with saturated necine bases are considered to be non-toxic.

4. 1,2-unsaturated PAs themselves are not toxic in their original form but require metabolic activation to exert their toxicities. Toxicity of the 1,2-unsaturated PAs in animal studies is characterised by hepatotoxicity, carcinogenicity and genotoxicity. The liver is the primary site for genotoxicity.

Nonetheless, there are no human epidemiological data suggesting a link of PA exposure and cancer in humans to date.

5. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated PAs in 2015 and opined that the genotoxic mode of action of PAs does not allow derivation of a health-based guidance value for chronic toxicity. Instead, JECFA chose the Margin of Exposure (MOE) approach to estimate the potential cancer risk of PA exposure in food, establishing a lower limit on the benchmark dose for a 10% excess cancer risk ($BMDL_{10}$) of 182 μg/kg body weight (bw) per day for liver haemangiosarcoma in female rats treated with a PA known as riddelliine. In general, an MOE of ≥ 10 000, if it is based on the $BMDL_{10}$ from an animal study, would be of low concern from a public health point of view and might be considered as a low priority for risk management actions.

Results

6. A total of 234 samples (involving 48 food items) was tested for the twenty-eight individual 1,2-unsaturated PAs. Of the 234 samples analysed, 118 samples (50%) were detected with at least one 1,2-unsaturated PA. Among these 118 samples, majority (i.e. 91 samples) belonged to the food groups "dried spices", "honey" and "tea leaves (infusion)". Other items with PAs detected included wheat and rye flour, duck eggs, yoghurts, cheeses, tea beverages, etc.

7. As regards the concentrations of PAs in different food groups, "dried spices" contained the highest levels of total 1,2-unsaturated PAs (total PAs), followed by "honey" and "tea leaves (infusion)". The upper bound mean

concentrations of "dried spices", "honey" and "tea leaves (infusion)" were 300 μg/kg, 7.5 μg/kg and 0.46 μg/kg respectively.

8. Regarding the dietary exposure to PAs of the local adult population, the lower bound (LB) and upper bound (UB) exposure estimates of total PAs for average consumers were found to be 0.00033 and 0.0015 μg/kg bw/day respectively. For high consumers, the LB and UB exposure estimates were found to be 0.0015 and 0.0043 μg/kg bw/day. In relation to the total PAs in food, the corresponding MOEs (determined by using the $BMDL_{10}$ of 182 μg/kg bw per day) for adults were in the ranges of 560 000 – 120 000 (LB-UB) and 120 000 – 42 000 (LB-UB) for the average and high consumers of the population respectively.

9. The major contributor to the exposure of total PAs was "tea leaves (infusion)" which contributed up to 50.3% (i.e. 0.00016 μg/kg bw/day at the LB) of the total exposure. The relatively high exposure contribution from "tea leaves (infusion)" in the Hong Kong adult population was likely due to high consumption amount and the relatively low contamination levels of PAs detected in other food groups. The food groups "cereal and cereal products" (i.e. 0.000079 μg/kg bw/day at the LB) and "honey" (i.e. 0.000077 μg/kg bw/day at the LB) altogether contributed less than 48% to the total exposure.

10. In this study, all "common teas" (i.e. fully-fermented, semi-fermented and non-fermented teas) were found to contain relatively low concentrations of PAs. However, some "specific teas" (such as rooibos tea, verbena tea and peppermint tea) and "dried spices" (such as cumin seed, oregano and tarragon) were found to contain relatively higher levels of PAs. At present, it is not possible to individually assess the long-term health effects of consuming of these

products because there are insufficient data on the causes of contamination, batch-to-batch variations of PAs in the products, the consumption patterns of the products in the local population, etc. When compared with overseas studies, the contamination levels of PAs in these "specific teas" were found to be significantly lower and therefore a lower level of health concern to local consumers was expected. However, it is worthwhile to point out that the German Federal Institute for Risk Assessment (BfR) has reported the detection of high levels of PAs in some teas sampled from the markets of Germany and opined that these teas could pose a health risk to consumers over a long period of time. The BfR advised the public to widen and alternate their choices of food by including different types of food in their diets in order to avoid excessive exposure to any contaminants, including PAs, from a small range of food items. As regards PAs in some dried spices, a significant contribution of PAs from dried spices to overall PAs dietary exposure in the local population is not expected since dried spices are normally used in small amounts as minor ingredients during food preparation. Nonetheless, because of the mutagenic and carcinogenic effects in animal experiments, PA contamination of foods should be reduced as low as possible.

Conclusions and Recommendations

11. In relation to the total PAs in food, the MOEs for the average and high consumers of the adult population are well above 10 000; hence, health concern for the general population is considered to be low from a public health point of view.

12. Because of the genotoxic nature of PAs, efforts should be made to minimise PA contents in food. To achieve this, the causes of contamination must be identified and source-directed measures aimed at the prevention and reduction

of contamination of food with PAs must be undertaken. Companies involved in the production of dried spices and tea leaves should investigate the causes of contamination and make reference to the Codex Code of Practice for Weed Control to Prevent and Reduce Pyrrolizidine Alkaloid Contamination in Food and Feed with a view to improving their cultivation, harvesting and cleaning methods so as to reduce PA content in their products.

13. The findings of the dietary exposure to total PAs in the present study did not provide sufficient justifications to warrant changes to the basic dietary advice on healthy eating. The public is advised to maintain a balanced and varied diet which includes a wide variety of fruit and vegetables so as to avoid excessive exposure to any contaminants from a small range of food items.

Risk Assessment Studies –

Pyrrolizidine Alkaloids in Food

OBJECTIVES

This study aims to determine the total sum of 1,2-unsaturated pyrrolizidine alkaloids (PAs) in selected food items, to estimate the dietary exposure to PAs of the Hong Kong adult population and to assess the associated health risks.

BACKGROUND

2. Pyrrolizidine alkaloids (PAs) are a group of secondary compounds that are produced by plants all over the world as a defense mechanism against herbivores. To date, over 660 PAs and their corresponding N-oxide derivatives have been identified from more than 6000 plant species that correspond approximately to 3% of the world's flowering plants[1-5]. PAs are reportedly the most widely distributed natural toxins and there is a possibility of risk to humans from the consumption of PA-contaminated food[2]. Cases of human toxicity include the use of toxic plant species as herbal teas or traditional medicines[6,7] and the consumption of grain or grain products (flour or bread) contaminated with PA-containing seeds (e.g. the outbreaks of poisonings affecting rural populations in Afghanistan and India.)[8]. Other possible food sources of exposure include honey, milk, tea, eggs and meat which have been reported to contain PAs in some studies; however, cases of human poisoning resulting from exposure through these sources

have not been reported[1,9,10].

3. PAs are present in more than 12 plant families, in particularly Boraginaceae, Asteraceae and Fabaceae[5]. All genera of the Boraginaceae (e.g. *Heliotropium* or *Echium* species) are known to contain PAs while the genera of *Senecio* and *Eupatorium* of the Asteraceae are the mostly known to contain PAs[2]. Of the Fabaceae, the *Crotalaria* genus hosts the majority of PA-containing species[2]. Although over 6 000 plant species are reported to contain PAs, direct poisonings in man and animals seem to be associated with only a few species[2].

Chemistry of PAs

4. PAs are esters composed of a necine base and one or more necic acids (Figure 1(a)). The necine base comprises two five-membered rings which share a nitrogen atom at position 4. In many cases the necine base has a hydroxymethyl group at C-l and a hydroxyl group at C-7 (Figure 1(b)). These hydroxyl groups can be esterified with necic acid(s) giving monoester, open-chain diester, or macrocyclic diester alkaloids (Figure 2). The necic acids found in PAs, excluding acetic acid, possess 5 to 10 carbon atoms[11]. They can be mono- or dicarboxylic acids with branched carbon chains, bearing hydroxy, epoxy, carboxy, acetoxy, methoxy or other alkoxy groups as substituents.

5. The necine base can either be saturated or contain a double bond (i.e. unsaturated) in the 1,2 position. The unsaturated necine base can further be classified into retronecine, heliotridine and otonecine type alkaloids and the former two are diastereomers at position C-7 (Figure 3). Toxic PAs are esters of 1,2

unsaturated necines whereas the ones with saturated necine bases (i.e. the platynecine type PAs) are considered to be non-toxic[5,11].

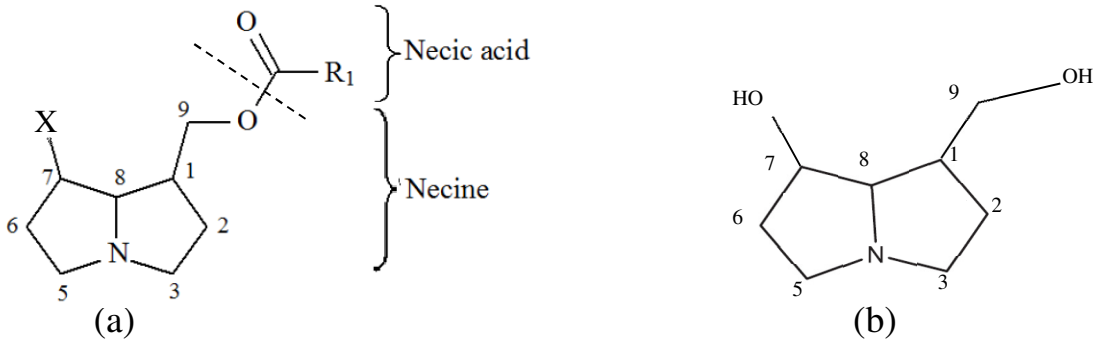


Figure 1. General structure of (a) a PA molecule and (b) a necine base. X = RCO=O, HO or O.

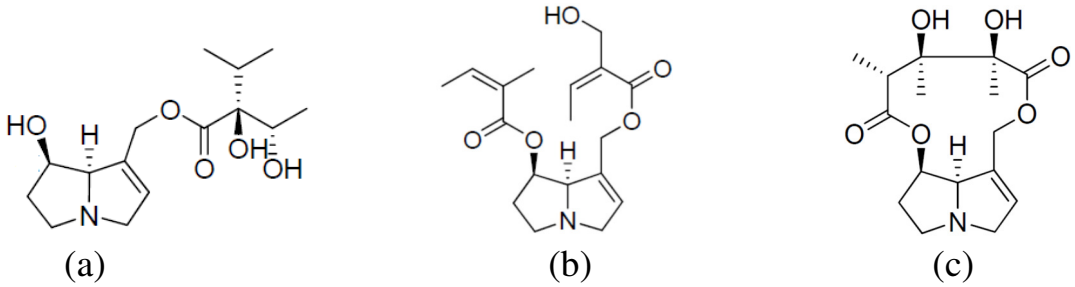


Figure 2. (a) Monoester, (b) Open-chain diester, (c) Macrocyclic diester alkaloids

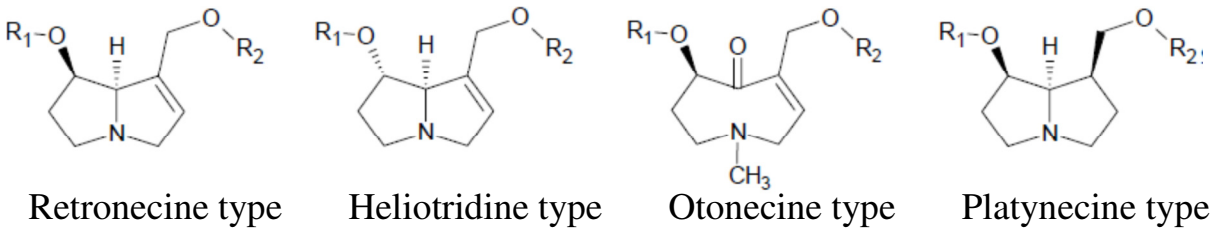
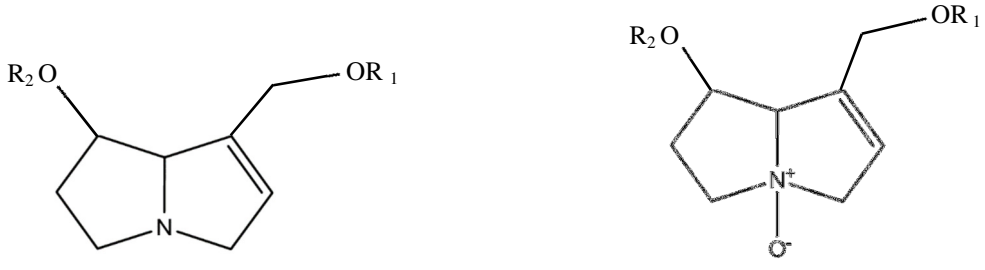


Figure 3. The necine bases of pyrrolizidine alkaloids. Retronecine, heliotridine and otonecine are toxic PAs while platynecine is non-toxic.

6. To date, approximately 660 different PA structures are known. This rich diversity of PAs is due to the combination of a pool of necine bases (Figures 3) with an even larger pool of necic acids. The diversity is further increased by the possible formation of monoesters at different positions (e.g. C-7 or C-9) and open or cyclic diesters (Figure 2). This complexity is further amplified by their existence in plants as both free bases and as N-oxides (Figure 4), the latter predominating in many plants species[8].



(a) (b)

Figure 4. (a) A pyrrolizidine alkaloid, and (b) its N-oxide

7. The minimum structural requirements for toxicity of PAs are[8,11]:

- presence of the 1-2 double bond in the necine base;
- presence of one or preferably two hydroxyl groups in the C-7 and C-9 position in the necine base;
- esterification of at least one of the hydroxyl groups in the necine base; and
- esterification of the hydroxyl group(s) with branched mono- or dicarboxylic acid(s).

8. Upon ingestion, PA N-oxides can be converted in the alimentary tract to the PAs which are then able to cause typical PA toxicity[8].

Sources of PAs

9. Although over 6 000 plant species are reported to contain PAs, direct poisonings in man and animals seem to be associated with only a few species. The plants reported to be associated with poisonings in humans are *Crotalaria*, *Gynura*, *Heliotropium*, *Senecio*, *Symphytum* and *Trichodesma* species[11]. The PA content of plants has been reported as generally varying from 100 mg/kg dry weight to 40 000 mg/kg, although the highest reported is 180 000 mg/kg in *Senecio riddelli*[10]. Both the composition and concentration of PAs may fluctuate according to climatic and environmental conditions, the age and the variety of the plants[12]. Furthermore, various parts of plants have different levels of PAs, some of which may be present predominantly in N-oxide form. For example, in *Senecio vulgaris* and *Senecio jacaobaea*, parts ranked in decreasing concentration of PAs are: flowers and seeds > leaves > stems > roots[13]. Other researchers reported that the roots of *Symphytum officinale* concentrate more PAs (from 1400 to 8300 mg/kg PAs) than the leaves (from 15 to 55 mg/kg)[6].

Sources of Dietary Exposure

10. Humans are thought to be exposed to PAs through plant products (i.e. either herbal products or contamination of grain crops). In developing countries, direct human cases of poisoning due to the consumption of grain or grain products (flour or bread) contaminated with seeds from PA-containing weed species are well documented[2]. The carry-over of plant PAs to other food products such as honey, milk, eggs and offal is also reported to be possible[1,2,5,8-10,12]. Available limited

data indicate that the transfer of PAs from contaminated animal feed into milk and eggs appears to be approximately 0.1 and 1 %, respectively[2]. The transfer of PAs to animal tissues (such as meat and liver) appears to be low and mostly poorly characterised protein adducts appear to be present, but the contribution to carry-over has not been quantified[1,2]. To date, no comprehensive studies for these products are available that would allow further risk evaluation.

11. In the risk assessment conducted in 2001, Australia New Zealand Food Authority (ANZFA) concluded that the major human dietary source of exposure is contaminated grains, with eggs, offal and honey being minor contributors. Table 1 summarises the contamination levels of 1,2-unsaturated PAs in retail honey and tea leaves (infusion) reported in recent European Food Safety Authority (EFSA)'s publications[2,9]. To date, despite studies on dietary exposures to PAs through consumption of a few individual food items (e.g. honey and tea) were reported, estimations on overall dietary exposures to PAs were not identified probably because of insufficient available data[2,14,15].

Table 1. Average content of PAs in honey and tea leaves (infusion) reported in EFSA's publications.

	Average (μg/kg)	
	Lower bound	Upper bound
Retail honey[#]	16	26
Tea leaves (infusion)[*]	6.04	6.38

[#]the mean concentration of 8 PAs.

[*]expressed in μg/L in the original study. Assuming the density of the infusion is equal to that of water, the weight of 1 liter of tea infusion is 1 kilogram.

Toxicity

12. 1,2-unsaturated PAs themselves are not toxic in their original forms but require metabolic activation to exert their toxicities[3,13,16]. Toxicity of the 1,2-unsaturated PAs in animal studies is characterised by hepatotoxicity, genotoxicity and carcinogenicity as well as developmental toxicity. There are also studies showing pulmonary toxicity. The liver is the primary site for genotoxicity of 1,2-unsaturated PAs[2]. 1,2-unsaturated PAs may differ in potency; however, available data are not sufficient to identify relative potency factors for different PAs in order to evaluate the possible effects of combined exposure[15].

Kinetics and metabolism

13. Upon oral exposure, PAs are rapidly absorbed across the gastrointestinal tract and distributed to the liver where metabolism may occur; distribution of PAs to other organs such as kidney and lung has also been reported. The PAs and their N-oxides are highly hydrophilic in nature and are excreted unchanged in the urine within a day[14]. Studies have also shown that once absorbed, three main metabolic pathways which lead to detoxification or to activation of PAs may also occur in the liver[2,8,17,18] (Figure 5):

(i) hydrolysis of the ester group(s) of PAs to form necine bases and necic acids

Necine bases and necic acids are non-toxic products and, due to their high water solubility, can be readily excreted in the urine. Hence, this is regarded as a detoxification pathway. It has been reported that the more highly branched (i.e. the more bulky) the necic acids of the PAs are, the more difficult for hydrolysis of the ester groups to occur. Hence, macrocyclic diesters with more complex acid moieties are more toxic because of their lower rate of hydrolytic detoxification. It

can be generalized that macrocyclic PAs are more toxic than diester PAs, which are more toxic than monoester PAs[5].

(ii) oxidation of PAs (heliotridine type and retronecine type) to their corresponding N-oxides

Being charged molecules, N-oxides are highly water soluble and are rapidly excreted in the urine. Hence, the formation of N-oxides from their corresponding PAs is also considered to be a detoxification pathway. However, this pathway is metabolically reversible and thus does not appear to fully prevent the formation of reactive toxic metabolites from PAs.

(iii) oxidation of PAs to reactive dehydropyrrolizidine ester metabolites (pyrrolic ester).

Pyrrolic esters can further undergo ester hydrolysis leading to the formation of 6,7-dihydropyrrolizine (DHP). Once formed, both the pyrrolic ester metabolites or DHP can rapidly bind with DNA, leading to DNA cross-linking, DNA-protein cross-linking, and DNA adduct formation. Protein binding can alter cell functions and cause cell damage and death while cross-linking to DNA may initiate carcinogenesis.

14. Pyrrolic esters can also react with glutathione (GSH), another pathway of detoxification. Finally, excretion of PAs occurs mainly within 24 hours after exposure via urine and faeces.

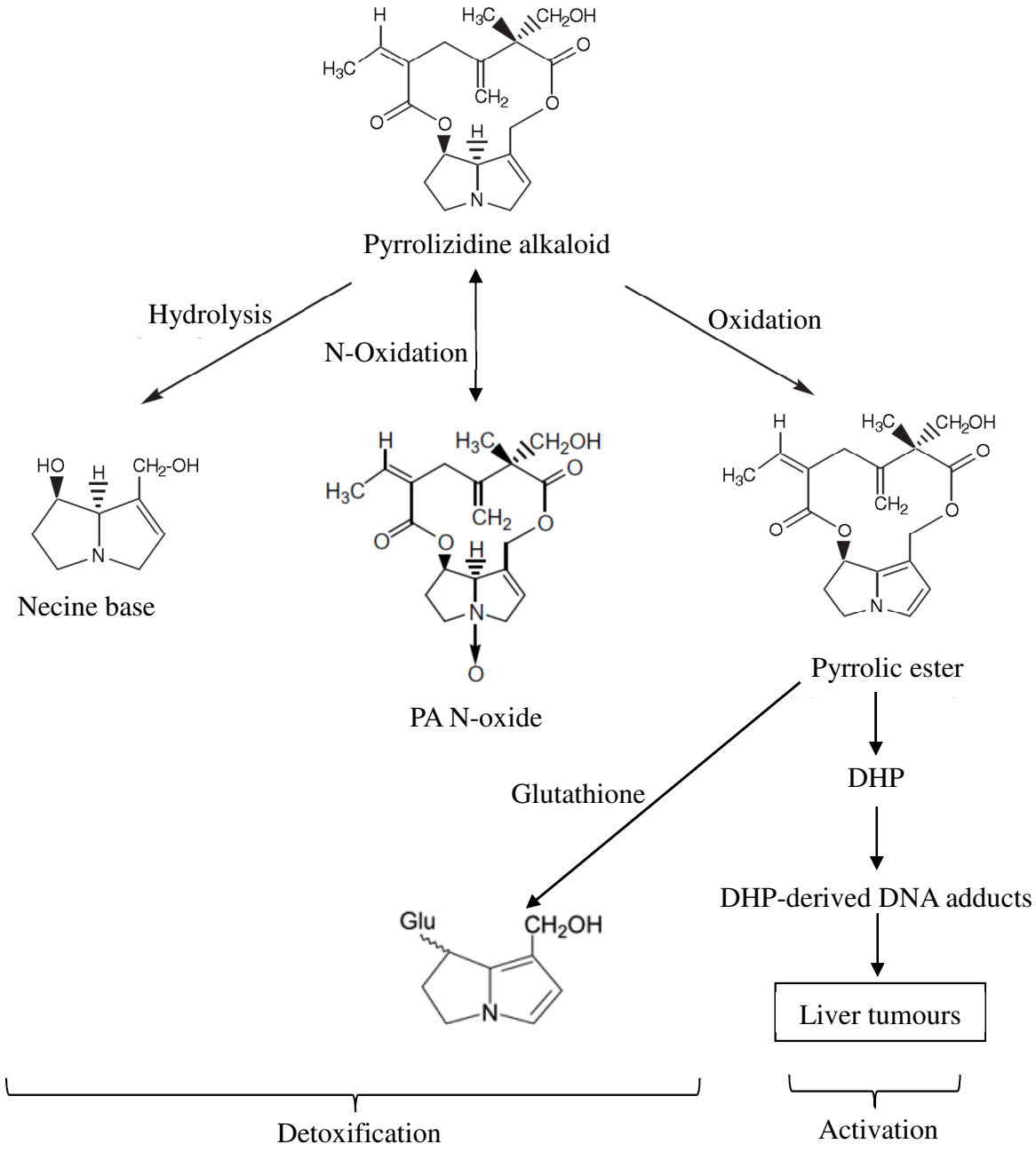


Figure 5. Metabolism pathways of a pyrrolizidine alkaloid.

Acute toxicity

15. In human, liver is the primary site for acute toxicity which is described as acute veno-occlusive disease (VOD). VOD is characterised by hepatomegaly, ascites, massive pleural effusion, and in many cases progressing to cirrhosis[2,19].

The lowest known doses associated with acute toxicity in humans are reported to be 3 mg PA/kg body weight (bw) per day (exposure of a boy for 4 day-period, lethal outcome) and 0.8 -1.7 mg PA/kg b.w. per day (exposure of a girl for a 2 week-period, VOD).[2]. Mortality due to acute poisoning can be high with death due to hepatic failure in the acute phase or due to hematemesis resulting from ruptured oesophageal varices caused by cirrhosis[11]. In man, it is reported that some 50% of patients, following acute poisoning, will recover completely and 20% will die rapidly. Of the survivors, about 20% may go on to develop cirrhosis and liver failure years later. Others may develop subacute liver pathological changes, which will either eventually resolve or go on to cirrhosis and liver failure[14].

Carcinogenicity and genotoxicity

16. The main carcinogenic target site for 1,2-unsaturated PAs in experimental animals is the liver although tumours have been reported in many other tissues, e.g. lung, kidney, skin, bladder, brain and spinal cord, pancreatic islets and adrenal gland[20]. Nonetheless, there are no human epidemiological data suggesting a link of PA exposure and cancer in humans to date[2,20]. The genotoxicity of PAs has been extensively evaluated in *in vitro* and *in vivo* studies. The observations that 1,2-unsaturated PAs from different structural classes (i.e., retronecine, heliotridine, and otonecine) undergo metabolic activation to common reactive pyrrolic intermediates (pyrrolic esters or DHP) and form the same DNA adducts, suggest that a genotoxic carcinogenic mechanism is applicable for all 1,2-unsaturated PAs and their N-oxides, which are metabolically converted into PAs[2].

17. Several PAs have been evaluated by the International Agency for Research on Cancer (IARC) and classified either as Group 2B (i.e. possibly carcinogenic to humans) or Group 3 (i.e. not classifiable as to its carcinogenicity to humans). Lasiocarpine, monocrotaline and riddelliine have been classified by IARC as Group 2B while isatidine, retrorsine, senkirkine, hydroxysenkirkine, jacobine, seneciphylline and symphytine have been classified as Group 3[21-23].

Reproductive toxicity

18. The ability of PAs to cross the placental barrier in the rat and to induce premature delivery or death of litters has been demonstrated. In addition, the embryo in utero appeared to be more resistant to the toxic effects of PAs than the neonate and that PAs were known to have passed through the mother's milk to the sucklings[19]. In 2011, European Food Safety Authority (EFSA) concluded that developmental toxicity of PAs has mainly been observed following parenteral administration in experimental animals, and it is not possible to determine if it is related to maternal toxicity[2].

19. There were reports in the literature showing that exposure of pregnant women to PAs in herbal teas or herbal medicines has caused fatal VOD in neonates. In 2003, a preterm neonate, in Germany, who was symptomatic with hepatomegaly and ascites was delivered by caesarean section for threatening foetal asphyxia and died shortly afterwards. Postmortem examination revealed VOD typical for PA poisoning. The content PAs in the liver was confirmed. Analysis of a herbal mixture which was used daily for cooking in the victim's family revealed high amounts of respective PAs[24].

Health-Based Guidance Values (HBGVs)

20. In 2015, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated PAs and concluded that the genotoxic mode of action of PAs does not allow derivation of a health-based guidance value for chronic toxicity[15]. Instead, JECFA considered the long-term carcinogenicity study on riddelliine (i.e. a PA) more appropriate for dose–response modelling and established a lower limit on the benchmark dose for a 10% excess cancer risk ($BMDL_{10}$) of 182 μg/kg bw per day for liver haemangiosarcoma in female rats treated with riddelliine. The $BMDL_{10}$ was used as the point of departure in the Margin of Exposure (MOE) approach to assess safety concerns arising from the presence of PAs in food[15].

21. The MOE is a tool used for considering possible safety concerns arising from the presence in food of substances which are both genotoxic and carcinogenic. An MOE is defined as the ratio of the $BMDL_{10}$ from an animal study to the level of dietary exposure to the substance considered. In general an MOE of 10 000 or higher (equivalent to the dietary exposure of 0.0182 μg/kg bw/day in the present study), if it is based on the $BMDL_{10}$ from an animal study, would be of low concern from a public health point of view; the magnitude of an MOE however only indicates a level of concern and does not quantify risk[2, 25-27].

Regulatory Control

22. There is currently no international standard for the maximum allowable level of PAs in foods. Some countries (e.g. Australia, Japan, Belgium, etc.) ban the sale or use of certain PA-containing plants (e.g. comfrey and borage) while

others (e.g. Canada) advise consumers not to use or to ingest them[28]. In the Netherlands, a limit value of 1 μg/kg for PAs applies to herbal teas and other food products and beverages containing herbs or herbal extracts, such as soft drinks or sweets containing herbal extracts[29]. Many years ago, Food Standards Australia New Zealand (FSANZ) established a safe level of intake for PAs of 1 μg/kg bw/day based on the known toxicity in humans; however, FSANZ has not established a regulatory level because there is no evidence of harm from normal consumption[30].

23. There is no specific regulation on PAs in foods in Hong Kong. Nevertheless, all foods for sale in Hong Kong must be fit for human consumption.

SCOPE OF STUDY

24. To estimate the dietary exposures to toxic PAs of Hong Kong adult population, this study analysed the amount of twenty-eight individual 1,2-unsaturated PAs (i.e. those with commercially available standard substances) in selected food items – items reported to have been involved in previous human PA poisoning cases or were found to be more likely contaminated with PAs in the literature. These included cereal and cereal products, milk and milk products, eggs, meat and meat products, honey, dried spices, tea beverages and tea leaves (infusion). Tea leaves were classified broadly into two types: common tea leaves and specific tea leaves. Common tea leaves include fermented, semi-fermented and non-fermented tea leaves which are commonly consumed by the general public. Specific tea leaves refer to peppermint tea, Melissa tea, chamomile tea, rooibos tea,

fennel tea, linden tea, verbena tea, nettle tea, cinnamon tea and so on which are less commonly consumed.

METHODOLOGY AND LABORATORY ANALYSIS

Methodology

25. A total of 234 food samples, involving 48 different food items, were collected between August and October 2015 in the local retail markets. The list of food items analysed is provided in Appendix 1. For the sake of estimation of the dietary exposures, all samples except 3 flour items (i.e. wheat flour, barley flour and rye flour) were prepared in a form of food as consumed prior to analysis. The analytical results, where appropriate, were then combined with food consumption information of the population, which were captured from the Hong Kong Population-based Food Consumption Survey (2005-2007)[31] to obtain the dietary exposures of Hong Kong adult population.

26. Dietary exposure estimation, involving food mapping and weighting of data, was performed with the aid of an in-house developed web-based computer system, Exposure Assessment System (EASY). The mean and 95th percentile exposure levels were used to represent the dietary exposures of the average and high consumer of the population respectively.

Laboratory Analysis

27. Laboratory analysis of PAs was conducted by the Food Research Laboratory (FRL) of CFS. A total of 234 samples have been tested for the presence of twenty-eight individual 1,2-unsaturated PAs (Table 2).

28. The homogenised samples were ultrasonic extracted by dilute sulfuric acid. Isotopically labelled PAs were added as internal standards for quantification. The extract was neutralised and purified by C18 solid phase extraction. The PAs were determined by ultra-performance liquid chromatography - tandem mass spectroscopy (UPLC-MS/MS). Limit of Detection (LOD) and Limit of Quantitation (LOQ) of PAs in general food are 0.0050 μg/kg and 0.050 μg/kg respectively. LOD and LOQ of PAs in dried spices are 0.050 μg/kg and 0.50 μg/kg respectively.

Table 2. Twenty-eight 1,2 unsaturated PAs analysed.

Pyrrolizidine alkaloid	
Echimidine	Echimidine N-oxide
Erucifoline	Erucifoline N-oxide
Europine	Europine N-oxide
Heliotrine	Heliotrine N-oxide
Intermedine	Intermedine N-oxide
Jacobine	Jacobine N-oxide
Lasiocarpine	Lasiocarpine N-oxide
Lycopsamine	Lycopsamine N-oxide
Monocrotaline	Monocrotaline N-oxide
Retrorsine	Retrorsine N-oxide
Senecionine	Senecionine N-oxide
Seneciphylline	Seneciphylline N-oxide
Senecivernine	Senecivernine N-oxide
Senkirkine	
Trichodesmine	

Treatment of Analytical Values Below the LOD

29. In this study, data (i.e. concentrations of PAs in each sample as well as dietary exposure estimations) were treated by the lower bound (LB) and upper bound (UB) approach. That is, at the LB, results below the LOD were replaced by zero whilst at the UB, results below the LOD were replaced by the value reported as the LOD[32]. This approach compares the two extreme scenarios, based on the consideration that the true value for results < LOD may actually be any value between zero and the LOD. The LB scenario assumes that the chemical is absent; therefore, to results reported as < LOD a value of zero is assigned. The UB scenario assumes that the chemical is present at the level of the LOD; thus, to results reported as < LOD a value of the corresponding LOD is assigned. In addition, the total 1,2-unsaturated PAs (total PAs) were obtained by summing up the levels of the twenty-eight individual 1,2-unsaturated PAs.

RESULTS AND DISCUSSION

Total PAs in Different Food Groups

30. Of the 234 samples analysed, 118 samples (50%) were detected with at least one 1,2-unsaturated PA. Among these 118 samples, majority (i.e. 91 samples; 77%) belonged to the three food groups, namely “dried spices”, “honey” and “tea leaves (infusion)”. Other items with PAs detected include wheat and rye flour, duck eggs, yoghurts, cheeses, tea beverages, etc. In contrast, all samples in the food group “meat and meat products” were not detected with any PAs.

31. As regards the concentration of PAs in different food groups, “dried spices” contained the highest levels of total PAs, followed by “honey” and “tea

leaves (infusion)". The upper bound mean concentrations of "dried spices", "honey" and "tea leaves (infusion)" were 300 μg/kg, 7.5 μg/kg and 0.46 μg/kg respectively. The concentrations of total PAs in different food groups are summarised in Table 3 and the results of 48 food items are shown in Appendix 1.

Table 3. Estimated total PAs (sum of twenty-eight individual 1,2 unsaturated PAs) in different food groups (mean concentrations (μg/kg) are presented as lower bound (LB) and upper bound (UB)).

Food group	No. of composite samples	% of samples < LOD*	Mean (μg/kg) [range]			
			LB #		UB #	
Cereals and cereal products	21	43	0.17	[0 – 2.5]	0.30	[0.14 – 2.7]
Meat and meat products	35		< LOD in all samples			
Eggs	18	67	0.019	[0 – 0.19]	0.16	[0.14 – 0.33]
Milk and milk products	18	72	0.0040	[0 – 0.048]	0.14	[0.14 – 0.18]
Honey	6	0	7.4	[0.21 – 16]	7.5	[0.31 – 17]
Dried spices	82	26	300	[0 –11 000]	300	[1.4 – 11 000]
Tea leaves (infusion)	48	50	0.33	[0 – 2.6]	0.46	[0.14 – 2.7]
Tea beverages	6	33	0.016	[0 – 0.043]	0.15	[0.14 – 0.17]
Total	234	50				

* LOD: limit of detection; values rounded off to whole figure.
# Values rounded off to two significant figures.

32. Under the food group "dried spices", cumin seed was found to contain the highest level (3.5 – 11 000 μg/kg; mean: 1 900 μg/kg at the UB), followed by oregano (2.5 – 5 100 μg/kg; mean: 1 400 μg/kg at the UB) and tarragon (8.7 – 3300 μg/kg; mean: 1 100 μg/kg at the UB). In fact, these food items were the top three items with the highest levels of total PAs detected in this study (Appendix 1). It is worthwhile to highlight that there was a high variation in the results for the total PA concentration among the samples of the same food item. For example, among the six cumin seed samples, one contained a relatively high level of total

PAs (11 000 μg/kg) while the remaining five samples contained total PAs levels < 100 μg/kg.

33. For the food group "honey", the levels found in this study (0.31 – 17 μg/kg; mean: 7.5 μg/kg at the UB) were low when compared with the concentrations of total PAs reported in the EFSA's publications (Table 1)[2,9]. A recent study showed that honey samples taken from supermarkets in Australia contained PAs with the mean total sum of PAs being 153 μg/kg[34]. PAs may get into the honey when bees forage on the flowers that are rich in PAs (e.g. *Echium plantagineum*, commonly known as Paterson's curse) and the level of contamination depends on the geographical and botanical origin of the honey. According to FSANZ, for people in Australia who normally eat honey derived from flowers other than Paterson's curse, the levels of PAs would not be a cause for concern[30].

34. For the food group "tea leaves (infusion)", while the concentrations of total PAs of all samples of non-fermented tea were below the LOD, rooibos tea (0.36 – 2.7 μg/kg; mean: 1.7 μg/kg at the UB), verbena tea (0.14 – 2.1 μg/kg; mean: 0.87 μg/kg at the UB), peppermint tea (0.14 – 1.4 μg/kg; mean: 0.44 μg/kg at the UB) and chamomile (0.14 – 1.8 μg/kg; mean: 0.43 μg/kg at the UB) had higher concentrations of total PAs (Appendix 1). In fact, rooibos, peppermint and chamomile teas were also reported to contain higher levels of total PAs in other studies and rooibos tea was always found to be the most contaminated[9,35,36]. The levels found in "tea leaves (infusion)" (mean: 0.46 μg/kg at the UB) were higher than those in "tea beverages" (mean: 0.15 μg/kg at the UB); nevertheless, they were relatively low when compared with the concentrations of total PAs reported in other studies (Table 1)[2,9]. Overseas studies have showed that the presence of

PAs in tea samples were most likely the result of contamination caused by co-harvesting of PA-producing plants[37,38]. The German Federal Institute for Risk Assessment (BfR) also reported that the content of PAs in tea samples from different batches may vary considerably in terms of the total PA concentration as well as the PA composition[38].

Dietary Exposure to PAs

35. The LB and UB dietary exposure estimates to total PAs for the average and high consumers of the local population and their corresponding MOEs are shown in Table 4. Dietary exposures to total PAs in the adult population, using LB and UB concentrations, were estimated to range from 0.00033 to 0.0015 μg/kg bw/day and from 0.0015 to 0.0043 μg/kg bw/day for the average and high consumers of the population respectively. The corresponding MOEs (determined by using the $BMDL_{10}$ of 182 μg/kg bw per day for liver haemangiosarcoma in female rats treated with riddelliine) are in the ranges of 560 000 – 120 000 (LB-UB) and 120 000 – 42 000 (LB-UB) for the average and high consumers of the adult population respectively. These MOEs are well above 10 000, indicating a low health concern.

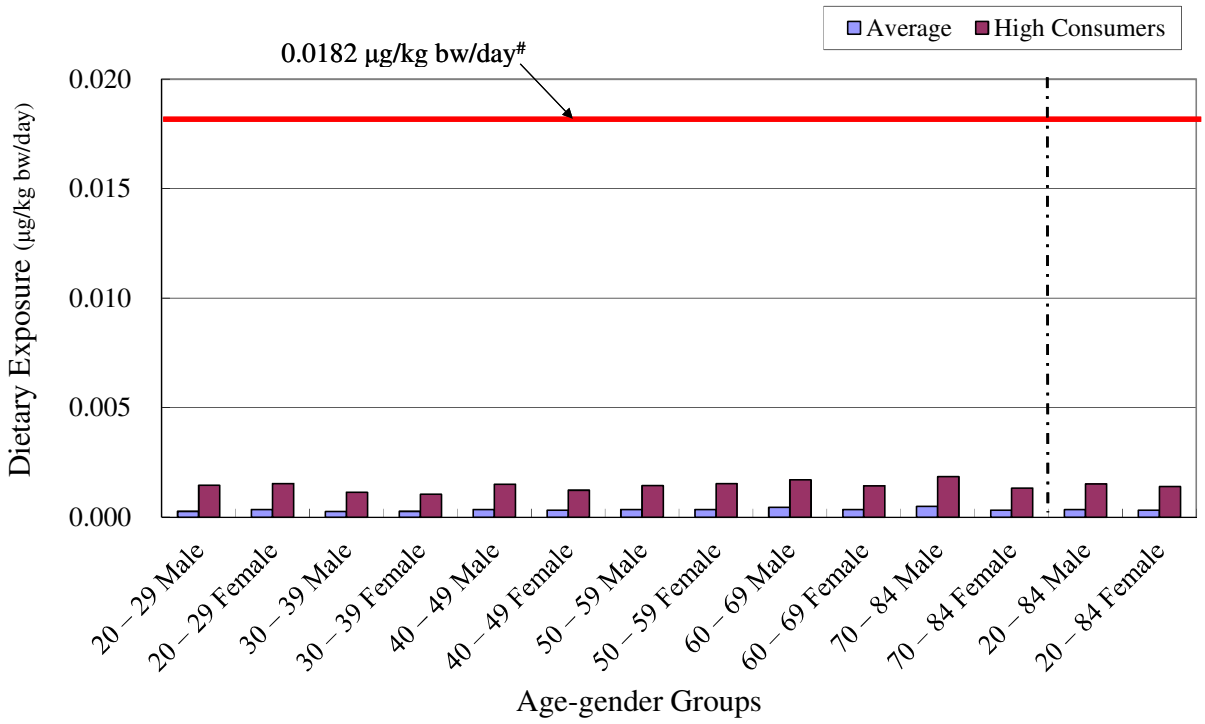
Table 4. Dietary exposures to PAs for the average and high consumers of the local adult population and their corresponding margin of exposures (MOEs).

	Average	High consumer
Dietary exposure (μg/kg bw/day)(LB-UB)	0.00033 – 0.0015	0.0015 – 0.0043
MOE (LB-UB)	560 000 – 120 000	120 000 – 42 000

36. Further analysis of dietary exposures of the individual age-gender population subgroups was performed and the results are shown in Figures 6 and 7

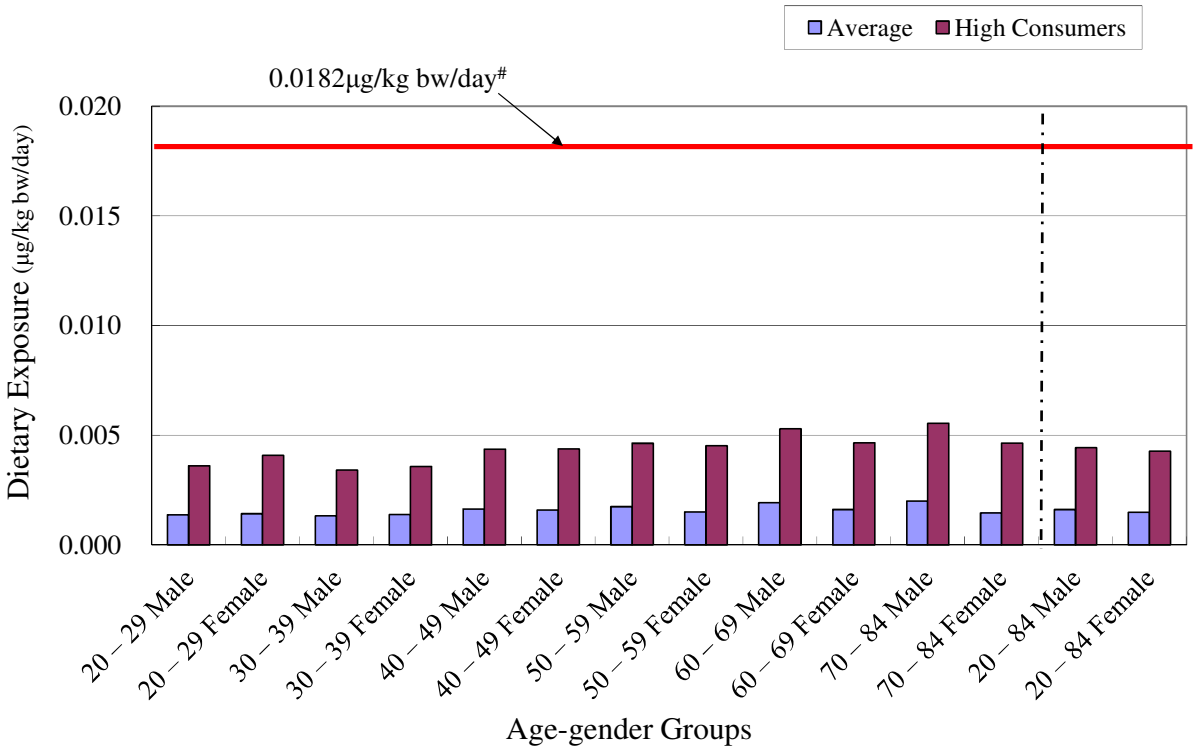
and Appendix 2. The highest dietary exposure among all age-gender population subgroups was found in males aged 70 – 84 (0.00050 – 0.0020 μg/kg bw/day (LB-UB) for the average of the population; 0.0018 – 0.0055 μg/kg bw/day (LB-UB) for the high consumers.

37. All in all, among the various age-gender population subgroups, all the estimated MOEs of the average and high consumers of the population were well above 10 000, indicating a low health concern for all age-gender subgroups.



Level of exposure equivalent to an MOE of 10 000.

Figure 6. Dietary exposures (lower bound) to the total PAs for the average and high consumers of the individual age-gender groups.



Level of exposure equivalent to an MOE of 10 000.

Figure 7. Dietary exposures (upper bound) to the total PAs for the average and high consumers of the individual age-gender groups.

Major Food Contributors

38. Relative contribution of each food group to overall LB PAs dietary exposure for an average consumer of the population is shown in Figure 8. The LB is considered to better reflect the actual food category contribution to overall PA exposure since it is not influenced by the high numbers of samples below the LOD in some food groups[33].

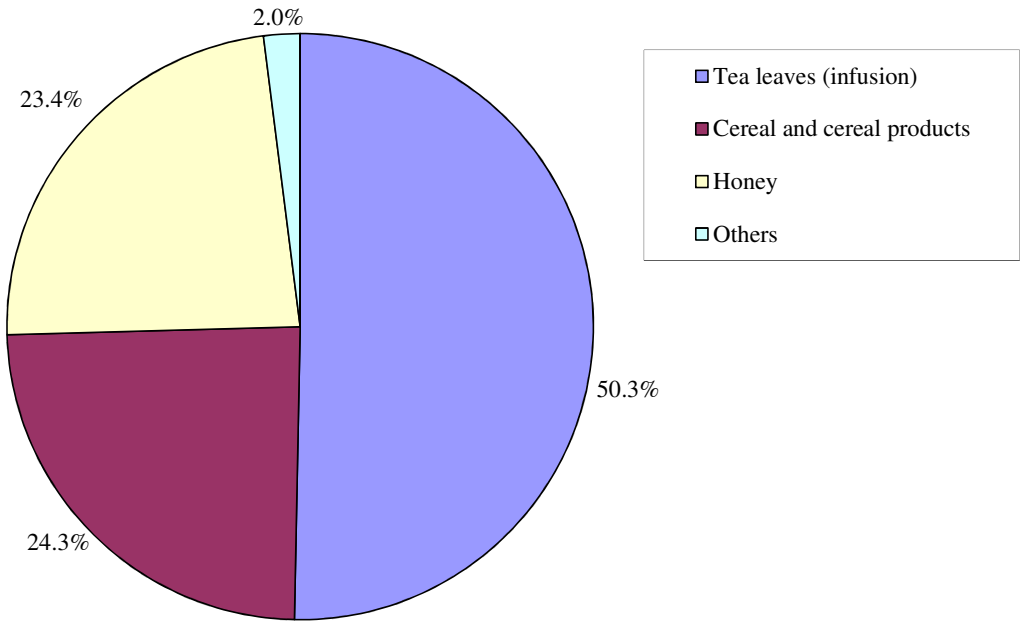


Figure 8. Relative contribution of each food group to overall lower bound total PAs exposure of average consumers in local adult population.

39. In this study, the major contributor to the exposure of total PAs was "tea leaves (infusion)" which contributed up to 50.3% (i.e. 0.00016 μg/kg bw/day at the LB) of the total exposure. The relatively high exposure contribution from "tea leaves (infusion)" in the Hong Kong adult population was likely due to high consumption amount and the relatively low contamination levels of PAs detected in other food groups. The food groups "cereal and cereal products" (i.e. 0.000079 μg/kg bw/day at the LB) and "honey" (i.e. 0.000077 μg/kg bw/day at the LB) altogether contributed less than 48% to the total exposure.

Food Items Not Covered By Exposure Estimation

40. It is necessary to point out that some food items under the food groups "dried spices" and "tea leaves (infusion)" (highlighted in Appendix 1) were not included in the dietary exposure estimation because these food items were not captured in the Hong Kong Population-based Food Consumption Survey (2005-2007). Nonetheless, the conclusions of the dietary exposure estimation to PAs of the local adult population are expected to be similar. This is because food items not captured in the food consumption survey are those that are less commonly consumed by the population and hence exposure to PAs of the general population due to them is probably low. At present, it is not possible to individually assess the long-term health effects of consuming of these products because there are insufficient data on the causes of contamination, batch-to-batch variations of PAs in the products, the consumption patterns of the products in the local population, etc.

Specific teas

41. In this study, all "common teas" (i.e. fully-fermented, semi-fermented and non-fermented teas) and some "specific teas" (e.g. nettle tea, cinnamon tea, fennel tea, linden tea and melissa tea) were found to contain relatively low concentrations of PAs. Of the various types of "specific teas", rooibos tea contained the highest concentration of total PAs (mean: 1.7 μg/kg at the UB), followed by verbena tea (mean: 0.87 μg/kg at the UB) and peppermint tea (mean: 0.44 μg/kg at the UB) (Appendix 1). When compared with overseas studies, the contamination levels of PAs in these specific teas were found to be significantly lower[9,38,39] (Table 5) and therefore a lower level of health concern to local

consumers was expected. However, the BfR has reported that unexpectedly high PA contents were detected in some teas sampled from the markets of Germany and the content of PAs in the tea samples from different batches might also vary considerably[38]. The BfR opined that such teas could pose a health risk to consumers over long period of time and advised the public to widen and alternate their choices of food by including different types of food in their diets in order to avoid excessive exposure to any contaminants, including PAs, from a small range of food items.

Table 5. Average content of total PAs in specific teas (infusion) reported in this study and in EFSA's publications[9,39].

	Tea infusion Average (μg/kg) (lower bound – upper bound)		
	Hong Kong	EFSA(2015)	EFSA(2016)
Rooibos tea	1.60 – 1.70[#]	7.96 – 8.29[*@]	4.1 – 6.3[*@]
Peppermint tea	0.33 – 0.44[#]	6.59 – 6.91[*@]	3.5 – 6.2[*@]
Chamomile tea	0.30 – 0.43[#]	3.63 – 3.96[*@]	2.3 – 4.8[*@]
Verbena tea	0.74 – 0.87[#]	NR[^]	NR[^]

tea leave infusion is prepared either according to the instructions on the label or, if no instructions, with tea leaves (2 grams or 1 tea bag) in 150 mL boiling distilled water for 5 minutes.

* expressed in μg/L in the original study. Assuming the density of the infusion is equal to that of water, the weight of 1 liter of tea infusion is 1 kilogram.

@ to obtain the concentration in infusion: either a 2 grams of tea was extracted with 150 mL of boiling water for 5 minutes or the concentration of PAs in the dry product was divided by a factor of 75.

^ NR = not reported

Dried spices

42. This study also found that under the category "dried spices", cumin seed (mean: 1 900 μg/kg at the UB) contained the highest mean concentration of total PAs, followed by oregano (mean: 1 400 μg/kg at the UB) and tarragon (mean: 1 100 μg/kg at the UB). Since no similar overseas results in dried spices were

identified, a comparison of PAs in dried spices in different places could not be made. Although at present there are insufficient data to assess the possible long-term health effects of consuming of these products, a significant contribution of PAs from dried spices to the overall PAs dietary exposure in the local population is not expected because dried spices are normally used in small amounts as minor ingredients during food preparation.

43. Because of their mutagenic and carcinogenic effects in animal experiments, PAs are undesired in foods and PA contamination in all foods, including tea leaves and dried spices, should be reduced as low as possible. These include the investigation of the causes of contamination and reduction of contamination through improved cultivation, harvesting and cleaning methods during the production of specific tea leaves and dried spices.

Uncertainties and Limitations of the Study

44. Although more accuracy and precision in exposure estimation could be achieved with more samples analysed, compromises had to be made in relation to the use of finite laboratory resources. In this study, only selected food items that were reported more likely to contain PAs were sampled. Furthermore, as the numbers of replicates were limited in this study, the results of this study represented only a snapshot of the PA levels in certain locally available foods.

45. In the Hong Kong Population-based Food Consumption Survey (2005-2007), a set of two non-consecutive days of 24-hour dietary intake questionnaires was used to obtain food consumption information (e.g. the types

and amounts of food consumed) among individuals in Hong Kong. Some food items, likely to be less commonly consumed ones (e.g. those mentioned in para. 40), were inevitably not covered by respondents and hence excluded in the survey, introducing some uncertainties in the results of the overall dietary exposures in this study.

46. There are differences among individual 1,2-unsaturated PAs in terms of their carcinogenic potential and their toxicity; however, available limited data are insufficient to identify their relative potency in order to accurately evaluate their possible combined effects arising from dietary exposure. Hence, the assumption of all 1,2-unsaturated PAs with equal carcinogenic potential and treatment of them as a group of substances with cumulative effect with respect to their carcinogenic effect would likely overestimates the risk.

CONCLUSIONS AND RECOMMENDATIONS

47. The dietary exposures to total PAs in the adult population, using LB and UB concentrations, were estimated to range from 0.00033 to 0.0015 μg/kg bw/day and from 0.0015 to 0.0043 μg/kg bw/day for the average and high consumers of the population respectively and their corresponding MOEs were all well above 10 000, indicating a low health concern from a public health point of view.

48. Some food items under the food groups “specific teas” and “dried spices” were not included in the dietary exposure estimation because these food items were not captured in the Hong Kong Population-based Food Consumption

Survey (2005-2007). For the "specific tea", rooibos tea, verbena tea and peppermint tea were found to contain higher amount of PAs. When compared with overseas studies, the contamination levels of PAs in these specific teas were found to be significantly lower and a lower level of health concern is expected. Some dried spices (such as cumin seed and oregano) also contained PAs. Since dried spices are normally used in small amounts as minor ingredients during food preparation, a significant contribution of PAs from dried spices to the overall PAs dietary exposure in the local population is not expected.

49. Because of their mutagenic and carcinogenic effects in animal experiments, PA contamination of foods should be reduced where possible. Efforts should be made to minimise PA contents in teas and spices. To achieve this, the causes of contamination must be identified and source-directed measures aimed at the prevention and reduction of contamination of food with PAs must be undertaken. Codex Alimentarius Commission has developed the Code of Practice for Weed Control to Prevent and Reduce Pyrrolizidine Alkaloid Contamination in Food and Feed (the Code) with a view to providing guidance on good management practices for weed control of PA-containing plants in order to prevent and reduce the contamination of food and feed with PAs. Generally speaking, companies involved in the production of dried spices and tea leaves should investigate the causes of contamination and make reference to the Code with a view to improving their cultivation, harvesting and cleaning methods so as to reduce PA content in their products.

50. The findings of the dietary exposure to total PAs in the present study did not provide sufficient justifications to warrant changes to the basic dietary advice on healthy eating. The public is advised to maintain a balanced and varied

diet which includes a wide variety of fruit and vegetables so as to avoid excessive exposure to any contaminants from a small range of food items.

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Appendix 1

Sum of twenty-eight 1,2 PAs (μg/kg) in food groups and food items.

Food Item	No. of samples	% of sample < LOD	Mean of total sum of PAs (μg/kg) [range]			
			Lower bound		Upper bound	
Cereals and cereal products:	**21**	**43**	**0.17**	**[0 – 2.5]**	**0.30**	**[0.14 – 2.7]**
Wheat flour	6	17	0.061	[0 – 0.16]	0.19	[0.14 – 0.28]
Barley flour	3		< LOD in all samples			
Rye flour	6	17	0.53	[0 – 2.5]	0.65	[0.14 – 2.7]
Bread, plain *	6	67	0.0034	[0 – 0.015]	0.14	[0.14 – 0.15]
Meat and meat products:	**35**					
Beef	6					
Cattle liver	5					
Pork	6		< LOD in all samples			
Pig liver	6					
Chicken meat	6					
Chicken liver	6					
Eggs:	**18**	**67**	**0.019**	**[0 – 0.19]**	**0.16**	**[0.14 – 0.33]**
Egg, chicken	6	83	0.0082	[0 – 0.049]	0.15	[0.14 – 0.18]
Egg, duck	6	33	0.047	[0 – 0.19]	0.18	[0.14 – 0.33]
Egg, quail	6	83	0.0017	[0 – 0.010]	0.14	[0.14 – 0.15]
Milk and milk products:	**18**	**72**	**0.0040**	**[0 – 0.048]**	**0.14**	**[0.14 – 0.18]**
Milk, whole	6		< LOD in all samples			
Yoghurt	6	50	0.0030	[0 – 0.0070]	0.14	[0.14 – 0.14]
Cheese	6	67	0.0092	[0 – 0.048]	0.15	[0.14 – 0.18]
Honey:	**6**	**0**	**7.4**	[0.21 – 16]	**7.5**	**[0.31 – 17]**
Dried Spices:	**82**	**26**	**300**	**[0 –11 000]**	**300**	**[1.4 – 11 000]**
Oregano #	6	0	1 400	[1.5 – 5 100]	1 400	[2.5 – 5 100]
Rosemary #	6	33	0.25	[0 – 0.99]	1.6	[1.4 – 2.3]
Tarragon #	3	0	1 100	[8.0 – 3 300]	1 100	[8.7 – 3 300]
Thyme #	6	0	6.1	[0.18 – 11]	7.0	[1.5 – 12]
Basil	6	17	1.4	[0 – 5.2]	2.6	[1.4 – 6.2]
Mint	3	67	0.72	[0 – 2.2]	2.0	[1.4 – 3.3]
Marjoram #	2	50	0.22	[0 – 0.43]	1.6	[1.4 – 1.7]
Dill Weed #	4	0	37	[0.33 – 85]	38	[1.5 – 86]
Herbs de Provence #	4	0	360	[18 – 1 300]	370	[18 – 1 300]
Bay leaf #	5	20	0.17	[0 – 0.27]	1.5	[1.4 – 1.5]
Black pepper	6	33	0.19	[0 – 0.63]	1.5	[1.4 – 1.9]
White pepper	6	83	0.11	[0 – 0.68]	1.5	[1.4 – 1.9]
Five spices powder	5	40	2.3	[0 – 4.7]	3.5	[1.4 – 5.7]
Cumin Seed #	6	0	1 900	[2.5 – 11 000]	1 900	[3.5 – 11 000]
Fennel seed #	4	50	0.90	[0 – 2.6]	2.2	[1.4 – 3.8]
Cloves	5	60	0.024	[0 – 0.062]	1.4	[1.4 – 1.4]

Food Item	No. of samples	% of sample < LOD	Mean of total sum of PAs (μg/kg) [range]			
			Lower bound		Upper bound	
Sage #	**5**	0	34	[3.5 – 78]	34	[4.2 – 79]
Tea leaves (infusion)[@]:	**48**	**50**	**0.33**	**[0 – 2.6]**	**0.46**	**[0.14 – 2.7]**
Common tea						
Fully-fermented tea	6	50	0.088	[0 – 0.43]	0.22	[0.14 – 0.55]
Semi-fermented tea	4	50	0.021	[0 – 0.079]	0.15	[0.14 – 0.20]
Non-fermented tea	6		< LOD in all samples			
Specific tea						
Peppermint tea #	5	60	0.33	[0 – 1.3]	0.44	[0.14 – 1.4]
Melissa tea #	1		< LOD			
Chamomile Tea #	6	50	0.30	[0 – 1.7]	0.43	[0.14 – 1.8]
Rooibos tea #	6	0	1.6	[0.23 – 2.6]	1.7	[0.36 – 2.7]
Fennel tea #	2	0	0.035	[0.016 – 0.053]	0.16	[0.15 – 0.17]
Linden tea #	2		< LOD in all samples			
Verbena tea #	3	33	0.74	[0 – 2.0]	0.87	[0.14 – 2.1]
Nettle tea #	1	0	0.053	–	0.18	–
Cinnamon tea #	3	67	0.0040	[0 – 0.012]	0.14	[0.14 – 0.15]
Others #^	3	33	0.025	[0 – 0.067]	0.16	[0.14 – 0.19]
Tea beverages:	**6**	**33**	**0.016**	**[0 – 0.043]**	**0.15**	**[0.14 – 0.17]**

* Consumption data of bread items under cereal and cereal products were disaggregated into their ingredients. Therefore, analytical results of "bread, plain" were not included in the dietary exposure where dietary exposure from bread was estimated based on the analytical results of various types of flours.

@ tea leave infusion is prepared either according to the instructions on the label or, if no instructions, with tea leaves (2 grams or 1 tea bag) in 150 ml boiling distilled water for 5 minutes.

Analytical results of food items, where appropriated consumption data are not available in the Population-based Food Consumption Survey, were not included in the dietary exposure assessment.

^ include spearmint tea, marjoram tea and mixed tea.

Appendix 2

Lower Bound (LB) and Upper Bound (UB) Dietary Exposure to the sum of 1,2 unsaturated PAs by Age-Gender Group (Average and High Consumer of the Population)

Age-gender Groups	Dietary Exposure (µg/kg bw/day)	
	Average (LB-UB)	High Consumer* (LB-UB)
Male aged 20 – 29	0.00026 – 0.0014	0.0015 – 0.0036
Female aged 20 – 29	0.00035 – 0.0014	0.0015 – 0.0041
Male aged 30 – 39	0.00026 – 0.0013	0.0011 – 0.0034
Female aged 30 – 39	0.00026 – 0.0014	0.0010 – 0.0036
Male aged 40 – 49	0.00035 – 0.0016	0.0015 – 0.0044
Female aged 40 – 49	0.00032 – 0.0016	0.0012 – 0.0044
Male aged 50 – 59	0.00035 – 0.0017	0.0014 – 0.0046
Female aged 50 – 59	0.00034 – 0.0015	0.0015 – 0.0045
Male aged 60 – 69	0.00044 – 0.0019	0.0017 – 0.0053
Female aged 60 – 69	0.00034 – 0.0016	0.0014 – 0.0046
Male aged 70 – 84	0.00050 – 0.0020	0.0018 – 0.0055
Female aged 70 – 84	0.00032 – 0.0015	0.0013 – 0.0046
Male aged 20 – 84	0.00034 – 0.0016	0.0015 – 0.0044
Female aged 20 – 84	0.00032 – 0.0015	0.0014 – 0.0043
Adult aged 20 – 84	0.00033 – 0.0015	0.0015 – 0.0043

*Exposures of high consumers refer to the exposures at 95th percentile.