Risk Assessment Studies
Report No. 39

Ethyl Carbamate in Local Fermented Foods

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GLOSSARY

Adduct	A complex that forms when a chemical binds to a biological molecule, such as DNA or a protein.
BMDL10	Benchmark Dose Lower Confidence Limit 10%: the lower bound of a 95% confidence interval on a benchmark dose corresponding to a 10% incidence of a response (e.g. tumour) in experimental animals.
Genotoxicity	The capacity of an agent to cause damage to DNA.
LD_{50}	Lethal Dose 50: the dose of an agent expected to kill 50% of organisms in a sample population under a defined set of conditions.
Multisite Carcinogen	An agent that causes cancer in multiple organ or tissue sites.
Mutagenicity	The capacity of an agent to cause permanent heritable changes to the genetic material in a cell.
MOE	Margin of Exposure: the ratio of the BMDL10 to the estimated intake in humans.

EXECUTIVE SUMMARY

The Centre for Food Safety (CFS) has conducted a study to examine the level of ethyl carbamate (EC) in local fermented foods and beverages, and to assess the associated health risk posed to the population through dietary exposure to EC. On the basis of the EC levels measured in this study and reported overseas, advice to the trade and the public was formulated.

Ethyl carbamate, also known as urethane, is naturally formed in fermented foods during the fermentation process or during storage. Variable levels of EC have been found in different fermented foods such as bread, soy sauce and yogurt, and in alcoholic beverages such as spirits, grape wine and beer. While studies carried out overseas mainly focused on the food items of the Western diet, little is known about the amount of EC in common local fermented foods.

Public health concerns regarding EC in foods are related to its carcinogenic potential. In 2007, the International Agency for Research on Cancer (IARC) reassessed EC and up-graded its classification from Group 2B ("possibly carcinogenic to humans") to Group 2A ("probably carcinogenic to humans"). The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) evaluated EC in 2005 and concluded that intake of EC from foods excluding alcoholic beverages would be of low concern. However, dietary exposure to EC from both food and alcoholic beverage was of concern and measures to reduce concentrations of EC in some alcoholic beverages were recommended.

In this study, sampling of fermented foods was carried out from late December 2007 to March 2008. The level of EC was analysed in 276 food and beverage samples including 70 alcoholic beverages. Laboratory analysis was conducted by the Food Research Laboratory of the CFS.

Results

Ethyl carbamate was detected in 202 / 276 samples analysed (73%). Levels of EC ranged from not-detected to 650 μg/kg. Fermented soy products (fermented red bean curd, fermented bean curd) and alcoholic beverages (yellow wine, sake and plum wine) were among the food items found with relatively high EC levels, while other fermented foods such as fermented cereals and grains products, preserved vegetables, fermented dairy products, fermented fish products (salted fish) and fermented tea (Chinese tea) contained low or non-detectable levels.

Dietary exposure to EC was estimated for the average population in Hong Kong using the mean EC concentrations. When the estimated average dietary exposure to EC was compared to the Benchmark Dose Lower Confidence Limit 10% (BMDL10) (i.e. a benchmark dose causing 10 % tumour incidence in experimental animals), a large margin of exposure (MOE) of around 3.6×10^4 was found indicating a low potential health concern for the average local population. However, for high consumers of alcoholic beverages such as distilled spirits, plum wine and grape wine, lower MOE values of 1.9×10^3 to 3.5×10^3 were observed suggesting potential health concern for this subpopulation.

Conclusion and recommendation

Results of this study show that EC was present in varying amounts in different local fermented food and beverage items at generally low levels. The food group "alcoholic beverages" was identified as the main dietary source of EC, followed by the "fermented cereals and grains products (bread/rolls/buns and crackers)" and "legumes (fermented soy products)". For the general population, dietary exposure to EC from normal consumption of fermented foods and beverages are unlikely to pose health concern. However, for high consumers of alcoholic beverages, health risk of EC cannot be ruled out.

Advice to consumers

1. Maintain a balanced diet. Avoid overindulgence of fermented foods and beverages, in particular alcoholic beverages.
2. Store fermented foods and beverages in a cool place under low light conditions.
3. Avoid stocking up excessive fermented foods and beverages to minimise the duration of storage.

Advice to the trade

1. Manufacturers should follow good manufacturing practice (GMP). Develop mitigation measures to reduce the levels of EC in fermented foods and beverages, e.g. identifying and reducing the amount of precursors.
2. Use proper containers to protect fermented foods and beverages from light exposure.
3. Shippers, distributors, wholesalers and retailers should minimise heat and light exposure during transportation and storage of fermented food and beverage products.
4. Obtain fermented foods and beverages from reliable suppliers.
5. Keep stock according to the first-in-first-out principle.

Risk Assessment Studies –

Ethyl Carbamate in Local Fermented Foods

OBJECTIVES

1. The study aims to

(i) examine the level of ethyl carbamate in local fermented foods and alcoholic beverages, and

(ii) assess the associated health risk posed to the population through dietary exposure to ethyl carbamate.

INTRODUCTION

2. Ethyl carbamate (EC), also known as urethane, is a chemical contaminant naturally formed in fermented foods during the fermentation process or during storage. Measurable levels of EC have been found in foods such as bread, soy sauce and yogurt, and in alcoholic beverages such as spirits, wine and beer. The health concern of EC in food is related to its carcinogenic potential. Ethyl carbamate has a history of use in industry, medicine and veterinary applications. Its use in human medicine was later banned due to toxicological concerns and lack of efficacy.

3. The levels of EC present in different alcoholic beverages and fermented foods have been extensively reviewed in overseas countries. Studies demonstrated that foods produced at least partially by yeast fermentation seemed to be much more likely to contain EC than those fermented by lactic acid bacteria, acetic acid bacteria

and moulds, while the non-yeast fermented products (e.g. cheese) usually contained low to non-detectable levels of EC.

4. There is currently no international standard for the maximum allowable level of EC in foods. However, some countries have established maximum levels of EC in alcoholic beverages (Annex I). [2,15,16] Canada was the first country to introduce maximum levels for ethyl carbamate in a variety of alcoholic beverages, from 30 μg/L for wine to 400 μg/L for fruit brandies. [2] The US has voluntary target levels of EC for her national food products, and has notified all countries exporting wines to the US that they must develop programs to meet these target levels. There are currently no harmonized maximum levels of EC in foods in the European Union, although some member states have recommended maximum EC levels in alcoholic beverages. Recently, Korea has also established a maximum EC level of 30 μg/L in wine. [15, 16]

Local situation

5. At present, there is no subsidiary legislation governing the maximum level of EC in fermented foods and alcoholic beverages in Hong Kong. While previous studies carried out overseas mainly focused on food items of the Western diet, no comprehensive studies on the level of contamination by EC in commonly consumed local fermented foods and beverages have been conducted. The health risk associated with dietary exposure to EC upon consumption of fermented foods and beverages for the local population is not clear.

6. It is important that a risk assessment study on EC be conducted, particularly on the local fermented foods such as preserved vegetables, fermented soy products, local condiments and sauces, and alcoholic beverages including Chinese fermented

wines. The study would be of value to both the local and international community, for providing first-hand information on the level of contamination of EC in the commonly consumed local fermented foods in Hong Kong, for assessment of the associated health risk of dietary exposure to EC of the local population, and for submission of the EC contamination data to the JECFA database.

HAZARD IDENTIFICATION

7. Ethyl carbamate is the ester of carbamic acid. It is naturally formed in fermented foods mainly as a byproduct of fermentation, primarily from the reaction of alcohol (ethanol) with urea and its break-down products. Cyanate is probably the ultimate precursor in most cases, reacting with ethanol to form the carbamate ester.

8. Ethyl carbamate can be formed from various substances derived from fermented foods and beverages, including urea, hydrogen cyanide, citrulline and other N-carbamyl compounds. For example, in stone fruit spirits, cyanogenic glycosides from the stones may be degraded through enzymatic action to cyanide which is then oxidised to cyanate and reacts with ethanol to form EC. Another example is the formation of urea from the degradation of arginine by yeast fermentation, which is then broken down to isocyanate and reacts with ethanol to form EC. The chemical reaction between urea and ethanol has been found to accelerate exponentially with increase in temperature. Besides temperature, the presence of light and duration of storage are the other two key factors influencing the formation of ethyl carbamate in fermented foods. [1,2]

9. The main source of dietary exposure to EC in human population is through the consumption of fermented foods and beverages containing EC. Alcoholic beverage is the known main contributor to EC exposure. [4,5]

HAZARD CHARACTERISATION

10. The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) evaluated EC in 2005 and concluded that the chemical was genotoxic and a multisite carcinogen in all animal species tested and a potential carcinogen in human.[5] In 2007, the International Agency for Research on Cancer (IARC) re-classified EC as "probably carcinogenic to humans" (Group 2A).[3]

Kinetics and metabolism

11. Ethyl carbamate is absorbed rapidly and almost completely from the gastrointestinal tract and is evenly distributed throughout the body. It is also eliminated quickly. In mice, more than 90% of the absorbed EC is eliminated as carbon dioxide within 6 hours.[4]

12. Ethyl carbamate is metabolised via three main metabolic pathways, namely, hydrolysis, hydroxylation and side-chain oxidation. Vinyl carbamate epoxide, one of the metabolic intermediates of EC via side-chain oxidation, is considered the main metabolite responsible for its carcinogenicity. It binds covalently to nucleic acids and proteins, resulting in the formation of adducts including those that have been shown to induce base-pair substitutions in DNA from tumour tissue. Vinyl carbamate epoxide is further metabolised to carbon dioxide and ammonia and excreted in the urine.[2,4]

Acute toxicity

13. The acute oral toxicity of ethyl carbamate is low, with oral lethal dose 50 (LD_{50}) in rodents being approximately 2000 mg/kg bw. In rodents, single doses of 1000 mg/kg bw cause anaesthesia.[4]

Developmental toxicity

14. While no multigeneration studies that meet currently accepted standard protocols are available, dose-related increases in skeletal anomalies have been observed in mice given single oral doses of EC at 300 – 1000 mg/kg bw on day 11 of gestation. Increases in external malformations and skeletal abnormalities have also been reported in rats given EC at daily doses of 1000 mg/kg bw by gavage for 1, 2, 6 or 7 consecutive days during the period of organogenesis (days 6 – 13 of gestation).[4]

15. Treatment of female mice with single or multiple doses of EC during gestation or lactation was found to increase the incidence or multiplicity of tumours in the adult offspring compared with untreated controls.[4]

Mutagenicity and genotoxicity

16. Ethyl carbamate is considered a genotoxic mutagen in experimental animals. It has been tested in a large number of genotoxicity studies in vitro and in vivo. The results of assays for point mutations were uniformly negative for mouse lymphoma cells, while assays in bacterial, yeast and other types of mammalian cells produced variable results. Results of assays in somatic cells in vivo (including tests for induction of chromosomal aberrations, micronucleus formation and sister chromatid exchange) were almost uniformly positive. The assay for micronucleus formation in

mice showed the strongest positive response, and co-administration of ethanol delayed, but did not inhibit the genotoxicity of EC in this assay. The results of assays for dominant lethal mutations or specific locus tests in mice, given EC either by intraperitoneal injection or in drinking-water, revealed no evidence for genotoxicity in mammalian germ cells in vivo.[4]

Carcinogenicity

17. Ethyl carbamate is a multisite carcinogen with a short latency period. Single doses or short-term oral dosing at 100 – 2000 mg/kg bw have been shown to induce tumours in mice, rats and hamsters. The upper range of these doses overlaps with the standard anaesthetic dose (1000 mg/kg bw) and the values of LD_{50} in rodents. In a 2-year oral study in mice, dose-dependent increase in the incidence of multisite tumours (including alveolar and bronchiolar, hepatocellular and haederian gland adenoma or carcinoma, hepatic haemangiosarcoma, and mammary gland adenoacanthoma or adenocarcinoma) was observed.[4]

18. In addition, non-human primates administered EC orally at a dose of 250 mg/kg bw/day for 5 years were found to develop, over an observation period of up to 22 years , a variety of tumour types analogous to those observed in rodents including adenocarcinoma of lung, hepatocellular adenoma and carcinoma and hepatic haemangiosarcoma.[4]

19. In 2007, the IARC updated the classification of EC from "possibly carcinogenic to humans" (Group 2B) to "probably carcinogenic to humans" (Group 2A). The reclassification is based on the findings that (i) experimental evidence suggests great similarities in the metabolic pathways of EC activation in rodents and humans; and (ii) the formation of DNA-reactive proxi-mate carcinogens which are

thought to play a major role in the EC-induced carcinogenesis in rodents probably also occurs in human cells.[3]

Observation in human

20. There are at present very few EC data in humans. JECFA considered in its 2005 evaluation that the human data available were not of a quality that could be used for hazard characterisation.[4]

Risk assessment for ethyl carbamate

21. JECFA evaluated EC in its 64th meeting in 2005 and concluded that EC was genotoxic and a multisite carcinogen in all animal species tested and a potential carcinogen in human.[5]

22. The pivotal toxicological study for risk assessment was a carcinogenicity study of EC in mice. The increased incidence of lung tumour (alveolar and bronchiolar adenoma or carcinoma) was considered the critical toxicological end-point of concern. The associated dose-response data were analysed and the Benchmark Dose Lower Confidence Limit 10% ($BMDL_{10}$) for EC was determined. The $BMDL_{10}$ represents the lower bound of a 95% confidence interval on a benchmark dose corresponding to a 10% tumour incidence (i.e. 10% extra risk of tumours) in experimental animals. The EC margin of exposure (MOE), defined as the ratio of $BMDL_{10}$ to the estimated dietary intake of EC in humans, was then calculated and used by the Committee as an index for the assessment of the potential health risk posed by EC to humans.[5]

23. When the estimated intake of EC in foods excluding alcoholic beverages

(15 ng/kg bw/day) was compared with the lower end (0.3 mg/kg bw/day) of the range of $BMDL_{10}$ values (0.3 to 0.5 mg/kg bw/day) for alveolar / bronchiolar neoplasms in mice, a MOE of 20,000 was estimated. With the inclusion of alcoholic beverages in the estimated intake of EC (80 ng/kg bw/day), the resulting MOE was only 3,800. The Committee concluded that exposure to EC in foods other than alcoholic beverages would be of low concern, but the estimated health risk posed by total dietary exposure to EC (in all foods and beverages) would be of potential concern and therefore mitigation measures to reduce concentrations of EC in some alcoholic beverages should be continued.[5]

EXPOSURE ASSESSMENT

Scope of study

24. To estimate the dietary exposure to EC, this study focused on the local fermented foods and beverages, both prepackaged and non-prepackaged, readily available in Hong Kong. Nine major food groups were included in this study, namely (i) fermented cereals and grains products (bread/rolls/buns and crackers); (ii) legumes (fermented soy products); (iii) preserved/dried vegetables; (iv) meat products (fermented pork products); (v) fermented dairy products; (vi) fermented fish products; (vii) condiments and sauces; (viii) non-alcoholic beverages; and (ix) alcoholic beverages.

Methodology

Sampling

25. Health inspectors of the Food Surveillance Unit undertook sampling from late December 2007 to March 2008. The food samples were obtained from various local sources, including wet markets, supermarkets, bakery shops, etc., in different districts of Hong Kong.

26. A total of 276 samples of fermented products in the 9 major food groups were collected for EC analysis. The types and number of individual food items sampled are shown in Annex II.

Laboratory analysis

27. Determination of EC was conducted by the Food Research Laboratory (FRL) on an individual sample basis. A total of 276 food and beverage samples were analysed.

28. For perishable food items, samples were kept at – 20 °C until analysis. A control standard was included in each batch of 20 analyses. The limit of detection for both solid and liquid samples was 0.4 µg/kg.

29. The food sample was extracted with dichloromethane. After extraction, the sample fraction was concentrated by solvent evaporation. The concentrate then underwent a florisil SPE cleanup to remove interfering compounds from the sample matrix. The EC content in the cleaned-up sample was determined by GC-MS operated in the Selective Ion Monitoring (SIM) mode. For trace level of EC, the HPLC-MS/MS technique was applied instead and the results were further confirmed by GC-HRMS. [7,8,9,10]

30. For tea samples, the level of EC was first determined for the tea leaves.

For those tea leaf samples with detectable levels of EC, the level of EC in the brewed tea samples was further determined. Brewed tea was normally prepared and consumed at a concentration of 2 grams tea leaves in 200 ml water.[14] For the present chemical analysis, a 5-times more concentrated liquid tea sample was prepared so that measurable quantities of EC could be obtained. 200 ml of distilled water at 90 ˚C was added to 10 grams of tea leaves and the tea was brewed for 30 minutes. The tea liquid for EC determination was obtained after decanting the tea leaves.

Assessment of dietary exposure

31. To estimate dietary exposure to EC in the population, the mean EC concentration was used for each food item. Considering that a number of test samples had EC levels below the detection limit and the median EC values were in most cases lower than the mean EC values (Annex II), the mean EC values were used for exposure estimate as a conservative approach for the protection of public health. The mean contamination levels were also used for exposure assessment in the JECFA 2005 evaluation since it was assumed that the chronic nature of the hazard posed by EC would allow a consumer to be exposed to an average amount of EC in any given foodstuff over a lifetime.[4,5]

32. The food consumption data used in the present study was extracted from the Hong Kong Population-Based Food Consumption Survey 2005-2007 commissioned by FEHD. This survey investigated the food consumption of a population-based sample of 5,008 Hong Kong adults aged 20 to 84 years, selected through an anonymous and scientific household address sampling procedure. Food consumption data were collected by both 24-hour dietary recalls and food frequency questionnaire. The survey results have been age- and gender- weighted and represent a population of

5,394,072 Hong Kong residents aged 20-84.

33. For this study, matching food items for which preliminary weighted consumption data were available from the Hong Kong Population-Based Food Consumption Survey 2005-2007 were used in the exposure assessment. Daily dietary exposure to EC from each food item was obtained by combining the weighted population mean consumption data and the mean EC level of that food item. Total exposure for an individual was obtained by summing EC exposures from all food items investigated.

Results

Concentrations of EC in local foods and beverages

34. The mean, median and concentration range of EC levels in individual food and beverage items are listed in Annex II. Food group means and concentration ranges of EC contamination are summarised in Table 1. A total of 276 food samples including 70 alcoholic beverage samples were analysed for individual EC levels. Ethyl carbamate was detected in 202 samples (73%). The mean and median EC levels for all food samples investigated were 25.2 μg/kg and 1.5 μg/kg, respectively. Concentrations of EC ranged from non-detected (ND) to 650 μg/kg.

Table 1: Concentrations of ethyl carbamate in various food groups

Food/ beverage group	No. of samples	EC concentration (µg/kg) Mean	Range
Fermented cereals and grains products	**25**	**2.01**	**ND – 8.6**
➢ Bread/ Rolls/Buns	15	2.63	ND – 8.6
• Chinese steamed bun	5	0.20	ND
• Bread and toasted bread	10	3.85	1.0 – 8.6
➢ Crackers	10	1.08	ND – 5.1
Legumes (fermented soy products)	**20**	**121**	**ND – 650**
• Fermented bean curd	6	80.7	11 – 130
• Fermented red bean curd	5	386	150 – 650
• Fermented black soy bean	5	2.22	ND – 7.0
• Stinky tofu	4	0.20	ND
Preserved /dried vegetables	**45**	**3.03**	**ND – 10**
Meat products (fermented pork products)	**5**	**18.0**	**12 – 29**
Fermented dairy products	**11**	**0.39**	**ND – 1.1**
• Cheese	5	0.44	ND – 1.1
• Yogurt	3	0.50	ND – 1.1
• Dairy-based fermented beverages	3	0.20	ND
Fermented fish products (salted fish)	**5**	**0.20**	**ND**
Condiments and sauces	**55**	**5.11**	**ND – 44**
• soy sauce	5	6.84	1.8 – 17
• oyster sauce	5	0.54	ND – 1.1
• vinegar	18	9.32	ND – 37
• condiment and savory sauces	27	2.84	ND – 44
Non-alcoholic beverages	**40**	**1.09**	**ND – 15**
➢ Vinegar drink (fruit vinegar)	5	1.54	0.4 – 3.0
➢ Tea (tea leaves)	35	1.03	ND – 15
• fully fermented tea (black tea)	5	3.26	ND – 15
• semi-fermented tea (Chinese tea)	30	0.65	ND – 5.1
Alcoholic beverages	**70**	**55.9**	**ND – 390**
➢ Beer/ale	15	1.13	ND – 5.8
➢ Wine or spirit made from cereals and grains	30	93.7	2.0 – 390
• Yellow wine	6	265	140 – 390
• Sorghum-based spirit	3	54.3	37 – 66
• Rice wine	21	50.4	2.0 – 330
- Chinese rice wine	12	32.1	3.3 – 62
- Sake	9	74.7	2.0 – 330
➢ Wine made from fruit			
• Grape wine	10	21.2	6.7 – 47
- Red wine	5	17.7	8.3 – 35
- White wine	5	24.7	6.7 – 47
• Plum wine	5	110	0.4 – 230
• Cider	5	6.90	ND – 31
➢ Compound alcoholic beverages	5	57.6	17 – 150
➢ Distilled spirits (Chinese distilled spirits)*	9	36.5	20 – 66
Total	**276**	**25.2**	**ND – 650**

ND = non-detect

*Included distilled rice wine and distilled sorghum-based spirit listed above

35. Among the different food groups, legumes (fermented soy products) showed the highest mean EC concentration (121 μg/kg), followed by alcoholic beverages (55.9 μg/kg). For individual food items, highest EC levels were observed in fermented red bean curd (mean: 386 μg/kg; range: 150 – 650 μg/kg) and Chinese yellow wine (mean: 265 μg/kg; range: 140 – 390 μg/kg). Ethyl carbamate was not detected in the following food items tested: Chinese steamed bun, stinky tofu, dairy-based fermented beverages, salted fish and shouwei tea.

Food consumption data

36. Food consumption data for the nine groups of local fermented foods and beverages are given in Table 2. The mean quantities of matched food items consumed per capita, weighted by age and gender, for the Hong Kong population were obtained from the average food consumption data of 5008 respondents.

Table 2: Weighted population mean consumption data for the 9 groups of local fermented foods and beverages (preliminary data)

Food Group	**Mean per capita consumption (g / day)**
Fermented cereals and grains products (bread/rolls/buns and crackers)	48.2
Legumes (fermented soy products)	0.66
Preserved /dried vegetables	2.13
Meat products (fermented pork products)	0.47
Fermented dairy products#	3.97
Fermented fish products	0.40
Condiments and sauces#	6.16
Non-alcoholic beverages#	345
Alcoholic beverages*	33.1

* Mean per capita consumption for liquid item is expressed in ml/day.

Food group composed of solid and liquid items. The weight of liquid food was assumed to be 1g per 1ml when calculating the amount of food group consumption.

Dietary exposure to EC

37. Table 3 shows the estimated dietary exposure to EC for the average population and the percentage contributions from different food groups to the total dietary exposure. Based on the preliminary weighted population mean consumption data from the Hong Kong Population-Based Food Consumption Survey, dietary exposure to EC excluding alcoholic beverage was estimated to be 5.42 ng/kg bw/day, while the total dietary exposure from all foods and alcoholic beverages was estimated to be 8.27 ng/kg bw/day. Among the 9 food groups, alcoholic beverages (34.5%) and fermented cereals and grains products (33.5%) were the major contributors to dietary exposure to EC while fermented fish products (0.1%) and dairy products (0.3%) contributed the least.

Table 3: Estimated dietary exposure to ethyl carbamate for average population and percentage contributions from various food groups

Food Groups	**Dietary Exposure (ng/kg bw/day)**	**% contribution**
Fermented cereals and grains products (bread/rolls/buns and crackers)	2.77	33.5
Legumes (fermented soy products)	0.98	11.8
Preserved /dried vegetables	0.09	1.1
Meat products (fermented pork products)	0.14	1.7
Fermented dairy products	0.02	0.3
Fermented fish products	0.01	0.1
Condiments and sauces	0.59	7.2
Non-alcoholic beverages	0.82	9.9
Alcoholic beverages	2.85	34.5
Total	**8.27**	**100**

RISK CHARACTERISATION

Margin of exposure

38. When the estimated dietary exposures of the average local population to EC from local fermented foods and alcoholic beverages were compared with the $BMDL_{10}$ (i.e., for 10% extra risk of tumours) of 300 µg/kg bw/day for EC in experimental animals, large margins of exposure (MOEs) were observed. The MOEs for total exposure and exposure excluding alcoholic beverages were in the range of 3.6×10^4 and 5.5×10^4 respectively, indicating that EC exposure from consumption of local fermented food and beverage items would be of low health concern for the average local population.

39. However, the potential health risk of dietary EC exposure for the subpopulation consuming large amounts of fermented foods and beverages, especially alcoholic beverages, would need to be further assessed. In the JECFA evaluation of food contaminants at its 64th meeting in 2005, the estimated intake of certain contaminants with MOE values of $\geq 10^4$ was considered to be of low concern for human health.[5]

40. The MOE values for high consumers of local fermented food items which are major contributors to dietary EC exposure, and those with high EC contamination levels are presented in Table 4. The MOE values for high consumers (95th percentile consumption pattern) of the majority of local fermented food items tested were in the order $10^4 – 10^6$, indicating low health concern for high consumers of these individual food items. Exceptions were the high consumers of alcoholic beverages

such as distilled spirits, plum wine and grape wine. The MOE values for estimated dietary EC exposure of this subpopulation were at the low end of the order of 10^3, suggesting potential health concern for high consumers of these fermented alcoholic beverages due to the high levels of EC contamination of these food items. Two other food items showed MOE values at the high end of the order of 10^3; they were the fermented red bean curd and the beer/ale. The former had the highest level of EC contamination but very low consumption value even for the high consumers, while the latter had the highest consumption value but very low level of EC contamination.

Table 4: Dietary exposure to ethyl carbamate of high consumers of various local fermented food and beverage items

Food/ beverage item	95^{th} percentile consumption (g)	Mean EC level (µg/ kg)	Exposure (ng/kg bw/day)	MOE
Bread/ Rolls/ Buns	144	2.63	6.18	4.9×10^4
Crackers	58.4	1.08	1.03	2.9×10^5
Fermented bean curd	15.0	80.7	19.7	1.5×10^4
Fermented red beancurd	5.00	386	31.5	9.5×10^3
Fermented black soybean	3.92	2.22	0.14	2.1×10^6
Preserved vegetables	24.6	3.03	1.22	2.5×10^5
Chinese pork sausage	30.0	18.0	8.81	3.4×10^4
Cheese	29.4	0.44	0.21	1.4×10^6
Yogurt	250	0.50	2.04	1.5×10^5
Soy sauce*	18.3	6.84	2.04	1.5×10^5
Vinegar*	12.5	9.32	1.90	1.6×10^5
Vinegar drink*	375	1.54	9.42	3.2×10^4
Fully fermented tea*	1350	0.18	3.96	7.6×10^4
Semi- fermented tea*	1500	0.08	1.96	1.5×10^5
Beer/ale*	1,650	1.13	30.4	9.9×10^3
Yellow wine*	0.03	265	0.13	2.3×10^6
Sorghum-based spirit*	0.59	54.3	0.52	5.7×10^5
Chinese rice wine*	0.43	32.1	0.23	1.3×10^6
Grape wine*	250	21.2	86.5	3.5×10^3
Plum wine*	76.0	110	136	2.2×10^3
Distilled spirits*	270	36.5	161	1.9×10^3

* For liquid item, consumption are expressed as mL

Discussion

Levels and patterns of ethyl carbamate in food commodities

41. The majority of food items tested showed low or undetected levels of EC. Ethyl carbamate was not detected in local fermented foods such as Chinese steamed bun, stinky tofu, dairy-based fermented beverages, salted fish and fermented Chinese tea. Highest levels of EC were found in the fermented red bean curd and Chinese yellow wine (Shaoshing wine/ huadiao). Considering that these food items are usually consumed infrequently and in small amounts or are added only as seasoning in Chinese cooking, they would unlikely be a cause of significant health concern for the general population.

42. A large range of EC concentration was measured among individual samples of each food item tested. The wide variation could be the result of difference in precursors used, fermentation methods employed or storage conditions. It is therefore important for the industry to continue closely monitor the manufacturing process, identify potentially problematic conditions and develop methods that would prevent or reduce the formation of EC and maintain EC contamination at the lowest level possible during processing and storage.

43. Table 5 presents a comparison of EC levels in fermented foods measured in the present study with those previously reported by overseas countries.[5] Results of this study are in general agreement with previous reports from other countries, with the exception of distilled spirits for which the local samples show a narrower range at the low end of the overseas reported EC ranges. The smaller range of EC levels found in local samples is likely due to the smaller number of items and different kinds of distilled spirits (Chinese distilled spirits) analysed in this study.

Table 5: Comparison of ethyl carbamate levels in fermented foods measured in this study with those previously reported

Food/ beverage group	Range of EC concentration (µg/kg) This study	Range of EC concentration (µg/kg) Previously reported by overseas countries [a]
Fermented cereals and grains products	**ND – 8.6**	**ND – 12**
➢ Bread/ Rolls/Buns	ND – 8.6	
• Chinese steamed bun	ND	
• Bread and toasted bread	1.0 – 8.6	
➢ Crackers	ND – 5.1	
Legumes (fermented soy products)	**ND – 650**	
• Fermented bean curd	11 – 130	
• Fermented red bean curd	150 – 650	
• Fermented black soy bean	ND – 7.0	
• Stinky tofu	ND	
Preserved / dried vegetables	**ND – 10**	**ND – 16 (kimchi)**
Fermented meat products (Chinese pork sausage)	**12 – 29**	
Fermented dairy products	**ND – 1.1**	**ND – 1.3**
• Cheese	ND – 1.1	ND
• Yogurt	ND – 1.1	ND – 1.3
• Dairy-based fermented beverages	ND	
Fermented fish products (salted fish)	**ND**	
Condiments and sauces	**ND – 44**	**ND – 84**
• soy sauce	1.8 – 17	ND – 84
• oyster sauce	ND – 1.1	
• vinegar	ND – 37	0.3 – 26
• condiment and savory sauces	ND – 44	ND – 8 (soybean paste)
Non-alcoholic beverages	**ND – 15**	
➢ Vinegar drink (fruit vinegar)	0.4 – 3.0	
➢ Tea (tea leaves)	ND – 15	
• fully fermented tea (black tea)	ND – 15	
• semi-fermented tea (Chinese tea)	ND – 5.1	
Alcoholic beverages	**ND – 390**	**ND – 262** [a]
➢ Beer/ales	ND – 5.8	ND – 5
➢ Wine or spirit made from cereals and grains	2.0 – 390	
• Yellow wine	140 – 390	
• Sorghum-based white spirit	37 – 66	
• Rice wine	2.0 – 330	
– Chinese rice wine	3.3 – 62	
– Sake	2.0 – 330	ND – 202
➢ Wine made from fruit	0.4 – 230	
• Grape wine	6.7 – 47	ND – 61
– Red wine	8.3 – 35	
– White wine	6.7 – 47	
• Plum wine	0.4 – 230	
• Cider	ND – 31	ND – 3
➢ Compound alcoholic beverages	17 – 150	
➢ Distilled spirits (excluding fruit brandy)	20 – 66	ND – 243 [b]

[a] Source of information: WHO.Food and Nutrition Paper 82, 2006

[b] Range excluding the single highest value of 6131 µg/kg reported

Exposure assessment

44. When compared to $BMDL_{10}$ of 0.3 mg/kg bw/day, the estimated total dietary exposure to EC for the average local consumers of fermented foods and alcoholic beverages gives a large margin of exposure (MOE) of 3.6 x 10^4. Therefore, their potential health risk of EC exposure via dietary intake is likely to be low. However, the risk to adverse effects of EC would be expected to be greater (MOE in the order of 10^3) for high consumers of alcoholic beverages especially those who are habitual drinkers of the alcoholic types known to have high EC contents (see Table 4).

45. In the Hong Kong Population-Based Food Consumption Survey, there was no reported consumption of fruit brandy which was known to contain a much higher mean level of EC (EFSA: 744 – 747 μg/ kg was reported).[2] In this study, exposure from distilled spirits was therefore estimated using the mean EC level of 36.5 μg/kg for Chinese distilled spirits. Similar mean EC levels for distilled spirits excluding fruit brandy have been reported by overseas countries (JECFA: 37 – 64 μg/ kg; EFSA: 64 – 66 μg/ kg),[2,5] giving comparable estimated exposure and calculated MOE for the average population. If the distilled spirits consumed solely consisted of fruit brandies, the estimated exposure would be increased 20 folds, giving a calculated MOE of around 10^2.

46. The estimated exposure due to the food group "fermented cereals and grains products" (33.5%) is likely to be overestimated for the following reasons. First, not all bread/rolls/buns and crackers available on the market are fermented. While a large number of products are made from yeast fermentation, some products may use baking soda instead. Since no data were available for estimating the proportion of fermented products, this study assumed that all the bread/rolls/buns and crackers consumed

were fermented. Second, in the absence of detailed ingredients breakdown, the gross weight of the bread/ rolls/ buns consumed including the non-fermented ingredients (e.g. sausage, raisins) were used to estimate the EC exposure. Therefore, the net quantity of fermented foodstuff consumed and hence the actual exposure to EC from the group “fermented cereals and grains products” would likely be substantially lower and contribute a much smaller percentage to the total EC exposure.

Mitigation measures

47. It has been reported that distilled alcoholic beverages consistently contain the highest residual EC level. Among the various wines and spirits of grain or fruit origin, stone fruit brandies were found to be of particular concern for high consumers. A MOE of about 600 (i.e. a value of 10-fold less than those of other alcoholic beverages) for high consumers of stone fruit spirits was reported by European Food Safety Authority (EFSA) in 2007.[2] The observed high level of EC in these distilled spirits would likely be the result of continued EC formation when ethanol and appropriate precursors in the spirits remain in contact for extended period during the ageing process. Efforts made by the alcoholic beverage industry to develop mitigation procedures to lower the formation of EC have lead to a steady decrease in the level of EC in distilled spirits and fruit brandies reported over the last decade.

48. The key to successful prevention of EC production in foods and beverages is the identification of the main precursor substances for the formation of EC and our better understanding of the influence of main external factors, namely the light, temperature and time. Ethyl carbamate can be formed from various substances derived from food and beverage, including hydrogen cyanide, urea, citrulline and other N-carbamyl compounds. It is therefore possible to devise appropriate mitigation

measures to curb its production. Internationally, major reduction in the level of EC contamination over the past years has been achieved using two approaches: i) by reducing the concentration of the main precursor substances in the food or beverage; and ii) by reducing the tendency for these substances to react to form cyanate, e.g. by the exclusion of light from bottled stone-fruit brandies.

49. Diethylpyrocarbonate, an inhibitor of fermentation, has also been reported to form EC. On the basis of this observation, the previous acceptance of diethylpyrocarbonate was revoked by the JECFA Committee at its 17th meeting. Another exogenous precursor for EC, azodicarbonamide, which has been used as a blowing agent to make sealing gaskets, is no longer recommended for bottling alcoholic beverages. Although the use of azaodicarbonamide as a dough maturing agent is permitted in some countries, it should be noted that at the maximum usage level, it would result in a slight increase in the formation of EC in bread. [4]

LIMITATIONS

50. A total of 276 fermented food and beverage samples from 9 food groups were analysed in this study. Increasing both the number of food items and the number of samples per individual food commodity for laboratory analysis could provide a more comprehensive coverage and a more precise estimate of the range and average EC concentration of fermented foods in the Hong Kong market.

51. The fermented food types studied were not exhaustive. Certain fermented food types, particularly those not commonly consumed by the Hong Kong population, were not covered. For the food group "meat products", only Chinese pork sausage was tested. Other common fermented meat products (e.g. salami) were not included.

52. Not all consumption data relevant to EC exposure were available. For example, no data were available for the proportion of bread/rolls/buns and crackers consumed that were fermented by yeast and no ingredients breakdown was available to assess the proportion of bread consumption only. For distilled spirits, mean per capita consumption data were available only as a group. Individual consumption data for Western distilled spirits (e.g. brandy, whisky, rum, vodka, gin) and other alcoholic beverages such as sake, cider and fortified wine known to contain high levels of EC were not available.

CONCLUSION AND RECOMMENDATIONS

53. Results of this study show that EC may be present in varying amounts in different local fermented food and beverage items at generally low levels. The food group "alcoholic beverages" was identified as the main dietary source of EC, followed by "fermented cereals and grains products (bread/rolls/buns and crackers)" and "legumes (fermented soy products)". For the general population, dietary exposure to EC from consumption of fermented foods and beverages are unlikely to pose health concern. However, for high consumers of alcoholic beverages, health risk of EC cannot be ruled out.

Advice to consumers

1. Maintain a balanced diet. Avoid overindulgence of fermented foods and beverages, in particular alcoholic beverages.
2. Store fermented foods and beverages in a cool place under low light conditions.
3. Avoid stocking up excessive fermented foods and beverages, to minimise the duration of storage.

Advice to the trade

1. Manufacturers should follow good manufacturing practice (GMP). Develop mitigation measures to reduce the levels of EC in fermented foods and beverages, e.g. identifying and reducing the amount of precursors.
2. Use proper containers to protect fermented foods and beverages from light exposure.
3. Shippers, distributors, wholesalers and retailers should minimise heat and light exposure during transportation and storage of fermented food and beverage products.
4. Obtain fermented foods and beverages from reliable suppliers.
5. Keep stock according to the first-in-first-out principle.

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Annex I

Maximum Levels of Ethyl Carbamate in Alcoholic Beverages

Country	Ethyl carbamate concentration (µg/L)				
	Wine	Fortified wine	Distilled spirits	Sake	Fruit brandy
Canada	30	100	150	200	400
USA	15	60	-	-	-
Czech Republic	30	100[a]	150	200	400[b]
France	-	-	150	-	1000
Germany	-	-	-	-	800
Korea	30	-	-	-	-

[a] Fruity wines and liqueurs

[b] Fruity distillates and fruity , mixed and other spirits

Annex II

Ethyl Carbamate Levels in Fermented Foods and Beverages

Food Group / Food Items	No. of samples	Mean concentration (µg/kg)	Median concentration (µg/kg)	Range (µg/kg)
FERMENTED FOODS				
Fermented cereals and grains products	25	2.01	0.90	ND – 8.6
Bread/ Rolls/ Buns	15	2.63	1.70	ND – 8.6
Chinese steamed bun *	5	0.20	0.20	ND
White bread *	5	5.04	5.10	1.5 – 8.6
Toasted bread	5	2.66	2.50	1.0 – 5.4
Crackers	10	1.08	0.40	ND – 5.1
Crackers *	5	1.86	0.90	ND – 5.1
Biscuit sticks	5	0.30	0.20	ND – 0.7
Legumes (fermented soy products)	20	121	45.0	ND – 650
Fermented bean curd *	6	80.7	83.0	11 – 130
Fermented red bean curd *	5	386	320	150 – 650
Fermented black soy bean *	5	2.22	1.40	ND – 7.0
Stinky tofu	4	0.20	0.20	ND
Preserved/ dried vegetables	45	3.03	1.70	ND – 10
Preserved leaf mustard *	5	2.28	1.80	ND – 6.6
Preserved rakkyo *	5	0.86	0.80	ND – 1.7
Preserved cabbage/ "Dong Choy" *	5	8.34	7.40	7.0 – 10
Preserved turnip *	5	1.06	0.90	0.8 – 1.7
Preserved mustard greens *	5	0.48	0.60	ND – 0.6
Preserved Sichuan mustard *	5	2.36	1.90	0.9 – 4.9
Dried raddish *	5	3.80	3.20	1.5 – 7.2
Preserved mustard/ "Mui Choy *	5	7.14	7.30	4.9 – 10
Kimchi *	5	0.96	0.20	ND – 3.8
Meat products (fermented pork products)	5	18.0	18.0	12 – 29
Chinese pork sausage *	5	18.0	18.0	12 – 29
Fermented dairy products	11	0.39	0.20	ND – 1.1
Cheese *	5	0.44	0.20	ND – 1.1
Yogurt *	3	0.50	0.20	ND – 1.1
Dairy-based fermented beverage *	3	0.20	0.20	ND
Fermented fish products	5	0.20	0.20	ND
Salted fish *	5	0.20	0.20	ND
Condiments and sauces	55	5.11	1.20	ND – 44
Soy sauce *	5	6.84	6.10	1.8 – 17
Oyster sauce *	5	0.54	0.20	ND – 1.1
Vinegar *	18	9.32	3.10	ND – 37
Rice vinegar	10	7.50	2.30	ND – 27
Sorghum vinegar	3	25.0	29.0	9.0 – 37
Wine vinegar	5	3.54	2.30	1.9 – 8.6
Condiments and savory sauces	27	2.84	0.80	ND – 44
Fish sauce *	5	0.60	0.50	ND – 1.2
Worcestershire sauce *	2	1.85	1.85	1.5 – 2.2
Shrimp paste *	5	1.18	0.20	ND – 4.9
Broad bean paste *	5	10.4	2.80	0.8 – 44
Miso *	5	1.10	0.80	0.6 – 2.2
Soybean paste *	5	1.28	0.80	ND – 3.1

Food Group / Food Items	No. of samples	Mean concentration (µg/kg)	Median concentration (µg/kg)	Range (µg/kg)
FERMENTED BEVERAGES				
Non-alcoholic beverages	40	1.09	0.20	ND – 15
Vinegar drink (fruit vinegar) *	5	1.54	1.30	0.4 – 3.0
Tea (Tea leaves)	35	1.03	0.20	ND – 15
Fully fermented tea * (black tea)	5	3.26	0.20	ND – 15
Semi-fermented tea * (Chinese tea)	30	0.65	0.20	ND – 5.1
Pu-er tea	5	0.70	0.20	ND – 2.7
Oolong tea	5	0.76	0.20	ND – 3.0
Jasmine tea	5	1.38	0.20	ND – 5.1
Tieguanyin tea	5	0.64	0.20	ND – 2.1
Shueixian tea	5	0.24	0.20	ND – 0.4
Shouwei tea	5	0.20	0.20	ND
Alcoholic beverages	70	55.9	20.7	ND – 390
Beer/ ale *	15	1.13	0.70	ND – 5.8
Draft (draught) beer	5	0.54	0.60	0.4 – 0.7
Regular Beer	5	0.70	0.70	ND – 1.5
Dark (black) beer	5	2.16	1.40	1.0 – 5.8
Wine/ spirit made from cereals and grains	30	93.7	40.0	2.0 - 390
Yellow wine * (Shaoshing wine /huadiao)	6	265	275	140 – 390
Sorghum-based spirit *	3	54.3	60	37 – 66
Rice wine *	21	50.4	28.0	2.0 – 330
Chinese rice wine	12	32.1	28.5	3.3 – 62
Sake	9	74.7	28.0	2.0 – 330
Wine made from fruit	20	39.7	18.5	0.4 – 230
Grape wine	10	21.2	18.5	6.7 – 47
Red wine *	5	17.7	15.0	8.3 – 35
White wine *	5	24.7	19.0	6.7 – 47
Plum wine *	5	110	91.0	0.4 – 230
Cider	5	6.90	1.40	ND – 31
Compound alcoholic beverage	5	57.6	32.0	17 - 150
Distilled spirit * # (distilled rice wine and distilled sorghum-based spirit)	9	36.5	37.0	20 – 66
Total	276	25.2	1.50	ND – 650

Note:
ND: non-detect
LOD for both solid and liquid samples = 0.4 µg/kg
The value of 1/2 LOD was assigned to non-detects (results below limit of detection) for the calculation of mean levels
* Food items for which consumption data are available in the Population Based Food Consumption Survey used in exposure assessment
# Distilled spirits include distilled rice wine and distilled sorghum-based spirit listed above
Brewed tea (10 g of tea leaves in 200 ml water):

1. Black tea sample (EC at 15 µg/kg before brewing): 0.9 µg/kg
2. Pu-er tea sample (EC at 2.7 µg/kg before brewing): ND
3. Jasmine tea sample (EC at 5.1 µg/kg before brewing): ND