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Exposure to Ethyl Carbamate by Consumption of Alcoholic Beverages Imported in Korea

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Abstract Determination of ethyl carbamate content in imported alcoholic beverages in Korea and an exposure assessment were conducted. In gas chromatography/mass spectrometry/selected ion monitoring (GC/MS/SIM) analysis, 2.5-39, 8-263, 6.3-112, 11.3-23.5, 53-94, 8.5-38.5, 7-9.5, 21.3-31.5, 5-832.5, and 10.5-364.8 μg/L of ethyl carbamate were detected in imported beers, *sakes*, whiskies, vodkas, Chinese liquors, cognacs, tequilas, rums, liqueurs, and wines, respectively. The exposure assessment indicated that the exposure of Korean adults to ethyl carbamate were lower than 20 ng/kg BW per day, (the virtual safe dose) indicating that the amount of ethyl carbamate exposed through fermented food and alcoholic beverages including imported products are currently in the 'no significant risk level'. However, the present low exposure to ethyl carbamate through the imported alcoholic products was not due to the low contents of ethyl carbamate in imported products, but low consumption of the imported products. Therefore, given increasing importation of alcoholic beverages in Korea, reductions of ethyl carbamate content in imported alcoholic beverages, especially non-distilled products, should be required by regulating limits on the ethyl carbamate content in the imported alcoholic beverages.

Keywords: ethyl carbamate, wine, beer, liqueur, *sake*

Introduction

Recently, ethyl carbamate (urethane) has received much attention due to its presence in diverse alcoholic beverages such as wines, sherries, liqueurs, and distilled spirits and its classification as a probably human carcinogen (class 2A) by the International Agency for Research on Cancer (1). Previous studies has suggested that the carcinogenicity of ethyl carbamate was initialized by oxidation to vinyl carbamate, and then to an epoxide, a metabolite proposed to be the ultimate carcinogen, which occurs in most fermented foods and alcoholic beverages (2-9). As it was recognized as a carcinogen, the Canadian Health Protection Branch set mandatory limits of ethyl carbamate levels in wine and alcoholic products imported into Canada (10). In Canada, the Department of Health and Welfare also issued guidelines limiting the levels of ethyl carbamate to 30 ng/g in table wine, 100 ng/g in fortified wines (such as sherries and ports), 150 ng/g in distilled spirits, and 400 ng/g in fruit brandies and liqueurs. The Food and Drug Administration in the United States (FDA-US) published an ethyl carbamate prevention action manual outlining methods to reduce ethyl carbamate formation (10). Although the International Agency for Research on Cancer (IARC) and the Environmental Protection Agency (EPA) have not established a Reference Concentration (RfC) or a Reference Dose (RfD) for ethyl carbamate, Schlatter and Luts (11-13) estimated a 'virtually safe dose of 20 to 80 ng/kg BW/day on the basis of sex and organ specific tumor data and with a linear extrapolation to a negligible increase of the lifetime tumor incidence by 0.0001% (one additional tumor in one million individuals exposed for life). The California State Government (US) used a 'no significant risk level' of 0.7 μg/body/day of ethyl carbamate in California Proposition 65 (14).

However, in Korea, despite the popularity and high consumption of alcoholic beverages, there are currently no limits on the ethyl carbamate content of alcoholic beverages. Therefore, the concentrations of ethyl carbamate in Korean domestic alcoholic beverages were determined and daily exposure to ethyl carbamate through consumption of Korean domestic alcoholic beverages was calculated as a preliminary study for ultimate use in the regulation of limits on the ethyl carbamate content in alcoholic beverages (11, 12, 15). In this study, the concentrations of ethyl carbamate in the alcoholic beverages imported into Korea were measured to obtain a more accurate ethyl carbamate exposure assessment. Based on our findings, we suggest imposing limits on the ethyl carbamate content in alcoholic beverages imported into Korea, especially considering the increasing importation of alcoholic beverages into its domestic markets.

Materials and Methods

Sample collection One-hundred-forty-three brands of commercially imported alcoholic beverages were selected for sampling, including 12 brands of beer, 8 brands of *sake*, 19 brands of whiskey, 4 brands of vodka, 3 brands of Chinese liquor, 4 brands of cognac, 2 brands of rum, 2 brands of tequila, 18 brands of liqueur, and 71 brands of wine.

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Sample preparation Ethyl carbamate (Sigma Aldrich, Milwaukee, WI, USA) and butyl carbamate (Sigma Aldrich) were used as the external and internal standards. Each standard solution was prepared by gradual dilution of standard stock solution (1,000 μg/mL) with ethyl acetate (Sigma Aldrich).

A sample clean-up procedure was conducted using the method of Henry *et al.* (16) with some modification. Twenty mL samples were weighed into a 250-mL centrifuge bottle, and 10 g sodium chloride was added and swirled 1-2 min to saturate solution. Eighty mL of dichloromethane (J. T. Baker, Palo Alto, CA, USA) was added and shaken for 1 min. The solution was centrifuged at 3,000×g until phase separation occurred. The dichloromethane layer was drawn off using a suitable suction device. The remaining compound was filtered into a 500-mL evaporator flask through 40 g anhydrous sodium sulfate on Whatman No. 1 filter paper in a conical glass funnel. This extraction step was repeated 2 more times. One mL ethyl acetate was added to the dichloromethane extract, and concentrated in a rotary evaporator below 30°C at reduced pressure until the volume decreased to 1 mL. Dichloromethane was completely removed at this step, and the extract was not evaporated to dryness or to near dryness to minimize ethyl carbamate loss. The liquid was transferred to a 2-mL vial, and the flask was rinsed with 1 mL of ethyl acetate and mixed well. Butyl carbamate (4 μL) was added as an internal standard. The mixture (2 μL) was injected into gas chromatograph for analysis.

A recovery test was performed with the ethyl carbamate standard solution as the external standard. The limits of detection and quantification were calculated at signal-to-noise ratio of 3 and 10, respectively.

Gas chromatography/mass spectrometry (GC/MS) detection An Agilent 6890 gas chromatograph with a 5973N mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) was used for separation and determination of ethyl carbamate in the extracts. Determination of ethyl carbamate content in each sample was performed in triplicate and cross-checked again at Sang-joo National University (Gyeongbuk, Korea) to improve analytical accuracy and reduce errors.

GC conditions were as follows: carrier gas flow, helium at 0.9 mL/min; injection volume, 2 μL; column, i.d. 30 m×0.25 mm, DB-Innowax capillary column; oven temperature, 15 min at 115°C, increasing to 240°C at 20 °C/min, and held for 25 min; injector temperature, 280°C. MS conditions were as follows: electron impact ionization potential at 70 eV; selected ion monitoring (SIM), *m/z* of 44, 62, 74, and 89; and injection mode, splitless. Peaks containing all 4 ions (*m/z*; 44, 62, 74, 89) were regarded as ethyl carbamate.

Exposure assessment of ethyl carbamate The concentration of ethyl carbamate was described as a mean± standard deviation. The mean value was calculated from the data excluding the upper and lower 5%. Exposure assessment of ethyl carbamate through imported alcoholic beverages was estimated based on the average ethyl carbamate concentration of each sample and from the diets of Korean male and female aged 20-64 years obtained from the National Health and Nutrition Survey (17), and then, calculated using following equation:

$$\text{Average daily intake (ng/kg bw/day)} = \sum_{i=1}^{n} (C_i \times CR_i/\text{BW})$$

where Ci is the contamination level of i food (mean, μg/kg); CRi is the contact rate of i food (g/day); BW is the average body weight (kg) over exposure period obtained from the Korea Research Institute of Standards and Science; and n is the number of tested samples.

Results and Discussion

Ethyl carbamate content in alcoholic beverages There are a large number of studies describing the mechanisms of formation of ethyl carbamate (18-29). The formation of ethyl carbamate is a spontaneous chemical reaction involving ethanol and a compound that contains a carbamyl group. The precursor of ethyl carbamate in fermented foods and beverages is supposed to be urea, cyanate, carbamyl phosphate, or diethyl pyrocarbonate (DEPC), an antimicrobial food additive. In certain alcoholic beverages, urea is the most important precursor of ethyl carbamate. Moreover, L-arginine is one of the amino acids present in alcoholic beverage and is catabolized by the yeast or lactic acid bacteria to ornithine, ammonia, and carbon dioxide during fermentation.

Peaks of ethyl carbamate and butyl carbamate at *m/z* 62 are shown in Fig. 1. The retention time of butyl carbamate was longer than that of ethyl carbamate. The recoveries of the external standard ranged from 88.4 to 106.7%. Although the recoveries of wine and beer were relatively low, recoveries of the rest of the samples were near 100%. Quantification of the internal standard, butyl carbamate, also confirmed the accuracy of the measurement. The limits of detection and quantification were 2.3 and 10.4 μg/L at the signal to noise ratios of 3:1 and 10:1, respectively. This limit of detection was somewhat lower than that described by Koh and Kwon (22).

Ethyl carbamate content in distilled hard liquors were described in Fig. 2, which were 6.3-112, 11.3-23.5, 53-94, 8.5-38.5, 7-9.5, and 21.3-31.5 μg/L for whiskey, vodka, Chinese liquor, cognac, tequila, and rum, respectively. Dennis *et al.* (30) also reported the similar content of ethyl carbamate (19-90 μg/L) in distilled liquors. Comparing the concentrations of alcohol in the distilled products with those in non-distilled products, the content of ethyl carbamate in the distilled products should have been higher. These results were likely due to the relatively low volatility of ethyl carbamate during distillation. French liquor had the largest amount of ethyl carbamate among the distilled liquors. Although most of whiskies had lower contents of ethyl carbamate than Chinese liquor, one brand of whisky had an extraordinarily high content (112 μg/L) of ethyl carbamate, however, it was also lower than the legal level (125 ppb) suggested by U.S. FDA (31). The differences in the amount of ethyl carbamate in the same types of samples were seemingly due to differences in the manufacturing processes and storage conditions. For instance, some sherries are baked and bourbons are distilled at high temperatures. Both baking and heating

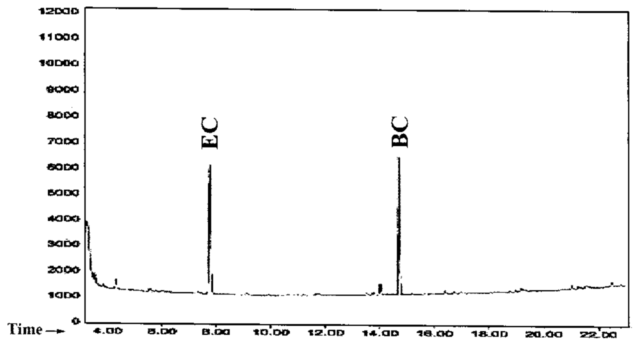

Fig. 1. GC/MS chromatogram of ethyl carbamate and butyl carbamate.

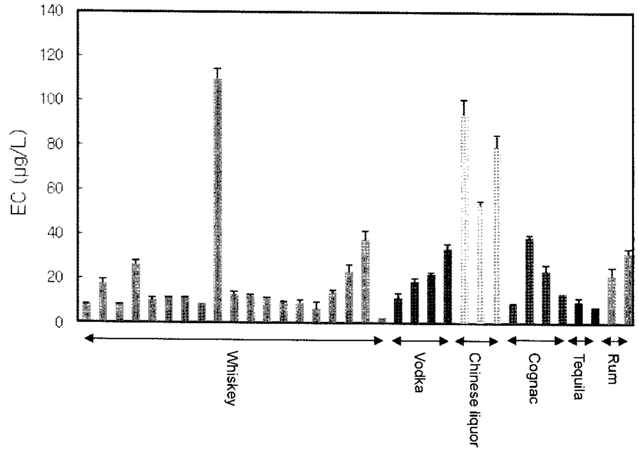

Fig. 2. Ethyl carbamate (EC) content of imported distilled alcoholic beverages.

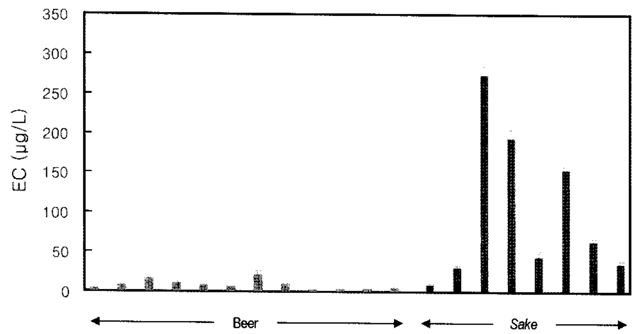

Fig. 3. Ethyl carbamate (EC) content of imported non-distilled alcoholic beverages made from cereals.

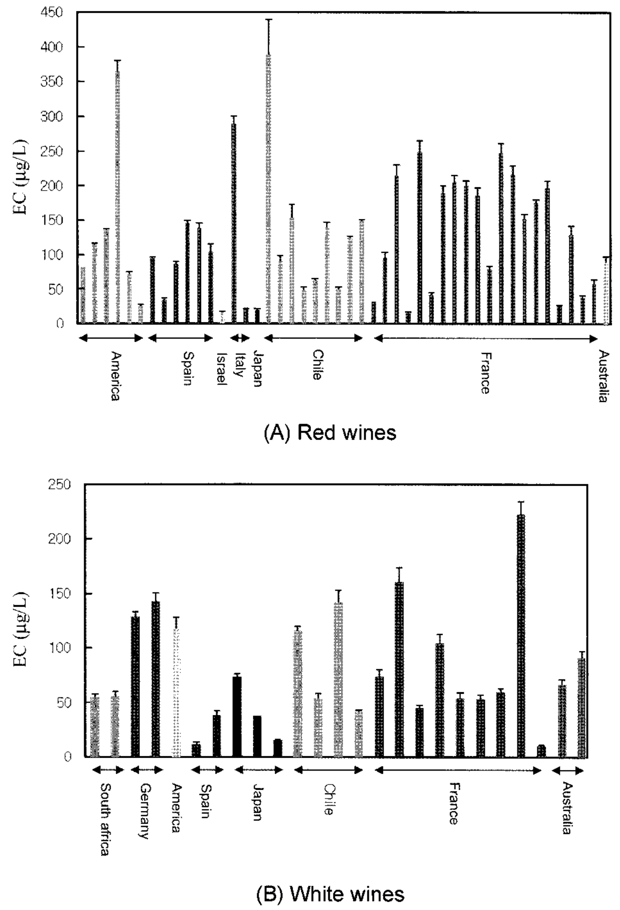

Fig. 4. Ethyl carbamate (EC) content of imported red (A) and white (B) wines.

processes may raise ethyl carbamate levels since heating seems to accelerate the production of ethyl carbamate. Also levels can differ significantly, even among different bottles of the same type or brand (11, 32).

The ethyl carbamate contents in the non-distilled alcoholic beverages made from cereals including beer and *sake*, were compared in Fig. 3. *Sakes* contained larger amounts (8-273 μg/L) of ethyl carbamate than beers and Korean domestic rice wines (11, 12). One sample of *sakes* showed higher content of ethyl carbamate than the legal limit (200 ppb) in Canada, however, much lower than findings of Sansan *et al.* (33) reporting ethyl carbamate content ranging 899 to 1,200 μg/L in non-distilled spirits.

White wines (Fig. 4B) contained a similar amount of ethyl carbamate compared with *sake*, however, this amount was less than that of red wines (Fig. 4A). Although the mean content of ethyl carbamate present in red wines imported from France was relatively high, the highest content of ethyl carbamate was found in the wines imported from Chile, America, and Italy. The Department of Health and Welfare in Canada (11, 16, 19, 23, 34) established regulatory limits on the ethyl carbamate content at 30 and 100 ng/g for table wines and fortified wines, such as sherries and ports. Many of red wines had higher levels of ethyl carbamate than these legal limits. Even the ethyl carbamate content in 8 red wines and 1 white wine were higher than the U.S. FDA's regulatory limits for imported wines, 200 μg/L.

Some of liqueurs made from fruits, except grapes, had the highest content of ethyl carbamate (5-832.5 μg/L) among the tested samples in this study (Fig. 5). Two of them had higher contents than the regulatory limits of the Canadian Health and Welfare Department for fruit brandies and liqueurs (400 μg/L). These liqueurs compare to Korean domestic plum brandies containing 607 μg/L of

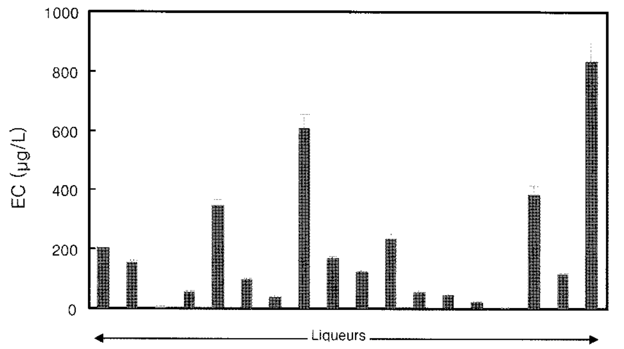

Fig. 5. Ethyl carbamate (EC) content of imported liqueurs made from fruits except grape.

ethyl carbamate (11). Ma *et al.* (35) also reported high ethyl carbamate content (124.6 μg/L) in liqueurs, however, our results were even higher than those reported. The high content of ethyl carbamate in liqueurs, plum brandies, and wines are probably due to large amount of cyanate present in fruits including plum and grape, as the 'Preventative Action Manual' published by U.S. FDA suggested removal of grape seeds containing cyanate during fermentation to reduce the risk of natural formation of ethyl carbamate in wine production (34).

Exposure assessment of ethyl carbamate The average daily intake of alcoholic beverages by Koreans and the associated ethyl carbamate intake are shown in Table 1 and 2. The estimated daily intake of ethyl carbamate through alcoholic beverage was calculated by multiplying the average consumption with the mean ethyl carbamate content. Our previous study (11) for fermented foods and domestic alcoholic beverage were also used in this total exposure assessment. Exposure to ethyl carbamate was calculated by dividing estimated daily intake into average body weight (17, 36). Because there was no consumption data of Chinese liquor, tequila, and rum, these samples were excluded from the exposure assessment.

Although the ethyl carbamate content in *sake* was not higher than wine and liqueur (Fig. 3-5), the contribution of *sake* to total exposure to ethyl carbamate was much higher than wine and liqueur (Table 2). This result was due to relatively high consumption of *sake* (Table 1). As shown in Table 2, although the ethyl carbamate content in the imported alcoholic beverages were much higher than those in Korean domestic alcoholic beverages, the contribution of the imported products to total exposure were small due to their much lower consumption level compared with Korean domestic products.

The total exposure of all adult Koreans to ethyl carbamate, including exposure through the imported alcoholic beverages was grouped into the 'no significant risk level', which is lower than the 'virtually safe dose'. The 'virtually safe dose' for a lifetime risk level of 10^{-6} had been calculated to be 20 ng/kg BW/day using the estimation of Schlatter and Luts (13). However, the low exposure to ethyl carbamate through the imported alcoholic products was likely not due to the low content of ethyl carbamate in imported products, but due to the low consumption of the imported products. The daily intake of wine is 1.0 g/person/day, which is relatively small especially compared to *soju* consumption (73.8 g/person/day; Table 1). However, the importation of wine and the number of wine consumers have recently shown to be rapidly growing, therefore the total carbamate exposure/intake of Koreans stands to increase in the future.

In addition to wine, the imported beer can also increase the total exposure to ethyl carbamate. Considering its large amount of daily intake (Table 1), beer merely contributes to total exposure due to the low content of ethyl carbamate in Korean domestic beer (Table 2). However, the ethyl carbamate content in imported beer was much higher (7.3 μg/L) than that in a Korean domestic beer (0.6 μg/L).

Table 1. Daily intake of alcoholic beverages of adult Koreans and average content of ethyl carbamate consumed

Alcoholic beverage \ Sex / Age	Average content of ethyl carbamate (μg/L)	Female (g/person/day)			Male (g/person/day)		
		20-29	30-49	50-64	20-29	30-49	50-64
Soju	3.0	11.2	7.9	8.6	67.9	73.8	66.8
Takju	0.6	0.0	1.4	8.0	9.4	6.6	20.6
Beer (Domestic)	0.6	87.0	28.7	13.5	117.1	82.8	52.7
Beer (Imported)	7.3						
Chinese liquor	75.5	-	-	-	-	-	-
Tequila	8.3	-	-	-	-	-	-
Rum	26.4	-	-	-	-	-	-
Vodka	21.4	0.0	0.1	0.0	0.0	0.0	0.3
Whiskey	13.7	0.2	0.1	0.0	1.3	2.1	0.3
Cognac	20.9	0.0	0.0	0.0	0.0	0.0	1.0
Sake	100.4	0.3	0.5	0.5	2.6	1.8	1.4
Liqueur	166	0.3	0.2	0.1	0.3	0.9	0.1
Wine	102.9	0.2	1.3	0.1	0.0	1.0	0.0

Table 2. Exposure to ethyl carbamate through intake of alcoholic beverages

Item \ Sex / Age	Female (ng/person/day)			Male (ng/person/day)		
	20-29	30-49	50-64	20-29	30-49	50-64
Fermented foods	241.7	295.7	293.9	309.6	361.9	351.6
Soju	33.6	23.7	25.8	203.7	221.4	200.4
Takju	0.0	0.8	4.8	5.6	4.0	12.4
Beer (Domestic)	52.2	17.2	8.1	70.3	49.7	31.6
Beer (Imported)	-	-	-	-	-	-
Chinese liquor	-	-	-	-	-	-
Tequila	-	-	-	-	-	-
Rum	-	-	-	-	-	-
Vodka	0.0	2.1	0.0	0.0	0.0	6.4
Whiskey	2.7	1.4	0.0	17.8	28.8	4.1
Cognac	0.0	0.0	0.0	0.0	0.0	20.9
Sake	30.1	50.2	50.2	261.0	180.7	140.6
Liqueur	49.8	33.2	16.6	49.8	149.4	16.6
Wine	20.6	133.8	10.3	0.0	102.9	0.0
Total	430.7	558.1	409.7	917.8	1,098.8	784.6
BW (kg)	54	55	57	67	68	68
Total exposure (ng/kg BW/day)	8.0	10.1	7.2	13.7	16.2	11.5

Therefore, considering increasing consumption of imported beer, the effort to reduce the ethyl carbamate in the imported beer is also needed.

In conclusion, given the increasing importation of alcoholic beverages in Korea, a reduction in the ethyl carbamate content in imported alcoholic beverages, especially non-distilled products, appears to now be required to protect Korean adults from consuming unhealthy levels of ethyl carbamate. In contrast to western countries where exposure to ethyl carbamate due to consumption of alcoholic beverages, ethyl carbamate exposure is mainly due to consumption of *kimchi* and soy sauce in Korea (15). Alcoholic beverages supplement ethyl carbamate levels from *kimchi* and soy sauce consumption in Korea. Therefore, we strongly suggest that the regulatory limits in Korea should be set at much lower levels than the current international limits.

Acknowledgments

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