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Exposure Assessment of Ethyl Carbamate in Alcoholic Beverages

HA, MI-SUN, KI-SUNG KWON[1], MEEHYE KIM[1], HEE RA PARK[1], SOO JUNG HU[1], HYOMIN LEE[1], KYUNG-MI KIM, EUN-JUNG KO, SANG-DO HA[2], AND DONG-HO BAE*

Division of Bioscience & Biotechnology, Konkuk University, Seoul 143-701, Korea
[1]Korea Food & Drug Administration, Seoul 122-704, Korea
[2]Department of Food Science and Technology, Chung-Ang University, Ansung 456-756, Korea

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Abstract Ethyl carbamate, a by-product produced naturally during fermentation and contained in fermented foods and beverages, is a carcinogen. Thus, due to the high consumption of alcoholic beverages in Korea, the ethyl carbamate concentrations in popular alcoholic beverages were determined, and the daily intake of ethyl carbamate through alcoholic beverages was estimated. The major Korean alcoholic beverages, *Soju*, beer, and *Takju*, with the highest market share were sampled and their ethyl carbamate concentrations determined by GC/MS/SIM. The ranges of ethyl carbamate contained in *Soju*, beer, and *Takju* was 0.83–10.07, 0.45–0.77, and 0.40–0.93 ppb, respectively. These results and data on the average daily intake of alcoholic beverages were then used to calculate the average and maximum estimated daily intakes (EDI) of ethyl carbamate through alcoholic beverages. As a result, a relatively high EDI of ethyl carbamate through alcoholic beverages was found for Korean males over 30 years old, indicating the need to reduce the ethyl carbamate content in alcoholic beverages.

Key words: Ethyl carbamate, urethane, alcoholic beverage, exposure assessment

Ethyl carbamate (Urethane: $NH_2COOCH_2CH_3$), which occurs naturally in most fermented foods and beverages, such as distilled spirits, wine, *sake*, whisky, *kimchi*, soy sauce, *natto*, yogurt, cheese, and bread [2, 5–7, 13, 14, 19, 20], is known to be carcinogenic to a number of species including mice, rats, hamsters, and monkeys, suggesting a potentially carcinogenic risk to humans. Based on experimental and epidemiological data, ethyl carbamate has also been classified as a possible human carcinogen (class 2B) by the International Agency for Research on Cancer (IARC, 1987). Acute exposure to high levels of ethyl carbamate may cause injury to various organs, especially the liver, central nervous system, and hemopoietic system. Previous reports have suggested that the carcinogenicity of ethyl carbamate appears to be mediated through a metabolic pathway involving sequential cytochrome P-450 catalyzed oxidation into vinyl carbamate and vinyl carbamate epoxide, the latter of which reacts with DNA to yield DNA adducts [3, 10, 17]. In certain alcoholic beverages, urea is the most important precursor of ethyl carbamate. In addition, L-arginine is one of the amino acids present in alcoholic beverages and is catabolized by the yeast or latic acid bacteria to ornithine, ammonia, and carbon dioxide during fermentation, by means of the arginase enzyme [1, 9, 12]. In 1985, the discovery of ethyl carbamate in distilled spirits and wines attracted international attention. As a result, the Canadian Health and Welfare Department established regulatory limits on the ethyl carbamate content at 30 ng/g for table wines, 100 ng/g for fortified wines (such as sherries and ports), 150 ng/g for distilled spirits, and 400 ng/g for fruit brandies and liqueurs. The wine and distilled spirits industry in the United States also sets voluntary limits on the ethyl carbamate content for imported alcoholic products [8].

However, in Korea, despite the popularity and consumption of alcoholic beverages, there are currently no limits on the ethyl carbamate content in alcoholic beverages. Therefore, the present study determined the concentrations of ethyl carbamate in the major Korean alcoholic beverages, plus the daily intake.

Among the numerous kinds of commercial alcoholic beverages in Korea, *Soju*, beer, and *Takju* were selected for sampling, since they were previously found to be included in 100 major Korean diets, representing 91.3% of the total diets [11]. Seven brands of *Soju* and 6 brands of beer, representing more than 90% of the market share, were purchased at grocery stores, whereas 7 brands of *Takju* from 7 major cities across Korea were purchased at traditional

*Corresponding author
Phone: 82-2-450-3756; Fax: 82-2-450-7011;
E-mail: donghoya@konkuk.ac.kr

Table 1. Ethyl carbamate content in alcoholic beverages.

Item	Brand	Alcohol content (%)	Ethyl carbamate content (ppb)	
			Range	Mean
Soju	1	21	2.9–4.57	3.65
	2	21	0.9–1.73	1.28
	3	21	4.0–15.37	10.07
	4	21	1.7–2.93	2.55
	5	21	0.6–3.37	1.7
	6	21	0.63–1.0	0.83
	7	21	0.63–1.07	0.91
Beer	1	4.5	0.37–0.8	0.51
	2	4.4	0.43–0.73	0.54
	3	4.5	0.5–0.67	0.61
	4	4.2	0.33–0.77	0.5
	5	5.0	0.4–0.47	0.45
	6	5.0	0.33–0.7	0.54
Takju	1	6.0	0.92–0.93	0.93
	2	6.0	0.55–0.71	0.63
	3	6.0	0.53–0.67	0.60
	4	6.0	0.48–0.60	0.54
	5	6.0	0.53–0.55	0.54
	6	6.0	0.40–0.60	0.50
	7	6.0	0.40–0.44	0.40

markets. Three samples of each brand of alcoholic beverages were collected at monthly intervals.

The sample clean-up procedure was conducted using the method of Conacher *et al.* [6] with some modification. For the ethyl carbamate standard solution, a stock solution of ethyl carbamate in ethyl acetate (1 mg/ml) was prepared, and then working standard dilutions of 100 μg/ml were prepared by diluting the solution tenfold with ethyl acetate. An Agilent 6890 gas chromatograph with a 5973N mass spectrometer was used to separate and determine the ethyl carbamate content in the extracts. The GC conditions were as follows: carrier gas flow, helium at 0.7 ml/min; injection volume, 2 μl; column, i.d. 30 m×0.25 mm, DB-INNOWAX capillary column; oven temperature, 1 min at 80°C, increase to 120°C at 5°C/min and hold for 15 min, increase to 250°C at 20°C/min; injector, 250°C. The mass spectrometer conditions were as follows: ion source, 200°C; electron impact ionization potential, 70 eV; SIM, m/z of 62, 74, and 89; injection mode, splitless. Peaks containing all three ions (m/z: 62, 74, 89) were regarded as ethyl carbamate.

The ethyl carbamate contents in the tested alcoholic beverages ranged from 0.40 to 15.37 ppb (Table 1). The highest average content of ethyl carbamate was found in *Soju* with a range of 0.6–15.4 ppb, depending on the brand. Considering the fact that *Soju* companies are supplied with the same spirits, the differences in the amounts of ethyl carbamate contained in each *Soju* brand was seemingly due to differences in the manufacturing processes and storage conditions between the *Soju* companies, implying that the ethyl carbamate content in *Soju* can be reduced by modifying such manufacturing processes and storage conditions. Although the ethyl carbamate content in *Soju* was not particularly high, the high consumption of *Soju* among Koreans still means it can be an important source of ethyl carbamate. Meanwhile, the ethyl carbamate content in beer ranged from 0.33 to 0.80 ppb, which was much lower than the finding (10 ppb) of Battaglia *et al.* [2], who also found the highest ethyl carbamate content in stone-fruit brandies (100–20,000 ppb). This study excluded stone-fruit brandies because of the minimal intake by Koreans.

The average daily intakes of *Soju*, beer, and *Takju* [11] is shown in Table 2. As such, the estimated daily intake (EDI) of ethyl carbamate through *Soju*, beer, and *Takju* was calculated from Tables 1 and 2, and the results are presented in Table 3. The estimated daily intakes of ethyl carbamate through alcoholic beverages was calculated by multiplying the average consumptions with the mean ethyl carbamate content. Although the daily intakes of *Soju* was not as high as beer (Table 2), the high ethyl carbamate concentration contributed significantly to the total intake of ethyl carbamate. Conversely, although beer had the highest consumption, its lower concentration meant that it contributed less to the total intakes of ethyl carbamate.

The EDI was only based on the ethyl carbamate levels found in the alcoholic beverages analyzed in this study. Thus, if more brands and samples from different regions with distinct manufacturing processes and storage conditions were analyzed, the estimated ethyl carbamate exposure would surely be refined.

For a Korean adult with an average body weight of 60 kg, the "virtually safe dose" for a lifetime risk level of 10^{-6} was calculated to be between 1.2 and 4.8 μg/day using

Table 2. Average intakes of alcoholic beverages (g/capita/day).

Item	Average	Age								
		3–6	7–12	13–19	20–29	30–49	50–64	>65	Males	Females
Soju	20.2	0.0	0.0	8.9	21.3	31.2	30.3	16.6	35.6	6.4
Beer	24.9	0.0	0.0	6.8	54.9	45.4	11.0	0.4	35.2	15.7
Takju	4.9	0.0	0.0	0.2	4.2	3.9	13.7	10.4	7.4	2.6
Total	50	0.0	0.0	15.9	80.4	80.5	55	27.4	78.2	24.7

Table 3. Estimated daily intakes (EDI) of ethyl carbamate through alcoholic beverages according to age (ng/capita/day).

Item	Age								
	3–6	7–12	13–19	20–29	30–49	50–64	>65	Mean	Max.
Soju	0.0	0.0	26.70	63.90	93.6	90.9	49.80	60.6	310.47
Beer	0.0	0.0	3.60	29.97	24.06	5.83	0.21	12.20	19.97
Takju	0.0	0.0	0.12	2.48	2.30	8.08	6.14	2.89	4.56
Total	0.0	0.0	30.42	96.35	119.96	104.81	56.15	75.69	335.0

the estimation (20–80 ng/kg body weight) by Schlatter and Lutz [16]. However, the California State Government used a "no significant risk level" of 0.7 μg/day of ethyl carbamate [15] in California Proposition 65. As such, the accepted daily intake of ethyl carbamate has not yet been well-defined.

Therefore, the maximum EDI of ethyl carbamate through *Soju*, beer, and *Takju* has been assumed in Table 4. Thus, if a Korean male were to drink *Soju* brand 3, beer brand 1, and *Takju* brand 1 everyday, his daily intake of ethyl carbamate would be 582.21 ng, which is lower than both the "no significant risk level" and the "virtually safe dose". However, this EDI is only based on the consumption of popular Korean alcoholic beverages and excludes fermented foods such as *kimchi*, soy sauce, yogurt, and soy paste, all of which are popularly consumed in Korea and believed to contain ethyl carbamate.

Nonetheless, the present results still reveal that alcoholic beverages represent a significant part of the intakes of ethyl carbamate. Therefore, the alcoholic beverage-producing industries need to make efforts to reduce the amount of ethyl carbamate in their products. For instance, if the ethyl carbamate content in *Soju* brand 1 were reduced to the average content in the other *Soju* brands, the EDI of ethyl carbamate from alcoholic beverages would be reduced to 200 ng/capita/day.

In order to find ways to reduce the ethyl carbamate content in foods, it is important to identify the formation mechanism of ethyl carbamate during fermentation and investigate the effect of processing and storage conditions on this formation. In addition, regulations and nationwide monitoring of ethyl carbamate levels in fermented foods should be implemented to protect the health of the nation.

Table 4. Estimated daily intakes (EDI) of ethyl carbamate through alcoholic beverages according to sex (ng/capita/day).

Item	Male		Female	
	Mean[a]	Max.[b]	Mean[a]	Max.[b]
Soju	106.8	547.17	19.2	96.37
Beer	18.66	28.16	8.32	12.56
Takju	4.37	6.88	1.53	2.42
Total	129.83	582.21	29.05	111.35

[a]Mean EDI is the sum of ethyl carbamate intakes from each type of alcoholic beverage.
[b]Max. EDI was calculated by multiplying the average food intake with maximum concentration of ethyl carbamate contained in each alcoholic beverage.

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