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Assessment of the Human Risk by an Intake of Ethyl Carbamate Present in Major Korean Fermented Foods

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Abstract Levels of ethyl carbamate, a potential carcinogen produced naturally during fermentation, in major Korean fermented foods and alcoholic beverages were determined by GC/MS/SIM, and their average daily intake and excess cancer risk in Korean people were estimated. In GC/MS/SIM analysis n.d.–4.26, 1.40–58.90, n.d.–3.76, n.d.–1.87, and 0.40–10.07 μg/kg of ethyl carbamate were detected in *kimchi*, soy sauces, fermented pastes, fermented dairy products, and alcoholic beverages, respectively. The average daily intake of ethyl carbamate and excess cancer risk through major Korean fermented foods and alcoholic beverage consumption were 6.0 ng/kg bw/day and 3.0×10^{-7}, respectively for the average Korean person aged 3–64 years, and were mainly contributed by Chinese cabbage *kimchi*, soy sauces, and *Soju*.

Key words: Ethyl carbamate, average daily intake, excess cancer risk, risk assessment

Based on experimental and epidemiological data, ethyl carbamate has been classified as a possible human carcinogen (Group 2B and Group B2) by the International Agency for Research on Cancer [15] and U.S. Environmental Protection Agency [33]. Previous reports [4, 5, 7, 13, 14, 22, 29, 30] have suggested that the carcinogenicity of ethyl carbamate appears to be mediated through a metabolic pathway involving sequential cytochrome P-450 that turns it into vinyl carbamate and vinyl carbamate epoxide, the latter of which reacts with DNA to yield DNA adducts. This reaction is probably the mode of the mutagenic action observed in many cellular and animal systems. The mechanisms of ethyl carbamate formation have been investigated in many studies [1, 2, 25, 31].

Ethyl carbamate occurs naturally in most fermented foods and beverages, such as distilled spirits, wine, sake, whisky, *kimchi*, soy sauce, *natto*, yogurt, cheese, and bread [3, 6, 9, 16, 26, 27, 34]. In Korea, fermented foods, such as *kimchi*, soy sauce, and soybean pastes, take a large part (15.5%) of the Korean daily diet and the popularity and consumption of alcoholic beverages are high [17]. However, there are currently no limits on the ethyl carbamate content in fermented foods including alcoholic beverages, and moreover, the recent information regarding average daily intake of ethyl carbamate is very scarce. Although an assessment of ethyl carbamate exposure by alcoholic beverages was conducted in our previous study [10], it could not supply adequate data in assessing the human risk by the intake of ethyl carbamate. Therefore, this study was conducted to determine the concentrations of ethyl carbamate in Korean fermented foods, estimate average daily intake, and assess the risk of ethyl carbamate for the general population in Korea.

Materials and Methods

Sample Collection

All the fermented foods and alcoholic beverages, 5 types of commercial *kimchi*, 2 types of soy sauces, 3 types of fermented soybean pastes, 3 types of fermented dairy products, and 3 types of commercial alcoholic beverages, included in 100 major Korean diets, representing 91.3% of total diets [17], were selected for sampling. Several brands of each food, representing more than 90% of the market share, were purchased at grocery stores and traditional

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Table 1. Fermented foods including alcoholic beverages in 100 major Korean diets and their daily consumption (g/day).

Food	Number of brands	Daily consumption (age/sex)											
		3–6		7–12		13–19		20–29		30–49		50–64	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Chinese cabbage *kimchi*	14	24.7	22.4	57.2	48.6	90.6	61.9	106.0	80.0	128.1	102.1	126.0	109.1
Radish root *kimchi*	2	3.4	4.8	9.4	7.8	10.0	11.7	14.6	11.2	20.0	10.3	18.6	9.3
Pickled young radish *kimchi*	11	2.0	1.2	2.9	2.9	4.3	4.5	5.6	5.0	10.0	12.0	8.7	12.4
Watery radish *kimchi*	1	0.8	2.6	2.7	1.9	2.4	3.7	3.9	6.4	7.9	8.4	11.0	12.4
Watery *kimchi*	7	0.6	1.3 .	1.2	1.0	2.1	0.5	1.5	2.5	3.6	4.1	6.4	4.9
Japanese soy sauce	7	2.4	3.1	5.3	4.3	5.3	3.8	7.3	5.8	11.3	9.0	16.1	15.4
Traditional soy sauce	4	2.7	2.5	4.1	4.1	4.2	4.1	6.0	4.7	6.4	5.0	5.3	4.1
Soybean paste	12	1.1	1.2	3.3	3.5	4.7	5.1	7.4	4.8	7.3	4.1	4.8	3.0
Mixed paste	4	0.4	0.2	0.6	0.5	1.9	0.7	2.1	1.3	2.9	1.8	1.6	1.3
Korean hot pepper paste	7	0.8	0.7	1.1	1.1	1.0	0.8	1.4	1.2	1.7	1.8	2.4	1.8
Plain yogurt	19	19.3	21.5	9.4	8.9	8.4	5.2	6.7	5.7	5.2	5.4	3.4	5.8
Liquid yogurt	11	5.8	4.5	3.4	2.9	2.3	1.5	2.0	4.2	1.2	2.1	1.5	1.4
Processed cheese	9	0.7	1.1	0.7	0.7	0.8	1.0	0.5	0.5	0.5	0.5	0.2	0.1
Beer	6	–	–	–	–	8.3	5.3	80.6	33.8	63.8	28.8	17.7	5.4
Soju (diluted spirits)	7	–	–	–	–	15.0	2.7	37.6	8.0	54.9	9.8	57.9	7.7
Takju (rice wine)	7	–	–	–	–	0.4	-	9.4	–	6.6	1.4	20.6	8.0
Body weight (kg)		19	19	33	33	59	53	67	54	68	55	64	54

markets in major cities across Korea. Collected samples are listed in Table 1.

Preparations of Sample and Standard Solution

Sample clean-up procedures were conducted using the methods of Canas *et al.* [6], Koh and Kwon [18], Kim *et al.* [16], and Conacher *et al.* [9] with some modification.

Alcoholic beverage samples were adjusted to 10% of alcohol content with distilled water and mixed to maintain approximately the same level of alcohol in all samples. Fifty g of diluted sample was weighed into a 250-ml centrifuge bottle, and 30 g sodium chloride was added and the mixture was swirled 1–2 min to saturate the solution. Seventy-five ml dichloromethane was added and the mixture vigorously shaken for 1 min. The solution was centrifuged at 2,000 ×*g* until phase separation occurred. The dichloromethane layer was drawn off using a suitable suction device. The remnant 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. The extraction step was repeated three more times. Three ml ethyl acetate was added to the dichloromethane extract, and the solution was concentrated in a rotary evaporator below 28°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 transfered to a 2-ml vial, and the flask was rinsed with 1 ml ethyl acetate and mixed well. The mixture (2 μl) was injected into a gas chromatograph for analysis.

For the analysis of solid samples such as *kimchi* and pastes, 20 g of sample was homogenized with distilled water. The same amount of ethyl acetate was added and the mixture was vigorously shaken for 1 min. The solution was centrifuged at 2,000 ×*g* until phase separation occurred. The ethyl acetate layer was drawn off using a suitable suction device. The remnant 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. The extraction step was repeated three more times. The combined ethyl acetate extract was concentrated in a rotary evaporator below 28°C at reduced pressure until the volume decreased to 10 ml, and then 15 g of celite was added. The fraction containing ethyl carbamate was eluted with 150 ml methylene chloride through a glass column (3×40 cm) packed with deactivated alumina, sodium sulfate anhydrous, and celite-concentrated sample mixture and evaporated at reduced pressure until the volume decreased to 1 ml. The liquid was transferred to a 2-ml vial, and the flask was rinsed with 1 ml ethyl acetate and mixed well. The mixture (2 μl) was injected into a gas chromatograph for analysis.

For the analysis of soy sauces and fermented milk products, 15 g of samples were cleaned up through the same procedure with analysis for solid samples except for the omission of ethyl acetate extraction. For cheese analysis, the Sep-Pak florisil cartridge (Waters Associates, Milford, MA, U.S.A.) clean-up method was applied to remove the fat in cheese.

Stock solution (1 mg/ml) was prepared as follows. In a 100-ml volumetric flask, 100 mg of ethyl carbamate (Sigma, U.S.A.) was dissolved in ethyl acetate, diluted, and mixed thoroughly. Then, a 100 μg/ml of working standard solutions was prepared by diluting the stock solution (1 mg/ml) ten-fold with ethyl acetate.

Gas Chromatography/Mass Spectrometry Detection

The Agilent 6890 gas chromatograph with a 5973N mass spectrometer (Agilent Technologies, Palo Alto, CA, U.S.A.) was used for the separation and determination of ethyl carbamate in the extracts, according to Ma *et al.* [23], Lachenmeier [20], and Lachenmeier *et al.* [21]. Determination of ethyl carbamate in each sample was triplicated and cross-checked again at the Cooperate Center for Research Facility in Sangjoo National University (Sangjoo, Korea) to reduce analytical errors.

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; and injector, 25°C.

Mass spectrometer conditions were as follows: ion source, 200°C; electron impact ionization potential at 70 eV; SIM, m/z of 62, 74, and 89; and injection mode, splitless. Peaks containing all three ions (m/z 62, 74, 89) were regarded as ethyl carbamate.

Average Daily Intake and Excess Cancer Risk Assessment

Average daily intake of ethyl carbamate through fermented foods and alcoholic beverages was estimated based on the average ethyl carbamate concentration of each sample and data of the diets of Korean males and females aged 3–64 years (Table 1) obtained from the National Health and Nutrition Survey [17], and then, calculated using the following equation:

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

where C_i is the contamination level of i food (mean, µg/kg); CR_i is the contact rate of i food (g/day); BW is the average body weight (kg) over an exposure period, obtained from the Korea Research Institute of Standards and Science [19] and described in Table 1; and n is the number of tested samples.

Excess cancer risk assessment was conducted because previously mentioned studies [1, 15, 32, 33] clearly indicated that ethyl carbamate was an animal carcinogen with respect to tumor induction in different species, organs, and stage of development of the animals. Currently, a reference dose (RfD) for acceptable human exposure levels to ethyl carbamate is not yet known. However, on the basis of sex and organ specific tumor data 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), a "virtually safe dose (VSD)" of 20 to 80 ng/kg bw/day was estimated by Schlatter and Lutz [28]. The VSD of 20 ng/kg bw/day and lifetime risk level of 10^{-6}, therefore, were used for the calculation of cancer potency and excess cancer risk as follows:

Cancer potency (Q_1^*)=10^{-6}/20 ng/kg bw/day (VSD)
= 5×10^{-2} pg/kg bw/day

Excess cancer risk
=Cancer potency (Q_1^*) Average daily intake

RESULTS AND DISCUSSION

Ethyl Carbamate Levels in Korean Fermented Foods

For quantification of ethyl carbamate in most samples, the signal intensity (Fig. 1) at m/z of 62 for ethyl carbamate was compared with that for the internal standard, propyl carbamate. Propyl carbamate was added to water used for diluting or homogenizing the samples. Ethyl carbamate concentration was calculated from the standard curve constructed with varying ethyl carbamate/propyl carbamate ratios. The recovery ratio of ethyl carbamate to propyl carbamate was 0.72, regardless of the food type, and this recovery ratio was incorporated into constructing a standard curve. Ethyl carbamate content in the 35 brands of 5 types of *kimchi* ranged from a not detectable (n.d.) level to 4.26 µg/kg (Table 2). Among the 5 types of *kimchi* products, the highest ethyl carbamate concentration was found in Chinese cabbage *kimchi*, implying that it can be an important supplier of ethyl carbamate because of its high consumption in Korea (Table 1). This result is in accordance with the finding of Koh and Kwon [18], but lower than the finding of Kim *et al.* [16]. They reported that *kimchi* prepared with relatively large amount of fermented fish (*Jeotgal*), like Chinese cabbage *kimchi*, had a higher concentration of ethyl carbamate than *kimchi* prepared with a small amount of or without fermented fish, such as radish *kimchi* and watery *kimchi*, because the fermented fish supplied an abundant amount of amino acids that can turn into ethyl

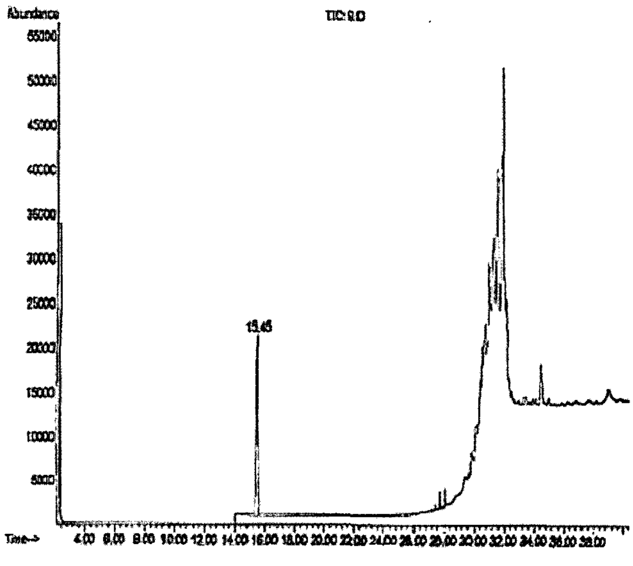

Fig. 1. GC/MS chromatogram of ethyl carbamate.

Table 2. Amounts of ethyl carbamate present in major Korean fermented foods including alcoholic beverages.

Food	Ethyl carbamate concentration (μg/kg)	
	Range	Mean
Chinese cabbage *kimchi*	0.12–4.26	1.41
Radish root *kimchi*	n.d.–0.34	0.17
Pickled young radish *kimchi*	n.d.–2.17	0.72
Watery radish *kimchi*	n.d.	n.d.
Watery *kimchi*	n.d.–0.06	0.01
Japanese soy sauce	3.60–58.9	19.44
Traditional soy sauce	1.4 0–49.2	16.69
Soybean paste	n.d.–3.76	1.06
Mixed paste	n.d.–1.00	0.46
Korean hot pepper paste	n.d.–1.10	0.56
Drinking fermented milk	n.d.–0.33	0.10
Stirred style fermented milk	n.d.–1.87	0.64
Liquid yogurt	n.d.–0.14	0.02
Processed cheese	n.d.–0.18	0.08
Beer	0.51–0.77	0.50
Soju (diluted spirits)	0.83–10.1	3.00
Takju (rice wine)	0.40–0.93	0.60

n.d.= not detected

carbamate in the *kimchi*. The highest average content of ethyl carbamate in the samples was found in the soy sauces. Matsudo *et al.* [24] suggested that ethyl carbamate formed chemically in soy sauce from some ethyl carbamate precursors by heating (pasteurization), because the ethyl carbamate concentration in soy sauce was directly proportional to its heating time. Chung and Kwon [8] reported that the soybean and storage length of soy sauce had the most profound effect on the formation of ethyl carbamate in soy sauce. Although irradiation of visible light for 12 h a day during storage lowered the concentration of ethyl carbamate compared with the soy sauce kept in the dark, both irradiation during the brining and the salt amount showed little effect on ethyl carbamate formation [12]. The fermented dairy products had negligible amounts of ethyl carbamate. Ethyl carbamate contents of *Soju*, the Korean popular commercial alcoholic beverage produced by dilution of distilled spirits, were relatively high and varied greatly depending on the brands. 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 [11] among 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, a high consumption of *Soju* among Koreans shows it can be an important source of ethyl carbamate. The ethyl carbamate content of beer ranged from 0.51 to 0.77 μg/kg, which was much lower than the finding (10 μg/kg) of Battaglia *et al.* [3].

Risk Assessment

For assessment of the average daily intake of ethyl carbamate for the Korean general population, aged 3–64 years, the daily consumption, mean body weight per age, and sex were considered under the assumption that an individual consumes a certain kind of fermented food in a day. The average daily intakes of ethyl carbamate through fermented

Table 3. Average daily intake of ethyl carbamate by consumption of each fermented food (ng/kg bw/day).

Food	3–6		7–12		13–19		20–29		30–49		50–64	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Chinese cabbage *kimchi*	1.83	1.66	2.44	2.08	2.17	1.65	2.23	2.09	2.66	2.62	2.61	2.70
Radish root *kimchi*	0.03	0.04	0.05	0.04	0.03	0.04	0.04	0.04	0.05	0.03	0.05	0.03
Pickled young radish *kimchi*	0.08	0.05	0.06	0.06	0.05	0.06	0.06	0.07	0.11	0.16	0.09	0.16
Watery radish *kimchi*	–	–	–	–	–	–	–	–	–	–	–	–
Watery *kimchi*	–	–	–	–	–	–	–	–	–	–	–	–
Japanese soy sauce	2.76	2.56	2.42	2.42	1.38	1.50	1.74	1.69	1.86	1.77	1.83	1.40
Traditional soy sauce	0.70	0.88	0.56	0.56	0.28	0.25	0.35	0.37	0.42	0.55	0.59	0.53
Soybean paste	0.13	0.17	0.17	0.14	0.10	0.08	0.12	0.10	0.18	0.17	0.25	0.29
Mixed paste	0.01	n.d.	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01
Korean hot pepper paste	0.03	0.04	0.06	0.06	0.04	0.05	0.06	0.05	0.06	0.04	0.04	0.03
Plain yogurt	0.02	0.02	0.01	0.01	–	–	–	–	–	–	–	–
Liquid yogurt	0.01	0.09	0.04	0.03	0.01	0.01	0.01	-	0.01	0.01	0.01	0.01
Processed cheese	–	–	–	–	–	–	–	–	–	–	–	–
Beer	–	–	–	–	0.07	0.05	0.64	0.33	0.50	0.28	0.14	0.05
Soju (diluted spirits)	–	–	–	–	0.76	0.15	1.68	0.44	2.42	0.53	2.55	0.41
Takju (rice wine)	–	–	–	–	–	–	0.08	–	0.06	0.01	0.18	0.08
Total	5.62	5.51	5.81	5.40	5.00	3.86	7.03	5.19	8.33	6.18	8.36	5.69

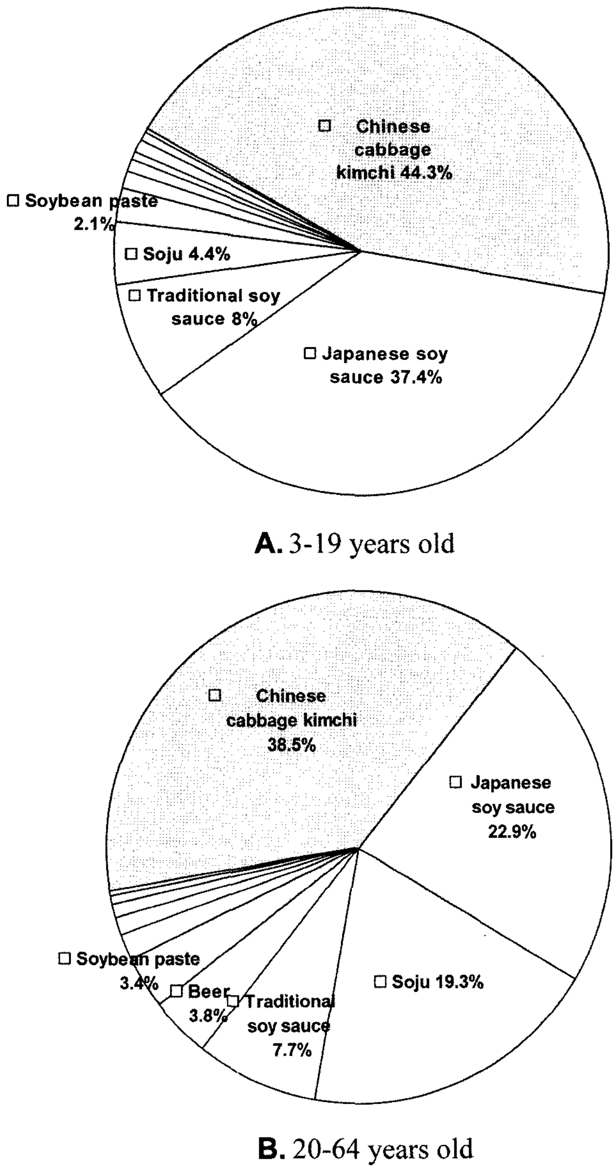

Fig. 2. The contributions of major Korean fermented foods and alcoholic beverages to human expose to ethyl carbamate.

foods and alcoholic beverages were calculated by multiplying the consumptions with the mean ethyl carbamate content and are shown in Table 3. The contributions of the major foods influencing average daily intakes are described in Fig. 2. The average daily intakes of ethyl carbamate were mainly caused by the Chinese cabbage *kimchi*, soy sauces, and *Soju* for adults. Although the daily intakes of *Soju* were not higher than beer (Table 1), the high content of ethyl carbamate in *Soju* (Table 2) contributed significantly to the total intake of ethyl carbamate. Conversely, although beer had the highest consumption among the alcoholic beverages (Table 1), its lower ethyl carbamate content meant that it contributed less to the total intakes of ethyl carbamate. Exposure of males to ethyl carbamate was higher than females because of the higher consumption of alcoholic beverages by males . The mean of the calculated average daily intakes for 3–64-year-old males and females (6 ng/kg bw/day) was lower than the VSD (20–80 ng/kg bw/day) suggested by Schlatter and Lutz [28]. However, in the case of consuming certain brands of the fermented foods with high ethyl carbamate contents, the maximum intakes, instead of mean intakes, of ethyl carbamate could be higher than the VSD. In addition, this value is only based on the 100 major Korean fermented foods and alcoholic beverages, and excludes minor foods such as coffee, whisky, fruit brandy, wine, and sake, which are highly suspected to contain ethyl carbamate [3]. In fact the highest average content of ethyl carbamate had been found in the fruit bandy, particularly stone fruit brandies made of plum (up to 689.9 μg/kg) in our preliminary study [10]. Therefore, the total intake of ethyl carbamate by Koreans through not only 100 major foods but also minors has a high possibility to be higher than the VSD of 20 ng/kg bw/day. The average excess cancer risk was calculated to be 3.0×10^{-7} (3 outbreaks of 1 million) and the highest excess cancer risk (4.2×10^{-7}) was found in 30–64-year-old males (Table 4), regardless of any possibilities increasing the average daily intakes of ethyl carbamate mentioned previously, except for the 100 major Korean diets, which is not far from the lifetime risk level of 10^{-6}. Nonetheless, the present results still reveal that Chinese cabbage *kimchi*, *Soju*, and soy sauces represent a significant part of the intakes of ethyl carbamate (Fig. 2). Since ethyl carbamate formation is avoidable, related industries need to make an effort to reduce the amount of ethyl carbamate in their products. Identification of the mechanism involved in the ethyl carbamate formation during fermentation and the effects of processing and storage conditions on the formation are important to achieve reduction of ethyl carbamate in foods. Regulations of ethyl carbamate levels in fermented

Table 4. Excess cancer risk of ethyl carbamate by consumption of fermented foods including alcoholic beverages.

Sex \ Age (years)	3–6	7–12	13–19	20–29	30–49	50–64
Male	2.8×10^{-7}	2.9×10^{-7}	2.5×10^{-7}	3.5×10^{-7}	4.2×10^{-7}	4.2×10^{-7}
Female	2.8×10^{-7}	2.7×10^{-7}	1.9×10^{-7}	2.6×10^{-7}	3.1×10^{-7}	2.8×10^{-7}
Average	3.0×10^{-7}					

foods should also be implemented to protect the health of the consumers. In this study, we determined the concentrations of ethyl carbamate in major Korean fermented foods and commercial alcoholic beverages. We hope that this work contributes to reducing the intake of ethyl carbamate in Korea.

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