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Ethyl carbamate and hydrocyanic acid in food and beverages[1]

Scientific Opinion of the Panel on Contaminants

(Question N° EFSA-Q-2006-076)

Adopted on 20 September 2007

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[2] After adoption of the opinion, EFSA was notified that the data it had been supplied with relating to ethyl carbamate levels in tequila were preliminary unconfirmed data derived during a research project and erroneously sent to EFSA. As a result, in the revision all references to tequila have been removed from the opinion as there was no other data available on tequila. This change does not affect the conclusions of the opinion. To avoid confusion, the original version of the opinion has been removed from the website, but is available on request as is a version showing all the changes made.

SUMMARY

Ethyl carbamate occurs naturally in fermented foods and alcoholic beverages such as bread, soy sauce, yoghurt, wine, beer, and spirits, particularly in stone-fruit brandies. A number of precursors present in food and beverages such as hydrocyanic acid, urea and ethanol can lead to the formation of ethyl carbamate during food processing and storage.

Ethyl carbamate is genotoxic and a multisite carcinogen in animals and probably carcinogenic in man. The European Commission asked the CONTAM Panel for a scientific opinion on the risks to human health related to the presence of ethyl carbamate and hydrocyanic acid in food and alcoholic beverages, in particular stone-fruit brandies.

In response, EFSA in late September 2006 issued a call for submission of data on levels of ethyl carbamate and hydrocyanic acid in food and beverages. Seven EU Member States, the Liquor Control Board of Ontario and the Wine Institute of California responded to EFSA's call for data on ethyl carbamate and submitted results covering analyses from 1998 to 2006. Three Member States submitted data on hydrocyanic acid in alcoholic beverages.

Only very few food (excluding alcoholic beverages) results for ethyl carbamate were reported to EFSA and of the results 41% were below the limit of detection. In the 2005 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) review it was concluded that food products in general would contribute less than 1 µg/person per day and this figure was used in exposure assessment calculations.

In contrast to the few food results, EFSA received over 33,000 testing results of alcoholic beverages. For almost 93% of the beer samples, 42% of the wine samples, but fewer than 15% of the spirit samples, the results were below the limit of detection. Median levels of ethyl carbamate in alcoholic beverages of up to 5 µg/L for beer and wine, 21 µg/L for spirits other than fruit brandy and 260 µg/L for fruit brandy were calculated. From these data, a dietary exposure of 17 ng/kg b.w. per day[3] was estimated from food for an average 60 kg person who does not consume alcohol, whereas this would increase up to 65 ng/kg b.w. for consumers of a variety of different alcoholic beverages. The highest exposure to ethyl carbamate can be expected for persons exclusively consuming fruit brandy with exposure at a 95th percentile consumption level of 558 ng/kg b.w. per day.

The estimated dietary exposure to hydrocyanic acid was about 1.6 µg/kg b.w. per day for a 60 kg person. The main contributor to hydrocyanic acid exposure in average consumers was food products, with alcoholic beverages contributing only minor amounts. At the 95th percentile consumption level of fruit brandy and the 95th percentile concentration level for hydrocyanic acid a peak dietary exposure of 24 µg/kg b.w. per day would be possible, which is undesirable. .

[3] JECFA arrived at a figure of 15 ng/kg b.w. per day when rounding intake calculations.

A risk characterisation was performed using the Margin of Exposure (MOE) approach comparing a BMDL10[4] derived from animal cancer data with scenarios for exposure to ethyl carbamate. A value of 10,000 and above was considered to be of low concern for public health. The MOEs were calculated using the estimated intake of ethyl carbamate at the median levels in alcoholic beverages and the BMDL10 value of 0.3 mg/kg b.w per day (10% incidence of alveolar and bronchiolar neoplasms in male and female mice).

The Panel concluded that the MOE of almost 18,000 calculated for exposure to ethyl carbamate in food excluding alcoholic beverages indicates a low concern for human health. However, the MOE was in the region of 5,000 for food consumed together with a variety of alcoholic beverages, and for high consumers of fruit brandy the MOE was less than 600. Based on these MOEs the Panel concluded that ethyl carbamate in alcoholic beverages indicates a health concern, particularly with respect to stone fruit brandies. The Panel noted that for consumers of particular brands of stone fruit brandy, with higher than average levels of ethyl carbamate, the MOEs could be even lower.

Mitigation measures should be taken to reduce the levels of ethyl carbamate in certain alcoholic beverages such as fruit brandies. Such measures should include focus on hydrocyanic acid and other precursors of ethyl carbamate to prevent the formation of ethyl carbamate during shelf-life of these products.

Key words:

Ethyl carbamate, hydrocyanic acid, food, alcoholic beverages, margin of exposure (MOE), pubic health, occurrence and exposure

[4] The BMDL10 (benchmark dose lower confidence limit 10%) represents the lower bound of a 95% confidence interval on a BMD (benchmark dose) corresponding to a 10% tumour incidence.

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BACKGROUND AS PROVIDED BY REQUESTOR

Ethyl carbamate is a compound that can occur naturally in fermented foods and beverages, such as spirits, wine, beer, bread, soy sauce and yoghurt. Therefore, the major source of dietary exposure to ethyl carbamate in the human population is through the consumption of fermented foods and beverages, e.g. as a consequence of its unintentional formation during the fermentation process or during storage.

Ethyl carbamate can be formed from various substances derived from food and beverages, including hydrogen cyanide, urea, citrulline, and other N-carbamyl compounds. Cyanate is probably the ultimate precursor in most cases, reacting with ethanol to form the carbamate ester.

Ethyl carbamate forms in stone fruit distillation, when exposed to light, from the natural precursors of fruit mash and ethyl alcohol. Hydrocyanic acid or the salts produced therefrom, the cyanides, are regarded as the most important precursors in the process. Hydrocyanic acid initially occurs in bound form in the stones of the fruits and is released through enzymes during the maturation process and after the harvest.

At the 64th meeting in February 2005, the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organisation (WHO) Expert Committee on Food Additives (JECFA) evaluated ethyl carbamate. JECFA concluded that ethyl carbamate is genotoxic and a multisite carcinogen in all animal species tested and is considered to be a potential carcinogen in humans (FAO/WHO, 2006).

The JECFA estimated that the MOE (margin of exposure) is 20,000 when the estimated intake of ethyl carbamate in foods (15 ng/kg b.w. per day) is compared with the BMDL (benchmark dose lower confidence limit) of 0.3 mg/kg b.w. per day (obtained for a 10 % incidence of alveolar and bronchiolar neoplasms in male and female mice). With the inclusion of alcoholic beverages in the estimated intake (80 ng/kg b.w. per day), the resulting MOE was 3,800. On the basis of these considerations, the JECFA concluded that the intake of ethyl carbamate from foods excluding alcoholic beverages would be of low concern[5]. However, the MOE of 3,800 for all intakes, food and alcoholic beverages combined, was of concern and therefore mitigation measures to reduce concentrations of ethyl carbamate in some alcoholic beverages should be continued.

[5] In the Opinion of the EFSA Scientific Committee on a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic, the Scientific Committee concluded that "in general a margin of exposure of 10,000 or higher, if it is based on the BMDL (10 % incidence) from an animal study, and taking into account the uncertainties in the interpretation, would be of low concern from a public health point of view and might be reasonably considered as a low priority for risk management actions".

TERMS OF REFERENCE AS PROVIDED BY REQUESTOR

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide a scientific opinion on the risks to human health related to the presence of ethyl carbamate in foods and beverages, in particular alcoholic beverages (stone fruit brandies). As hydrocyanic acid and its salts are important precursors for ethyl carbamate formation, possible health risks related to the presence of cyanides should also be considered.

ACKNOWLEDGEMENTS

The Panel on contaminants in the food chain (CONTAM) wishes to thank the EFSA units on data collection and exposure (DATEX) and on contaminants for the preparation of this opinion.

ASSESSMENT

1. Introduction

Ethyl carbamate or urethane (CAS number 51-79-6) is the ethyl ester of carbamic acid. It can be found in fermented foods and beverages like spirits, wine, beer, bread, soy sauce and yoghurt (Conacher and Page, 1986; Dennis *et al.,* 1989; Battaglia *et al.,* 1990; Schlatter and Lutz, 1990; Zimmerli and Schlatter, 1991; Sen *et al.,* 1992; Sen *et al.,* 1993; Benson and Beland, 1997; Kim *et al.,* 2000). The structure and two routes of the formation of ethyl carbamate are illustrated in Figure 1.

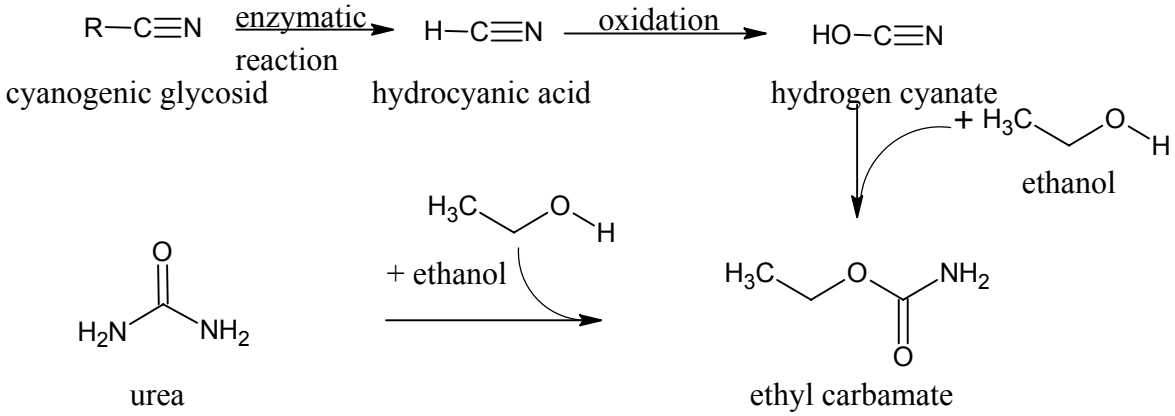


Figure 1: The formation of ethyl carbamate from ethanol and hydrogen cyanate (top) or from ethanol and urea (bottom).

There are a number of precursors in food and beverages that can form ethyl carbamate including hydrocyanic acid, urea, citrulline, cyanogenic glycosides and other N-carbamyl compounds. Hydrocyanic acid or hydrogen cyanide (CAS number 74-90-8) a gas that is a weak acid when dissolved in water. Cyanide (CAS number 57-12-5) is the anion of hydrocyanic acid. In stone fruit spirits, ethyl carbamate is formed when cyanogenic glycosides (such as amygdaline) from the stones are degraded through enzymatic action (mainly beta-glucosidase) to cyanide, which is then oxidised to cyanate and reacts with ethanol (Lachenmeier, 2005b; Schehl *et al.,* 2007). Another source of ethyl carbamate is urea, resulting from the degradation of arginine by yeasts, which reacts with ethanol as illustrated in Figure 1.

Although there is an extensive literature dating back to the 1940s on the genotoxicity and carcinogenicity of ethyl carbamate, public health concerns related to its detection were only raised in 1985 when relatively high levels were found in alcoholic beverages by Canadian authorities. Major reviews of the available data pertaining to carcinogenicity were performed in 1989 by the California Department of Health Services (Salmon *et al.,* 1991). The International Agency for Research on Cancer (IARC) classified ethyl carbamate as "possibly carcinogenic to humans" (Group 2B) in 1974 (IARC, 1974), and updated the classification to probably carcinogenic to humans (Group 2A) in 2007 (IARC, 2007). The Report on Carcinogens of the U.S. National Toxicology Program (NTP) also concluded on the compound's probable carcinogenicity to humans from a 2-year study in rodents (NTP, 2004).

At its 64th meeting in February 2005, the Joint Food and Agriculture Organization of the United Nations (FAO) World Health Organisation (WHO) Expert Committee on Food Additives (JECFA) evaluated ethyl carbamate. The JECFA concluded that ethyl carbamate is genotoxic and is a multisite carcinogen in all animal species tested and is considered to be a potential carcinogen in humans (FAO/WHO, 2006). The JECFA calculated several margins of exposure (MOE) and concluded that the intake of ethyl carbamate from foods excluding alcoholic beverages would be of low concern. However, dietary exposure to ethyl carbamate from both food and alcoholic beverages was of concern and mitigation measures to reduce concentrations of ethyl carbamate in some alcoholic beverages were recommended.

This opinion presents an updated risk assessment of ethyl carbamate in food and beverages from Europe with particular attention to alcoholic beverages such as stone-fruit brandies. In addition, the possible health risks related to the combined exposure of ethyl carbamate and hydrocyanic acid in food and beverages are discussed.

2. Legislation of ethyl carbamate and hydrocyanic acid

2.1 Ethyl carbamate

There are currently no harmonised maximum levels for ethyl carbamate in the European Union (EU). However, some Member States and Third Countries recommend maximum levels for ethyl carbamate in alcoholic beverages (Table 1).

Table 1: Maximum levels for ethyl carbamate in alcoholic beverages.

Country	Ethyl carbamate concentration µg/L in legislation				
	Wine	Fortified wine	Distilled spirits	Sake	Fruit brandy
Canada	30	100	150	200	400
USA	15	60			
Czech Republic	30	100a)	150	200	400b)
France			150		1,000
Germany					800

a) Fruity wines and liqueurs
b) Fruity distillates and fruity, mixed and other spirits

In 1986, Canada was the first country to introduce maximum levels for the presence of ethyl carbamate in a variety of alcoholic beverages. These limits varied from 30 µg/L for wine to 400 µg/L for fruit brandies. The USA has voluntary targets for products produced in the country, and notified all countries exporting wines to the USA that they must develop programs to meet these target levels. The Czech Republic closely followed the Canadian limits although the nomenclature for the alcoholic beverages is slightly different.

2.2. Hydrocyanic acid

Council Regulation (EEC) No 1576/89 of 29 May 1989[6] lays down general rules on the definition, description and presentation of spirit drinks and a maximum content for hydrocyanic acid in stone fruit spirits is established at a level of 10g/hL (equivalent to 100mg/L) pure (100% volume) alcohol. The same maximum content is established for stone fruit marc spirits in Commission Regulation (EEC) No 1014/90 of 24 April 1990[7] laying down detailed implementing rules on the definition, description and presentation of spirit drinks.

In Council Directive 88/388/EEC of 22 June 1988[8] on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production a maximum limit for hydrocyanic acid of 1 mg per % volume of alcohol in alcoholic beverages is established. Hydrocyanic acid may not be added as such to

[6] OJ L 160, 12.6.1989, p. 1
[7] OJ L 105, 25.4.1990, p. 9
[8] OJ L 184, 15.7.1988, p. 61

foodstuffs or to flavourings, but it may be present in a foodstuff either naturally or following the addition of flavourings prepared from natural raw materials.

3. Sampling and methods of analysis

3.1 Ethyl carbamate

Sampling for ethyl carbamate analysis is straightforward. The substance itself is relatively stable in most matrices. Because ethyl carbamate can be formed after the processing and packaging of the food or beverage, the time of sampling of a product in relation to usual consumption is important. Samples should not be unduly exposed to heat or light since this can induce ethyl carbamate formation.

Over the past 30 years, methods have been developed for the extraction and analysis of ethyl carbamate in all the food and beverage types in which the substance is known to be formed. The use of gas chromatography (GC) coupled with mass spectrometry (MS) gives confidence in the analytical aspects of correct identification and quantification in food and beverages. These methods have been tested in two international collaborative trials, for application to beer and whisky (Dennis *et al.*, 1989) and to wine, fortified wine, spirits and soy sauce (Canas *et al.*, 1994).

A Community method for the analysis of ethyl carbamate in wines is laid down in Commission Regulation (EC) No 761/1999[9] of 12 April 1999 amending Regulation (EEC) No 2676/90[10]. In the prescribed GC-MS method using fragmentometry in SIM (selected ion monitoring) mode, propyl carbamate is added as an internal standard. The method is capable of routinely detecting ethyl carbamate at concentrations of 1 µg/kg, and has limits of quantification (LOQ) in the range of 3 to 5 µg/kg in food and beverages. Sensitivity can be further improved if extra sample clean-up and concentration steps are performed.

Lachenmeier and co-workers recently reported results from combining GC and tandem MS using a triple-quadruple instrument (GC/MS/MS Multi Reaction Monitoring - MRM) to determine ethyl carbamate in stone-fruit spirits. A good agreement was found for analytical results of a GC/MS SIM method and the GC/MS/MS MRM procedure (Lachenmeier *et al.*, 2005a).

3.2 Hydrocyanic acid

Several methods have been reported for detection of hydrocyanic acid in environmental, water and biological samples. Commission Regulation (EC) No 761/1999[8] of 12 April 1999 amending Regulation (EEC) No 2676/90[9] lays down a Community method for the

[9] OJ L 99/4, 12.4.1999, p. 1
[10] OJ L 272, 3.10.1990, p. 1

analysis of hydrocyanic acid in wines. In the prescribed method, total free hydrocyanic acid in wine is released by acid hydrolysis and separated by distillation. After reacting with chloramine-T and pyridine, the glutaconic dialdehyde formed is determined by colorimetry on the basis of the blue coloration it gives with 1,3-dimethyl-barbituric acid.

4. Occurrence of ethyl carbamate and hydrocyanic acid in food and beverages

4.1 Results reported previously

The JECFA undertook a review of ethyl carbamate in food and beverages in 2005. Of the 6,376 sample results reported to the JECFA, the 372 food samples showed mean values from non-detected to 16 µg/kg with an overall maximum of 84 µg/kg in a soy sauce product. Among the 6,004 samples of alcoholic beverages there were some very high values of up to 6,131 µg/kg reported in the group of cordials, liqueurs and brandies, presumably confined to brandies. The highest mean of 122 µg/kg was reported in sake (FAO/WHO, 2006). It should be noted that some food and beverages groups contained very few samples as shown in Table 2.

Table 2: Concentrations of ethyl carbamate in food and beverages (FAO/WHO, 2006).

Product	Country	No. of samples	Mean (µg/kg)	Range (µg/kg)
Alcoholic beverages				
Wine	Various	5,431	4-10	ND–61
Fortified wine	Various	140	32-41	ND–262
Whisky	Various	235	29-32	ND–239
Cordial, liqueur, brandy	Various	14-31	37-64[a)]	ND–243; 6,131
Sake	Japan	92	73-122	ND–202
Beer	Various	62	ND[b)]-1	ND-5
Food				
Bread	UK	157	ND-2	ND-4.5
	Denmark	33	4	0.8–12
Kimchi	South Korea	20	4	ND–16
Yogurt	UK	4	—	ND
	Various	9	1	ND–1.3
	Denmark	19	0.2	ND–0.3
Cheese	Various	17	—	ND
Soy sauce	Japan	48	ND-16	ND-84

Note that the results have been aggregated into product groups with the same country assignment when possible

a) This mean concentration excludes the single highest value reported, 6,131µg/kg.

b) ND: not detected, i.e. results ≤ LOD/LOQ of the method used.

Many edible plants contain cyanogenic glycosides, with concentrations varying widely as a result of genetic and environmental factors (Ermans *et al.*, 1980; FAO/WHO, 1993). Concentrations of hydrocyanic acid in some foodstuffs are shown in Table 3 (modified from Simeonova and Fishbein, 2004).

Table 3: Hydrocyanic acid concentrations in food products (mg/kg) and beverages (mg/L).

Type of product	Hydrocyanic acid concentrations (mg/kg or mg/L)
Cereal grains and their products	0.001-0.45
Soy protein products	0.07-0.3
Soybean hulls	1.24
Apricot pits	89-2,170
Fruit juice (cherry, apricot, prune)	1.9-4.6
Cassava	300-2,360
Sorghum (immature)	2,400
Bamboo (immature shoot tip)	7,700
Lima beans	2,000-3,300

4.2 Current results

Because of the sparse number of results reported to the JECFA for ethyl carbamate in some important beverage categories, EFSA in late September 2006 issued a call for submission of data on levels of ethyl carbamate and hydrocyanic acid in food and beverages. Seven EU Member States (see Table 4) responded to EFSA's call for data on ethyl carbamate and submitted results covering analyses for 1998 to 2006. Three Member States (Germany, France and Austria) also submitted results from cyanide testing. The sensitivity of the methods used for ethyl carbamate as reported by the LOD ranged from 0.1 µg/kg to 400 µg/kg (in a few instances). The LOD for hydrocyanic acid varied from 10 to 100 µg/kg. During evaluation of data, incomplete and duplicate records were omitted, as well as samples with a LOD for ethyl carbamate at 100 µg/kg or above as such levels would influence the accuracy of the risk assessment. The number of valid results incorporated in the further analyses is illustrated in Table 4.

Of the 4,203 results for ethyl carbamate, 137 covered food samples and 4,066 covered alcoholic beverage samples. Testing for hydrocyanic acid included 715 results covering spirits other than fruit brandy and fruit brandy, and one further result covering wine. Ethyl carbamate was detected in 59% of food samples and 88% of alcoholic beverage samples, and hydrocyanic acid was detected in 41% of alcoholic beverage samples. No food sample was tested for hydrocyanic acid. Test results for whisky from 1998 and 1999 were incorporated in the material since very few whisky results were received from the call for data covering years 2000 to 2006.

Table 4: The number of samples analysed for ethyl carbamate by respective Member State for the years 1998 to 2006.

Country	Number of analytical results by year									Total
	1998	1999	2000	2001	2002	2003	2004	2005	2006	
Austria	139	16	20	5	2	4	2	-	-	188
Belgium	-	-	-	-	-	-	-	-	11	11
Czech Republic	-	-	-	-	-	-	-	-	92	92
France	-	-	48	42	41	27	65	19	40	282
Germany	22	-	181	387	278	377	499	620	845	3,293
The Netherlands	-	-	-	-	-	-	-	67	-	67
United Kingdom	-	205	-	-	-	-	-	149	-	354
Total	161	221	249	434	321	408	566	855	988	4,203

The EFSA also received submissions from the Liquor Control Board of Ontario, Canada and the Wine Institute of California, USA covering analyses of a range of alcoholic beverages produced in or imported to North America. Products originating from an EU Member State were extracted from this material and data covering the years of 2002-2006 as presented in Table 5 were incorporated in the further analyses.

All of the 28,858 results from North America comprised alcoholic beverages. The sensitivity of the methods used for ethyl carbamate in the North American material, as reported by the limit of detection, was 5 µg/kg.

Ethyl carbamate was found in 54 % of the samples. The bulk of the sample was comprised of wines, usually with a lower incidence of ethyl carbamate compared to some spirits, which can explain the lower overall incidence in the North American material compared to sample results submitted by EU Member States.

Table 5: The number of analytical results submitted from North America for the years 2002 to 2006 for products originating from EU Member States.

Country	Number of analytical results by year					Total
	2002	2003	2004	2005	2006	
Austria	18	80	94	86	103	381
Belgium	23	100	104	119	82	428
Bulgaria	21	12	17	24	11	85
Croatia	11	9	20	4	4	48
Cyprus	2	16	14	6	8	46
Czech Republic	5	9	11	26	12	63
Denmark	5	6	17	14	14	56
Estonia	0	0	2	2	0	4
Finland	3	5	6	7	5	26
France	740	2,510	2,717	2,730	2,733	11,430
Germany	78	308	231	230	222	1,069
Greece	34	110	110	115	113	482
Hungary	15	45	62	45	44	211
Ireland	27	24	43	35	25	154
Italy	407	1,937	2,206	2,233	2,164	8,947
Latvia	0	3	7	4	1	15
Lithuania	0	1	2	1	1	5
Luxembourg	0	0	0	2	2	4
Netherlands	12	21	32	31	23	119
Poland	21	23	28	29	20	121
Portugal	89	357	403	483	355	1,687
Romania	2	6	28	16	10	62
Slovakia	2	2	1	4	0	9
Slovenia	2	55	13	20	24	114
Spain	86	304	304	467	453	1,613
Sweden	6	4	15	8	16	49
United Kingdom	125	327	387	453	338	1,630
Total	1,734	6,274	6,874	7,194	6,783	28,858

4.2.1 Ethyl carbamate results

Statistical descriptors for the concentration of ethyl carbamate in various products were calculated separately for submissions from EU Member States and from North America. Samples at or below the limit of detection were either entered as zero for lower bound values or the actual limit of detection for upper bound values. Ethyl carbamate findings from the data submitted by the Member States are summarised in Table 6.

Table 6: Concentrations of ethyl carbamate in food and beverages as submitted by EU Member States.

Product	No. of samples	Ethyl carbamate in µg/kg beverage			
		Median	Mean	P95[a)]	Range
Alcoholic beverages					
Beer	13 (1)[b)]	-	-	-	ND[c)]-1
Cider	1 (0)	-	-	-	ND[d)]
Wine	17 (11)	11	10-11[e)]	21	ND–24
Fortified wine	15 (15)	29	32	49	14-60
Sake	2 (2)	-	123	-	81–164
Brandy	42 (19)	0-30	123-129	395	ND–2,100
Cachaça	19 (19)	110	229	478	40–730
Distillate	13 (8)	1210	1,425-1,435	4500	ND–4500
Gin	1 (1)	-	-	-	580
Liqueur	4 (2)	6-7	45-47	146	ND–170
Miscellaneous spirits	86 (64)	290	590	1,745	ND–6,000
Other fruit brandy	328 (281)	215	663-667	4,187	ND–7,920
Rum	11 (10)	280	325-328	755	ND–1,020
Stone-fruit brandy	3,244 (2,912)	330	848-851	3,399	ND–22,000
Vodka	60 (57)	365	386-387	846	ND–2,140
Whisky	210 (196)	22	41	78	ND–1,000
Food					
Bakery	50 (49)	5	6	13	ND-20
Dairy	22 (0)	-	-	-	ND
Fermented beans	6 (0)	-	-	-	ND
Fermented olives	3 (0)	-	-	-	ND
Fermented sauce	44 (28)	2-3	3-4	14	ND-18
Sauerkraut	1 (1)	-	-	-	29
Vinegar	10 (1)	-	-	-	ND-33
Yeast extract	1 (1)	-	-	-	41

[a)] P95 is the 95th percentile of values.

[b)] Sample number in brackets indicates the number of positive samples.

[c)] ND is value at or below the limit of detection.

[d)] One value only in range field indicates a single result or all values below the limit of detection.

[e)] When two levels are shown in the median or mean field it illustrates the lower and upper bound values with results set to zero or the limit of detection, respectively, for samples with no detectable levels.

As expected, ethyl carbamate levels were particularly high for stone-fruit brandy as reported previously. However, the highest mean level was recorded in the few samples of distillate tested, seemingly liquid sampled at an interim step of alcohol production. Nine of the categories of spirits tested had individual samples at or exceeding 1,000 µg/kg, while only five categories had the 95th percentile above this level. Fruit brandies made from a mixture of fruits or from unspecified fruits were included in "other fruit brandy". It is thus possible that the high values recorded in this category could come from inclusion of stone fruits in the fruit mix. All fruit brandy results were combined into one category for the exposure assessment since the consumption statistics do not record them separately. As the number of other fruit brandy samples tested were only 10% of stone-fruit brandy samples, the higher levels found in stone-fruit brandy dominated the combined result. This provided a conservative estimate for the level of ethyl carbamate in fruit brandy.

Food products in general had non-detectable levels of ethyl carbamate, although bread and soy sauces consistently showed some detectable low levels.

In comparing results from the Member States (Table 6) with previous results from the JECFA (Table 2), the maximum level was about three times higher than any level recorded by the JECFA for alcoholic beverages. However, sample profiles are quite different. The JECFA reported mainly wine results with a few brandy results aggregated into a broader group, while the present review was dominated by results from testing of stone-fruit brandies.

Ethyl carbamate findings from the data submitted by organisations in North America using officially recognised analytical methods are summarised in Table 7 (LCBO, 2006). Only two of the categories tested had individual samples exceeding 1,000 µg/kg, fruit brandies and miscellaneous spirits. There is no further specification of fruit brandies, but it is assumed that stone-fruit brandies could be included in the sample and possibly responsible for the highest values. Armagnac had the highest calculated mean value and also by far the highest 95th percentile. In more than half of the wine samples no ethyl carbamate was recorded.

Table 7: Concentrations of ethyl carbamate in alcoholic beverages as submitted from North America for product originating in EU Member States.

Product	No. of samples	Ethyl carbamate in µg/kg beverage			
		Median	Mean	P95[a)]	Range
Spirits					
Armagnac	71 (69)[b)]	219	246	503	ND[c)]-630
Brandy	137 (135)	45	78	345	ND–642
Cognac	256 (247)	24	30	67	ND-191
Cooler	93 (14)	ND-5[d)]	3-7	13	ND–68
Fruit Brandy	186 (168)	27	100	284	ND–3,133
Gin	53 (30)	6	9-11	28	ND–60
Grappa	270 (242)	24	32	87	ND–192
Liqueur	356 (252)	9	21-22	74	ND-405
Miscellaneous spirits	632 (370)	7	17-19	58	ND–1,060
Rum	19 (14)	12	16-17	45	ND–57
Vodka	101 (33)	ND-5	4-8	17	ND–49
Whisky	1,122 (1,076)	30	40	106	ND–509
Beer and Wine					
Beer	1,208 (88)	ND-5	0.6-5	6	ND-33
Cider	26 (3)	ND-5	0.9-5	8	ND–9
Fortified Wine	1,000 (965)	26	39	113	ND-404
Fruit Wine	44 (8)	ND-5	2-6	10	ND–17
Wine	23,278 (12,001)	5	5-7	78	ND–180

[a)] P95 is the 95th percentile of values.

[b)] Sample number in brackets indicates the number of positive samples.

[c)] ND is value at or below the limit of detection.

[d)] When two levels are shown in the median or mean field it illustrates the lower and upper bound values with results set to zero or the limit of detection, respectively, for samples with no detectable levels.

To get an overview of the overall material, products from both sample groups were aggregated into six broader categories for closer analyses of distribution patterns of ethyl carbamate as presented in Table 8.

Table 8: Ethyl carbamate results split across concentration ranges for aggregated product categories from the combined North American and EU Member State samples.

Product	Per cent of analytical results in concentration ranges (µg/kg beverage)						
	ND	>ND-30	>30-100	>100-200	>200-400	>400-1,000	>1,000
Food	40.9	57.7	1.5	0	0	0	0
Beer	92.7	7.2	0.1	0	0	0	0
Wine	48.4	51.3	0.3	0	0	0	0
Fortified Wine	9.4	51.6	36.1	2.9	0	0	0
Fruit Brandy	10.6	8.2	12.4	13.1	17.7	17.9	20.2
Other Spirits	20.3	44.0	25.4	4.0	3.7	2.0	0.7
Total	41.3	43.7	5.4	2.1	2.6	2.4	2.5

As shown in Table 8, two of the six aggregated product categories had samples exceeding 100 µg/kg and only fruit brandies and other spirits had samples of more than 1,000 µg/kg.

Levels of ethyl carbamate in food samples varied from not detected to 41 µg/kg. There were only two products with a value over 30 µg/kg, a yeast extract with 41 µg/kg and sherry vinegar with 33 µg/kg, both tested in the United Kingdom. Among the further 6.5% of samples above 10 µg/kg were Sauerkraut, Christmas pudding, three soy sauce products and three bread products in decreasing order.

Several different beers were tested from light to strong originating from 23 different countries with very little ethyl carbamate recorded in any sample. Only one sample from Belgium (33 µg/kg) exceeded a level of 30 µg/kg.

Levels of ethyl carbamate in wine varied between not detected and 180 µg/kg. There was a large difference between white and red wine in that white wine had almost double the number of samples with no detected levels of ethyl carbamate, 68% compared to 37% for red wine. Of the 531 test results for rosé wine, 78% contained no detected levels of ethyl carbamate. Of the results above 30 µg/kg, 54 (0.36%) were red wine and 7 (0.09%) white wines samples. It has been hypothesised that more ethyl carbamate will be found in aged wines. However, there was no clear correlation between the age of the wine and the ethyl carbamate recorded in the submitted material with a coefficient of determination (R^2) of 0.099 calculated for the correlation coefficient for all wines and just a slightly higher value of 0.125 for red wines when analysed separately. The highest value of 180 µg/kg was recorded in a red wine of unspecified age.

For fortified wine samples, almost 3% of results exceeded 100 µg/kg with a maximum of 404 µg/kg. Also more than 90% of samples had detectable levels of ethyl carbamate, the highest proportion of any product category.

Fruit brandies, in particular stone-fruit brandies, had higher levels of ethyl carbamate with 68.9% of results exceeding 100 µg/kg, 20.2% above 1,000 µg/kg and seven results

of more than 10,000 µg/kg. The fruit brandies sampled were manufactured in 18 specified countries or were of unknown origin, with Germany the dominating manufacturing country. Of the 477 samples from France, 97% contained ethyl carbamate above the detection limit with a mean of 1,357 µg/kg. For Germany similar figures from the 2,698 samples were 90% and 863 µg/kg, for Austria from 197 samples were 81% and 962 µg/kg, for Italy from 69 samples were 87% and 250 µg/kg, and for the Czech Republic from 83 samples were 76% and 521 µg/kg. Of the seven samples with values above 10,000 µg/kg, six were plum brandies produced in France and one cherry brandy produced in Germany. The seven samples were spread over time in that one was produced in 2000, two in 2004, three in 2005 and one in 2006. Although there was no clear reduction in concentration over time, as has been reported previously, the seven samples are too few to draw any conclusion in this respect.

The other spirit category is a disparate mixture of brandy, cachaça, distillate, gin, liqueur, miscellaneous spirits, rum, vodka, and whisky. About 10.4% of samples in this category were reported with values above 100 µg/kg and 0.7% above 1,000 µg/kg. There were 14 miscellaneous samples, seven distillate samples and one each of a brandy and a rum sample above the 1,000 µg/kg level.

Concentrations of ethyl carbamate in different alcoholic beverages submitted by North America (originating from Europe) and by Member States are summarised in Table 9. These data have been used in the exposure assessment.

Table 9: Ethyl carbamate concentrations in different alcoholic beverages from North America (originating from Europe) and EU Member States.

	Ethyl carbamate concentration in µg/kg beverage			
	Beer	**Wine**	**Spirits other**	**Fruit brandy**
Median	0-5[a)]	5	20-21	260
Mean	1-5	5-7	64-66	744-747
P95[b)]	6	16	290	3,180
Maximum	33	180	6,000	22,000

[a)] When two levels are shown in the median or mean field it illustrates the lower and upper bound values with results set to zero or the limit of detection, respectively, for samples with no detectable levels.

[b)] P95 is the 95th percentile of values.

There is not much difference between the lower and upper bound values and therefore the upper bound values were used for the exposure assessment, except for beer where both lower and upper bound were used because of the many samples below the limit of detection.

4.2.2 Hydrocyanic acid results

A limited number of European samples were also tested for the presence of hydrocyanic acid with results given in Table 10.

Of the 716 samples tested for hydrocyanic acid, the dominating proportion, or 685 samples, was from testing of fruit brandy where relatively high levels of hydrocyanic acid have been shown previously. In positive samples levels of hydrocyanic acid varied from 13µg/kg in one sample of whisky, 149 µg/kg in one wine sample, 300 µg/kg in one sample of cachaça, between 130-880 µg/kg for eight brandy samples, 120-5,000 µg/kg for four distillate samples, to 25-70,000 µg/kg for fruit brandy. Two results, or 0.3% of the tested samples, exceeded the current maximum limit for hydrocyanic acid of 100 mg/L pure alcohol (i.e. 40 mg/L for a spirit beverage containing 40% alcohol).

Table 10: Concentrations of hydrocyanic acid in some alcoholic beverages.

	Hydrocyanic acid concentration in µg/kg beverage		
	Wine	**Spirits other**	**Fruit brandy**
Number of samples	1	30	685
Median		0-30[2a]	0-30
Mean	149	319-334	1,755-1,780
P95[b)]		1,500	10,800
Maximum		5,000	70,000

a) When two levels are shown in the median or mean field it illustrates the lower and upper bound values with results set to zero or the limit of detection, respectively, for samples with no detectable levels.

b) P95 is the 95th percentile of values

4.3 Factors influencing the concentration of ethyl carbamate

Since the identification of high levels of ethyl carbamate in alcoholic beverages there has been a considerable focus on reducing levels by a range of different methods (Lachenmeier *et al.,* 2005b). The key to successful prevention and control has been the identification of the main precursor substances responsible for the formation of ethyl carbamate in food and beverages, together with an understanding of the influence of the main external factors of light (for spirits only), time and temperature. This information has led to a mechanistic understanding from which control measures have been devised. Over the past years, major reductions in concentrations of ethyl carbamate have been achieved by reducing the concentration of the main precursor substances in the food or beverage and by reducing the tendency for these substances to react to form cyanate, e.g. by the exclusion of light from bottled stone-fruit brandies.

The reaction mechanisms that give rise to ethyl carbamate vary significantly between foods (Ough *et al.*, 1988). In the case of wine, the use of urea as a yeast nutrient can result in an elevated level of ethyl carbamate in the finished product while light has little influence. Ethyl carbamate formation increases over time with the reaction rate being exponentially accelerated at elevated temperatures. Urea is formed when the wine yeast metabolises arginine, a major alpha-amino acid in grape juice available to yeast. This reaction is yeast strain dependent. Yeasts differ in their ability to produce urea and to re-use urea secreted into the must/wine. Lactic acid bacteria also metabolise arginine and

liberate citrulline, an amino acid, which then reacts with ethanol to form ethyl carbamate. Over-fertilised vineyards, in general, will yield wines with higher urea and thus ethyl carbamate concentrations (Butzke and Bisson, 1997).

Much higher ethyl carbamate concentrations were detected in spirits derived from stone fruit like cherries, plums, mirabelles, or apricots (Battaglia *et al.*, 1990; Zimmerli and Schlatter, 1991). The formation of cyanogenic glycosides such as amygdalin in stone fruit by enzymatic action leads to the generation of cyanide, which is the most important precursor of ethyl carbamate in these spirits. Cyanide is oxidised to cyanate, which reacts with ethanol to form ethyl carbamate (Wucherpfennig *et al.*, 1987; Battaglia *et al.*, 1990; MacKenzie *et al.*, 1990; Taki *et al.*, 1992; Aresta *et al.*, 2001). The wide range of ethyl carbamate concentrations in stone-fruit spirits reflects its light-induced and time-dependent formation after distillation and during storage (Andrey, 1987; Mildau *et al.*, 1987; Baumann and Zimmerli, 1988; Zimmerli and Schlatter, 1991; Suzuki *et al.*, 2001). This is a major problem in that ethyl carbamate continues to be formed in spirits during storage so analytical results might not provide an accurate estimate of the potential levels which might be encountered at a later point in time. Some methods thus incorporate a light inducing step to determine the ethyl carbamate formation potential. Results reported to EFSA covered the use of methods both with and without light induction. However, results presented here only include "actual" levels detected without light induction.

4.4 Relationship between ethyl carbamate and hydrocyanic acid

Since hydrocyanic acid is a precursor to the formation of ethyl carbamate it has been hypothesised that a relationship exists between the respective amounts of ethyl carbamate and hydrocyanic acid found in alcoholic beverages. To test this hypothesis, a linear regression between the log transformed amounts of hydrocyanic acid and ethyl carbamate detected in 260 samples of alcoholic beverages with values for both compounds above the respective limit of detection was undertaken.

A statistical analysis predicted the following linear regression equation also illustrated in Figure 2:

Log (ethyl carbamate µg/kg) = 1.877 + 0.284 * Log (hydrocyanic acid µg/kg)

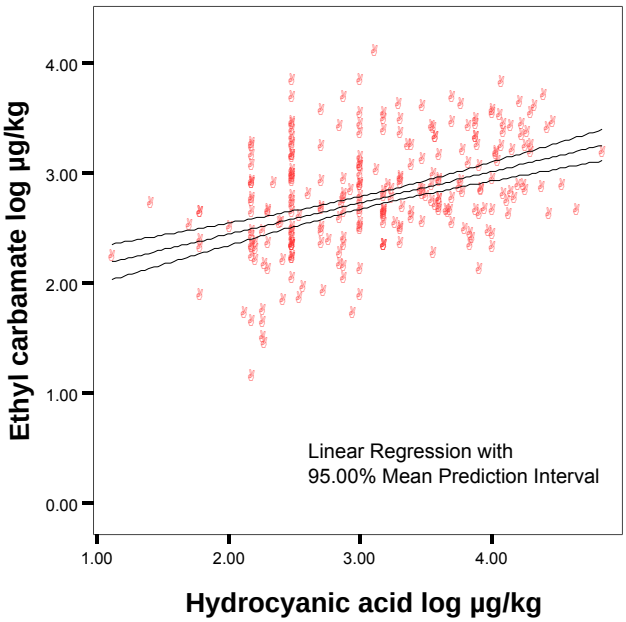


Figure 2: Distribution of results in relating hydrocyanic acid and ethyl carbamate log transformed concentrations and illustration of the linear regression equation.

The regression coefficient was 0.284 with a correlation coefficient (R) of 0.419 for the log transformed concentrations. There is thus some relationship between the two compounds as judged by the correlation coefficient, but with some considerable spread in the data. A coefficient of determination (R^2), indicating the ratio of explained variation to the total variation, of 0.175 showed that only 17.5% of changes in ethyl carbamate levels can be explained by changes in hydrocyanic acid concentrations. This is not an unexpected finding since ethyl carbamate formation is multifactorial, among many other factors time and light dependent in most cases, and there was a lack of detailed knowledge about the handling of the product after manufacturing.

However, comparing the distribution of samples with ethyl carbamate concentrations above and below 200 or 400 µg/kg for different levels of hydrocyanic acid provided a better fit (Table 11).

Table 11: Distribution of samples across given limits for ethyl carbamate of 200 or 400 μg/kg for different levels of hydrocyanic acid.

Hydrocyanic acid μg/kg	Ethyl carbamate μg/kg beverage				
	All	<200	≥200	<400	≥400
<5,000	645	278 (43%)	367 (57%)	405 (63%)	240 (37%)
≥5,000	71	4 (6%)	67 (94%)	14 (20%)	57 (80%)
≥10,000	42	2 (5%)	40 (95%)	5 (12%)	37 (88%)
≥20,000	12	0	12 (100%)	1 (8%)	11 (92%)
≥28,000	5	0	5 (100%)	1 (20%)	4 (80%)
≥40,000	2	0	2 (100%)	0	2 (100%)

The number of samples in the matrix diminishes as the limit for hydrocyanic acid increases and thus also the accuracy in the prediction. It can nevertheless be seen that at hydrocyanic acid concentrations at or above 20,000 μg/kg there are no samples with ethyl carbamate below 200 μg/kg. Further, with hydrocyanic acid concentrations at or above 28,000 μg/kg there is only one sample below 400 μg/kg.

5. Consumption of alcoholic beverages

In relation to alcoholic beverage consumption across EU Member States, few detailed statistics are available. Aggregated estimates were available from the World Health Organization Global Status Report on Alcohol (WHO, 2004; WHO, 2007) covering total yearly consumption of beer, wine and spirits per person in 25 EU Member States (Table 12). The consumption estimates are based on either FAO or WDT (World Drink Trends) data, except for a few countries in Europe where the data came directly from governments. Where both FAO and WDT data exist, a choice was made by FAO in favour of what they considered to be the more accurate and reliable data.

When using WDT data the per capita is recalculated into adult per capita consumption. The FAO data consist of estimates of production and trade in metric tonnes for the following beverages: wine, vermouth, must of grape, fermented beverages, spirits, sorghum beer, millet beer, maize beer, barley beer, wheat fermented and rice fermented. All the beverages were converted into pure alcohol and then combined into the categories of beer, wine and spirits so that all beers make up the beer category, and all other beverages, besides spirits, belong to the wine category.

Table 12: Yearly consumption in the adult population (15+ years) of beer, wine and spirits in litres of pure alcohol per person in 25 EU Member States (WHO, 2007).

Country	Yearly consumption of pure alcohol L/capita			
	Beer	Wine	Spirit	Total
Austria	6.6	3.9	1.6	12.1
Belgium	5.8	3.1	1.6	10.5
Bulgaria	0.5	2.7	2.5	5.7
Czech Republic	9.3	2.2	4.5	16.0
Denmark	5.9	4.4	1.4	11.7
Estonia	4.5	0.4	1.6	6.5
Finland	4.6	2.4	2.9	9.9
France	2.2	6.6	2.9	11.7
Germany	6.9	3.1	2.4	12.4
Greece	2.4	4.3	1.9	8.6
Hungary	4.3	4.9	4.2	13.4
Ireland	8.9	2.1	2.5	13.5
Italy	1.8	6.1	0.5	8.4
Latvia	2.2	0.5	7.2	9.9
Lithuania	4.5	1.1	4.3	9.9
Luxembourg	6.3	9.0	2.0	17.3
Netherlands	4.8	2.6	1.8	9.2
Poland	4.8	1.6	1.6	8.0
Portugal	3.5	5.6	1.7	10.8
Romania	4.0	3.0	2.4	9.4
Slovakia	5.4	1.7	4.3	11.4
Slovenia	3.8	2.0	1.0	6.8
Spain	4.6	3.9	2.8	11.3
Sweden	3.3	2.2	1.1	6.6
United Kingdom	6.2	2.7	2.2	11.1

The methodology to convert alcoholic drinks to pure alcohol may differ across countries. Typically beer is weighted as 4-5%, wine as 11-16% and spirits as 40% of pure alcohol equivalent (Anon, 2007). For beer a concentration of 5% was assumed, for wine 12% and for spirits 40%. Translated to daily consumption of total alcoholic beverages in the adult population aged 15 or over, the mean beer intake varied from 27 mL in Bulgaria to 510 mL in the Czech Republic, the mean wine intake varied from 9 mL in Estonia to 206 mL in Luxembourg, and the mean spirit intake varied from 3 mL in Italy to 49 mL in Latvia. A mean intake of 29 mL of pure alcohol per person and day across the 25 EU States with available statistics has, as a rough estimate, been equated to an average consumption of 257 mL of beer, 75 mL of wine and 17 mL of spirits. This calculation was not stratified according to population size.

The numbers above also include the varying proportions of the population that do not drink alcohol at all. This has been estimated to a low of 2.5% in Luxembourg and a high of 38% in Romania with an average across 21 European countries examined of 14.2% (WHO, 2004). The mean intake among consumers of alcohol would thus be about 16.5% (100/[100-14.2]) higher than indicated by the figures above. Further, it is anticipated that there is some consumption of unrecorded alcohol in many countries. One study that attempted to document the extent of unrecorded alcohol consumption within the EU is the European Comparative Alcohol Study which involved 13 EU Member States. According to this study, the approximate level of unrecorded alcohol average consumption (litres of pure alcohol per year per inhabitant aged 15 or over) ranged from about 0.5 litres (Netherlands and Belgium), around 1 L (Austria, France, Germany, Ireland, Portugal and Spain), between 1 and 2 L (Italy) and approximately 2 L (Finland, Sweden, Denmark and the United Kingdom) (Leifman, 2001). This indicates an increase in mean per capita consumption in the adult population 15 years and over of between 5 and 30% or an average of 12%.

Average daily per capita intake figures for consumers of alcohol to be used in the exposure calculation have thus been adjusted to 335 mL of beer, 98 mL of wine and 22 mL of total spirits.

There is very little indication of types of spirit consumed. Of particular interest to the exposure assessment is consumption of fruit brandies. Limited information from North America and Germany (personal communication G. Soleas and O. Lindtner, respectively) both indicate that fruit brandy consumption could be around 4% of the total spirits consumption, but with large variations. Thus a daily consumption of 0.7 or 0.9 mL of fruit brandies as a subset of total spirits can be estimated for the mean population and mean consumers only with other spirits at 17 or 21 mL, respectively.

It has been estimated that 79% of the adult population drink alcohol up to 20g (women) or 40 g (men) per day, 15% consume 20-40 g (women) or 40-60 g (men) per day, and 6% of the adult population drink over 40 g (women) or 60 g (men) per day (Anderson and Baumberg, 2006). The 95th percentile for pure alcohol consumption across gender translated to respective alcoholic beverage would be close to about 1,000 mL of beer or 417 mL of wine or 125 mL of spirits. Under some local circumstances all of the spirit consumption could consist of fruit brandy. Table 13 summarises possible consumption scenarios.

Table 13: Summary of calculated per capita alcoholic beverage consumption in the adult population 15 years and older.

	Alcohol consumption in mL/person per day			
	Beer	**Wine**	**Spirits other**	**Fruit brandy**
Mean population	257	75	17	0.7
Mean consumers only	335	98	21	0.9
P95[a)]	1,000	417	125	125

[a)] P95 is the 95th percentile of values assuming that the person consumes all alcohol as either beer, wine, spirit or fruit brandy.

6. Exposure scenarios

As only few data on ethyl carbamate in foods were reported to EFSA, and of the results about 41% were below the LOD, it was not possible to estimate the dietary exposure of ethyl carbamate from foods in EU member states. Therefore, the JECFA estimate, assuming that food products in general would contribute less than 1 µg/person per day when using mean concentrations of ethyl carbamate found in food (FAO/WHO, 2006) and corresponding to 16.7 ng/kg b.w. per day for a 60 kg person, was used in this opinion.

EFSA received many results covering alcoholic beverage concentrations of ethyl carbamate. The potential dietary contribution of ethyl carbamate through the consumption of alcoholic beverages is given in Table 14. Ethyl carbamate concentrations expressed as µg/L have been assumed to be the same expressed as µg/kg. The Panel noted that the specific gravity for individual products will vary from just below 0.9 to just above 1.1, but the data have not been adjusted accordingly.

Three main scenarios were developed for the exposure assessment with all using the median concentration of ethyl carbamate found in beer, wine, spirits other than fruit brandy, and in fruit brandy, respectively. They differ from each other in that they use alcoholic beverage consumption across subjects 15 years and older: across the population (S1), in consumers of alcoholic beverages only (S2), and in consumers at the 95th percentile consumption level for any one of the alcoholic beverage groups (S3 A to D). Lower and upper bound concentrations are only shown for beer consumption where it had an impact.

Table 14: Dietary exposure estimates for ethyl carbamate in µg per day per person from the consumption of alcoholic beverages in three different scenario settings (S1-S3A-D).

			Beer[a)]	Wine	Spirit	Fruit brandy	Food	Total
S1	Consumption	mL/person/day	257	75	17	0.7		
	Concentration	µg/L	0-5	5	22	260		
	Dietary exposure	µg/person/day	0-1.3	0.4	0.4	0.2	1	2-3[b)]
	Dietary exposure	ng/kg b.w. per day day						33-55
S2	Consumption	mL/person/day	335	98	21	0.9		
	Concentration	µg/L	0-5	5	21	260		
	Dietary exposure	µg/person/day	0-1.7	0.5	0.4	0.2	1	2-4
	Dietary exposure	ng/kg b.w. per day						35-65
S3	Consumption	mL/person/day	1,000	417	125	125		
	Concentration	µg/L	0-5	5	21	260		
A	Dietary exposure	µg/person/day	0-5.0				1	1-6
	Dietary exposure	ng/kg b.w. per day						17-100
B	Dietary exposure	µg/person/day		2.1			1	3
	Dietary exposure	ng/kg b.w. per day						52
C	Dietary exposure	µg/person/day			2.6		1	4
	Dietary exposure	ng/kg b.w. per day						60
D	Dietary exposure	µg/person/day				32.5	1	34
	Dietary exposure	ng/kg b.w. per day						558

[a)] The two values in concentration and dietary intake are lower and upper bound, respectively
[b)] Total rounded to nearest whole number

In the whole population 15 years and older (scenario S1) ethyl carbamate exposure can be estimated to be 2-3 µg per day per person or 33-55 ng/kg b.w. per day for a 60 kg person. For the part of the population that doesn't consume alcoholic beverages, ethyl carbamate dietary exposure would only come from other food and would most often be less than 17 ng/kg b.w. per day depending on the food consumed. On the other hand, looking at alcohol consumers only (scenario S2) the estimated average exposure increases slightly to 2-4 µg per day per person or 35-65 ng/kg b.w. per day. At the 95th percentile consumption level of different types of alcoholic beverages in scenarios S3 A to D the dietary exposure would be between 1-34µg per day per person or 17-558 ng/kg bodyweight per day.

Consumption of fruit brandy will vary considerably between countries and regions. It is only when a large proportion of the alcoholic beverages is in the form of fruit brandy that the dietary exposure of ethyl carbamate escalates. Considerable fluctuations in ethyl carbamate levels were noted between different brands of fruit brandy. It has been speculated that high levels are more common in small-scale production facilities or in home-brewed fruit brandy.

Data on the presence of hydrocyanic acid in alcoholic beverages were submitted to the EFSA but no data for food. However, in the opinion issued by the EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (EFSA, 2004) reference is given to a Norwegian dietary survey showing a mean exposure to hydrocyanic acid of 95 µg/person per day or 1.4 µg/kg b.w. per day. This figure is used for food in the exposure assessment.

The three previous scenarios (S1-S3 C-D) were also applied to the hydrocyanic acid dietary exposure assessment (Table 15). A further scenario (S4 C-D) was introduced to combine 95th percentile occurrence and consumption for exposure assessment in relation to possible acute toxicity. These scenarios could be relevant in some limited situations also for the chronic toxicity situation because of brand loyalty. Locally or home produced spirits with high levels of hydrocyanic acid could be consumed for a longer period of time. The single wine result was not considered representative and therefore contribution to the dietary intake was confined to spirits other than fruit brandies, and fruit brandies.

Table 15: Dietary exposure assessment of hydrocyanic acid in µg per day per person from the consumption of alcoholic beverages in three different scenario settings (S1-S4 C-D).

			Spirit[a)]	Fruit brandy	Food	Total
S1	Consumption	mL/person/day	17	0.7		
	Concentration	µg/L	0-30	0-30		
	Dietary exposure	µg/person/day	0-0.51	0-0.02	95	95-95.53
	Dietary exposure	µg/kg b.w./day				1.58-1.59
S2	Consumption	mL/person/day	21	0.9		
	Concentration	µg/L	0-30	0-30		
	Dietary exposure	µg/person/day	0-0.63	0-0.03	95	95-95.66
	Dietary exposure	µg/kg b.w./day				1.58-1.59
S3	Consumption	mL/person/day	125	125		
	Concentration	µg/L	0-30	0-30		
C	Dietary exposure	g/person/day	0-3.75		95	95-98.75
	Dietary exposure	µg/kg b.w/day				1.58-1.65
D	Dietary exposure	µg/person/day		0-3.75	95	95-98.75
	Dietary exposure	µg/kg b.w./day				1.58-1.65
S4	Consumption	mL/person/day	125	125		
	Concentration	µg/L	1,500	10,800		
C	Dietary exposure	µg/person/day	188		95	283
	Dietary exposure	µg/kg b.w./day				4.72
D	Dietary exposure	µg/person/day		1,350	95	1445
	Dietary exposure	µg/kg b.w./day				24.08

[a)] The two values in concentration and dietary intake are lower and upper bound, respectively.

Irrespective of the scenarios the total dietary exposure to hydrocyanic acid with median concentration levels in alcoholic beverages was mainly influenced by levels in food and

averaged 1.58-1.65 µg/kg b.w. per day for a 60 kg person. However, a consumption intake at the 95th percentile level of fruit brandy and at the 95th percentile concentration level for hydrocyanic acid (scenario S4) would result in a peak dietary exposure of 24 µg/kg b.w. per day.

7. Hazard identification and characterisation

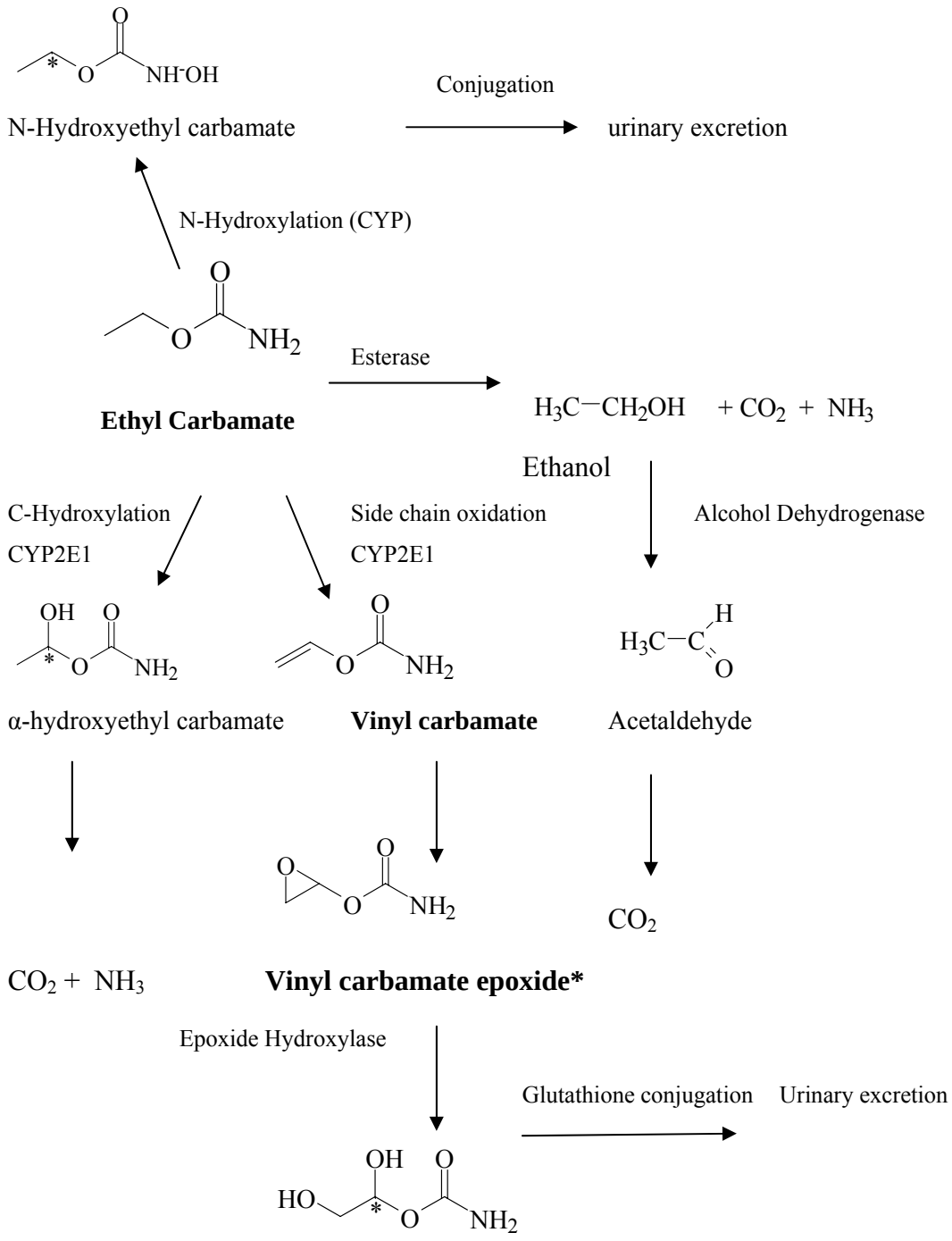
The toxicology of ethyl carbamate is well documented and there is a considerable amount of literature published on the subject in peer reviewed journals. Moreover, recently the JECFA performed a risk assessment on ethyl carbamate (FAO/WHO, 2005; 2006). This opinion will only briefly describe the pivotal studies used for the risk characterisation of ethyl carbamate in humans. New *in vitro* and *in vivo* toxicological studies including mechanistic studies published since the last the JECFA meeting in 2005 are outlined.

As hydrocyanic acid (and its salts) is one of several precursors of ethyl carbamate formation, possible health risks related to the presence of hydrocyanic acid were also considered by the Panel. The EFSA Panel on food additives, flavourings, processing aids and materials in contact with food (AFC Panel) has published an opinion on hydrocyanic acid in flavourings and other food ingredients with flavouring properties, (EFSA, 2004) and the CONTAM Panel refers to its conclusions as well as other relevant data which have become available since 2004.

7.1 Ethyl carbamate

7.1.1 Toxicokinetics

Ethyl carbamate is absorbed rapidly and nearly completely from the gastro-intestinal tract and the skin. It is evenly distributed in the body, followed by fast elimination with more than 90% eliminated as carbon dioxide within 6 h in mice. Ethyl carbamate metabolism involves three main pathways: hydrolysis, N-hydroxylation or C-hydroxylation and side chain oxidation. Hydrolysis is mediated by esterases and leads to the production of ethanol, carbon dioxide and ammonia. The N-hydroxylation, C-hydroxylation and side chain oxidation have been shown to be mediated by cytochrome P-450 2E1 (CYP2E1) to form N-hydroxycarbamate, α-hydroxy ethyl carbamate and vinyl carbamate, respectively. Hydroxycarbamate is conjugated and excreted in the urine, α-hydroxy ethyl carbamate is metabolised to ammonia and carbon dioxide and vinyl carbamate is oxidised to vinyl carbamate epoxide. The vinyl carbamate epoxide is further metabolised via glutathione conjugation to carbon dioxide and ammonia (Guengerich *et al.*, 1991; Lee *et al.*, 1998; Hoffler *et al.*, 2003) and has been recognised as the main metabolite responsible for the carcinogenicity of ethyl carbamate since it binds covalently to nucleic acids (DNA, RNA) and proteins (Park *et al.*, 1993; FAO/WHO, 2006). Figure 3 summarises the main metabolic routes involved in the metabolism of ethyl carbamate.



* DNA, RNA, protein adducts 1, 2-Dihydroxyethyl carbamate

Figure 3: Metabolism of ethyl carbamate (adapted from Hoffler and Ghanayem, 2005 and FAO/WHO, 2006).

The metabolism of ^{14}C-ethyl carbamate in CYP2E1-/- and CYP2E1+/+ mice was investigated after a single dose of 10 or 100 mg/kg b.w. or at 100 mg/kg b.w. per day for five consecutive days by gavage. For both treatments, CYP2E1+/+ mice exhaled 78 to 88% of the dose as $^{14}CO_2$ whereas CYP2E1-/- mice eliminated 30 to 38% of a single dose as $^{14}CO_2$ in 24 h with a plateau after day 3 at approximately 52% of dose (Hoffler and Ghanayem, 2005). Pharmacokinetic modelling using $^{14}CO_2$ exhalation data showed

that 96% of ethyl carbamate was metabolised by CYP2E1 in wild-type mice while metabolism by other enzymes accounted for the remaining 4% (Hoffler *et al.*, 2003).

Co-administration of ethyl carbamate and ethanol has also been looked at as a potential meaning of reducing CYP2E1-mediated activation and shifting the elimination of ethyl carbamate towards esterase-mediated hydrolysis. Delayed elimination as carbon dioxide was observed when high doses of ethanol (4 mL/kg b.w. or 5 g/kg b.w.) were administered to mice in conjunction with, or 1 h before, ethyl carbamate administration. In contrast, pre-treatment with 10% ethanol in drinking-water for 3 weeks had no effect (FAO/WHO, 2005).

7.1.2 Toxicology

The JECFA and the IARC have evaluated acute and chronic toxicity of ethyl carbamate and the critical findings are summarised below (FAO/WHO, 2005; 2006; IARC 1974; 2007; Baan *et al.*, 2007).

Acute oral toxicity of ethyl carbamate is low, with an LD_{50} in rodents of around 2,000 mg/kg b.w. whereas single doses of 1,000 mg/kg b.w. induce anaesthesia. In relation to chronic toxicity, a two year carcinogenicity study was performed by the NTP, with groups of 48 male and female B6C3F1 mice given drinking water containing ethyl carbamate at concentrations of 0, 10, 30 or 90 mg/L together with ethanol at a concentration of 0, 2.5% or 5%. Intakes of ethyl carbamate were approximately 0, 1, 3 or 9 mg/kg b.w. per day, respectively, and resulted in dose-dependent increased incidences of alveolar and bronchiolar, hepatocellular and harderian gland adenoma or carcinoma, hepatic haemangiosarcoma, and mammary gland adenoacanthoma or adenocarcinoma (in females only). Statistically significant but smaller increases in incidence of haemangiosarcoma of the heart (males only) and spleen (females only), squamous cell papilloma or carcinoma of the forestomach and skin (males only) and benign or malignant ovarian granulosa cell tumours were also observed as well as dose-related increases in non-neoplastic lesions affecting liver, heart and uterine blood together with hepatic eosinophilic foci. Those sites for which a significant increase in tumours was observed at the lowest dose tested, were the lung and the Harderian gland. The incidence of combined alveolar and bronchiolar adenoma or carcinoma were 5/48, 18/48, 29/47, 37/48 (males); and 6/48, 8/48, 28/48, 39/47 (females). The incidences of combined Harderian gland adenomas or carcinomas were 3/47, 12/47, 30/47, 38/47 (males); and 3/48, 11/48, 19/48, 30/48 (females). There was also a treatment-related increase in the combined incidence of any tumour type at any site (males: 33/48, 39/48, 46/47, 47/48; females: 37/48, 35/48, 45/48, 47/48). The co-administration of ethyl carbamate and ethanol had no consistent effect on the carcinogenicity of ethyl carbamate (FAO/WHO, 2005; NTP, 2004).

The JECFA used the increased incidence of alveolar and bronchiolar adenoma or carcinoma together with Harderian gland tumours from the 2 year carcinogenicity study

in mice as the critical response (NTP, 2004). The dose response data were analysed using eight different statistical models and fitted to the experimental data considered relevant to derive the benchmark doses (BMD) and its lower confidence 95% limit (BMDL) for a 10% extra risk of tumours. Dose–response relationships did not differ between sexes so that the fitted models could be combined (FAO/WHO, 2005; 2006). The JECFA chose lung tumours as the critical end-point with values for BMDL10 ranging from 0.3 to 0.5 mg/kg b.w. and decided to use the lower end of the range for the evaluation to be conservative. This value of 0.3 mg/kg will be used in the risk characterisation section of this opinion.

New data which have become available since the last JECFA evaluation

Recent studies evaluated the ability of both ethyl carbamate and vinyl carbamate to induce gene mutations in the lung and extrapulmonary tissues of the F1 (Big Blue x A/J) transgenic mice expressing the lambda cII transgene. Vinyl carbamate was mutagenic in the lung and intestine at doses of 45, 60 and 75 mg/kg whereas ethyl carbamate was mutagenic in the lung at 500 and 1,000 mg/kg respectively (Hernandez and Forkert, 2007a, b). The mutations at these doses further confirm the mechanism of mutagenesis seen in earlier studies (FAO/WHO, 2005).

CYP2E1 -/- and +/+ -/- mice were administered ethyl carbamate by gavage at 1, 10, or 100 mg/kg/day, 5 days/week, for 6 weeks and then kept without further treatment for 7 months before microscopic examination of tissues and assessment of carcinogenicity. A significantly lower incidence of liver hemangiomas, hemangiosarcomas, lung nodules, lung adenomas, hyperplasia and adenomas of the Harderian gland was seen in CYP2E1-/- compared to CYP2E1+/+ mice. Lung nodules increased in a dose-dependent manner and with a lower incidence in CYP2E1-/- compared to CYP2E1+/+ mice (Ghanayem, 2007).

The IARC reclassified ethyl carbamate recently from possible carcinogenic to humans (Group 2B) to "probably carcinogenic to humans (Group 2A) and noted that "(i) experimental evidence suggests great similarities in the metabolic pathways of the activation of ethyl carbamate in rodents and humans, and (ii) the formation of proximate carcinogens that are DNA-reactive and are thought to play a major role in ethyl carbamate-induced carcinogenesis in rodents probably also occurs in human cells" (IARC, 2007).

7.2 Hydrocyanic acid

7.2.1 Toxicokinetics

After oral administration, cyanide forms hydrocyanic acid under the stomach's acidic conditions. The hydrocyanic acid enters mucous, and cell membranes and 99% binds to methaemoglobin in erythrocytes. In humans, maximum body methaemoglobin of an adult can bind up to 10 mg of hydrocyanic acid. Hydrocyanic acid is metabolised in the liver to less toxic species such as thiocyanates by three different routes. The main route involves hepatic conversion of hydrocyanic acid to thiocyanate by the enzyme rhodanese with a rate of detoxification in humans of about 1µg hydrocyanic acid/kg b.w./min followed by thiocyanate excretion in the urine (Schultz, 1984; Nelson, 2006). Species differences in rhodanese activity have been shown with lower activities, and thus a lower rate of detoxification, in dogs, rhesus monkeys and rabbits compared to rats (EFSA, 2004).

Two other minor routes exist in humans: direct chemical combination of cyanide with sulphur (thiosulphate or 3-mercaptopyruvate) in the form of an amino acid (di-cysteine) to form 2-aminothiazoline-4-carboxylic acid and cysteine; and combination of cyanide with hydroxycobalamin to form cyanocobalamin (vitamin B12) (EFSA, 2004; Nelson, 2006). After oral exposure to $S^{14}CN^-$ and ^{14}C-labelled cyanide, rats excreted most of the dose in the form of thiocyanates in the urine whereas excretion in the faeces and expired air were minor (Okoh and Pitt, 1982; Okoh, 1983).

7.2.2 Toxicology

LD_{50} values for oral acute toxicity of potassium cyanide (KCN) in dogs and rats were 5.3 mg/kg b.w. and 10-15 mg KCN/kg b.w., equivalent to 2.1 and 4.0-6.0 mg CN^-/kg b.w., respectively. In humans, the reported acute oral lethal doses of hydrocyanic acid (HCN) range from 0.5-3.5 mg CN^-/kg b.w. corresponding to 1.0-7.0 mg KCN/kg b.w.

In stone fruits, cyanogenic glycosides, such as amygdalin and prunasin in almonds, apricots, plums and peaches, are hydrolysed by the gut microflora to slowly and incompletely release cyanide with subsequent absorption and therefore the acute toxicity for cyanogenic glycosides will be lower (EFSA, 2004).

A 15 day short term study was performed in rats with KCN in the drinking water providing doses equivalent to 0, 0.3, 0.9, 3.0 or 9.0 mg/kg b.w. per day. Rats treated with the highest cyanide dose showed lower body weight gain and a hydropic degeneration of hepatocytes whereas a hydropic degeneration of the renal tubular epithelial cells was observed at doses of 3.0 and 9.0 mg/kg per day. A dose-dependent increase in the number of re-absorption vacuoles on follicular colloid was also observed in the thyroid gland of all animals treated with cyanide including the lowest dose. This dose dependent increase in the number of reabsorption vacuoles in the thyroid follicles has been speculated to indicate an early phase of a goitrogenic effect of a metabolite of cyanide,

thiocyanate. However, these mechanisms remain unclear and further investigation has been recommended (Sousa *et al.*, 2002).

Subchronic oral studies (13 weeks) using sodium cyanide (NaCN) in drinking water have been performed in rats and mice (NTP, 1993). Male rats were given doses of 0, 0.3, 0.9, 2.7, 8.5 and 23.6 mg NaCN/kg b.w. per day and female rats were given doses of 0, 0.3, 1.0, 3.2, 9.2 and 23.5 mg NaCN/kg b.w. per day. The highest dose-level resulted in no significant adverse effects on body weights, organ weights, histopathology, or clinical pathology parameters. No evidence of neurological or thyroid gland damage was seen. A reported reduction in sperm motility was minor and not dose related and therefore of doubtful significance. However, a significant reduction of sperm heads per testis was observed at the highest dose level in rats. Based on these effects in male rats, the NOAEL was 8.5 mg NaCN/kg b.w. per day, corresponding to 4.5 mg CN^-/kg b.w. per day.

Male mice received doses of 0, 0.5, 1.8, 5.1, 16.2 and 45.9 mg NaCN/kg b.w. per day via drinking water and female mice 0, 0.6, 2.1, 6.2, 19.1 and 54.3 mg NaCN/kg b.w. per day. The only effect with toxicological significance was a slight reduction in cauda epididymal weight together with reduced sperm motility at the highest dose level of 46 mg sodium cyanide/kg b.w. per day in males. Based on these effects in mice, a NOAEL of 16 mg NaCN/kg b.w. per day was identified, corresponding to 8.5 mg CN^-/kg b.w. per day (NTP, 1993).

Another 13 week study in rats using dietary doses equivalent to 0, 0.15, 0.3, or 0.6 mg KCN/kg b.w. per day demonstrated no lesions in the pancreas or thyroid but revealed the presence of spheroids on the ventral horn of the spinal cord, neuron loss in the hippocampus, damaged Purkinje cells, and loss of cerebellar white matter. The authors concluded that cyanide administration could promote neuropathological lesions in rats without affecting pancreas or thyroid gland metabolism (Soto-Blanco *et al.*, 2002).

In a limited study dogs were fed a rice-based diet supplemented with sodium cyanide for 14 weeks, resulting in doses equivalent to 0 and 1.08 mg/kg b.w. per day. Significant effects were observed on the kidney, adrenal, testis and spermatogenesis, suggesting that dogs are more sensitive to cyanide than rats or mice (EFSA, 2004).

The effects of prenatal exposure to KCN were investigated in pregnant Wistar rats receiving doses of 1, 3 and 30mg KCN/kg or 0.8, 2.4 from day 6 to day 20 via the drinking water. There was a loss of cells in the Islets of Langerhans and an increase in the number of biliary ducts in dams treated with the highest doses of cyanide and in their pups. The authors concluded that the cyanide and/or thiocyanate promoted toxic effects in the foetuses that can also be observed at weaning (de Sousa *et al.*, 2007).

A toxicity study has also been performed recently in growing-finishing swine. Twenty-four pigs, 45 days of age, were divided into four equal groups and fed a diet containing different doses of KCN: 0, 2.0, 4.0 or 6.0 mg per kg b.w. per day for 70 consecutive days. Results showed a significant alteration in thiocyanate, creatinine and urea levels

and in alanine aminotransferase activity at 4.0 and 6.0 mg/kg KCN. Thyroid weight was significantly increased in those pigs from the 4.0 mg/kg KCN group. No changes in cholesterol, triiodothyronine or thyrosine levels and body weight gain were observed. The histopathology demonstrated an increase in the number of vacuoles in the colloid of thyroid follicles, a degeneration of cerebellar white matter and Purkinje cells, degeneration of renal tubular epithelial cells, caryolysis and pyknosis in hepatocytes, and disturbance of the normal lobular architecture of the liver in all treated pigs (Manzano *et al.*, 2007).

Overall, the mutagenicity tests conducted with hydrocyanic acid and cyanides at gene and/or chromosome level did not reveal a genotoxic potential (EFSA, 2004).

In view of the lack of adequate data from which to derive a tolerable daily intake (TDI) the AFC Panel supported the continued application of limits for the presence of hydrocyanic acid in foods and beverages (EFSA, 2004).

The AFC Panel concluded that the data for hydrocyanic acid were not adequate to identify a NOAEL for chronic exposure in humans, because they were highly confounded by other nutritional and environmental factors (EFSA, 2004). Adequate long-term toxicity studies in animals fed a diet containing hydrocyanic acid or cyanogenic glycosides to derive a NOAEL were also lacking and therefore a TDI could not be derived. In 1993, the JECFA concluded: "Because of a lack of quantitative toxicological and epidemiological information, a safe level of intake of cyanogenic glycosides could not be established". However, the JECFA also concluded that, at the CODEX standard for of 10 mg hydrocyanic acid/kg, cassava flour (CAC, 1991) is not associated with acute toxicity (FAO/WHO, 1993). Cassava flour is used as a staple food mainly outside Europe; a consumption of 200 g/person would lead to an estimated intake of 30 µg hydrocyanic acid/kg b.w. for a 60 kg adult.

8. Risk characterisation

The following three scenarios were applied to estimate the dietary exposure to ethyl carbamate from the consumption of food and alcoholic beverages for adults (15 years and older):

- whole population (S1);
- consumers of alcoholic beverages only (S2);
- high consumers (at the 95th percentile consumption level) for any one of the alcoholic beverages beer, wine, spirits other than fruit brandy, and fruit brandy (S3 A-D).

For the contribution of food to the exposure to ethyl carbamate an estimate of 1 µg/person per day as calculated by the JECFA (FAO/WHO, 2006) was included in all the above listed exposure scenarios. Table 16 presents also as a base scenario the contributions from food only. Exposure to ethyl carbamate from food was combined with median concentrations of ethyl carbamate found in beer, wine, spirits other than fruit brandy, and fruit brandy (Table 14).

The Panel estimated dietary exposure for three scenarios in adults (15 years and older):

- in the whole population daily dietary exposure of 2.0 to 3.3 µg per person corresponding to 33-55 ng/kg b.w. per for a 60 kg person (S1);.
- in consumers of alcoholic beverages daily dietary exposure of 2.2 to 3.9 µg per person corresponding to 36.7-65 ng/kg b.w. for a 60 kg person (S2); and
- in consumers at the 95th percentile consumption level for any one of the alcoholic beverages (beer, wine, spirit, fruit brandy) a daily dietary exposure of 1-6.0 µg per person (S3A-beer), 3.1 µg per person (S3B-wine) and 3.8 µg per person (S3C-spirit), 33.5 µg per person (S3D-fruit brandy) corresponding to 16.7-100 (beer), 51.7 (wine), 60.0 (spirit), 558 (fruit brandy) ng/kg b.w. for a 60 kg person.

The margin of exposure (MOE) approach was used for the risk characterisation, comparing animal cancer data with human exposure scenarios (EFSA, 2005; Barlow *et al.*, 2006). Using this approach, the EFSA Scientific Committee considered that an MOE of 10,000 or more, based on a BMDL10 derived from animal cancer bioassay data, "*would be of low concern from a public health point of view and might reasonably be considered as a low priority for risk management actions*" (EFSA, 2005, O' Brien *et al.*, 2006). Table 16 reports the MOE values calculated using the estimated intake of ethyl carbamate and the BMDL10 value obtained by the JECFA (FAO/WHO, 2006) for the incidence of alveolar and bronchiolar neoplasms in male and female mice (0.3 mg/kg b.w. per day).

Table 16: Estimated MOEs for different exposure scenarios to ethyl carbamate in subjects 15 years and older.

Exposure Scenario	Ethyl carbamate intake (ng/kg b.w/day)[a)]		MOE[b)]	
	LB	UB	LB	UB
Food excluding alcoholic beverages	17	17	17,600	17,600
S1 Overall population	33	55	9,090	5,450
S2 Consumers of alcoholic beverages	37	65	8,110	4,620
S3 Consumers of alcohol beverages at the 95th percentile				
A. Beer	17	100	17,600	3,000
B. Wine	52		5,770	
C. Spirit	60		5,000	
D. Fruit brandy	558		540	

a) Based on information presented in Table 14.

b) Rodent BMDL10 of 300,000 ng/kg b.w. per day divided by the estimated lower bound-upper bound intake, LB = lower bound, UB = upper bound.

The Panel concluded that this MOE of almost 18,000 for exposure to ethyl carbamate in food excluding alcoholic beverages indicates a low concern for human health.

The Panel noted that because of the high proportion of the population that consumes alcohol there is not much difference whether dietary exposure to ethyl carbamate is distributed across the whole population (S1) or in alcohol consumers only (S2).

In relation to the large part of the general population consuming a variety of alcoholic beverages (S2) as well as high percentile consumers of mainly beer, wine or spirits other than fruit brandy (S3 A-C), MOEs were lower and on average in the region of 5,000. This MOE indicates a potential health concern. The Panel also concluded that an MOE of below 600 for high level consumers who primarily drink fruit brandies is of particular concern.

Overall, the panel concluded that ethyl carbamate in alcoholic drinks is of human health concern, particularly for consumers of stone fruit brandies.

Using the same consumption data scenarios to estimate hydrocyanic acid intake in the whole population (S1), consumers of alcoholic drinks (S2), and high percentile consumers of individual alcoholic beverages (S3), daily exposure at median concentration levels would be on average 1.6-1.7 µg/kg b.w. for a 60 kg person, respectively. Exposure from alcoholic beverages at median concentration levels are far below the dietary exposure to hydrocyanic acid from other food sources as indicated by the exposure assessment performed by the AFC Panel for hydrocyanic acid in flavouring ingredients, and therefore are not expected to present a risk of acute toxicity.

Because of the acute toxicity of hydrocyanic acid and possible brand loyalty at a local level, a further scenario (S4) was tested with both the concentration of hydrocyanic acid and the consumption of alcoholic beverages set at the 95th percentile levels. This resulted in a possible high dietary exposure of 24 µg/kg b.w. for a 60 kg person, which is 16 times more than hydrocyanic exposure from other foods. Although this high level exposure is about 20 times below the lower end of the range of reported human lethal doses, it is uncertain if this margin is sufficient to discount a risk for some people. However, it is also below the dietary exposure to hydrocyanic acid from cassava identified by the JECFA as not associated with acute toxicity.

The CONTAM Panel concluded that levels of hydrocyanic acid as such found in most alcoholic beverages do not pose a risk of acute toxicity. In relation to the terms of reference considering the importance of hydrocyanic acid as a precursor of ethyl carbamate, the panel considered the correlation between the levels of ethyl carbamate and hydrocyanic acid in alcoholic beverages. Although no strong correlation was found, samples with high levels of hydrocyanic acid always contained higher levels of ethyl carbamate.

CONCLUSIONS

- Ethyl carbamate occurs naturally in fermented foods and alcoholic beverages, such as fruit brandies. It can be formed from various substances such as hydrocyanic acid found in fruit stones or through reaction between urea and ethanol during yeast fermentation.

- Ethyl carbamate is genotoxic and a multisite carcinogen in animals and probably carcinogenic to humans (group 2A).

- Median levels of ethyl carbamate in alcoholic beverages of 0-5 µg/L for beer, up to 5 µg/L for wine, 21 µg/L for spirits other than fruit brandy and 260 µg/L for fruit brandy were found. Daily dietary exposures of 17 ng/kg b.w. from food excluding alcoholic beverages up to 65 ng/kg b.w. when also consuming different alcoholic beverages were calculated. The highest exposure to ethyl carbamate was shown for adults primarily consuming fruit brandy at the 95th percentile consumption level with 558 ng/kg b.w. per day.

- Using the MOE approach with a BMDL value of 0.3 mg/kg b.w. per day (10% incidence of alveolar and bronchiolar neoplasms in male and female mice), MOEs were calculated for food consumption excluding alcoholic beverages only at close to 18,000, for food consumed together with a variety of alcoholic beverages in the region of 5,000, and for high consumers of fruit brandy at less than 600. Based on these MOEs the Panel concluded that ethyl carbamate in alcoholic beverages indicates a health concern, particularly with respect to stone fruit brandies.

- The Panel noted that for consumers of particular brands of stone fruit brandy, with higher than average levels of ethyl carbamate, the MOEs could be even lower.

- The main contributor to hydrocyanic acid exposure in average consumers was food products, with alcoholic beverages contributing only minor amounts. For high consumers of fruit brandy containing high levels of hydrocyanic acid a higher exposure would be possible, which is undesirable.

RECOMMENDATIONS

- Mitigation measures should be taken to reduce the levels of ethyl carbamate in certain alcoholic beverages such as fruit brandies. Such measures should include focus on hydrocyanic acid and other precursors of ethyl carbamate to prevent the formation of ethyl carbamate during shelf-life of these products.

- Measures should be introduced to reduce concentrations of hydrocyanic acid in certain types of beverages

REFERENCES

Anderson, P. and Baumberg, B. 2006. Alcohol in Europe, A public health perspective. Report to the European Commission. Available at URL: http://dse.univr.it/addiction/documents/External/alcoholineu.pdf.

Andrey, D. 1987. A simple gas chromatography method for the determination of ethyl carbamate in spirits. Zeitschrift fuer Lebensmittel-Untersuchung und -Forschung, 185, 21–23.

Anon 2007. Total alcohol consumption. Available at URL: http://ec.europa.eu/health/ph_information/dissemination/echi/echi_3_en.htm#24

Aresta, M., Boscolo, M. and Franco, D. W. 2001. Copper (II) catalysis in cyanide conversion into ethyl carbamate in spirits and relevant reactions. Journal of Agricultural and Food Chemistry, 49, 2819–2824.

Baan,R., Straif,K., Grosse,Y., Secretan,B., El,G.F., Bouvard,V., Altieri,A., and Cogliano,V. 2007. Carcinogenicity of alcoholic beverages. Lancet Oncol. 8, 292-293.

Barlow,S., Renwick,A.G., Kleiner,J., Bridges,J.W., Busk,L., Dybing,E., Edler,L., Eisenbrand,G., Fink-Gremmels,J., Knaap,A., Kroes,R., Liem,D., Muller,D.J., Page,S., Rolland,V., Schlatter,J., Tritscher,A., Tueting,W., and Wurtzen,G. 2006. Risk assessment of substances that are both genotoxic and carcinogenic report of an International Conference organized by EFSA and WHO with support of ILSI Europe. Food Chem.Toxicol. 44, 1636-1650.

Battaglia, R., Conacher, H. B. S. and Page, B. D. 1990. Ethyl carbamate (urethane) in alcoholic beverages and foods – a review. Food Additives and Contaminants, 7, 477-496.

Baumann, U. and and Zimmerli, B. 1988. Accelerated formation of ethyl carbamate in spirits. Mitteilung Gebiete Lebensmittel Hygeinik, 22, 175-185.

Benson, R.W. and Beland, F.A. 1997. Modulation of urethane (ethyl carbamate) carcinogenicity by ethyl alcohol: a review. International Journal of Toxicology, 16, 521–544.

Butzke, C.E. and Bisson, L.F. 1997. Ethyl carbamate preventative action manual. US Food and Drug Administration, Washington D.C., USA. Available at URL: http://vm.cfsan.fda.gov/~frf/ecaction.html.

CAC (Codex Alimentarius Commission) 1991. Codex Standard for Edible Cassava Flour (African Regional Standard. Codex Alimentarius, Vol. XII, Suppl. 4. Rome, FAO, (CODEX STAN 176).

Canas, B.J., Joe, F.L., Jr., Diachenko, G.W. and Burns, G. 1994. Determination of ethyl carbamate in alcoholic beverages and soy sauce by gas chromatography with mass selective detection: collaborative study. J.AOAC Int. 77, 1530-1536.

Conacher, H.B.S. and Page, B.D. 1986. Ethyl carbamate in alcoholic beverages: A Canadian case history. Proceedings of Euro Food Tox II, European Society of Toxicology, Schwerzenbach, Switzerland. pp 237–242.

de Sousa, A.B., Maiorka, P.C., Gonçalves, I.D., Marques de Sá, L.R. and Górniak, S.L. 2007. Evaluation of effects of prenatal exposure to the cyanide and thiocyanate in wistar rats. Reproductive Toxicology, 23 (4), 568-577.

Dennis. M.J., Howarth, N., Key, P.E., Pointer, M., and Massey, R.C. 1989. Investigation of ethyl carbamate levels in some fermented foods and alcoholic beverages. Food Additives and Contaminants 6, 383-389.

EFSA (European Food Safety Authority). 2004. Opinion of the Scientific Panel AFC on hydrocyanic acid in flavourings and other food ingredients with flavouring properties. Adopted on 19 November 2004. The EFSA Journal 105, 1-28. Available at URL: http://www.efsa.europa.eu/en/science/afc/afc_opinions/698.html.

EFSA (European Food Safety Authority) 2005. Opinion of the Scientific Committee on a request from EFSA related to A Harmonised Approach for Risk Assessment of Substances Which are both Genotoxic and Carcinogenic. The EFSA Journal 282, 1-31. Available at URL: http://www.efsa.europa.eu/EFSA/Scientific_Opinion/sc_op_ej282_gentox_en3.pdf

Ermans, A.M., Mbulamoko, N.M., Delange, F. and Ahluwalia, R. 1980. Role of cassava in the etiology of endemic goitre and cretinism. International Development Research Centre, 182 pp, Ottawa, Ontario

FAO/WHO (Food and Agriculture Organisation of the United Nations/World Health Organisation). 1993. Cyanogenic glycosides. In: Toxicological evaluation of certain food additives and naturally occurring toxicants. 39th Meeting of the Joint FAO/WHO Expert Committee of Food Additives (WHO Food Additives Series 30) World Health Organisation, Geneva.

FAO/WHO (Food and Agriculture Organisation of the United Nations/World Health Organisation). 2005. Summary and conclusions of the sixty-fourth meeting of the Joint Meeting of the Joint FAO/WHO Expert Committee of Food Additives (WHO Food Additives Series 30) World Health Organisation, Geneva. WHO Tech. Rep. Ser. 928, 1-47. Available at URL: http://www.who.int/ipcs/food/jecfa/summaries/en/summary_report_64_final.pdf

FAO/WHO (Food and Agriculture Organisation of the United Nations/World Health Organisation). 2006. Safety evaluation of certain contaminants in food. Prepared by the Sixty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) FAO Food Nutr. Pap. 82:1-778.

Ghanayem,B.I. 2007. Inhibition of urethane-induced carcinogenicity in cyp2e1-/- in comparison to cyp2e1+/+ mice. Toxicol.Sci. 95, 331-339.

Guengerich,F.P., Kim,D.H., and Iwasaki,M. 1991. Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. Chem.Res.Toxicol. 4, 168-179.

Hoffler,U. and Ghanayem,B.I. 2005. Increased bioaccumulation of urethane in CYP2E1-/- versus CYP2E1+/+ mice. Drug Metab Dispos. 33, 1144-1150.

Hoffler,U., El-Masri,H.A., and Ghanayem,B.I. 2003. Cytochrome P450 2E1 (CYP2E1) is the principal enzyme responsible for urethane metabolism: comparative studies using CYP2E1-null and wild-type mice. J.Pharmacol.Exp.Ther. 305, 557-564.

IARC (International Agency for Research on Cancer). 1974. International Agency for Research on Cancer Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man: Some Anti-thyroid and Related Substances, Nitrofurans and Industrial Chemicals. Volume 7. World Health Organization, Lyon, France.

IARC (International Agency for Research on Cancer) 2007. International Agency for Research. Volume 96: Alcoholic Beverage Consumption and Ethyl Carbamate (Urethane) 6–13 February 2007 1-5. World Health Organization, Lyon, France. http://monographs.iarc.fr/ENG/Meetings/vol96-summary.pdf

Kim, Y.-K. L., Koh, E., Chung, H.-J. and Kwon, H. 2000. Determination of ethyl carbamate in some fermented Korean foods and beverages. Food Additives and Contaminants, 17, 469–475.

Lachenmeier, D. W., Frank, W. and Kuballa, T. 2005a. Application of tandem mass spectrometry combined with gas chromatography to the routine analysis of ethyl carbamate in stone-fruit spirits. Rapid Commun. Mass Spectrom. 19, 108–112.

Lachenmeier, D.W., Schehl, B., Kuballa, T., Frank, W. and Senn, T. 2005b. Retrospective trends and current status of ethyl carbamate in German stone-fruit sprits. Food Additives and Contaminants, 22, 397-405.

LCBO (Liquor Control Board of Ontario). 2006. Information supplied by the Liquor Control Board of Ontario, LCBO Corporate Communications, 55 Lake Shore Boulevard, East Toronto, ON M5E 1A4.

Lee,R.P., Parkinson,A., and Forkert,P.G. 1998. Isozyme-selective metabolism of ethyl carbamate by cytochrome P450 (CYP2E1) and carboxylesterase (hydrolase A) enzymes in murine liver microsomes. Drug Metab Dispos. 26, 60-65.

Leifman H. 2001. Estimations of unrecorded alcohol consumption levels and trends in 14 European countries. Nordic Studies on Alcohol and Drugs 18 (English Supplement), 54-69.

MacKenzie, W. M., Clyne, A. H. and MacDonald, L. S. 1990. Ethyl carbamate formation in grain based spirits. II. The identification and determination of cyanide related species involved in ethyl carbamate formation in Scotch grain whisky. Journal of the Institute of Brewing, 96, 223–232.

Manzano,H., de Sousa, A.B., Soto-Blanco, B., Guerra, J.L., Maiorka, P.C., and Gorniak, S.L. 2007. Effects of long-term cyanide ingestion by pigs. Vet.Res.Commun. 31, 93-104.

Mildau, G., Preuß, A., Frank, W. and Heering, W. 1987. Ethyl carbamate (urethane) in alcoholic beverages: Improved analysis and light-dependent formation. Deutsche Lebensmittel-Rundschau, 83, 69–74.

NTP (National Toxicology Program). 2004. Toxicology and carcinogensis. Studies of urethane, ethanol, and urethane/ethanol (urethane, CAS No. 51-79-6; ethanol, CAS No. 64-17-5) in B6C3F1 mice (drinking water studies). Natl. Toxicol. Program Tech. Rep. Ser. 510:1-346.

NTP (National Toxicology Program). 1993. NTP Toxicity Studies of Sodium Cyanide (CAS No. 143-33-9) Administered by Dosed Water to F344/N Rats and B6C3F1 Mice. Toxic.Rep.Ser. 37, 1-D3.

Nelson, L. 2006. Acute cyanide toxicity: mechanisms and manifestations. J.Emerg.Nurs. 32, S8-11.

O'Brien, J., Renwick, A.G., Constable, A., Dybing, E., Muller, D.J., Schlatter, J., Slob, W., Tueting, W., van, B.J., Williams, G.M., and Wolfreys, A. 2006. Approaches to the risk assessment of genotoxic carcinogens in food: a critical appraisal. Food Chem.Toxicol. 44, 1613-1635.

Okoh, P.N. 1983. Excretion of 14C-labeled cyanide in rats exposed to chronic intake of potassium cyanide. Toxicol.Appl.Pharmacol. 70, 335-339.

Okoh, P.N. and Pitt, G.A. 1982. The metabolism of cyanide and the gastrointestinal circulation of the resulting thiocyanate under conditions of chronic cyanide intake in the rat. Can.J.Physiol Pharmacol. 60, 381-386.

Ough, C.S., Crowell, E.A. and Gutlove, B.R. 1988. Carbamyl compound reactions with ethanol. Am. J. Enol. Vitic., 39:3, 239-242.

Park,K.K., Liem,A., Stewart,B.C., and Miller,J.A. 1993. Vinyl carbamate epoxide, a major strong electrophilic, mutagenic and carcinogenic metabolite of vinyl carbamate and ethyl carbamate (urethane). Carcinogenesis 14, 441-450.

Salmon, A. G., Painter, P., Dunn, A. J., Wu-Williams, A., Monserrat, L. and Zeise, L. 1991. Carcinogenic effects. In: Salmon, A. G. and Zeise, L., Risks of Carcinogenesis from Urethane Exposure, pp 48-77. CRC Press Inc., Boca Raton, Florida, USA.

Schehl, B., Senn, T., Lachenmeier, D.W., Rodicio, R., Heinisch, J.J. 2007. Contribution of the fermenting yeast strain to ethyl carbamate generation in stone fruit sprits. Applied Microbiology and Biotechnology, 74, 843-850.

Schlatter J. and Lutz, W. K. 1990. The carcinogenic potential of ethyl carbamate (urethane): Risk assessment at human dietary exposure levels. Food and Chemical Toxicology, 28, 205–211.

Schulz V. 1984. Clinical pharmacokinetics of nitroprusside, cyanide, thiosulphate and thiocyante. Clin. Pharmcokinetics, 9, 239-251.

Sen, N. P., Seaman, S. W. and Weber, D. 1992. A method for the determination of methyl carbamate and ethyl carbamate in wines. Food Additives and Contaminants, 9,149–160.

Sen, N. P., Seaman, S. W., Boyle, M. and Weber, D. 1993. Methyl carbamate and ethyl carbamate in alcoholic beverages and other fermented foods. Food Chemistry, 48, 359–366.

Simeonova, F.P. and Fishbein, L. 2004. Hydrogen cyanide and cyanides: Human health aspects. Concise International Chemical Assessment Document 61. World Health Organisation, Geneva.

Soto-Blanco, B., Marioka, P.C. and Górniak, S.L. 2002. Effects of long-term low-dose cyanide administration to rats. Ecotoxicol Environ Saf. 53(1), 37-41.

Sousa, A.B., Soto-Blanco, B., Guerra, J.L., Kimura, E.T., and Gorniak, S.L. 2002. Does prolonged oral exposure to cyanide promote hepatotoxicity and nephrotoxicity? Toxicology 174, 87-95.

Suzuki, K., Kamimura, H., Ibe, A., Tabata, S., Yasuda, K. and Nishijima, M. 2001. Formation of ethyl carbamate in umeshu (plum liqueur). Shokuhin Eiseigaku Zasshi, 42, 354–358.

Taki, N., Imamura, L., Takebe, S. and Kobashi, K. 1992. Cyanate as a precursor of ethyl carbamate in alcoholic beverages. Japanese Journal of Toxicology and Environmental Health, 38, 498-505.

WHO (World Health Organisation). 2004. Global status report on alcohol 2004. Website accessed on 22 May 2007: Available at URL: http://www.who.int/substance_abuse/publications/global_status_report_2004_overview.pdf

WHO (World Health Organisation). 2007. Global Alcohol Database. Website accessed on 17 March 2007: Available at URL http://www.who.int/globalatlas/default.asp

Wucherpfennig K, Clauss E, Konja G. 1987. Formation of ethyl carbamate in alcoholic beverages based on the maraschino cherry. Deutsche Lebensmittel-Rundschau 83, 344–349.

Zimmerli, B. and Schlatter, J. 1991. Ethyl Carbamate: analytical methodology, occurrence, formation, biological activity and risk assessment. Mutation Research, 259, 325-350.

LIST OF ABBREVIATIONS AND ACRONYMS

AFC	Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food of EFSA
BMD	Benchmark dose
BMDL	BMD lower confidence limit
BMDL10	The benchmark dose lower confidence limit 10% (BMDL10) represents the lower bound of a 95% confidence interval on a BMD (benchmark dose) corresponding to a 10% tumour incidence.
CAS	Chemical Abstracts Service
CN^-	Cyanide
CODEX	The Codex Alimentarius Commission was created in 1963 by FAO and WHO to develop food standards, guidelines and related texts such as codes of practice under the Joint FAO/WHO Food Standards Programme.
CONTAM	Scientific Panel on Contaminants in the Food chain of EFSA
CYPs	Cytochrome P-450 enzymes
DATEX	EFSA´s unit on data collection and exposure
DNA	Deoxyribonucleic acid
EC	European Commission
EU	European Union
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organisation
GC	Gas chromatography
HCN	Hydrocyanic acid or hydrogen cyanide
IARC	International Agency for Research on Cancer
JECFA	Joint FAO/WHO Expert Committee for Food Additives
KCN	Potassium cyanide
LB	Lower bound
LCBO	Liquor Control Board of Ontario
LD_{50}	The lethal dose 50 (LD 50) is defined as the amount of a substance which causes the death of 50% of a group of test animals
LOD	Limit of detection

LOQ	Limit of quantification
MOE	The margin of exposure (MOE) is defined as the reference point on the dose-response curve (usually based on animal experiments in the absence of human data) divided by the estimated intake by humans
MRM	Multi Reaction Monitoring
MS	Mass spectrometry
NaCN	Sodium cyanide
ND	Not detected, that is results at or below the limit of detection or the limit of quantification for the method used
NOAEL	No observed adverse effect level
NTP	National Toxicology Program of the U.S.
RNA	Ribonucleic acid
SIM	Selected ion monitoring
TDI	Tolerable daily intake
UB	Upper bound
Volume vs. mass	Ethyl carbamate concentrations expressed as µg/L have been assumed to be the same expressed as µg/kg. However, the specific gravity for individual products will vary from just below 0.9 to just above 1.1.
WDT	World Drink Trends
WHO	World Health Organisation