



Screening Assessment

Internationally Classified Substance Grouping

**Carbamic acid, ethyl ester
(Ethyl carbamate)**

**Chemical Abstracts Service Registry Number
51-79-6**

**Environment and Climate Change Canada
Health Canada**

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Synopsis

Pursuant to section 68 of the *Canadian Environmental Protection Act, 1999* (CEPA), the Ministers of the Environment and Climate Change and of Health have conducted a screening assessment on Carbamic acid, ethyl ester, hereinafter referred to as ethyl carbamate, Chemical Abstracts Service Registry Number (CAS RN) 51-79-6. This substance is part of the Internationally Classified Substance Grouping, which includes substances that were prioritised for screening assessment because they were classified by certain international agencies as potentially of concern for human health.

Ethyl carbamate is a by-product of the fermentation process and has been detected in many types of fermented foods and beverages. It is also a constituent of tobacco plants and is present in mainstream tobacco smoke.

Based on information obtained from stakeholder consultation in 2012-2013, no single company has been identified as having imported or used ethyl carbamate above the reporting threshold of 100 kg per year in Canada. In Canada and internationally, the current uses of ethyl carbamate are limited to medical laboratory research.

Ethyl carbamate has high water solubility, a very low octanol-water partition coefficient, and moderate vapour pressure. If released to the environment, the substance is not expected to significantly partition into air. Based on a low Henry's Law constant, the majority of ethyl carbamate is expected to reside in water and soil. Partitioning to sediments is expected to be limited; however, given that the substance is highly water soluble, it could be found in pore water.

Ethyl carbamate demonstrated low toxicity to aquatic organisms; however, some genetic and biochemical effects have been observed in worms and frogs. Considering the low quantity of ethyl carbamate in commerce in Canada and its limited uses, environmental releases of this substance are not expected to be significant. Therefore, environmental exposure of organisms is considered to be negligible, and ethyl carbamate is not expected to pose a risk to organisms in Canada.

Considering all available lines of evidence presented in this Screening Assessment, there is low risk of harm to organisms and the broader integrity of the environment from ethyl carbamate. It is concluded that ethyl carbamate does not meet the criteria under paragraphs 64(a) or (b) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

The critical effect for characterization of risk to human health associated with exposure to ethyl carbamate is carcinogenicity; ethyl carbamate is a multisite carcinogen in animal studies. The margins of exposure between upper-bounding estimates of dietary exposure for adults from alcohol consumption and the critical effect level for cancer are

potentially inadequate to address uncertainties in the health effects and exposure databases. Corresponding margins of exposure for the general population, excluding alcohol consumption, are considered adequate to address uncertainties in the health effects and exposure databases.

Based on the information presented in this Screening Assessment, it is concluded that ethyl carbamate meets the criteria under paragraph 64(c) of CEPA as it is entering or may enter the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

Overall conclusion

It is concluded that ethyl carbamate meets one or more of the criteria set out in section 64 of CEPA.

Ethyl carbamate does not meet the criteria for persistence or bioaccumulation as set out in the *Persistence and Bioaccumulation Regulations* of CEPA.

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1. Introduction

Pursuant to section 68 of the *Canadian Environmental Protection Act, 1999* (CEPA) (Canada 1999) the Minister of the Environment and the Minister of Health conduct screening assessments of substances to determine whether these substances present or may present a risk to the environment or to human health.

The Substance Groupings Initiative is a key element of the Government of Canada's Chemicals Management Plan (CMP). The Internationally Classified Substance Grouping consists of six substances that were identified as priorities for action, because

they met the categorization criteria under section 73 of CEPA and/or were considered as priority substances under the CMP based on human health concerns (Environment Canada and Health Canada 2013). Substances in this substance grouping have been identified by other jurisdictions as a concern for human health due to high hazard potential as recognized by international agencies.

The Internationally Classified Substance Grouping includes four cresol (phenol, methyl-) substances, as well as two other substances, Ethanol, 2-[(2-aminoethyl)amino]- (CAS RN 111-41-1) and Carbamic acid, ethyl ester (CAS RN 51-79-6). These substances are not necessarily similar in terms of chemical structure, physical and chemical properties, uses, or other assessment parameters. For this reason, three separate Screening Assessments have been conducted within the Internationally Classified Substance Grouping, with one Screening Assessment for the sub-grouping of the four cresols, and individual assessments for Ethanol, 2-[(2-aminoethyl)amino]- and Carbamic acid, ethyl ester.

Screening Assessments focus on information critical to determining whether a substance meets the criteria as set out in section 64 of CEPA. Screening Assessments examine scientific information and develop conclusions by incorporating a weight-of-evidence approach and precaution.¹

This Screening Assessment includes consideration of information on physical and chemical properties, quantity, uses, exposure and hazard, including additional information submitted by stakeholders. Relevant data were identified up to September 2014. Empirical data from key studies as well as some results from models were used to reach conclusions. When available and relevant, information presented in risk and hazard assessments from other jurisdictions was considered.

The Screening Assessment does not represent an exhaustive or critical review of all available data. Rather, it presents the most critical studies and lines of evidence pertinent to the conclusion.

The Screening Assessment was prepared by staff in the Existing Substances Programs at Health Canada and Environment and Climate Change Canada and incorporates input from other programs within these departments. The ecological and human health portions of this assessment have undergone external written peer review and consultation. Comments on the technical portions relevant to the environment were received from Tim Fletcher (Ministry of Environment in Ontario) and Pamela Welbourn (Queen's University). Comments on the technical portions relevant to human health were received from Penny Fenner-Crisp (consultant to Toxicology Excellence for Risk

¹ A determination of whether one or more of the criteria of section 64 are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposures in the general environment. For humans, this includes, but is not limited to, exposures from ambient and indoor air, drinking water, foodstuffs, and the use of consumer products. A conclusion under CEPA on the substances in the Chemicals Management Plan (CMP) is not relevant to, nor does it preclude, an assessment against the hazard criteria for the *Workplace Hazardous Materials Information System* (WHMIS) that are specified in the *Controlled Products Regulations* for products intended for workplace use. Similarly, a conclusion based on the criteria contained in section 64 of CEPA does not preclude actions being taken under other sections of CEPA or other Acts.

Assessment), Michael Jayjock (LifeLine Group Inc.), Jerry M. Rice (Georgetown University Medical Center) and Sue Ross (Toxicology Excellence for Risk Assessment). Additionally, the draft of this Screening Assessment report was subject to a 60-day public comment period. While external comments were taken into consideration, the final content and outcome of the Screening Assessment remain the responsibility of Health Canada and Environment and Climate Change Canada.

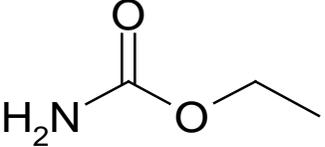
The critical information and considerations upon which the Screening Assessment is based are summarized below.

2. Substance Identity

This Screening Assessment focuses on the substance, Carbamic acid, ethyl ester (CAS RN 51-79-6). In this assessment, the substance is referred to as ethyl carbamate, which is the common name used in the International Union of Pure and Applied Chemistry (IUPAC) nomenclature. Ethyl carbamate has the trivial name *urethane*, which is sometimes also used to describe the unrelated substance polyurethane. Although ethyl carbamate and polyurethanes have the same synonym, they are distinct molecules. Polyurethanes are high-molecular-weight polymers. Ethyl carbamate is not used to synthesise polyurethanes, and polyurethanes do not decompose to ethyl carbamate. Polyurethanes are not the subject of this Screening Assessment.

The substance identity of ethyl carbamate is presented in Table 2-1 below.

Table 2-1. Substance identity for ethyl carbamate

CAS RN	Chemical structure	Molecular mass	Chemical formula	SMILES ^a
51-79-6		89.09	C ₃ H ₇ NO ₂	CCOC(N)=O

^a Simplified Molecular Input Line Entry System.

3. Physical and Chemical Properties

Experimental data for physical and chemical properties of ethyl carbamate have been identified and are summarized in Table 3-1.

Where there are no empirical data for a certain parameter, quantitative structure-activity relationship (QSAR) models are used to generate an estimate. These models are mainly based on fragment addition methods, i.e., they sum the contributions of sub-structural fragments of a molecule to make predictions for a property or endpoint. Most of these models rely on the neutral form of a chemical as input (in a SMILES string as CCOC(N)=O). Ethyl carbamate is a simple neutral chemical, and thus QSAR modelling can be simply applied to this compound.

Ethyl carbamate is a colourless, almost odourless columnar crystal solid or white granular powder at room temperature (Budavari 2000). It is miscible in water as well as in a variety of organic solvents (Speyers 1902). It is not likely to dissociate (modelled pK_a is 12), and it is expected that the substance would remain in a neutral form at environmentally relevant pH values of 6–9. Experimental data indicate that ethyl carbamate has a very low octanol-water partition coefficient (log K_{ow} = -0.15). Based on

the model prediction, the organic carbon-water partition coefficient is expected to be very low (modelled log K_{oc} is ~ 1).

Table 3-1. Physical and chemical properties of ethyl carbamate

Property	Type	Value	Temperature (°C)	Reference
Melting point (°C)	Experimental	46–50 ^a		Modarresi-Alam et al. 2007; Kurita et al. 1986; O'Neil 2006; Haynes 2011
Boiling point (°C)	Experimental	182–184		O'Neil 2006
Boiling point (°C)	Experimental	185		Haynes 2011
Density (kg/m ³)	Experimental	986		Haynes 2011
Density (kg/m ³)	Experimental	1 100		O'Neil 2006
Vapour pressure (Pa)	Experimental	1 333	77.8	Sigma-Aldrich 2013
Vapour pressure (Pa)	Extrapolated	34.9 ^b	25	Perry and Green 1984
Vapour pressure (Pa)	Modelled	55.3	25	MPBPWIN 2010
Henry's Law constant (Pa·m ³ /mol)	Extrapolated	6.52×10 ⁻³	25	HENRYWIN 2011
Log K_{ow} (dimensionless)	Experimental	-0.15	37	Houston et al. 1974
Log K_{oc} (dimensionless)	Modelled	0.76, 1.08		KOCWIN 2010
Water solubility (mg/L)	Experimental	6.62×10 ⁴	11.1	Speyers 1902
Water solubility (mg/L)	Experimental	4.37×10 ^{5b}	23.5	Speyers 1902
Water solubility (mg/L)	Experimental	7.56×10 ⁵	37.0	Speyers 1902
Solubility in methyl alcohol (mg/L)	Experimental	5.9×10 ⁵	22.5	Speyers 1902
Solubility in ethyl alcohol (mg/L)	Experimental	5.2×10 ⁵	21.7	Speyers 1902

Property	Type	Value	Temperature (°C)	Reference
Solubility in propyl alcohol (mg/L)	Experimental	5.3×10^5	21.6	Speyers 1902
Solubility in chloroform (mg/L)	Experimental	4.9×10^5	17.4	Speyers 1902
pK _a	Modelled	12		ACD/Percepta c1997-2012

Abbreviations: pK_a, acid dissociation coefficient expressed as a negative logarithm; K_{oc}, organic carbon–water partition coefficient; K_{ow}, octanol–water partition coefficient.

^aMP = 48°C was selected for modelling.

^bValue selected for modelling.

4. Sources

Ethyl carbamate is a by-product of the fermentation process and has been detected in many types of fermented foods (JECFA 2006a, 2006b) and beverages (Conacher and Page 1986; Sen et al. 1993; Velisek 1995). The formation of ethyl carbamate in alcoholic beverages and other foods is dependent on the chemical precursors and potential catalysts available in the raw material as well as the storage conditions during fermentation (i.e., light, temperature, pH and duration). In the case of wine, for example, arginine is a naturally occurring amino acid in grapes and is a food source for yeast (Zimmerli and Schlatter 1991). Yeast consumes arginine and produces urea, which reacts with ethanol produced during alcoholic fermentation to form ethyl carbamate. Ethyl carbamate formation from urea is favoured at high temperatures (Weber and Sharypov 2009); thus, temperatures during transport and storage can impact its levels. Hydrogen cyanide is a key precursor of ethyl carbamate in stone fruit brandies. Copper has been shown to be able to catalyze the formation of ethyl carbamate in whisky and it has been implicated in the conversion of cyanide to ethyl carbamate in distilled spirits (Aresta et al. 2001). It is also a natural constituent of tobacco plants and is present in mainstream tobacco smoke (Zimmerli and Schlatter 1991; NTP 2011).

In 2009, ethyl carbamate was included in a notice issued pursuant to section 71 of CEPA, aiming to update the Domestic Substances List (DSL) inventory for the reporting year 2008 (Canada 2009). According to responses to this notice, no manufacturing of this substance above the 100 kg reporting threshold was identified in Canada (Environment Canada 2009), but significant import quantities were reported. However, ethyl carbamate can also be described by its trivial name *urethane*, which is also a synonym for polyurethanes. At the time, it was unclear as to whether the reported 2008 import quantities included data for ethyl carbamate, polyurethane, or both. However, based on new information provided voluntarily through stakeholder consultation in 2012–2013 in follow-up to information submitted for the DSL inventory update (Canada 2009), no company was identified as importing or using ethyl carbamate above the

reporting threshold of 100 kg per year, and volumes reported in 2008 are not considered to be representative of import volumes in Canada.

This new information is supported by the data reporting low historical quantities of ethyl carbamate being used within Canada, and low current production capacity of ethyl carbamate globally. From 1984 to 1986, the total quantity of ethyl carbamate in commerce in Canada was reported to be 100 kg annually (Environment Canada 1988).

Since 1987, ethyl carbamate production in the United States has not exceeded 25 000 lbs (11 340 kg) per year (10 000 lbs or 4 536 kg per year prior to 2006) (US EPA 2012). And in 2009, worldwide production of ethyl carbamate was attributed to a single manufacturer located in the United States (production quantity not available) (SRI 2009).

5. Uses

In Canada and internationally, the current uses of ethyl carbamate are limited to medical research on laboratory animals where it is used for its anaesthetic (Hara and Harris 2002; Janssen et al. 2004) or neoplastic properties (Kawano et al. 1995; Hara and Harris 2002; Miller et al. 2003; Avanzo 2004, 2006; Minowada and Miller 2009).

No Canadian Material Safety Data Sheet listing ethyl carbamate as an ingredient was identified.

6. Releases to the Environment

Monitoring data have not been identified for ethyl carbamate in any environmental medium in Canada.

The National Pollutant Release Inventory (NPRI 1995) provides information on releases and transfers of key pollutants in Canada. However, ethyl carbamate is not on the list of NPRI reportable substances.

According to the results of the DSL Inventory Update (Environment Canada 2009) and the follow-up stakeholder consultation, ethyl carbamate was not manufactured or imported in Canada above the reporting threshold of 100 kg per year in 2008 and 2011. Therefore, releases of this substance to the environment due to anthropogenic activities are expected to be negligible.

In the United States, where ethyl carbamate is manufactured, the substance has been listed as a hazardous air pollutant under the US Environmental Protection Agency's (US EPA) Clean Air Act and has been subject to reporting since 1990 (CFR 1990). Detectable concentrations of ethyl carbamate in U.S. ambient air or water were not identified.

7. Environmental Fate

The environmental fate of a substance describes the processes by which it moves and is transformed in the environment. Based on its physical and chemical properties, the environmental fate of ethyl carbamate was predicted using Level III fugacity modelling (EQC 2011). This model simulates the environmental distribution of a chemical at a regional scale (i.e. 10 000 km² for water and sediment, and 100 000 km² for air and soil) and outputs the fraction of the total mass in each compartment from an emission into the unit world and the resulting concentration in each compartment. It also assumes non-equilibrium conditions between environmental compartments, but equilibrium within compartments.

The mass-fraction distribution of ethyl carbamate is presented in Table 7-1. These results represent the net effect of chemical partitioning, inter-media transport, and loss by both advection (out of the modelled region) and degradation/transformation processes.

Table 7-1. Results of the Level III fugacity modelling (EQC 2011) (Percentage of substance partitioning into each compartment)

Substance released to:	Air	Water	Soil	Sediment
Air (100%)	6	27	67	negligible
Water (100%)	negligible	100	negligible	negligible
Soil (100%)	negligible	22	78	negligible

When released to air, it is predicted that ethyl carbamate will reside mainly in soil (67 %) and, to a lesser extent, partition to water (27 %). A small quantity of the substance will remain in air (6 %), which is supported by its moderate vapour pressure. Ethyl carbamate is not expected to be found in sediment due to its very high water solubility and low adsorption potential.

When released to water, ethyl carbamate is likely to remain in this compartment (100%) as it has high water solubility. It is unlikely that the substance would partition to any other environmental compartments.

When released to soil, the substance is expected to remain in this compartment (78%), and be largely associated with soil pore water. The substance is expected to demonstrate high mobility in soil and could run-off from surface soils to the aquatic compartment (22%). Evaporation into air is not expected to be significant.

8. Environmental Persistence and Bioaccumulation

8.1 Environmental Persistence

Limited studies on the persistence potential of ethyl carbamate are available. Therefore, QSAR models are also used to estimate biodegradation potential for this substance.

8.1.1 Empirical data for persistence

No data for degradation of ethyl carbamate in the atmosphere were identified.

The substance contains no functional groups that can hydrolyze under environmental conditions; hydrolysis is therefore not expected to be an important fate process for the substance.

There were experimental studies to investigate biodegradation potential for ethyl carbamate; however, findings reported in these studies demonstrate a discrepancy.

Ethyl carbamate was classified as readily biodegradable in river die-away tests (HSDB 1983-). The Ministry of Economy, Trade and Industry (MITI) in Japan reported a half-life of 15 days in water for ethyl carbamate from a ready-biodegradation study (NITE 2002). However, as the original study is not accessible, key information of this study (e.g., the test concentration of the substance or the test conditions) is unknown. The current Chemical Risk Information Platform (CHRIP) database of National Institute of Technology and Evaluation (NITE) in Japan includes degradation data for this substance. In a screening test, inoculum containing activated sludge at 30 mg/L was exposed to ethyl carbamate at a concentration of 100 mg/L for 28 days, and no rapid degradation was reported in the end of the study (CHRIP c2008). Based on this result, the substance was then considered as "non-biodegradable" under the Chemical Substances Control Law in Japan (CHRIP c2008).

Lutin et al. (1965) conducted an oxidation study of ethyl carbamate and other selected carcinogenic chemicals. Each test chemical at a concentration of 500 mg/L was exposed to three sludge samples separately at 20°C for 144 hours. None of these test chemicals was significantly oxidized by any of the activated sludges (Lutin et al. 1965). In another study, biodegradation of ethyl carbamate was also reported to be slow at high concentrations (Malaney et al. 1967); however, the original study is not available for review.

Findings in these studies suggest that the biodegradation rate of ethyl carbamate is dependent upon its concentration in the test media. The substance may degrade rapidly at low concentrations; however, at higher concentrations, ethyl carbamate is toxic to the microbes, which can slow down the biodegradation (HSDB 1983-).

8.1.2 Modelling of persistence

A QSAR-based weight-of-evidence approach was applied and model predictions are summarized in Table 8-1 below.

Table 8-1. Modelled data for degradation of ethyl carbamate

Fate process	Model	Model result and prediction	Extrapolated half-life (days)
Atmospheric oxidation	AOPWIN 2008 ^a	Half-life = 17.46 hours	< 2
Ozone reaction	AOPWIN 2008 ^a	NA ^b	NA
Hydrolysis	HYDROWIN 2008 ^a	NA ^b	NA
Primary biodegradation (aerobic)	BIOWIN 2010 ^a Sub-model 4: Expert Survey (qualitative results)	3.91 ^c “biodegrades fast”	< 182
Ultimate biodegradation (aerobic)	BIOWIN 2010 ^a Sub-model 3: Expert Survey (qualitative results)	2.96 ^c “biodegrades rapidly”	< 182
Ultimate biodegradation (aerobic)	BIOWIN 2010 ^a Sub-model 5 (MITI linear model probability)	0.45 ^d “biodegrades rapidly”	< 182
Ultimate biodegradation (aerobic)	BIOWIN 2010 ^a Sub-model 6 (MITI non-linear model probability)	0.69 ^d “biodegrades rapidly”	< 182
Ultimate biodegradation (aerobic)	TOPKAT 2004 (probability of biodegradability)	1.00 ^d “biodegrades rapidly”	< 182
Ultimate biodegradation (aerobic)	CPOPs 2012 % BOD (biological oxygen demand)	28-day %BOD = 89.95% “biodegrades rapidly”	< 182

^a Sub-models in EPIsuite (2008)

^b Model does not provide an estimate for this type of structure.

^c Output is a numerical score from 0 to 5.

^d Output is a probability score from 0 to 1.

NA – not applicable

In air, ethyl carbamate has a predicted atmospheric oxidation half-life of 17.46 hours (see Table 8-1), which indicates that the substance is oxidized relatively quickly. However, the substance is not anticipated to react with other photo-oxidative species in the atmosphere, such as O₃, nor is it likely to degrade by direct photolysis. Therefore, it

is expected that reactions with hydroxyl radicals will be the most important fate process in the atmosphere for the substance. With a half-life of 17.46 hours via reactions with hydroxyl radicals, ethyl carbamate is considered not persistent in air.

In water, results for all of the BIOWIN biodegradation sub-models (BIOWIN sub-models 3, 4, 5 and 6) indicate a potential for rapid biodegradation of ethyl carbamate. In addition, the ultimate degradation predictions from TOPKAT (2004) and CPOPs (2012) also suggest a rapid biodegradation.

Products resulting from primary degradation are likely to be acetic acid (CAS RN 64-19-7) and carbamic acid (CAS RN 463-77-4) (CPOPs 2012). Carbamic acid is not stable and can further undergo hydrolysis, producing carbon dioxide and methyl amine. According to the empirical data, both acetic acid and methyl amine possess moderate toxicity to aquatic organisms. Combined with the limited source of the parent compound, these metabolites are of no significant concern to organisms.

8.1.3 Summary of persistence

Ethyl carbamate is not considered to be persistent in air, based on a model prediction (AOPWIN 2008). Available biodegradation studies on ethyl carbamate suggest that the substance biodegrades rapidly in water at low concentrations. At higher substance concentrations, some toxicity to micro-organisms was observed that resulted in slower biodegradation rates. All model results indicate that ethyl carbamate biodegrades quickly in water (see Table 8-1) (BIOWIN 2010; TOPKAT 2004; CPOPs 2012). Ethyl carbamate is also expected to undergo rapid biodegradation in soil and sediment, based on the extrapolation criteria from the half-life in water (Boethling et al. 1995).

Based on empirical and modelled data, ethyl carbamate is expected to have a limited persistence in environmental media, and it does not meet the persistence criteria in air, water, sediment or soil (half-life in air \geq 2 days, half-lives in water and soil \geq 182 days and half-life in sediment \geq 365 days) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

A low persistence potential for ethyl carbamate in Canada suggests that the substance is not expected to remain in the environment and cause long-term exposure.

8.2 Potential for Bioaccumulation

8.2.1 Empirically determined bioaccumulation

8.2.1.1 Bioconcentration factor (BCF)

There are limited empirical bioaccumulation data for ethyl carbamate. The substance was determined to have a low bioconcentration potential under the Chemical Substances Control Law in Japan (CHRIP c2008); the BCF was reported up to 6 L/kg

on fish (*Cyprinus carpio*) after a 28-day exposure to this substance at concentrations of 0.2 mg/L and 2 mg/L.

An experimental BCF (3.98 L/kg) was included in the training set of the Canadian POPs model (CPOPs 2012). These data were provided by Japan's Chemicals Inspection and Testing Institute (CITI 1992); however details of the original study are not available.

8.2.1.2 Bioaccumulation factor (BAF)

Bioaccumulation factors (BAFs) are measured under field conditions as the ratio of the whole body burden of chemical taken up from all exposures to that of the ambient water concentrations. Measures of BAF are the preferred metric for assessing the bioaccumulation potential of substances because they incorporate chemical exposures from all routes including the diet, which predominates for substances with $\log K_{ow} > \sim 4.0$ (Arnot and Gobas 2003). As the $\log K_{ow}$ for ethyl carbamate is -0.15, accumulation through dietary uptake is not expected to be an important process for this substance. Therefore, the value of BAF for ethyl carbamate is expected to be similar to its BCF.

No empirical BAF data were found for ethyl carbamate. Instead, the available QSAR model was used to estimate this endpoint (see Table 8-2).

8.2.2 Modelled bioaccumulation

QSAR models were used to provide an additional line of evidence for assessing bioaccumulation potential for ethyl carbamate. Predicted BCF and BAF values are presented in Table 8-2.

The low calculated BAF and BCF for ethyl carbamate correlate with the very low empirical $\log K_{ow}$ (-0.15). The model predictions suggest that the potential for bioconcentration in aquatic organisms is low.

Table 8-2. Modelled bioaccumulation and bioconcentration factors for ethyl carbamate

Test organism	Endpoint and value	Reference
Fish	BCF = 0.95 L/kg BAF = 0.95 L/kg (middle trophic level fish)	BCFBAF 2010
Fish	BCF = 3.53 L/kg	CPOPs 2012

Considering the low BCF of ethyl carbamate, the dietary intake of this substance is not significant in aquatic organisms. Therefore, the estimated BAF is same as the BCF for this substance (see Table 8-2).

8.2.3 Summary of bioaccumulation

Physical and chemical properties of ethyl carbamate including its high water solubility and the very low log K_{ow} value, suggest that the substance is unlikely to bioaccumulate in biota. There are limited experimental data for the bioconcentration potential of ethyl carbamate. The empirical BCF value is in agreement with model predictions, and the estimated BAF is similar to the BCF. All suggest a low bioaccumulation potential of the substance. As accumulation of the substance in organisms is unlikely, there is lower potential to demonstrate effects due to the internal toxicity threshold being exceeded. Biomagnification of ethyl carbamate in the food web is not expected to be significant either.

Based on the known properties of ethyl carbamate, and the available empirical and modelled bioaccumulation evidence, it is concluded that this substance does not meet the bioaccumulation criteria (BCF or BAF \geq 5000) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

9. Potential to Cause Ecological Harm

9.1 Ecological effects assessment

9.1.1 Ecotoxicity to aquatic organisms

The ecological effect of ethyl carbamate was characterized based on the empirical data available for this substance (see Table 9-1).

In general, ethyl carbamate demonstrated low toxicity to aquatic species. Most short-term studies reported median effects concentration / median lethal concentration (EC₅₀/LC₅₀) at or above 1000 mg/L (Tonogai et al. 1982; Schultz 1997; Russom et al. 1997; Geiger et al. 1990). Another study reported no observed effects in *Daphnia pulex* in a 1-hour exposure to ethyl carbamate at 10 700 mg/L (Gannon and Gannon 1975).

Table 9-1. Empirical acute ecotoxicity data for the aquatic compartment

Test organism	Test duration	Endpoint	Value (mg/L)	Reference
Japanese medaka (<i>Oryzias latipes</i>)	24 hours	LC ₅₀	1000	Tonogai et al. 1982
Japanese medaka (<i>Oryzias latipes</i>)	48 hours	LC ₅₀	1000	Tonogai et al. 1982
Fathead minnow (<i>Pimephales promelas</i>)	96 hours	LC ₅₀	5250	ECOTOX 2006; Russom et al. 1997
Fathead minnow (<i>Pimephales promelas</i>)	96 hours	LC ₅₀	5240	Geiger et al. 1990
Fathead minnow (<i>Pimephales promelas</i>)	96 hours	EC ₅₀ (behavior and reaction)	3770	Geiger et al. 1990
<i>Daphnia pulex</i>	1 hours	EC ₀ (movement)	> 10 700	Gannon and Gannon 1975
<i>Tetrahymena pyriformis</i>	48 hours	EC ₅₀ (growth)	3980	Schultz 1997

Abbreviations: LC₅₀, the concentration of a substance that is estimated to be lethal to 50% of the test organisms; EC₀, the concentration of a substance that is estimated to cause no toxic sublethal effect on all test organisms; EC₅₀, the concentration of a substance that is estimated to cause some toxic sublethal effect on 50% of the test organisms

In a chronic test, Japanese medaka (*Oryzias latipes*) were exposed to ethyl carbamate at a concentration of 3.86 mg/L. One hundred percent mortality was reported at the end of a 28-day study (Johnson et al. 1993). Having high water solubility, the substance is expected to reach equilibrium in fish very quickly; therefore for the baseline toxicity, the concentration that causes chronic effects is not expected to be much lower than that which causes acute effects. However, the original study (Johnson et al. 1993) was not available for review; the experiment and the reported data therefore cannot be evaluated.

In an amphibian toxicity study (*Xenopus laevis*), there was an LC₅₀ reported at a concentration of 5580 mg/L (Verschuere 2001). Details of this study are not available; the age of the test organism and the exposure period therefore remain unknown.

9.1.2 Ecotoxicity to terrestrial organisms

Roberts and Dorough (1984) conducted a study to compare the relative toxicities of 90 chemicals to earthworms and other soil invertebrates. These chemicals include a variety of pesticides, amino acids, solvents, mutagens/carcinogens, drugs, heavy metals and

other chemicals. Test organisms were exposed to deposits on filter paper for 48 hours, and exposure concentrations were expressed in $\mu\text{g}/\text{cm}^2$. In this 48-hour acute contact toxicity study, the LC_{50} for ethyl carbamate was reported as $>1000\mu\text{g}/\text{cm}^2$ to the earthworm (*Eisenia foetida*) (Roberts and Dorough 1984). Based on the findings from this study, ethyl carbamate was considered relatively non-toxic amongst the 90 chemicals.

There are two toxicity studies on the worm planaria (*Dugesia bengalensis*). In one study, some general biochemical effects were observed on the test worms following exposure to ethyl carbamate at a concentration of 1500 mg/L for 24 to 96 hours (Aditya and Mahapatra 1992). In another study, genetic effects were observed on test organisms after an exposure to ethyl carbamate at a concentration of 2500 mg/L for up to 96 hours (Aditya et al. 1994). However, the original studies (Aditya and Mahapatra 1992; Aditya et al. 1994) were not available for review; the experimental conditions and the incidence of biochemical effects and genetic changes therefore cannot be evaluated. Nevertheless, the effect observed on test organisms (Aditya et al. 1994) is consistent with the genotoxic potential of ethyl carbamate observed from mammalian data (see the section 10.2 Health effects assessment).

9.2 Ecological exposure assessment

No reports indicating the measurement or detection of ethyl carbamate in the Canadian environment were identified.

Based on the results of the DSL Inventory Update (Environment Canada 2009) and subsequent stakeholder follow-up and consultation, there was no manufacturing and import of the substance in Canada in quantities above the 100 kg per year reporting threshold. The known uses of this substance in Canada are limited to its application in laboratory research. Therefore, release of this substance due to anthropogenic activities in Canada is expected to be negligible. Exposure to the organisms in the Canadian environment is not expected.

9.3 Characterization of ecological risk

The approach taken in this ecological screening assessment was to examine various supporting information and develop conclusions based on a weight-of-evidence approach and using precaution as required under CEPA. Lines of evidence considered in the assessment of ethyl carbamate include information on physical and chemical properties, sources, uses, the potential for persistence and bioaccumulation, ecotoxicity to aquatic and terrestrial organisms, and presence of this substance in the Canadian environment.

There are a few experimental data for biodegradation of ethyl carbamate, which report biodegradation rates at different concentrations. At the same time, the available models suggest a rapid biodegradation of the substance in water. The metabolites are likely to

be acetic acid and carbamic acid; however, any significant release of these metabolites from ethyl carbamate would be low due to a limited source of the parent compound in Canada. Both empirical data and model predictions suggest a low bioaccumulation potential for the substance. In conjunction with the high water solubility (10^4 - 10^5 mg/L) and very low octanol-water partition coefficient ($\log K_{ow} = -0.15$), this substance is not anticipated to bioaccumulate in organisms.

Given the low quantities imported and used in Canada, and recognizing that its use is limited to laboratory research, it is expected that releases of ethyl carbamate to the environment are negligible. In addition, the substance is not acutely toxic to aquatic organisms. Therefore, a quantitative risk characterization was not performed for this substance.

Considering all available lines of evidence presented in this Screening Assessment, there is low risk of harm to organisms and the broader integrity of the environment from ethyl carbamate. It is concluded that ethyl carbamate does not meet the criteria in paragraphs 64(a) or (b) of CEPA as the substance is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity, or that constitute or may constitute a danger to the environment on which life depends.

9.4 Uncertainty in evaluation of ecological risk

There is uncertainty associated with persistence data reported for this substance. There have been only a few biodegradation studies on ethyl carbamate identified, and details of most of them are not available. There are discrepancies among findings in these experimental studies. A measured half-life of 15 days and a rapid biodegradation in river die-away tests were reported in one study, suggesting a rapid biodegradation of ethyl carbamate in water. However, outcomes from the other two studies indicate a slow degradation of this substance in activated sludge exposed to the substance at high concentrations. Ethyl carbamate might be toxic to the microbes at these high concentrations and therefore the degradation did not proceed rapidly. According to the reported sources and uses, the environmental concentration of ethyl carbamate is expected to be very low in Canada; therefore the substance is expected to biodegrade rapidly in water. To provide additional information, QSAR models were used. Estimates suggest a rapid biodegradation of ethyl carbamate if released to water. The structural class of this substance is within domains of these QSAR models used; model predictions are therefore considered reliable and adequate to conclude on the persistence potential for ethyl carbamate.

10. Potential to Cause Harm to Human Health

10.1 Exposure assessment

Environmental media

Data on the concentration of ethyl carbamate in the Canadian environment were not identified. Ethyl carbamate is not manufactured or imported in Canada at quantities greater than 100 kg per year, and use in Canada is limited to its application in laboratory research. Based on this, releases to the environment in Canada are expected to be negligible, and exposure to the general population from environmental media is not expected.

Food

Ethyl carbamate is a chemical that is formed naturally during the fermentation process or during storage of fermented foods (Health Canada 2008). While the highest levels are typically found in yeast-fermented foods like distilled alcoholic beverages, lower levels are found in other foods such as bread, soy sauce, cheese, yogurt, vinegar, soybean paste (e.g., miso), kimchi and other fermented food products (JECFA 2006a, 2006b; Kim et al. 2000; Tang et al. 2011; Wu et al. 2011).

As the exposure database for levels of ethyl carbamate in foods and beverages is robust, and the critical effect levels for ethyl carbamate are low, it was considered appropriate to conduct a probabilistic exposure assessment (using 2D Monte Carlo simulation).

The Monte Carlo simulation requires occurrence distribution and consumption distribution. For the occurrence distribution, measured ethyl carbamate levels in foods were primarily obtained from the scientific literature and included foods marketed in both Canada and internationally. Data on ethyl carbamate in alcoholic beverages were obtained from compliance monitoring results collected by the Liquor Control Board of Ontario (LCBO), the Société des alcools du Québec (SAQ) and the Canadian Food Inspection Agency (CFIA). Between 2000 and 2011, over 50 000 monitoring results for ethyl carbamate in a wide variety of alcoholic beverages were collected. Foods not identified in the literature as containing ethyl carbamate were excluded from intake simulations. Summary statistics for ethyl carbamate levels in food used to predict ethyl carbamate intake and the supporting literature references are provided in Appendix A.

For the consumption distribution, the frequency and amounts of foods and beverages consumed by the Canadian population were obtained from the Canadian Community Health Survey (CCHS), Cycle 2.2: Nutrition (Statistics Canada 2008). The CCHS was carried out in 2004–2005 and using stratified multistage cluster design, obtained a sample size of 35 107 respondents of all ages living in private occupied dwellings in all 10 provinces.

The CCHS provided individuals' measured or self-reported body weights for participants two years and older. Surrogate body weights for those younger than two years of age were obtained from the US National Health and Nutrition Examination Survey IV (Portier et al. 2007). Body weight distribution statistics based on CCHS data are shown in Appendix B.

Exposure to ethyl carbamate via food intake is long-term in duration and long-term consumption estimates are required to characterize the associated risk. However, long-run averages of single-day intakes for the Canadian population were not available. Alternatively, a distribution of intake was generated based on those respondents in the CCHS who consumed foods likely to contain ethyl carbamate on both 24-hour recall days (approximately 2000 respondents), using the method of Nusser et al. (1996). Dietary intake estimates for ethyl carbamate were generated for all age categories one year and older using this approach.

Intake estimates could not be generated from CCHS data for infants less than one year of age due to the limited number of respondents. Therefore, an upper-bound deterministic intake estimate of 13.1 ng/kg body weight per day (ng/kg-bw/d) was derived instead. This deterministic intake estimate was based on Canadian data (Health Canada 1998) for consumption of fruit juice, dairy products, breads and crackers by children less than one year old, assuming ethyl carbamate levels in food at the 95th percentile (Appendix A) and a body weight of 7.5 kg.

For other age groups, at the 90th percentile of the distribution, usual intakes of ethyl carbamate from all foods except alcoholic beverages ranged from 11.9 ng/kg-bw/d (males, aged 14<19 years) to 24.0 ng/kg-bw/d (males and females, aged 1<14 years), shown in Table 10-1.

Table 10-1. Estimated 50th, 90th and 95th percentile daily ethyl carbamate dietary intake (ng/kg-bw/d) and [95% confidence interval]

Age category (years)	Sex	P50 ^a	P90 ^a	P95 ^a
1<14	Male and female	9.6 [8.1, 12]	24.0 [16, 32]	32.3 [20, 48]
14<19	Male	6.6 [4.8, 11]	11.9 [7.4, 28]	14.0 [8.1, 40]
14<19	Female	7.6 [5.0, 15]	12.5 [7.8, 41]	14.4 [8.5, 62]
≥19	Male (all except alcohol)	7.2 [5.0, 8.8]	20.3 [11, 27]	28.0 [14, 41]
≥19	Male (alcohol only)	50.0 [37, 70]	106 [75, 149]	132 [90, 185]
≥19	Female (all except alcohol)	7.2 [5.4, 9.7]	20.0 [13, 33]	27.0 [17, 52]
≥19	Female (alcohol only)	28.8 [18, 40]	59.0 [38, 93]	73.3 [46, 121]

Abbreviation: bw/d, body weight per day.

^a 50th, 90th and 95th percentiles of ethyl carbamate intake, respectively.

For the males and females 19 years of age and older, intake from alcoholic beverage and intake from food were separated. Alcoholic beverages typically have the highest reported ethyl carbamate levels of all foods reported in the literature, although an established trend of declining ethyl carbamate levels over time has been observed globally (JECFA 2006a, 2006b). At the 90th percentile for both males and females, 19 years of age and older, intake of ethyl carbamate from alcoholic beverages was estimated to be 106.0 and 59.0 ng/kg-bw/d, respectively.

Confidence in the estimates of dietary exposure to ethyl carbamate is moderate to high. Although full data sets were not available for ethyl carbamate in most fermented foods on the Canadian market, there were extensive Canadian data for ethyl carbamate in alcoholic beverages. Where available, Canadian occurrence data were used, but for many food groups, the only data available were in a summarized form as presented in published literature from various countries (shown in Appendix A). Conversely, only part of the data from the CCHS were used so that only consistent (on both recall days) eaters of foods containing ethyl carbamate were selected, which may have led to an overestimate of ethyl carbamate exposure.

While it is possible that not all food containing ethyl carbamate was represented in the exposure assessment, these other food items that were not captured are not expected to contribute significantly to overall dietary exposure. The 90th percentile of the distribution of exposure estimates is considered to be upper-bound, and sufficiently conservative when considering chronic consumption of food and alcoholic beverages to account for these uncertainties, as is the deterministic exposure assessment for children less than one year of age.

10.2 Health effects assessment²

The International Agency for Research on Cancer (IARC) first evaluated the carcinogenic risk of ethyl carbamate in 1974 (IARC Monograph Vol. 7) and classified this substance as IARC 2B– the agent is “possibly carcinogenic to humans” (IARC 1974). This substance was re-evaluated by IARC in 2010, jointly with an evaluation of alcohol consumption (IARC Monograph Vol. 96), and was upgraded and reclassified as a group 2A carcinogen—the agent is “probably carcinogenic to humans” (IARC 2010). In addition to evaluation(s) by IARC, the National Toxicology Program (NTP) in the United States evaluated ethyl carbamate and listed it in their 12th Report on Carcinogens (ROC) as “Reasonably anticipated to be a human carcinogen.” Additionally, NTP specified that its ROC listing for ethyl carbamate was on the basis of sufficient evidence of carcinogenicity from studies in experimental animals. This substance was first listed by the NTP in 1983 in their Third Annual Report on Carcinogens (NTP 2011).

² Additional details on the health effects assessment described in this section can be found in supporting documentation available upon request (Health Canada 2013).

This substance was also classified under the European Union's (EU) Dangerous Substances Directive as a Carcinogen Category 2/R45,³ as listed in the 29th Adaptations to Technical Progress (ATP), which under this classification system indicates that this substance "may cause cancer" (Annex I of Directive 67/548/EEC). Subsequent changes to the classification schemes for the hazard class within the European Union's Classification, Labelling and Packaging (CLP) regulations (EC No 1272/2008) resulted in a change in the status of ethyl carbamate to "Category 1B—presumed to have carcinogenic potential for humans, classification is largely based on animal evidence" (European Commission 2008). It should be noted that this classification change in the EU from Category 2 to 1B is a reflection of the changes in the classification system than an actual change in the perceived severity of effect or adequacy of the evidence for carcinogenicity.

Toxicokinetics and metabolism

It has been recognized since the 1960's that ethyl carbamate is metabolically activated, generating reactive metabolites that interact and covalently bind with nucleic acids and proteins (WHO 2006). Ethyl carbamate is soluble in both water and lipids and evenly distributed in the body (it was the first water-soluble carcinogen discovered) and as a result the organs affected are not necessarily limited to sites where the substance is applied/administered (Mirvish 1968). It is well absorbed from both the gastrointestinal tract and skin (WHO 2006). The distribution of ethyl carbamate after dosing, regardless of route (i.e., intraperitoneal, oral, or dermal), appears to be even and no significant differences in the concentrations in various tissues were observed in either rats or mice. Ethyl carbamate is rapidly metabolized via multiple pathways. Toxicokinetics is considered to be an important factor in the dose-response for carcinogenicity and in the variability in sensitivity based on age, interstrain, interspecies, and so forth (Salmon and Zeise 1991).

Various studies have reported that 90-95% of ethyl carbamate is metabolized to ethanol, CO₂ and NH₃ prior to elimination, with 2-8% and 0.3-1% of the parent recovered in urine and feces, respectively, in mice and rats (Nomeir et al. 1989; NTP 2004). Metabolic pathways of potential importance include hydrolysis to ethanol and ammonia, and side-chain oxidation to vinyl carbamate (JECFA 2006a). On the basis of information available at the time, Salmon and Zeise (1991) stated that hydrolysis was the primary mechanism responsible for the metabolism of ethyl carbamate. However, more recent studies have demonstrated that CYP2E1 was the principal enzyme responsible for about 95-96% of ethyl carbamate metabolism to carbon dioxide (Carlson 1994; Hoffler et al. 2003; WHO 2006). In rats and mice this CYP2E1 activity is proposed to result in active metabolites that interact and bind covalently with nucleic acids and proteins. Ghanayem (2007) demonstrated a significant reduction of carcinogenicity in knockout (i.e., CYP2E1 -/-) mice compared to wild-type (CYP2E1 +/+) mice and demonstrated that CYP2E1-mediated oxidation plays an essential role in ethyl

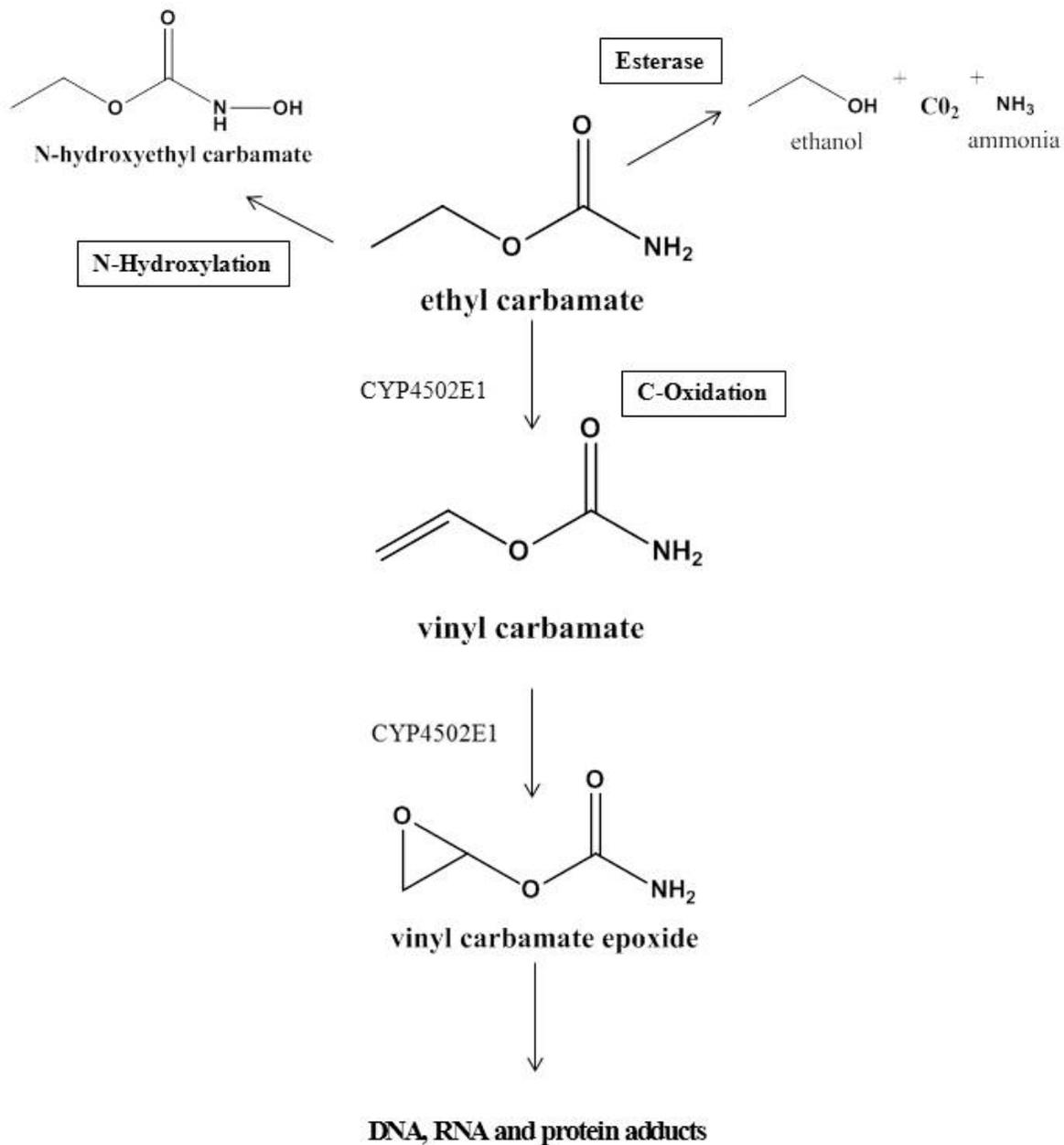
³ The R indicates "Risk Phrase" under the EU's Dangerous Goods Directive.

carbamate induced carcinogenicity (this study is described in more detail in the next section on carcinogenicity). The proposed activation pathway for ethyl carbamate proceeds via sequential oxidation by CYP2E1, starting with oxidation of the carbon side chain (i.e., C-oxidation) of ethyl carbamate, to form vinyl carbamate, followed by rapid oxidation to vinyl carbamate epoxide (Dahl et al. 1978, 1980) (see Figure 1).

Work by Dahl et al. (1978, 1980) explored the role that the C-oxidation pathway has in the metabolic activation of ethyl carbamate. The authors postulated that this pathway would lead to the formation of the proximate carcinogen metabolite, vinyl carbamate. It was then postulated that, with vinyl carbamate formed, this metabolite would be further oxidized to vinyl carbamate epoxide, the ultimate carcinogen. They observed that vinyl carbamate was 10-50 times more carcinogenic than ethyl carbamate in both the skin and lungs of female CD-1 mice. Subsequent investigations showed similar results in F344 rats and B6C3F1 mice (Dahl et al. 1980). It is considered that vinyl carbamate epoxide is responsible for reactions with DNA which ultimately yield DNA adducts, specifically etheno-type (Guengerich and Kim 1991).

The NTP (2004) considered that the oxidation of ethyl carbamate to vinyl carbamate, and the subsequent oxidation to generate the epoxide, accounts for the carcinogenic properties observed in ethyl carbamate in rodents. Furthermore, it was the conclusion of IARC that the available “experimental evidence suggests great similarities in the metabolic pathways of the activation of ethyl carbamate in rodents and humans,” with bio-activation expected to be predominantly via CYP2E1, and the subsequent generation of metabolites from these pathways (IARC 2010).

Figure 1. Metabolism of ethyl carbamate (adapted from NTP 2004 and Ghanayem 2007)



In the past, it has been proposed that ethyl carbamate may be metabolically activated via other pathways, such as further degradation of N-hydroxylated products. Boyland and Nery (1965) observed that 3-4% of ethyl carbamate is excreted in urine either as the parent compound (ethyl carbamate) or as one of a number of metabolites, including: N-hydroxyurethane; N-acetyl-N-hydroxyurethane; N-acetyl-S-ethylcystine and; N-acetyl-S-carbathoxycysteine. At one time, it was suggested that because metabolic activation of aromatic amines and amides was mediated via an N-hydroxylation pathway, this could mean that ethyl carbamate was metabolically activated via this same pathway (Boyland and Nery 1965, NTP 2004). However, studies have found that N-hydroxyurethane has about half the carcinogenic potency of ethyl carbamate, and also that about 70% of an administered dose of N-hydroxyurethane was metabolized by reduction to ethyl carbamate (Mirvish 1968). Therefore, this possible pathway for metabolic activation of ethyl carbamate is now seen as unlikely to be a major contributor to the carcinogenicity of ethyl carbamate (NTP 2004). The products of C-oxidation are now considered the most important for ethyl carbamate, as the products of N-hydroxylation (e.g., N-hydroxyurethane) are generally quite low, in addition to being transitory, with the majority converted back to ethyl carbamate (Mirvish et al 1969, Salmon and Zeise 1991, NTP 2004).

NTP (2004) also identifies other investigators (e.g., Ribovich et al. 1982; Miller and Miller 1983) who have demonstrated the presence of various adducts, such as 1,N(6)-ethenoadenosine and 3,N(4)-ethenocytidine in RNA (hepatic), following exposure to ethyl carbamate. It was noted that these adducts were derived from metabolic activation of ethyl carbamate, and not another compound (e.g., ethanol formed by hydrolysis). The conclusion therefore was that vinyl carbamate and its epoxide were the carcinogenic metabolites of ethyl carbamate (Ribovich et al 1982; Miller and Miller 1983). Vinyl carbamate forms far more of these adducts than ethyl carbamate, providing further evidence that vinyl carbamate is more carcinogenic than ethyl carbamate, and thus, likely to be its proximate carcinogenic metabolite (Park et al. 1990). Park et al. (1993) also found that vinyl carbamate epoxide was a stronger tumour initiator than either ethyl carbamate or vinyl carbamate on the skin of CD-1 mice.

It was demonstrated by Guengerich and Kim (1991) that human CYP2E1 played a major role in the oxidation of both ethyl and vinyl carbamate. This investigation further demonstrated that microsomal oxidation of both substances in the presence of adenosine resulted in the formation of 1,N(6)-ethenoadenosine. Furthermore, Guengerich and Kim (1991) showed that the rate of conversion forming these adducts was approximately 400 times faster for vinyl carbamate over ethyl carbamate. This rate difference appears to be the reason that previous metabolic studies failed to detect vinyl carbamate as a metabolite of ethyl carbamate, as such rapid oxidation of vinyl carbamate by CYP2E1 relative to ethyl carbamate would likely mean the steady-state level of vinyl carbamate would be extremely low (Guengerich and Kim 1991, NTP 2004).

Elimination of ethyl carbamate and its metabolites is rapid, and while prolonged at higher doses due to saturation of metabolic processes, is still complete in 24 and 72

hours in mice and rats, respectively. Significant bioaccumulation of ethyl carbamate (in the parent form) has not been reported.

Carcinogenicity

There have been extensive investigations into the carcinogenicity of ethyl carbamate, beginning with evidence in the early 1940's indicating that injections of ethyl carbamate induced lung adenomas in mice (Mirvish 1968). Subsequent studies also found that the carcinogenicity of ethyl carbamate is not limited to the lung and is a multi-site carcinogen, being found to induce tumours in skin, lymphomas of the thymus, hepatomas, and haemangiomas, amongst others (e.g., Pietra and Shubik 1960; Tannenbaum 1964; Mirvish 1968; Innes et al. 1969; Van Esch and Kroes 1972). Much of this early work is captured in a review of the literature by Mirvish (1968). The carcinogenicity of ethyl carbamate continued to be studied, with a two year bioassay conducted by NTP as recently as 2004. The evidence for the carcinogenicity of ethyl carbamate has culminated in this substance being used as a positive control in both cancer and *in vivo* genetic toxicity assays (e.g., Tomatis et al. 1972; Van Esch and Kroes 1972; Salmon and Zeise 1991; Dogan et al. 2005).

Ethyl carbamate has been found to induce tumours in multiple animal species (including rats, mice, hamsters, and primates), in animal newborns and adults, by oral, dermal, inhalation, subcutaneous and intraperitoneal routes of exposure (Mirvish 1968; WHO 2006; IARC 2010). This substance has been found to be a more potent carcinogen in newborn mice than in adults (Mirvish 1968). Mirvish (1968) also cites studies which indicate that newborn mice eliminate ethyl carbamate at a much slower rate than adult mice and that this may explain higher sensitivity rates to carcinogenesis observed in newborn mice. In addition, ethyl carbamate is regarded as being the first substance where transplacental carcinogenicity was observed in animals exposed prenatally to a chemical (WHO 2006).

It has been demonstrated that the induction of tumours by ethyl carbamate occurs regardless of the route of exposure (Schmahl et al. 1977; WHO 2006; IARC 2010). The main source of exposure to ethyl carbamate for humans, however, is considered to be through the diet and this health effects review focuses predominantly on studies that looked at a route of administration, treatment frequency and duration reflective of this exposure pathway. While there is extensive data on the toxicity of ethyl carbamate in several species of rodents, the literature suggests that mice are more sensitive than rats to the tumourigenic effects of ethyl carbamate (NTP 2004). As a consequence, more focus will be placed on data derived from mouse studies.

Oral

The NTP's 2-year mouse bioassay from 2004 is a pivotal study that looked at a variety of effects over a lifetime exposure to ethyl carbamate in drinking water. This study examined the incidence of neoplasms for all drinking water concentrations of ethyl

carbamate, alone or co-administered with 2.5% or 5% ethanol, and limited evidence was found that ethanol affected the tumourigenicity of ethyl carbamate. It was concluded that the changes in tumourigenicity observed in the presence of ethanol were modest and may have been due to normal biological variation (NTP 2004; Beland et al. 2005). The results for ethyl carbamate alone (i.e., 0% ethanol groups) were found by the authors to be associated with clear evidence of carcinogenic activity (in mice), in both males and females, whereas the effects of ethanol on the carcinogenicity of ethyl carbamate were considered insufficient to establish definitively; as overall there was weak evidence of an interaction between ethanol and the carcinogenicity of ethyl carbamate (NTP 2004; Beland et al. 2005; WHO 2006).

The NTP's 2-year bioassay administered ethyl carbamate in drinking water to groups of B6C3F₁ male and female mice at concentrations of 0 (controls), 10, 30 and 90 parts per million (ppm). This resulted in an average daily consumption of approximately 40, 115, and 360 µg of ethyl carbamate for males, and 35, 105, and 325 µg of ethyl carbamate in females (NTP 2004). WHO (2006) calculated that this equated to a dose of 0, 1.2/0.9, 3.3/2.8, and 10.1/8.2 mg/kg-bw/d in males/females, respectively. This was determined to be equivalent to a dosage of approximately 0.9, 2.7 and 8.7 mg/kg-bw/d in both sexes (Beland et al. 2005). There were 48 animals per sex per dose group. All animals were observed twice daily. Clinical findings were recorded weekly and water consumption was constant. Body weights were recorded weekly and at the end of the study. Complete necropsies and microscopic examinations were performed on all mice. The liver and lung were weighed, and all organs and tissues were examined for grossly visible lesions. For the study, a quality assessment pathologist evaluated slides from all tumors. In addition, slides were taken from the heart, liver, lung, Harderian gland, spleen, and thymus of males and females, the adrenal, coagulating, and preputial glands of males, and the ovary and uterus of females (NTP 2004).

At the lowest drinking water concentration of ethyl carbamate tested (10 ppm) there was a significant incidence of both lung and Harderian gland neoplastic lesions in male mice, and a significant incidence of neoplastic lesions in the Harderian gland in female mice. At these two sites, in both sexes, an increase in ethyl carbamate concentrations also caused dose-dependent increases in lung and Harderian gland adenoma or carcinoma. Table 10-2 provides a summary of the incidence of neoplasms, for male and female mice, in the lung (See Table 10-3 for Harderian gland).

Table 10-2. Incidence of lung neoplasms in male/female B6C3F1 mice

Sex	Neoplasm (alveolar/bronchiolar)	Control (0ppm)	Low dose (10 ppm)	Mid dose (30 ppm)	High dose (90 ppm)
Male	Adenoma	3 (6.2%)	15 (31.2%)	15 (31.9%)	14 (29.2%)
Male	Adenoma, multiple	1 (2.1%)	2 (4.2%)	7 (14.9%)	20 (41.7%)
Male	Carcinoma	1 (2.1%)	1 (2.1%)	8 (17.0%)	8 (16.7%)
Male	Carcinoma, multiple	-	-	1 (2.1%)	1 (2.1%)
Male	Overall rate (adenoma/carcinoma)	5/48 (10.4%)	18/48 (37.5%)*	29/47 (61.7%)*	37/48 (77.1%)*

Sex	Neoplasm (alveolar/bronchiolar)	Control (0ppm)	Low dose (10 ppm)	Mid dose (30 ppm)	High dose (90 ppm)
Female	Adenoma	4 (8.3%)	6 (12.5%)	13 (27.1%)	12 (25.5%)
Female	Adenoma, multiple	-	-	4 (8.3%)	17 (36.2%)
Female	Carcinoma	2 (4.2%)	4 (8.3 %)	10 (20.8%)	6 (12.8%)
Female	Carcinoma, multiple	-	-	3 (6.2%)	13 (27.7%)
Female	Overall rate (adenoma/carcinoma)	6/48 (12.5%)	8/48 (16.7%)	28/48 (58.3%)*	39/47 (82.9%)*

Abbreviation: ppm, parts per million

*=Statistically significant over controls

As indicated in Table 10-2, the overall incidence rate of lung neoplasms in male mice at the lowest drinking water concentration (10 ppm) was 18/48 (37.5%), and they were primarily alveolar/bronchiolar adenomas. Dose-dependent increases were observed in male mice with overall rates of 29/47 (61.7%) and 37/48 (77.1%) observed at 30 ppm and 90 ppm, respectively. In female mice a dose-dependent increase in incidence rate of lung neoplasms was observed with an overall rate of 8/48 (16.7%), 28/48 (58.3%) and 39/47 (82.9%) in animals given 10, 30 and 90 ppm ethyl carbamate in drinking water, respectively. However, the incidence of lung neoplasms was not significant compared to controls in the group treated with 10 ppm in drinking water. The overall rate for female controls was 6/48 (12.5%).

The incidence of neoplasms in the Harderian gland was significant in all dose groups for both male and female mice. A dose-dependent increase in neoplasms was observed in both sexes, primarily adenomas at the lower dose with carcinomas becoming more prevalent at higher doses. Table 10-3 provides the overall rate of neoplasms by dose, sex and tumour type.

Table 10-3. Incidence of Harderian gland neoplasms in male/female B6C3F1 mice

Sex	Neoplasm (Harderian gland)	Control (0 ppm)	Low dose (10 ppm)	Mid dose (30 ppm)	High dose (90 ppm)
Male	Adenoma	3 (6.4%)	10 (21.3%)	21 (44.7%)	21 (44.7%)
Male	Adenoma, multiple	-	1 (2.1%)	4 (8.5%)	7 (14.9%)
Male	Carcinoma	-	1 (2.1%)	6 (12.8%)	14 (29.8%)
Male	Carcinoma, multiple	-	-	1 (2.1%)	2 (4.2%)
Male	Overall rate (adenoma/carcinoma)	3/47 (6.4%)	12/47 (25.5%)*	30/47 (63.8%)*	38/47 (80.9%)*
Female	Adenoma	3 (6.3%)	10 (20.8%)	8 (16.7%)	20 (41.7%)
Female	Adenoma, multiple	-	-	-	1 (2.1%)
Female	Carcinoma	-	1 (2.1%)	11 (22.9%)	7 (14.6%)
Female	Carcinoma, multiple	-	-	-	4 (8.3%)
Female	Overall rate (adenoma/carcinoma)	3/48 (6.3%)	11/48 (22.9%)*	19/48 (39.6%)*	30/48 (62.5%)*

Abbreviation: ppm, parts per million

*=Statistically significant over controls

The overall rate of neoplasms at 10 ppm is similar in both males and females at 12/47 (25.5%) and 11/48 (22.9%), respectively. There were dose-dependent increases observed in both male and female mice. At the mid (30 ppm) and high (90 ppm) drinking water concentrations, the overall rate of adenoma or carcinoma was higher in males versus females, 63.8% versus 39.6% and 80.9% versus 62.5% at 30 ppm and 90 ppm, respectively.

In addition to neoplasms in the lung and Harderian gland, a significantly increased incidence of neoplasms were also observed at a number of other sites in both male and female mice, however, these sites were not as sensitive as the lung and Harderian gland. In particular, dose-dependent increases in hepatocellular adenomas or carcinomas in both male and female mice were observed, and these increases were significant at 30 ppm in both sexes. At 30 ppm, overall rates of hepatocellular adenomas or carcinomas in male and female mice were 24/46 (52.2%) and 20/47 (42.5%), respectively. Dose-dependent increases of hemangiosarcoma of the liver were also observed in both sexes, and in the mammary gland and ovarian tumours in females, as were dose-dependent increases in squamous cell papilloma or carcinoma of the skin and fore stomach in males. However, the increases of neoplasms in these sites were only statistically significant from controls at the high dose (90 ppm). Table 10-4 presents a summary of some of the more pertinent neoplasms observed in other sites/organs in male and female mice.

Table 1-4. Incidence of other significant neoplasms in male/female B6C3F1 mice

Sex	Site	Neoplasm	Control (0 ppm)	Low dose (10 ppm)	Mid dose (30 ppm)	High dose (90 ppm)
Male	Liver	Hemangiosarcoma (overall)	1/46 (2.2%)	2/47 (4.3%)	5/46 (10.9%)	13/44* (29.5%)
Male	Liver	Hepatocellular adenoma	5 (10.9%)	10 (21.3%)	13 (28.3%)	10 (22.7%)
Male	Liver	Hepatocellular adenoma, multiple	2 (4.3%)	3 (6.4%)	4 (8.7%)	7 (15.9%)
Male	Liver	Hepatocellular carcinoma	6 (13.0%)	6 (12.8%)	8 (17.4%)	8 (18.2%)
Male	Liver	Hepatocellular carcinoma, multiple	1 (2.2%)	0 (0%)	1 (2.2%)	1 (2.3%)
Male	Liver	Overall rate Hepatocellular adenoma or carcinoma	12/46 (26.1%)	18/47 (38.3%)	24/46* (52.2%)	23/44* (52.3%)
Female	Liver	Hemangiosarcoma (Overall)	0/48 (0%)	0/47 (0%)	1/47 (2.1%)	7/47* (14.9%)
Female	Liver	Hepatocellular adenoma	3 (6.2%)	8 (17.0%)	10 (21.2%)	2 (4.3%)

Sex	Site	Neoplasm	Control (0 ppm)	Low dose (10 ppm)	Mid dose (30 ppm)	High dose (90 ppm)
Female	Liver	Hepatocellular adenoma, multiple	2 (4.2%)	2 (4.3%)	9 (19.2%)	16 (34.0%)
Female	Liver	Hepatocellular carcinoma	0 (0%)	1 (2.1%)	2 (4.2%)	1 (2.1%)
Female	Liver	Overall rate hepatocellular adenoma and carcinoma	5/48 (10.4%)	11/47 (23.4%)	20/47* (42.6%)	19/47* (40.4%)
Female	Ovary	Granulosa cell tumour benign	0 (0%)	0 (0%)	2 (4%)	3 (6%)
Female	Ovary	Granulosa cell tumour malignant	0 (0%)	0 (0%)	0 (0%)	3 (6%)
Female	Ovary	Overall rate benign or malignant granulosa cell tumour	0/48 (0%)	0/46 (0%)	2/46 (4.3%)	5/39* (12.8%)
Female	Mammary Gland	Adenoacanthoma	0 (0%)	1 (2.2%)	1 (2.2%)	11 (22.9%)
Female	Mammary Gland	Adenocarcinoma	4 (8.5%)	3 (6.5%)	3 (6.5%)	11 (22.9%)
Female	Mammary Gland	Overall rate adenoacanthoma and adenocarcinoma	4/47 (8.5%)	4/46 (8.7%)	4/46 (8.7%)	22/48* (45.8%)
Male	Skin	Squamous cell carcinoma	0 (0%)	0 (0%)	2 (4.3%)	1 (2.1%)
Male	Skin	Squamous cell papilloma	0 (0%)	1 (2.1%)	1 (2.1%)	3 (6.2%)
Male	Skin	Squamous cell papilloma, multiple	0 (0%)	0 (0%)	0 (0%)	2 (4.2%)
Male	Skin	Overall rate papilloma or carcinoma	0/47 (0%)	1/48 (2.1%)	3/47 (6.4%)	6/48* (12.5%)

Abbreviation: ppm, parts per million

*=Statistically significant over controls (only shown for overall rates)

In a study conducted by Inai et al. (1991), groups of 50 male B6C3F₁ mice were administered ethyl carbamate in drinking water for 70 weeks. There were six groups of animals, each tested with a different dose, in drinking water available *ad libitum* (0 [concurrent controls], 0.6, 3, 6, 60 or 600 ppm, equivalent to 0, 0.1, 0.58, 1.0, 10, 100 mg/kg-bw/d). At the end of the 70-week treatment period all surviving mice were autopsied. Any mouse found dead or moribund during the treatment was autopsied. At

autopsy all major organs were weighed and all organs, tissues and tumour masses were examined by microscope. Amounts of drinking water consumed per cage over 3 consecutive days were measured once a week during the treatment period. Each mouse was weighed once every two weeks until the 14th week and thereafter once every four weeks (Inai et al. 1991).

Ethyl carbamate caused dose-related increases in lung, liver and heart tumours. A statistically significant increase in alveolar/bronchiolar adenoma ($P < 0.01$) was observed starting at 60 ppm. The incidence of this tumour in lower-dose groups (8-15%) was similar to, or lower than, that of controls (18%). The incidences of alveolar/bronchiolar adenoma at 60 ppm and in the highest dose group (600 ppm) were 68% and 95.5% (all mice in this group died at the 46th week), respectively. A significant incidence of lung alveolar/bronchiolar carcinoma was only observed in the highest dose group (600 ppm). Significant increases in other neoplastic lesions were also observed at 600 ppm at sites in the liver and heart (Inai et al. 1991).

Ghanayem (2007) studied groups of 28-30 CYP2E1 +/+ (wild-type) and CYP2E1 -/- (knockout) male mice that were administered ethyl carbamate by gavage at 1, 10, 100 mg/kg-bw/d. Matching controls were given 10 ml water/kg-bw/d by gavage. Doses were administered once per day, 5 days a week, for 6 weeks. Half of each group (14-15) were killed 24 hours after the last treatment. The other half were held for seven months. A complete gross necropsy and microscopic examination were performed on all mice, including in animals that died prior to the scheduled sacrifice. At the end of the seven month recovery period, macroscopic alterations were noted in the lungs, livers, and Harderian gland of treated mice. These alterations were more prevalent in treated wild mice than in treated knockout mice. In the wild-type mice, there was a statistically significant increase in the incidence of Harderian gland adenoma (14/14 and 13/15 at 10 and 100 mg/kg-bw/d versus 1/14 in controls), bronchioalveolar adenomas (14/14 and 14/15 at 10 and 100 mg/kg-bw/d versus 0/14 in controls) and liver tumours at the highest dose (hemangiosarcoma 8/15, and hemangioma 5/15 versus 0/14 in controls) (Ghanayem 2007).

Evidence of increased incidences of tumours in mice exposed to ethyl carbamate via drinking water was also observed in studies by Tomatis et al. (1972), Schmahl et al. (1977) and Mirsalis et al. (2005); in the diet by Van Esch and Kroes (1972); and in mice exposed via gavage by Carmichael et al. (2000). A study by Toth and Boreisha (1969) also observed significant increases in a number of tumour types in Syrian hamsters receiving ethyl carbamate in drinking water for their lifetime. Only one study on the carcinogenicity of ethyl carbamate in primates was identified. This study by Thorgeirsson et al. (1994) examined the toxicity of a variety of compounds to nonhuman primates (cynomolgus, rhesus and African green monkeys) over a 30-year period. Thirty-two monkeys from a mixed colony received a dose of 250 mg/kg-bw/d administered in sterile water five days a week for five years. Throughout their lifetime, clinical chemistry examinations were performed on the monkeys every three or six months, in addition to routine physical examinations. Complete necropsies were performed on all animals at the time of death. At least five animals were treated with

only sterile water as controls. Almost 20% (6 of 32) of the treated controls had malignant tumours at their death, including: adenocarcinomas of the lung, pancreas, and bile ducts, and hemangiosarcomas of the liver. Some of the treated monkeys also received radiation treatments (only two of the six monkeys with malignant tumours had been irradiated); however, the authors concluded that ethyl carbamate is carcinogenic regardless of irradiation (Thorgeirsson et al. 1994; WHO 2006).

Inhalation

There is a limited data set for this route of administration. Shorter-term repeat dose studies indicate that ethyl carbamate can induce tumours at multiple sites via this route of administration as well.

In a study by Nomura et al. (1990), groups of female JCL:ICR mice were continuously exposed to one of two concentrations of ethyl carbamate in air, with different groups being exposed for differing time lengths, ranging from 0.25 to 10 days. Groups of female mice were either exposed to air containing 0.25 µg/ml ethyl carbamate for 1, 3, 5 or 10 days, or 1.29 µg/ml ethyl carbamate for 0.25, 1, 2, 4 or 5 days. Groups of male mice were exposed to air containing 0.25 µg/ml of ethyl carbamate for 10 days, or 1.29 µg/ml of ethyl carbamate for 4 days. Concurrent controls were exposed to air only. Female mice were killed five months after the exposure period, and male mice were killed 12 months after exposure. A statistically significant increase in lung tumours in female mice was observed at 0.25 mg/L, after continuous exposure at this dose for one day. The lung tumour type was not specified by the authors. A significantly increased incidence in lung tumours (unspecified) was observed at both dose levels and in all exposure duration groups for female mice. In males, a high incidence of lung adenocarcinoma was observed and was significant compared to control in both low and high dose groups. However, these results were questioned by the IARC Working Group, as the high incidence of adenocarcinomas was associated with a high incidence of survival (IARC 2010). Table 10-5 summarizes the incidence of lung tumours in both male and female mice at various dose and exposure durations (Nomura et al. 1990).

Table 10-5. Incidence of lung tumours in male and female mice (Nomura et al. 1990)

Sex	Neoplasm	Exposure duration (days)	Controls (0 µg/mL)	Low concentration (0.25 µg/mL)	High concentration (1.29 µg/mL)
Female	not specified	0.25	-	-	38/79 (48.1%)*
Female	not specified	1	-	27/51 (52.9%)*	37/40 (92.5%)*
Female	not specified	2	-	-	66/70 (94.3%)*
Female	not specified	3	-	44/51 (86.3%)*	-
Female	not specified	4	-	-	81/86 (94.2%)*
Female	not specified	5	-	46/53 (86.8%)*	18/18 (100%)*
Female	not specified	7	-	-	75% mortality

Sex	Neoplasm	Exposure duration (days)	Controls (0 µg/mL)	Low concentration (0.25 µg/mL)	High concentration (1.29 µg/mL)
					within 7 d after exposure ended
Female	not specified	10	2/51 (3.9%)	9/11 (81.8%)*	-
Female	not specified	Pooled controls (untreated)	8/198 (4.0%)	-	-
Male	adenocarcinoma	10	1/51 (2.0%)	40/50 (50%)*	-
Male	adenocarcinoma	4	-	-	14/40 (35%)*

*=statistically significant over controls

In addition to the lung tumours, Nomura et al. (1990) also observed an increase in the incidence of leukemia in both male and female mice in both the high and low-dose concentrations. The increases were only significant in female mice exposed for 2 days or more in the high-dose group, and five days in the low dose group. The increases were significant in both the high and low dose groups for the male mice. The incidence of uterine haemangioma in mice exposed for four days was significantly greater than that in the control group.

Dermal

Significant increases in the incidences of tumours in mice have been observed after dermal applications of ethyl carbamate. Dahl et al. (1978) studied female CD-1 mice pre-treated with croton oil, a tumour promoter. Eighteen to 24 hours later, each mouse was treated topically with 5 or 60 mg ethyl carbamate or 5 mg vinyl carbamate in 200 µL acetone, or the solvent alone. The application of the carbamate compounds or solvent was repeated one week later. One week after the second application, all mice were treated twice weekly with 900 µg croton oil in 150 µL acetone. The negative controls received the croton oil pre- and post-treatment, but were given the vehicle only with no carbamate. The experiment lasted 32 weeks, at which time ≥ 88% of the mice were still alive. All animals were subjected to gross necropsy.

Dahl et al. (1978) observed a significant increase in the incidence of lung adenomas in mice treated with a total of 10 mg of ethyl carbamate (17/40, 42%), 120 mg of ethyl carbamate (33/40, 82%) or 10 mg of vinyl carbamate (34/35, 97%) compared to controls (7/40, 17%), with an average number of adenomas per mouse of 1.0, 8.8 and 18.9, respectively, compared to 0.4 in controls. There was also a significant increase in incidence of skin papillomas observed in mice treated with ethyl carbamate and vinyl carbamate (Dahl et al. 1978).

Park et al. (1993) examined the carcinogenicity of two metabolites of ethyl carbamate, vinyl carbamate and vinyl carbamate epoxide, in groups of female CD-1 mice treated dermally in a series of experiments. The three experiments all involved a topical application of either vinyl carbamate or vinyl carbamate epoxide in 200 µl of acetone and 15% dimethyl sulfoxide (DMSO) in the treated groups. This study involved a series of different groups of 30 female CD-1 mice, 6-8 weeks of age all being treated topically with various dose regimes of vinyl carbamate and vinyl carbamate epoxide after a pre-treatment with 12-*O*-tetradecanoylphorbol-13 acetate (TPA), a tumour promoter. The experiment was terminated 22 weeks after the first application of vinyl carbamate or vinyl carbamate epoxide. The number of skin papillomas per mouse was higher in vinyl carbamate epoxide-treated mice than in controls in all cases and the number of papillomas per mouse was significantly greater in vinyl carbamate epoxide mice than in vinyl carbamate-treated animals. Forty-two weeks after the first application of vinyl carbamate and vinyl carbamate epoxide in one experiment, a number of additional malignant tumours were observed (e.g., mammary adenocarcinomas, epidermoid carcinomas, etc.). There were no malignant tumours observed in the controls in this experiment (Park et al. 1993).

Genotoxicity

Ethyl carbamate is genotoxic (mutagenic and clastogenic), especially in the presence of metabolic activation (WHO 2006; IARC 2010). The genotoxicity of ethyl carbamate as well as its metabolites (e.g., vinyl carbamate) have been well studied, both *in vivo* and *in vitro*. Ethyl carbamate generally produces positive results *in vivo* in most test systems. Conversely, assays conducted *in vitro* usually yielded negative (or highly varied) results. The likely metabolites responsible for the genotoxicity of ethyl carbamate (e.g., vinyl carbamate and its epoxides) have also been studied *in vitro* and *in vivo* and were found to be genotoxic as well and, in addition, generally eliciting a stronger positive response (Cheng and Conner 1982; Allen et al. 1986; Leithauser et al. 1990; Park et al. 1990). A number of studies and reviews of the genotoxicity database for ethyl carbamate have been conducted dating from Allen et al. (1982) and Salmon and Zeise (1991) up to the NTP (1996) and WHO (2006), to as recently as the review by IARC (2010). A brief summary of some of the assay results from a variety of *in vitro* and *in vivo* test systems, for both ethyl carbamate and its metabolites, is provided below.

in vitro

Extensive reviews of the *in vitro* genotoxicity data available for ethyl carbamate have also been conducted recently by WHO and IARC, including studies conducted from 1991 onwards (WHO 2006; IARC 2010).

The review by the WHO (2006) of the genotoxicity data available for ethyl carbamate summarized the results of over 30 studies, and was consistent with what had been observed based on the earlier review undertaken by Salmon and Zeise (1991). The results from three assays using mouse lymphoma cells were uniformly negative;

however, the results from other mammalian cell assays were not uniform. Results were variable for chromosomal aberrations, sister chromatid exchange (SCE) and unscheduled DNA synthesis assays in rats, mice, hamsters and in human cells. Similarly, the results from non-mammalian *in vitro* assays, such as bacterial mutation (e.g., Ames), and on certain yeast strains, were varied (WHO 2006).

IARC (2010) also concluded that mutagenicity and genotoxicity varied greatly among different tester strains. Ethyl carbamate was found to appear weakly mutagenic in *Salmonella typhmuri*, *Escherichia coli*, *Bacillus subtilis* and in fungi. Results on the ability of ethyl carbamate to induce point mutations in mammalian cells *in vitro* were also varied, with positive responses, often occurring at high doses. The studies on clastogenicity of ethyl carbamate in human cells *in vitro* showed that it can induce SCE in human lymphocytes and cause DNA damage in human fibroblasts. The results, however, were negative for micronucleus formation and chromosomal aberrations in human lymphocytes and germ cells. In addition, no effects on gene mutations in a human lymphoblastoid cell test system were observed (IARC 2010).

Salmon and Zeise (1991), in addition to reviewing available *in vitro* genotoxicity data for ethyl carbamate, also tabulated results for a number of ethyl carbamate metabolites. Mutagenicity results using *Salmonella* generally showed negative results, both with and without metabolic activation, with ethyl carbamate; results on ethyl carbamate metabolites, however, tended to elicit a positive result in *Salmonella* tests. For instance, N-hydroxyurethane was positive with or without metabolic activation, while vinyl carbamate was only positive with metabolic activation (Salmon and Zeise 1991).

in vivo

The WHO (2006) undertook an extensive review of the *in vivo* genotoxicity assays available, and tabulated over 70 *in vivo* assays for ethyl carbamate. Study results cited indicate that ethyl carbamate is mutagenic to *Drosophila melanogaster*. Studies in mammalian *in vivo* tests are available for a wide variety of strains of rat and mouse, with a broad range of administered doses (primarily intraperitoneal injections) in a substantial number of test systems. Results were almost uniformly positive in micronucleus tests in a variety of tissue types (e.g., peripheral blood, bone marrow) and in SCE induction and chromosomal aberration assays in both mice and rats. Negative results were obtained in a number of mammalian germ cell assays (e.g., dominant lethal assay and specific locus tests).

In vivo germ cell genotoxicity was assessed in males or females mice exposed prior to mating (with untreated animals) to ethyl carbamate via either intraperitoneal or subcutaneous injection at high doses. A significant increase in the incidence of tumours in adult offspring (mainly in the lung) has been found in a number of these studies (WHO 2006).

The results for over 60 *in vivo* assays (including *D. melanogaster*) were examined by IARC in its assessment of the genetic effects of ethyl carbamate (IARC 2010). Based on

the review of the data, ethyl carbamate was found to be clearly mutagenic *in vivo* in *D. melanogaster*, inducing sex-linked recessive mutations or reciprocal translocations in germs cells in all eight studies cited. The results of *in vivo* assays in mammalian species were generally found to be positive. Genotoxic effects were induced over a wide range of doses in mice, rats and hamsters, as well as in a variety of tissues (e.g., liver, bone marrow and lungs). The types of genotoxic effects that were induced by ethyl carbamate included chromosomal aberrations, SCE, gene mutation, DNA damage and micronucleus formation (IARC 2010).

In addition to results from *in vivo* assays for ethyl carbamate, Salmon and Zeise (1991) also tabulated results from a number of *in vivo* assays for vinyl carbamate and N-hydroxyurethane. These assays showed that, like ethyl carbamate, these substances induce chromosome aberrations, micronuclei formation and SCE in all mice, rat and hamster strains tested (Salmon and Zeise 1991).

As mentioned previously, the majority of mammalian *in vivo* assays reviewed involved ethyl carbamate being administered by intraperitoneal injection. However, there are a number of studies that were conducted using oral exposure, more relevant to the primary route of general population exposure. The results of a number of these studies are provided below.

An NTP (1996) drinking water study was conducted with five dose groups (and a control group) of 10 male and 10 female B6C3F₁ mice. The mice were exposed seven days/week for 13 weeks to ethyl carbamate administered *ad libitum* in drinking water. The animals received doses of 0, 50, 200, 750 or 2000 ppm. Results were positive in micronucleus testing. A dose-dependent response in the frequencies of micronucleated erythrocytes (normochromatic [NCEs] and polychromatic [PCEs]) observed in peripheral blood obtained from male and female mice after 45 days of exposure was observed, and the increase was significant over controls at 200 ppm, in both males (NCEs and PCEs) and females (NCEs). Similar results were observed in peripheral blood analyses conducted at 13 weeks. In bone marrow, a dose-dependent response was also observed in the frequencies of PCEs observed, with the increases at 13 weeks being significant over controls at 200 ppm in females, and 750 ppm in males (NTP 1996).

Female C57BL mice received ethyl carbamate 7 days/week *ad libitum* for 18 weeks. Dose groups, consisting of five animals per group, were administered ethyl carbamate at concentrations of 0, 5 000, 10 000 or 15 000 ppm. Water consumption and body weights were recorded weekly. After 2 weeks of exposure mice receiving 10 000 and 15 000 ppm were removed from the study and given normal drinking water due to severe dehydration and decreased body weight. Responses observed in micronucleus tests found that in the low-dose group (5 000 ppm), micronucleated erythrocytes (NCEs) in peripheral blood were significantly increased over controls at all sampling points (Director et al. 1998).

In an oral gavage study, knockout and wild-type mice were administered ethyl carbamate at doses of 0 (just vehicle), 1, 10 or 100 mg/kg bw/d for 5 days/week for 6

weeks. A dose dependent increase in micronucleated erythrocytes was significant in wild-type mice. Effects were largely observed in CYP2E1-null mice with only a slight increase at the high dose level in micronucleated polychromatic erythrocytes (Hoffler et al. 2005).

Subchronic and chronic toxicity (non-cancer effects)

The non-cancer chronic and subchronic effects of ethyl carbamate were observed in a number of species of rodents. The effects generally occurred at doses that were equivalent to, or higher than, those observed to induce neoplastic effects. In a 13-week drinking water study in rodents (NTP 1996), a variety of non-cancer toxic effects were observed. Groups of both 10 females and 10 males F344/N rats and B6C3F₁ mice received doses of 0, 110, 330, 1 100, 3 300 and 10 000 ppm ethyl carbamate in drinking water. Ethyl carbamate was found to be much more lethal in mice than rats. All mice in the two highest dose groups (3 300 and 10 000 ppm) died during the study, whereas an increased mortality rate was only observed in the in the high-dose group (10 000 ppm) in the rats. The body weights of both sexes of mice were lower in the mid-dose (1 100 ppm) group than in controls. In the lower-dose groups of both rats and mice a number of non neo-plastic lesions were observed.

In female rats (at 110 ppm and greater) and in males (at 330 ppm and greater), there was a significant decrease over controls in the number of leukocytes (leukopenia) and lymphocytes (lymphopenia). The decreases were significant and observed in all treated female groups of rats, and in all treated male groups 330 ppm and greater. Female rats administered 330 ppm or greater of ethyl carbamate also had significantly higher incidence of cardiomyopathy than controls. Lymphoid depletion of the spleen, lymph nodes, and thymus was observed in male and female rats receiving 1 100 ppm and greater. Cellular depletion of the bone marrow occurred in males and females in the 10 000 ppm groups. Hepatocellular fatty changes and clear cell foci of alteration were noted in the liver of males and females that received 3 300 or 10 000 ppm. The incidences of nephropathy were significantly increased in female rats that received 1 100 ppm or greater; the severity of this lesion in exposed males and females was greater than that in the controls.

In mice, non-cancer lesions were observed at higher doses (3 300 and 10 000 ppm) (depletion of lymphocytes in the lymph node, spleen and thymus), with the incidence of lung inflammation in males and females being significant at 1 100 ppm. Alveolar epithelial hyperplasia occurred in the lungs of males in the 330 and 1 100 ppm groups and females in the 1 100 ppm group. Nephropathy was observed in females that received 1 100 or 10 000 ppm and in males in the 10 000 ppm group that died early; the lesions in female mice were more severe than those in male mice. Cardiomyopathy occurred in males and females that received 1 100 or 3 300 ppm. A concentration of 1 100 ppm effectively stopped estrous cycling. Clinical signs of toxicity were generally limited to the two highest dose groups in rats and mice (3 300 and 10 000 ppm), including lethargy and ruffled fur. (NTP 1996).

As part of the NTP's 2-year study (NTP 2004) on B6C3F₁ mice, a separate 4-week mechanistic study was also conducted for cell proliferation and apoptosis analyses in liver and lung, as was induction of glutathione, total CYP450s and CYP450 2E1 as well as DNA adduct formation. Groups of 4 male and 4 female mice were administered 0, 10, 30, or 90 ppm ethyl carbamate in drinking water, available *ad libitum*. In the mid and high-dose groups there were effects in the liver of females, with both decreases and increases in the percentage of hepatocytes observed, depending on the phase. The extent of apoptosis was significant in males liver in the low and mid-dose group (but not in the high-dose group), and the differences were not significant in females in any dose group. The percentage of PCNA-labeling (proliferating cell nuclear antigen) was decreased in the lung of males and females exposed to 30 or 90 ppm. Total cytochrome P450 content, cytochrome P450 2E1 activity, and glutathione content in the liver of males and females were not affected. DNA adduct (Etheno-dA) concentrations in hepatic DNA (but not in lung DNA) were significantly increased at the high-dose level (male and female mice combined). Body and lung weights were not affected by exposure to ethyl carbamate (NTP 2004).

In the mice exposed to the same concentrations (0, 10, 30, or 90 ppm) of ethyl carbamate for 2 years, the incidence in the liver of angiectasis was significantly increased compared to controls at 10 ppm in males and females, with a dose-dependent increase observed. Also in the liver, an increased incidence compared to controls of eosinophilic foci occurred at 10 ppm in females (30 ppm in males) with a positive trend observed at higher doses. In females the incidence of other non-cancer lesions was also significant in the liver (necrosis and thrombosis) in the high-dose group. Additionally, statistically significant increases in the incidence of non-cancer lesions in the heart occurred; the incidence of endothelial hyperplasia was significant over controls for males in the 30 and 90 ppm groups, and for females in the 90 ppm group. The incidence of angiectasis in the heart was significant in males in the 90 ppm group. In the uterus of females, significant increases in the incidences of angiectasis and thrombosis at 30 and 90 ppm were observed. The decrease in the number of animals surviving until study termination was significant in female mice in the mid and high-dose group, and in males in the highest dose group. Mean body weights of mice exposed to increasing concentrations of ethyl carbamate showed evidence of induced reductions, especially in females (NTP 2004).

In a 6-week study with male CYP2E1 +/+ mice, doses of 0, 1, 10, 100 mg/kg-bw/d of ethyl carbamate were given to the animals by oral gavage for 5 days/week. There was a significant increase of hyperplasia in the Harderian gland of animals at all doses. In the liver, an increased incidence of hepatocellular hypertrophy was observed in the mid and high-dose groups. Eosinophilic foci and angiectasis were statistically increased in the high-dose group. In the lung, there was a significant increase in the incidence of bronchioalveolar epithelial hyperplasia in the mid and high-dose groups (Ghanayem 2007). Inai et al. (1991) observed a significant decrease in survival time in B6C3F₁ mice at the highest dose level (600 mg/L) (administered via drinking water). Similarly, Swiss mice fed 150 mg/kg bw/d ethyl carbamate in the diet, 7 days/wk for 116 weeks, had an

average lifespan (both sexes) of 40 weeks, compared with 75 weeks in the control group (Van Esch and Kroes 1972).

Reproductive and developmental effects

There are data available on the toxicity of ethyl carbamate to the reproductive systems and fertility in both rats and mice. However, the data appears to indicate that these effects are minimal or secondary effects, and occur only in the high dose groups in these studies (IARC 2010).

A 13-week study on F344/N rats and B6C3F₁ mice found some reproductive effects, generally mild to minimal, at higher dose levels. In male rats, epididymal spermatozoal motility was significantly lowered in the 1 100 ppm and 3 300 ppm groups, and in spermatozoal concentration which was significantly lowered in the 3 300 ppm group. In mice, mild to minimal degeneration of the testes was observed in males dosed 10 000 ppm. There were significant differences in spermatozoal motility in males in the 1 100 ppm dose group. Epididymal spermatozoal concentrations were also lower in treated males than in controls, with the decreases being significant in the 110 and 1 100 ppm groups. Female mice that received 1 100 ppm effectively ceased to have an estrous cycle and degenerative changes (follicle) in the ovaries were seen in the 3 300 and 10 000 ppm groups. However, the authors considered that the histopathologic changes in the testes and ovaries were secondary to the generally debilitated state of the mice, and these effects were only observed in the mice that died prematurely (NTP 1996).

Reproductive effects were also observed in the 2-year NTP study (2004) in female B6C3F₁ mice. An increased incidence and positive trend in non-cancer effects (angiectasis and thrombosis) in the uterus was observed in the 2.7 mg/kg-bw/d (30 mg/L) and 8.7 mg/kg-bw/d (90 mg/L) dose groups. The study found no significant effects on the male reproductive system (NTP 2004). Other studies examining reproductive effects in male mice were reviewed and summarized by the WHO (2006). From that summary, no sperm abnormalities were observed in one study and only slight to borderline effects were observed in sperm development and litter sizes in the other.

With respect to developmental toxicity, there is evidence (in rodents) of developmental effects in the offspring of pregnant females exposed to ethyl carbamate, as well as effects when both males and females are exposed prior to mating (IARC 2010). The treatment of ethyl carbamate prior to mating mostly when administered via subcutaneous or intraperitoneal injection, and predominantly at high-dose concentrations, was found to result in an increased incidence of tumours in adult offspring, primarily lung tumours (WHO 2006). High rates of embryonic/fetal mortality and malformations in offspring have been observed in most studies where parental animals have been exposed to ethyl carbamate, via various routes of administration. However, studies measuring these effects when ethyl carbamate is administered orally are limited, but effects observed (e.g., increased skeletal abnormalities and malformations) are consistent with those observed in studies using other routes of

exposures (e.g. intraperitoneal injection). No well-conducted multigeneration reproduction studies are available (WHO 2006).

As an example, the study of Tomatis et al. (1972) is outlined here. Groups of 60 male and 60 female CF-1 mice (P generation) were given ethyl carbamate at a dose of 0 or 100 mg/L (0.01%) in drinking water, *ad libitum*, 7 days/week for a lifetime (starting at 6-7 weeks up to 140 weeks in the P generation and 130 weeks in the F1 generation). The dose was equivalent to approximately 15 mg/kg-bw/d. Twenty-three of the P generation females were mated at 9-10 weeks of age (not stated, but assumed to be with treated males) to obtain the F1 generation (61 males and 38 females). All mice were autopsied and histological examinations were performed on the lungs, heart, thymus, liver, kidney, spleen, gonads and brain, along with any organs showing gross abnormalities (WHO 2006). The incidence of lung tumours was significantly higher in the P generation at 83% (42% controls) and 70% (23% controls) in males and females, respectively. The increased incidence of lung tumours was also significant in the F1 generation at 92% (31% controls) and 82% (40% controls) in males and females, respectively. An increased incidence and earlier onset of lung tumours in both the P and F1 generations were observed at 15 mg/kg-bw/d (the only dose tested). It is also worth noting that ethyl carbamate was chosen as a positive control in this study intended to be a multigenerational investigation of the effects of dichloro-diphenyl-trichloroethane (DDT) (Tomatis et al. 1972). Details on several other studies can be found in IARC (2010).

Intravenous injections into female hamsters on gestation day 8 at 200 mg/kg bw led to abnormalities in 33% of the fetuses examined 1-3 days later (Ferm and Hanover 1966). Higher doses of 400, 800 or 1200 mg/kg bw given intraperitoneal or intravenously produced fetotoxicity, as well as fetal abnormalities. The malformations reported were exencephaly, *spina bifida*, convoluted cardiac tubes, non-closing of neural folds and marked degeneration of the anterior neural tube. Nomura (1988) observed a dose-dependent increase after a single 1 000, 1 500 or 2 000 mg/kg-bw subcutaneous injection of ethyl carbamate administered to female mice (at different intervals before conception or during gestation) in the incidence of malformations such as cleft palate, open eyelid and dwarfism in offspring from subsequent matings. Details on several other studies can be found in WHO (2006) and IARC (2010).

The effects on offspring after paternal exposures to ethyl carbamate have also been relatively well studied. Male mice administered a single subcutaneous injection of 1 500 mg/kg bw ethyl carbamate were mated with untreated female mice. The authors stated that the rate of malformation (e.g., open eyelids, cleft palates) in the offspring was significantly increased (Nomura 1975). Nomura (1988) found similar results again with a single subcutaneous injection (1 000, 1 500 or 2 000 mg/kg bw) in male mice leading to a dose-dependent increase in the incidence of malformations. No effects were observed by Edwards et al. (1999) in the offspring of male CD-1 mice exposed either acutely by intraperitoneal injection of 1 250 and 1 750 mg/kg bw, or subchronically in the drinking water at 190 mg/kg bw for 10 weeks and 370 mg/kg bw for 9 weeks.

Nomura (1988) showed that some ethyl carbamate-induced anomalies were heritable, by mating F1 offspring from treated parents with untreated mice to produce further generations. The total incidence of these anomalies in F3 generation was 9.9% (14 tail anomalies, 6 dwarfs, and 7 open eyelids in 274 F3 progeny). Among viable anomalies in the F3 generation, 1 of 4 tail anomalies tested was inherited with low expressivity (7%), and 3 of 6 open eyelids tested were inherited with 11, 5, and 50% expressivity, when these malformed F3 mice were crossed with normal ICR mice.

10.3 Characterization of Risk to Human Health

Based on the weight of evidence-based classifications from IARC (2010) and the NTP (2011), and on the available health effects data, the critical effect for characterization of risk to human health associated with exposure to ethyl carbamate is carcinogenicity. Evidence shows that the metabolic pathways for activation of ethyl carbamate observed in rodents are similar to those in humans, and that carcinogenicity observed in laboratory animals is relevant to humans (IARC 2010).

Ethyl carbamate induces tumours in multiple animal species (including rats, mice, hamsters, and primates) in both newborns and adults, by oral, dermal, inhalation, subcutaneous or intraperitoneal routes of exposure. In animal studies, it was shown that ethyl carbamate is evenly distributed in the body upon exposure and, as a result, the organs affected are not necessarily limited to the ones to which the substance is applied or administered. Ethyl carbamate was also found to be genotoxic *in vivo* in a wide range of mammalian assays. Therefore, although the mode of action has not been fully elucidated, there is strong evidence that ethyl carbamate is genotoxic and a multisite carcinogen, and that the induction of tumours can occur via any route of exposure.

The 2 year mouse bioassay study conducted by the NTP (2004) in which ethyl carbamate was administered to mice through drinking water was considered appropriate for risk characterization. Benchmark dose (BMD) modelling was applied to derive a point of departure (POD) for critical neoplastic effects from oral exposure, based on the chronic-lifetime oral toxicity study conducted by the NTP (2004). The dose-response curve was used to derive a lower one-sided 95% confidence limit for the benchmark dose (BMDL) predicted a 10% incidence of tumours (BMDL₁₀). The model selection criteria and results are provided in Appendix C.

The BMDL₁₀ levels were calculated for each tumour dataset from the nine models available in the US EPA Benchmark Dose Software (BMDS v.24), and a model was selected on the basis of fit. A dose-response analysis of each tumour site by BMDS shows that the lung (alveolar/bronchiolar) is the most sensitive organ. It was concluded that, on the basis of the incidence of lung tumours at all dose levels observed in both sexes, as well as the absence of a significant difference in the dose-response between sexes, that the tumourigenicity of ethyl carbamate in the lungs of B6C3F₁ is not gender specific. As a consequence, the BMD model results derived using the dataset of both males and females combined were selected. Based on the results of the BMD analysis,

a BMDL₁₀ of 0.3 mg/kg bw/d was selected as the point of departure for risk characterization.

The WHO (JECFA⁴) also undertook BMD modelling of ethyl carbamate in its assessment (WHO 2006) and selected a BMDL₁₀ of 0.3 mg/kg-bw/d for lung tumours in male and female mice combined. The lack of significant difference in dose-response between the sexes was also noted by the WHO, leading them to combine data from both males and females to derive a BMDL₁₀.

Margins of exposure resulting from a comparison of the critical neoplastic effect level for oral exposure (BMDL₁₀ for incidence of lung tumours estimated at 0.3 mg/kg-bw per day) with estimates of daily intake of ethyl carbamate for all groups are presented in Table 10-6.

Table 10-6. Margins of exposure (MOE) between dietary intake of ethyl carbamate and the BMDL₁₀ of 0.3 mg/kg-bw/d for lung tumours in mice

Age category, years	Sex	MOE ^a
<1	Male and female	22 900 ^b
1<14	Male and female	12 500
14<19	Male	25 200
14<19	Female	24 000
≥19	Male (all except alcohol)	14 800
≥19	Male (alcohol only)	2 800
≥19	Female (all except alcohol)	15 000
≥19	Female (alcohol only)	5 100

Abbreviations: BMDL₁₀, lower one-sided 95% confidence limit for the benchmark dose, which predicted a 10% incidence of tumours; bw/d, body weight per day; MOE, margin of exposure.

^aBased on a comparison of the critical effect level for oral exposure (0.3 mg/kg bw/d) with the 90th percentile of ethyl carbamate daily intakes (shown in Table 10-1).

^bBased on a comparison of the critical effect level for oral exposure (0.3 mg/kg bw/d) with a deterministic ethyl carbamate intake of 13.1 ng/kg-bw/d.

The margins of exposure between critical effects and the estimate of daily intake of ethyl carbamate from food (excluding alcohol) ranged from 12 500 to 25 200 for all age groups. These margins are considered adequate to account for uncertainties in the exposure and health effects databases. However, the margins of exposure between the BMDL₁₀ and daily intake of ethyl carbamate based on alcohol consumption alone are 2800 and 5100, for males and females aged 19 years and older, respectively. These margins of exposure are considered potentially inadequate to account for uncertainties in the databases.

⁴ The Joint FAO/WHO Expert Committee on Food Additives (JECFA) is an international expert scientific committee that is administered jointly by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO).

10.4 Uncertainties in Evaluation of Risk to Human Health

The confidence in the health effects database is considered to be high. This Screening Assessment does not include a full analysis of the mode of induction of effects, including cancer, associated with exposure to ethyl carbamate, nor does it take into account possible differences between humans and experimental species with respect to effects induced by this substance.

There is evidence in animal studies that ethyl carbamate is associated with developmental effects when administered during gestation. However, as noted by WHO (2006) and IARC (2010), the majority of the studies involved high single doses, and routes of exposure not directly applicable to anticipated exposure pathways for the general population. Although there is an uncertainty in identifying the lowest dose associated with developmental effects, doses tested are several orders of magnitude higher than the BMD for lung tumours in mice, and the most sensitive end-point for oral exposure to ethyl carbamate is likely carcinogenicity.

Confidence is moderate to high that the derived dietary exposure estimates are representative of the general population of Canada, as robust Canadian data were available for ethyl carbamate in alcoholic beverages (the predicted primary source of ethyl carbamate exposure to Canadians 19 years of age and older). In addition, all scenarios were based on two 24-hour recall surveys of consistent eaters (i.e., people who consumed foods or beverages containing ethyl carbamate) which is considered to be a conservative estimate of exposure to ethyl carbamate.

11. Conclusions

Considering all available lines of evidence presented in this Screening Assessment, there is low risk of harm to organisms and the broader integrity of the environment from ethyl carbamate. It is concluded that ethyl carbamate does not meet the criteria under paragraphs 64(a) or (b) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that has or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

Based on the information presented in this Screening Assessment, it is concluded that ethyl carbamate meets the criteria under paragraph 64(c) of CEPA as it is entering or may enter the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

It is concluded that ethyl carbamate meets one or more of the criteria set out in section 64 of CEPA.

Ethyl carbamate does not meet the criteria for persistence or bioaccumulation as set out in the *Persistence and Bioaccumulation Regulations* of CEPA.

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Appendix A. Estimated daily intake of ethyl carbamate in food and beverages

Appendix A1. Summary statistics of ethyl carbamate levels in alcoholic beverages that were used to estimate dietary intake of ethyl carbamate

Alcoholic Beverages	Mean (ng/g)	Median (ng/g)	P90 ^a (ng/g)	Range ^b (ng/g)
Beer ^c	5.1	5.0	6.0	4.0–6.4
Brandy	66.0	28.0	150	3.3–241
Liqueur	24.8	8.1	55.0	0.7–94.6
Wine ^d	7.1	6.0	12.6	2.3–15.6
Fortified wine	40.8	27.2	86.3	6.2–120
Distilled spirits ^e	39.8	28.2	81.8	7.2–111

^a 90th percentile of samples.

^b Reported as 5th–95th percentile.

^c Beer includes light, regular, strong and non-alcoholic varieties. Statistics representing only regular beer shown. Regular beer was considered representative of all varieties except non-alcoholic.

^d Wine includes red, white and rosé varieties. Statistics representing only red wine shown. Red wine was considered representative of all wine varieties.

^e Distilled spirits includes gin, rum, tequila, vodka and whisky (bourbon, Irish, rye and scotch) varieties. Statistics representing only scotch whisky shown. Distilled spirits had the greatest amount of variation in ethyl carbamate levels between varieties. Scotch whisky was considered representative of all distilled spirit varieties. Sources: Cañas et al. 1988; Groux et al. 1994; CFIA (unpublished survey data); LCBO (unpublished survey data); SAQ (unpublished survey data).

Appendix A2. Summary statistics of ethyl carbamate levels in non-alcoholic beverages that were used to estimate dietary intake of ethyl carbamate

Non-alcoholic Beverages	Mean (ng/g)	Median (ng/g)	P90 ^a (ng/g)	Range ^b (ng/g)
Fruit and vegetable juices	<0.1	<0.1	0.1	<0.1–0.1
Fruit juice drinks	<0.1	<0.1	0.1	<0.1–0.1
Non-alcoholic cider	3.1	2.3	6.1	0.6–8.2
Tea	2.3	0.1	2.4	<0.1–5.9

^a 90th percentile of samples.

^b Reported as 5th–95th percentile.

Sources : Cañas et al. 1988; Groux et al. 1994; Wang and Yen 1998; FSA NZ 2007; Tang et al. 2011

Appendix A3. Summary statistics of ethyl carbamate levels in dairy products that were used to estimate dietary intake of ethyl carbamate

Dairy products	Mean (ng/g)	Median (ng/g)	P90 ^a (ng/g)	Range ^b (ng/g)
Cheese	0.4	0.1	0.9	<0.1–1.7
Milk (other acidified)	0.4	0.2	0.9	<0.1–1.4
Yoghurt	0.4	0.1	0.9	<0.1–1.7

^a 90th percentile of samples.

^b Reported as 5th–95th percentile.

Sources: Ough 1976; Zimmerli et al. 1986; Cañas et al. 1988; Dennis et al. 1989; Hasegawa et al. 1990; Diachenko et al. 1992; Sen et al. 1993; Vahl 1993; Wang and Yen 1998; FSA UK 2005; EFSA 2007; FSA NZ 2007; Hasnip et al. 2007; Tang et al. 2011.

Appendix A4. Summary statistics of ethyl carbamate levels in breads and crackers that were used to estimate dietary intake of ethyl carbamate

Breads and crackers	Mean (ng/g)	Median (ng/g)	P90 ^a (ng/g)	Range ^b (ng/g)
Bread (white)	3.3	2.4	6.8	0.6–9.1
Bread (white, toasted)	6.0	4.5	12.0	1.3–15.8
Bread (whole grain)	3.1	2.7	5.4	1.1–6.7
Bread (whole grain, toasted)	6.3	5.4	11.3	2.1–13.9
Bread (rye, dark)	2.1	1.6	4.0	0.5–5.2
Bread (rye, dark, toasted)	3.8	3.2	6.7	1.2–8.2
Bread (rye, light)	4.8	4.7	6.1	3.4–6.6
Bread (rye, light, toasted)	9.0	8.8	11.1	6.5–11.9
Bread (rye, whole meal)	4.7	4.0	8.0	1.7–9.6
Bread (rye, whole meal, toasted)	8.7	7.6	14.7	3.2–17.8
Bread (other)	2.9	0.3	4.9	<0.1–10.2
Crackers and crispbreads	1.1	0.3	2.4	<0.1–4.2

^a 90th percentile of samples.

^b Reported as 5th–95th percentile.

Sources: Cañas et al. 1988; Sen et al. 1993; Vahl 1993; Haddon et al. 1994; Dennis et al. 1997a,b; Hamlet et al. 2005; Tang et al. 2011.

Appendix A5. Summary statistics of ethyl carbamate levels in other fermented foods that were used to estimate dietary intake of ethyl carbamate

Other fermented foods	Mean (ng/g)	Median (ng/g)	P90^a (ng/g)	Range^b (ng/g)
Bean curd products	82.0	1.6	59.5	<0.2–163
Pickled, preserved or dried vegetables	2.7	1.5	6.1	<0.2–9.2

^a 90th percentile of samples.

^b Reported as 5th–95th percentile.

Sources: Zimmerli et al. 1986; Diachenko et al. 1992; Wang and Yen 1998; Kim et al. 2000; FSA UK 2005; FSA NZ 2007; Hasnip et al. 2007; Park et al. 2009; Tang et al. 2011.

Appendix A6. Summary statistics of ethyl carbamate levels in sauces and condiments that were used to estimate dietary intake of ethyl carbamate

Sauces and condiments	Mean (ng/g)	Median (ng/g)	P90^a (ng/g)	Range^b (ng/g)
Soy sauces	17.0	3.3	33.2	0.3–64.6
Vinegar	23.4	3.9	43.9	0.2–86.7
Vinegar-based sauces	1.0	0.4	2.2	0.1–3.6
Other sauces and condiments	1.0	0.4	2.3	0.1–3.6

^a 90th percentile of samples.

^b Reported as 5th–95th percentile.

Sources: Zimmerli et al. 1986; Cañas et al. 1988; Hartman and Rosen 1988; Diachenko et al. 1992; Fauhl et al. 1993; Matsudo et al. 1993; Sen et al. 1993; Wang and Yen 1998; Kim et al. 2000; FSA UK 2005; FSA NZ 2007; Koh and Kwon 2007; Park et al. 2007, 2009; Tang et al. 2011.

Appendix B. Body weight distribution statistics

Appendix B1. Body weight distribution statistics

Age category, years	Sex	Mean (kg)	Range (kg) ^a
1<4	Male and female	15.1	13.5–16.6
4<9	Male and female	24.0	19.4–27.3
9<14	Male	45.7	35.0–53.7
9<14	Female	44.5	35.0–51.8
14<19	Male	70.3	59.7–78.0
14<19	Female	60.7	52.2–65.7
19<31	Male	80.1	69.7–87.8
19<31	Female	67.3	55.5–74.3
31<51	Male	84.1	73.1–93.2
31<51	Male	84.1	73.1–93.2
51<71	Male	85.0	73.9–93.6
51<71	Female	70.6	60.0–77.1
≥71	Male	79.0	69.9–87.2
≥71	Female	66.3	57.0–74.2

^a Reported as the 25th–75th percentile.

Sources: Body weight distribution statistics for infants younger than two years from Portier et al. (2007). All other body weight distribution statistics from Statistics Canada (2008).

Appendix C. Benchmark Dose (BMD) Modelling and identification of a point of departure for ethyl carbamate risk characterization

General Methodology

The US EPA Benchmark Dose Software (BMDS2.4) was used to calculate the benchmark dose (BMD) and the corresponding lower limit of a one-sided 95 % confidence interval (BMDL) (<http://www.epa.gov/ncea/bmds/about.html>) for characterization of the cancer risk associated with chronic exposure to ethyl carbamate. The BMD approach, which includes dose-response modeling, provides a quantitative alternative to the dose-response assessment which first defines the point of departure (POD), and then extrapolates the POD for relevance to human exposure. A dichotomous restricted model type is chosen for the BMD and BMDL analysis. A benchmark response of 10% extra risk above predicted background response for dichotomous data is chosen because 10% is at or near the limit of sensitivity in most cancer bioassays. In animal cancer studies, BMD₁₀ refers to a dose of a substance that produces 10% increase in the response rate of tumour relative to the background response rate of this tumour. BMDL₁₀ refers to a lower one-sided 95% confidence limit on the corresponding benchmark dose (BMD₁₀). BMD₁₀ and BMDL₁₀ levels are calculated for each tumour dataset from the nine models and a model is selected on the basis of best fit (see details in model section). Then, the lowest BMDL₁₀/BMD₁₀ from various tumour types is chosen as a reasonable conservative estimate for subsequent risk characterization. For derivation of a BMD and BMDL for ethyl carbamate, nine models were applied for analysis of each tumour type (described in table D1 below) reported in the 2004 NTP study (NTP, 2004). These models included Gamma, Logistic, LogLogistic, LogProbit, Multistage, Multistage-Cancer, Probit, Weibull and Quantal-Linear (see Table C2)

Table C1. Incidences of neoplasms in B6C3F1 mice exposed to ethyl carbamate in drinking water for 2 years (NTP 2004)

Drinking water (ppm)	0	10	30	90
Male B6C3F1 mice				
Calculated equivalent dose for male mice (mg/kg-bw/d)	0	1.2	3.3	10.1
Alveolar/bronchiolar adenoma or carcinoma (combined)	5/48	18/48	29/47	37/48
Harderian gland adenoma or carcinoma (combined)	3/47	12/47	30/47	38/47
Liver hemangiosarcoma	1/46	2/47	5/46	13/44
Hepatocellular adenoma or carcinoma (combined)	12/46	18/47	24/46	23/44

Forestomach squamous cell carcinoma or papilloma (combined)	0/46	2/47	3/44	5/45
Heart hemangiosarcoma	0/48	0/48	1/47	5/48
Skin papilloma or carcinoma (combined)	0/47	1/48	5/47	8/48
Female B6C3F1 mice				
Calculated equivalent dose for female mice (mg/kg-bw/d)	0	0.9	2.8	8.2
Alveolar/bronchiolar adenoma or carcinoma (combined)	6/48	8/48	28/48	39/47
Harderian gland adenoma or carcinoma (combined)	3/48	11/48	19/48	30/48
Liver hemangiosarcoma	0/48	0/47	1/47	7/47
Hepatocellular adenoma or carcinoma (combined)	5/48	11/47	20/47	19/47*
Mammary gland adenoacanthoma or adenocarcinoma (combined)	4/47	4/46	4/46	22/48
Ovary benign or malignant granulosa cell tumours (combined)	0/48	0/46	2/46	5/39

Abbreviation: bw/d, body weight per day

Abbreviation: ppm, parts per million

*The highest dose is omitted from modelling to improve model fit.

Model Selection

The best-fit model is selected from nine models for each tumour type generally based on the highest P-value of goodness of fit; and the lowest Akaike's Information Criterion (AIC) value (a measure of information loss from a dose-response model that can be used to compare a set of models). A fit was judged adequate based on the goodness-of-fit P-value, scaled residual closest to the BMR (10% extra risk) and visual inspection of the model fit. A goodness-of-fit P-value > 0.1 and an absolute value of scaled residual of interest (SRI); represents observed minus predicted response divided by standard errors) <2, is considered to be indicative of an acceptable fit. If the models for a given tumour type were not accepted (e.g., P-values < 0.1), then the results from the high - dose group were omitted and re-modelled.

The results for BMD10 and BMDL10 estimation (mg/kg-bw/d) for tumours induced by ethyl carbamate in the 2004 NTP study are shown in tables C2, C3 and C4.

Table C2. BMD₁₀ and BMDL₁₀ calculations (mg/kg-bw/day) for tumours induced by ethyl carbamate

Tumours	Model name	Number of groups	AIC	P-value	SRI	BMD ₁₀	BMDL ₁₀
Alveolar/bronchiolar adenoma or carcinoma (combined)	LogLogistic	7	409.39	0.213	-1.647	0.505	0.276
Alveolar/bronchiolar adenoma or carcinoma (combined)	LogProbit	7	411.725	0.039	-0.834	0.909436	0.75351
Alveolar/bronchiolar adenoma or carcinoma (combined)	Multistage	7	412.056	0.0486	-1.491	0.552728	0.46215
Alveolar/bronchiolar adenoma or carcinoma (combined)	Probit	7	429.556	0	1.259	1.24823	1.09661
Alveolar/bronchiolar adenoma or carcinoma (combined)	Weibull	7	412.056	0.0486	-1.491	0.552728	0.46215
Alveolar/bronchiolar adenoma or carcinoma (combined)	Quantal-Linear	7	412.056	0.0486	-1.491	0.552728	0.46215
Alveolar/bronchiolar adenoma or carcinoma (combined)	Gamma	7	0.0486	-1.491	0.1	0.462153	0.0486
Alveolar/bronchiolar adenoma or carcinoma (combined)	Logistic	7	0	1.236	0.1	1.07423	0

Abbreviations: AIC, Akaike's Information Criterion; BMR, basal metabolic rate; bw/d, body weight per day; SRI, scaled residual of interest

Table C3. BMD₁₀ and BMDL₁₀ calculations (mg/kg-bw/day) for tumours in male B6C3F1 mice induced by ethyl carbamate

Tumours	Model Name	Number of groups	AIC	P-value	SRI	BMD ₁₀	BMDL ₁₀
Alveolar/bronchiolar adenoma or carcinoma (combined)	LogLogistic	4	214.3 2	0.773	- 0.06	0.317	0.220
Harderian gland adenoma or carcinoma (combined)	LogLogistic	4	190.7 2	0.209	0.09	0.501	0.237
Liver hemangiosarcoma	Quantal-Linear	4	115.4 3	0.903	- 0.11	3.375	2.273
Hepatocellular adenoma or carcinoma (combined)	LogLogistic	4	246.7 5	0.246	0.31	1.702	0.809
Forestomach squamous cell carcinoma or papilloma (combined)	LogLogistic	4	75.42	0.440	0.63	6.788	3.847
Heart hemangiosarcoma	LogProbit	4	44.29	0.892	0.30	9.171	6.614
Skin papilloma or carcinoma (combined)	LogLogistic	4	87.85	0.777	0.90	4.692	2.986

Abbreviations: AIC, Akaike's Information Criterion; BMR, basal metabolic rate; bw/d, body weight per day; SRI, scaled residual of interest

Table C4. BMD₁₀ and BMDL₁₀ calculations (mg/kg-bw/day) for tumours in female B6C3F1 mice induced by ethyl carbamate

Tumours	Model Name	Number of groups	AIC	P-value	SRI	BMD₁₀	BMDL₁₀
Alveolar/bronchiolar adenoma or carcinoma (combined)	LogProbit	4	195.09	0.213	-0.71	0.859	0.669
Harderian gland adenoma or carcinoma (combined)	LogLogistic	4	206.29	0.895	0.38	0.570	0.395
Liver hemangiosarcoma	LogProbit	4	51.33	0.996	-0.07	6.350	4.650
Hepatocellular adenoma or carcinoma (combined)	Multistage	3	151.36	0.885	0.12	0.656	0.434
Mammary gland adenoacanthoma or adenocarcinoma (combined)	Multistage	4	152.05	0.943	-0.29	4.801	2.54
Ovary benign or malignant granulosa cell tumours (combined)	Quantal-Linear	4	49.63	0.876	0.24	7.018	3.994

Abbreviations: AIC, Akaike's Information Criterion; BMR, basal metabolic rate; bw/d, body weight per day; SRI, scaled residual of interest