ETHYL CARBAMATE

There appears to be no general consensus on a common trivial name for this substance: ethyl carbamate and urethane (or urethan) are both commonly used; however, a preference for ethyl carbamate was noted in the more recent literature. The name urethane is also sometimes applied to high-molecular-weight polyurethanes used as foams, elastomers and coatings. Such products are not made from and do not generate the chemical ethyl carbamate on decomposition. Due to this possible confusion, the term ethyl carbamate has been used in this monograph.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Synonyms*

CAS Registry No.*: 51–79–6*

Synonyms: Carbamic acid ethyl ester; ethylurethan; ethyl urethan; ethyl urethane; urethan; urethane

1.1.2 *Chemical formula and relative molecular mass*

$NH_2COOC_2H_5$ Relative molecular mass: 89.1

1.1.3 *Chemical and physical properties of the pure substance*

From Budavari (2000)

(a) Description: Colourless, almost odourless, columnar crystals or white granular powder; the pH of an aqueous solution is neutral

(b) Boiling-point: *182–184 °C*

(c) Melting-point: *48–50 °C*

(d) Solubility: Dissolves in water (1 g/0.5 mL), ethanol (1 g/0.8 mL), chloroform (1 g/0.9 mL), ether (1 g/1.5 mL), glycerol (1 g/2.5 mL) and olive oil (1 g/32 mL)

(e) Volatility: Sublimes readily at 103°C at 54 mm Hg; volatile at room temperature

1.1.4 *Technical products and impurities*

Trade names for ethyl carbamate include Leucothane, Leucethane and Pracarbamine.

The Chemical Catalogs Online database, produced by Chemical Abstracts Services, lists 37 suppliers for ethyl carbamate, which are predominantly situated in Europe, Japan and the USA. Technical grades with 98% purity as well as products with more than 99% purity (less than 0.1% ignitable residues) are available.

1.1.5 *Analysis*

The titration method described by Archer *et al*. (1948) was used to monitor patients who underwent therapy with ethyl carbamate. A gas chromatography–mass spectrometry (GC–MS) method to monitor ethyl carbamate in blood was developed by Hurst *et al*. (1990) to monitor the time course of elimination of ethyl carbamate in mice.

The methods developed to determine ethyl carbamate in various food matrices are summarized in Table 1.1; the analytical methodology was reviewed by Zimmerli and Schlatter (1991). GC coupled with MS seems to be the method of choice for this purpose. The overwhelming majority of methods involve quadrupole MS operating in selected-ion monitoring mode and the use of isotopically labelled internal standards. Validation data of collaborative studies are available (Dennis *et al*., 1990; Canas *et al*., 1994; Dyer, 1994; Hesford & Schneider, 2001; de Melo Abreu *et al*., 2005). In general, the validation results were judged to be satisfactory for the purpose of analysing ethyl carbamate in the lower microgram per kilogram range. The methods presented by Dyer (1994) and Canas *et al*. (1994) were adopted by the Association of Official Analytical Chemists International as part of their Official Methods. A collaborative analysis also led to the adoption of a method for the determination of ethyl carbamate in the European Community methods for the analysis of wine (European Commission, 1999).

The analysis of minor organic compounds in complex matrices, such as in spirit beverages, is difficult because of interferences by matrix components, even when extensive clean-up procedures are applied to the sample, e.g. extraction over diatomaceous earth columns, which is proposed by many authors. A possible approach to eliminate these interferences is the use of solid-phase extraction in combination with an improved chromatographic separation using multidimensional GC, as proposed by Jagerdeo *et al*. (2002) for the analysis of wine. However, this technique requires the time-consuming removal of ethanol before solid-phase extraction and specialized equipment consisting of GC with a flame-ionization detector and GC–MS, which are coupled using a cryo trap. As another approach, MS detection may be enhanced by application of tandem MS (MS–MS) to provide an improved sensitivity and specificity. Recently, it was demonstrated that low-cost bench-top triple quadruple mass spectrometers can be used in the routine analysis of ethyl carbamate in spirits (Lachenmeier *et al*., 2005a) or in bread (Hamlet *et al*., 2005).

Table 1.1 Methods for the analysis of ethyl carbamate in different matrices

Sample matrix	Internal standard	Extraction principle	Clean-up	Detection	Column	LOD (µg/L)	Reference
Alcoholic beverages	–	Dilution to 10% vol, dichloromethane extraction	–	GC–ECD	DBWAX-30W	Low µg/kg range	Bailey *et al.* (1986)
	Methyl carbamate	Dichloromethane extraction	Extrelut	GC-NPD	Durabond-Wax	20	Baumann & Zimmerli (1986a)
	–	Dilution to 5% alcohol	Chemtube or Extrelut	GC (1) TEA (2) ECD (3) MS	CP Wax 52 CB	 (1) 1 (2) 2–5 (3) 1	Dennis *et al.* (1986, 1988)
	1,4-Butanediol or *N*,*N*-dimethylformamide	Salting-out with potassium carbonate	–	GC-MS EI or PCI	Carbowax 20M	EI: 100 PCI: 10	Bebiolka & Dunkel (1987)
	–	Dichloromethane extraction	–	GC–ECD, GC–MS	DBWAX	ECD: 5–10 MS: 0.5	Conacher *et al.* (1987)
	–	Dichloromethane extraction	–	GC–MS	DBWAX	0.5	Lau *et al.* (1987)
	n-Butyl carbamate	Dichloromethane extraction	Extrelut	GC–MS	WCOT, DBWAX	10	Mildau *et al.* (1987)
	–	Dilution to 10% vol, dichloromethane extraction	–	Two-dimensional GC–FID	(1) CP-SIL 5 CB (2) CP-WAX 52	1	van Ingen *et al.* (1987)
	[^{13}C,^{15}N]-Ethyl carbamate	Dichloromethane extraction	Deactivated alumina	GC–TEA	DB-Wax	1.5	Canas *et al.* (1988)
	–	Dichloromethane extraction	–	GC–ion trap	Supelcowax 10	5	Clegg & Frank (1988)

Table 1.1 (continued)

Sample matrix	Internal standard	Extraction principle	Clean-up	Detection	Column	LOD (µg/L)	Reference
	Ethyl carbamate-d_5	Distillation, dichloromethane extraction	–	GC–MS	SGE BP 20	2-5	Funch & Lisbjerg (1988)
	tert-Butyl carbamate and *n*-butyl carbamate (GC–FID), [^{13}C,^{15}N]-ethyl carbamate	Dilution to 25% vol, dichloromethane extraction	Alumina clean-up	GC–FID GC–MS	DB-WAX Carbopack B/ Carbowax 20M	10-25 5	Pierce *et al*. (1988)
	Isopropyl carbamate	Dichloromethane extraction	–	Two-dimensional GC–TSD	BP-20, OV-1	1	Ma *et al*. (1995)
	–	Dilution to 20% vol	Derivatization with 9-xanthydrol	HPLC–fluorescence detection	HP AminoQuant	4.2	Herbert *et al*. (2002)
	Ethyl carbamate-d_5	Removal of ethanol	SPE (styrene–divinylbenzene copolymer)	GC–MS	HP-INNOWAX	3	Mirzoian & Mabud (2006)
Distilled spirits	Propyl carbamate	Evaporation with nitrogen	–	GC–MS	DB-Wax	10	Farah Nagato *et al*. (2000)
Grappa	Ethyl carbamate	Dichloromethane–ethyl acetate extraction	Derivatization with xanthydrol	GC–MS	DB 5	1	Giachetti *et al*. (1991)
Must and wine	–	–	–	FTNIR-screening	–	–	Manley *et al*. (2001)
Rice wine	Propyl carbamate	Chloroform extraction	Florisil	GC–MS	DB-Wax	–	Woo *et al*. (2001)
Spirits and mashes	–	Distillation	Chem-Elut 1020	GC–FID	(1) DB-Wax (2) DB-225	5	Wasserfallen & Georges (1987)

Table 1.1 (continued)

Sample matrix	Internal standard	Extraction principle	Clean-up	Detection	Column	LOD (µg/L)	Reference
Spirits	Pyrazole	Salting-out	–	GC–NPD	BC-CW 20 M	10	Adam & Postel (1987)
	n-Octanol	Ethyl acetate extraction	–	GC–FID	CP Wax 57 CB	10-20	Andrey (1987)
	tert-Butyl carbamate	Extraction with *n*-hexane–ethyl acetate mixture	Extrelut	GC–FID, GC–N-TSD	Stabilwax	50	Drexler & Schmid (1989)
	Propyl carbamate	–	–	GC–MS	FSOT	5	MacNamara *et al*. (1989)
	–	Salting-out	Filtration over activated carbon	GC–NPD, GC–FID	HP 19091 F-115 or Carbowax 20M	LOQ:1-5	Adam & Postel (1990)
	Ethyl carbamate-d_5	Dichloromethane extraction	Extrelut	GC–MS/MS	CP-wax	10	Lachenmeier *et al*. (2005a)
	–	–	–	FTIR screening	–	–	Lachenmeier (2005)
	Ethyl carbamate-d_5	Dilution 1:10	HS-SPME	GC–MS/MS	Stabilwax	30	Lachenmeier *et al*. (2006)
Whisky, sherry, port, wine	[^{13}C,^{15}N]-Ethyl carbamate	Dichloromethane extraction	–	GC–MS/MS CI.	Carbowax SP-10	1	Brumley *et al*. (1988)
Wines and spirits	[^{13}C,^{15}N]-Ethyl carbamate	Dichloromethane extraction	Florisil	GC–ECD, GC–MS/MS	Carbowax 20M Stabilwax		Cairns *et al*. (1987)
Wine	–	Chloroform extraction	Florisil	GC–ECD	GCQ, OV-17, Carbowax 1540	<100	Walker *et al*. (1974)

Table 1.1 (continued)

Sample matrix	Internal standard	Extraction principle	Clean-up	Detection	Column	LOD (µg/L)	Reference
	Propyl carbamate	Extraction with Soxhlet apparatus	–	GC–MS	DB-Wax	–	Fauhl & Wittkowski (1992)
	–	Dichloromethane extraction	Chem-Elut or Extrelut	GC–N-TEA	DB-Wax	1-2	Sen *et al*. (1992)
	Propyl carbamate	Dilution, dichloromethane extraction	Diatomaceous earth columns	GC–MS	Carbowax 20M	–	European Commission (1999)
	[^{13}C,^{15}N]-Ethyl carbamate	Removal of ethanol, dilution	SPE (styrene-divinylbenzene copolymer)	Two-dimensional GC–MS	HP-5MS DB-WAX	0.1	Jagerdeo *et al*. (2002)
	Propyl carbamate	–	MS–SPME	GC–MS	DB-Wax	9.6	Whiton & Zoecklein (2002)
Alcoholic beverages and foods	[^{13}C,^{15}N]-Ethyl carbamate	Dichloromethane extraction	–	GC–MI/FTIR	DBWAX-30W	10	Mossoba *et al*. (1988)
Alcoholic beverages, fermented foods	*n*-Butyl carbamate	Pre-extraction with petroleum ether, dichloromethane extraction	Deactivated alumina	GC–FID	DB-Wax	6,7	Wang *et al*. (1997); Wang & Gow (1998)
Bread	Ethyl carbamate-d_5	Dichloromethane extraction	Extrelut	GC–MS/MS	EC-WAX	0.6	Hamlet *et al*. (2005)
Fermented foods	–	Dichloromethane extraction	Acid–celite column	GC–MS	CBP-20	0.5	Hasegawa *et al*. (1990)
Fermented Korean foods and beverages	Propyl carbamate	Various procedures	Various procedures	GC–MS	DB-Wax	11	Kim *et al*. (2000)

Table 1.1 (continued)

Sample matrix	Internal standard	Extraction principle	Clean-up	Detection	Column	LOD (µg/L)	Reference
Soya sauce	Propyl carbamate	Dichloromethane extraction	Extrelut	GC–MS	DB-Wax	1	Fauhl *et al.* (1993)
	–	Dichloromethane extraction	Celite columns	GC–MS	Supelcowax	0.5	Matsudo *et al.* (1993)
Blood	–	Before and after alkaline hydrolysis	–	Titration with 0.1 N sodium thiosulfate	–	–	Archer *et al.* (1948)
	[^{13}C,^{15}N]-Ethyl carbamate	Dichloromethane extraction	Chem-Elut 1000M	GC–MS	DB-WAX, DB-1	20	Hurst *et al.* (1990)

CI., chemical ionization; ECD, electrolytic conductivity detector; EI, electron ionization; FID, flame ionization detection; FTIR, Fourier transform infrared spectroscopy; FTNIR, Fourier transform near-infrared spectroscopy; GC, gas chromatography; HPLC, high-performance liquid chromatography; LOD, limit of detection; MI, matrix isolation; MS, mass spectrometry; NPD, nitrogen/phosphorus detector; PCI, positive chemical ionization; SPME, solid-phase microextraction; TEA, thermal energy analyser; TSD, thermoionic-specific detection

Solid-phase microextraction has recently emerged as a versatile solvent-free alternative to conventional extraction procedures. Ethyl carbamate has been analysed by HS–solid-phase microextraction only in wine samples (Whiton & Zoecklein, 2002) and spirits (Lachenmeier *et al*., 2006).

The procedures that combine sample extraction and subsequent GC–MS or GC–MS–MS are regarded as references for the analysis of ethyl carbamate in alcoholic beverages (Lachenmeier, 2005). Increasing requirements and cost pressures have forced both government and commercial food-testing laboratories to replace traditional reference methods with faster and more economical systems. Fourier-transform infrared spectroscopy, in combination with multivariate data analysis, has shown great potential for expeditious and reliable screening analysis of alcoholic beverages. The analysis of ethyl carbamate found in wine samples using Fourier-transform near-infrared spectroscopy was evaluated by Manley *et al*. (2001). Fourier-transform infrared spectroscopy in combination with partial least squares regression was applied to the screening analysis of ethyl carbamate in stone-fruit spirits (Lachenmeier, 2005).

1.2 Production and use

Ethyl carbamate can be made by the reaction of ethanol and urea or by warming urea nitrate with ethanol and sodium nitrite (Budavari, 2000). Another possible method is via addition of ethanol to trichloroacetyl isocyanate (Kocovský, 1986).

Production of ethyl carbamate was predominantly reported in the first half of the twentieth century. Ethyl carbamate has been produced commercially in the USA for at least 30 years (Tariff Commission, 1945). A major use of methyl and ethyl carbamate has been for the manufacture of meprobamate (Adams & Baron, 1965), and the spectacular success of this drug as a tranquilizer in the 1950s resulted in a demand for the commercial production of these intermediates. Ethyl carbamate had been used as a crease-resistant finish in the textile industry, as a solvent, in hair conditioners, in the preparation of sulfamic acids, as an extractant of hydrocarbons from crude oil and as a food flavour-enhancing agent (Adams & Baron, 1965). No data on the present use of ethyl carbamate in industry were available to the Working Group.

Ethyl carbamate was used in medical practice as a hypnotic agent at the end of nineteenth century but this use was discontinued after barbiturates became available. It was also tested for the treatment of cancers (Paterson *et al*., 1946; Hirschboeck *et al*., 1948), or used as a co-solvent in water for dissolving water-insoluble analgesics used for post-operative pain (Nomura, 1975). Ethyl carbamate has also been used in human medicine as an antileukaemic agent at doses of up to 3 g per day for the treatment of multiple myeloma (Adams & Baron, 1965). No evidence was available to the Working Group that ethyl carbamate is currently used in human medicine.

Ethyl carbamate is widely used in veterinary medicine as an anaesthetic for laboratory animals (Hara & Harris, 2002).

1.3 Occurrence and exposure

The occurrence of and exposure to ethyl carbamate in food have been reviewed (Battaglia *et al*., 1990; Zimmerli & Schlatter, 1991).

Ethyl carbamate has been detected in many types of fermented foods and beverages. The levels in wine and beer are in the microgram per litre range (Tables 1.2 and 1.3). Higher levels have been found in spirits, especially stone-fruit spirits, up to the milligram per litre range (Table 1.4). Ethyl carbamate has also been found in bread (Table 1.5). It may occur in fruit and vegetable juices at very low concentrations (< 1 µg/L) (Table 1.6). Its occurrence in other fermented food products (most notably fermented Asian products, such as soy sauce) is shown in Table 1.7.

In the past 20 years, major research has been carried out to identify the precursors of ethyl carbamate (Table 1.8) and develop methods for its reduction. One of the most established sources of ethyl carbamate is urea, which may be formed during the degradation of arginine by yeast. Arginase hydrolyses l-arginine to l-ornithine and urea (Schehl *et al*., 2007), and urea is secreted by the yeast into the medium where it reacts with ethanol to form ethyl carbamate (Ough *et al*., 1988a; Kitamoto *et al*., 1991; An & Ough, 1993). The addition of urease has been shown to reduce the content of ethyl carbamate in wine and other fermented products (Kobashi *et al*., 1988; Ough & Trioli, 1988; Tegmo-Larsson & Henick-Kling, 1990; Kim *et al*., 1995; Kodama & Yotsuzuka, 1996).

Ethyl carbamate may also be formed from cyanide. This may explain its high concentrations in stone-fruit spirits. The removal of cyanogenic glycosides such as amygdalin in stone-fruit by enzymatic action (mainly β-glucosidase) leads to the formation of cyanide (Lachenmeier *et al*., 2005b). Cyanide is oxidized to cyanate, which reacts with ethanol to form ethyl carbamate (Wucherpfennig *et al*., 1987; Battaglia *et al*., 1990; MacKenzie *et al*., 1990; Taki *et al*., 1992; Aresta *et al*., 2001). The wide range of concentrations of ethyl carbamate in stone-fruit spirits reflects its light- and time-dependent formation after distillation and storage (Andrey, 1987; Mildau *et al*., 1987; Baumann & Zimmerli, 1988; Zimmerli & Schlatter, 1991; Suzuki *et al*., 2001).

1.4 Regulations, guidelines and preventive actions

Public health concern regarding ethyl carbamate in food, and especially in alcoholic beverages, began in 1985 when relatively high levels were detected by Canadian authorities in alcoholic beverages, mainly in spirit drinks imported from Germany (Conacher & Page, 1986). Subsequently, Canada established an ethyl carbamate guideline of 30 µg/L for table wines, 100 µg/L for fortified wines, 150 µg/L for distilled spirits and 400 µg/L for fruit spirits (Conacher & Page, 1986). The Canadian guidelines were adopted by many other countries. The *Codex alimentarius* gives no specific standards for ethyl carbamate in food.

Table 1.2 Occurrence of ethyl carbamate in wine and fortified wine

Product	Year	No. of samples	Ethyl carbamate (μg/L)		Reference
			Mean	Range	
Wine	1951–89	127	0–5	0–48.6	Sponholz *et al*. (1991)
Wine	1988				Clegg *et al*. (1988)
White wines		196		<10–>100	
Red wines		51		<10–100	
Sparkling wines		14		–	
Wine coolers		2		–	
Fortified wines					
Sherries		256		<10–>200	
Ports		57		<10–>200	
Vermouths		7		<10–200	
Sherry	1985–87	12	32–33	<5–60	Dennis *et al*. (1989)
Wine		31	6	1–18	
Wine	1993				Sen *et al*. (1993)
White wines		16		ND–24	
Red wines		7		1–14	
Sake		2		3–29	
Sherry		6		28–69	
Fortified wines	1988–90	14	30	7–61	Vahl (1993)
Wine		57	7	<3–29	
Italian wine	2000	90			Cerutti *et al*. (2000)
Red				6–22	
White				6–16	
Rosé				7–15	
Brazilian wine	2002				Francisquetti *et al*. (2002)
Cabernet Sauvignon		30	10.6	2–31.8	
Merlot		17	6.6	1.8–32.4	
Gamay		3	4.5	3.4–6.5	
Pinot blanc		5	7.4	2.7–10.1	
Generic reds		9	16.6	2.4–36.2	
Gewürztraminer		12	10.1	1.2–30.5	
Italian Riesling		10	13.0	1.0–39.1	
Chardonnay		5	19.3	1.7–70	
Semillon		3	14.5	3.5–20.5	
Generic whites		3	4.8	4.7–5.1	
Common reds		10	5.1	2.1–9	
Sparkling wines		17	7.6	2.1–24.6	
Spanish red wine	2004	36		0–25	Uthurry *et al*. (2004)
Wine	2006	3	4.9	1.7–11.7	Ha *et al*. (2006)

ND, not detected

Table 1.3 Occurrence of ethyl carbamate in beer

Product	Year	No. of samples	Ethyl carbamate (µg/L)		Reference
			Mean	Range	
Beer	1985–87	15	0.1–1.1	<1–1.8	Dennis *et al*. (1989)
Beer	1989				Canas *et al*. (1989)
Domestic		33	0.24	ND–0.8	
Imported		36	2.8	2.1–3.5	
Danish Beer	1988–90	50	3	<0.2–6.6	Vahl (1993)
Alcohol-free beer	1994	4	0.3	0.1–0.7	Groux *et al*. (1994)
Beer		5	2.7	0.9–4.7	Groux *et al*. (1994)
Beer	1997				Dennis *et al*. (1997)
Draught		20		<1	
Canned		26		0.4–2.5	
Bottled		51		<1–14.7	
Home-brewed beer		32		<1–9	
Beer	2006	6	0.5	0.5–0.8	Ha *et al*. (2006)

ND, not detected

However, the general standard for contaminants and toxins in foods demands that contaminant levels shall be as low as reasonably achievable and that contamination may be reduced by applying appropriate technology in food production, handling, storage, processing and packaging (FAO/WHO, 2008).

Many preventive actions to avoid ethyl carbamate formation in food and beverages have been proposed (Table 1.9). For beverages such as wine and sake, the preventive measures have concentrated on yeast metabolism, whereas for stone-fruit spirits, research has been centred on reducing the precursor, cyanide. In addition, measures of good manufacturing practice such as the use of high-quality, unspoiled raw materials and high standards of hygiene during fermentation and storage of the fruit mashes, mashing and distillation must be optimized. To avoid the release of cyanide, it is essential to avoid breaking the stones, to minimize exposure to light and to shorten storage time. Some authors have proposed the addition of enzymes to decompose cyanide or a complete de-stoning of the fruit before mashing. The mashes have to be distilled slowly with an early switch to the tailing-fraction. Further preventive actions are the addition of patented copper salts to precipitate cyanide in the mash, distillation using copper catalysts or the application of steam washers (Zimmerli & Schlatter, 1991).

Table 1.4 Occurrence of ethyl carbamate in spirits

Product	Year	No. of samples	Ethyl carbamate (µg/L)		Reference
			Mean	Range	
Canadian whiskey	1988	18		<50–150	Clegg *et al*. (1988)
Rum		20		<50–150	
Vodka		5		<50	
Gin		4		<50	
Scotch whisky		7		<50–150	
Bourbon whiskey		19		<50–>150	
Fruit spirits and liqueurs		123		<50–>400	
Scotch whisky	1985–87	11	44	19–90	Dennis *et al*. (1989)
Imported whiskey		7	69–70	<5–206	
Vodka		3	ND	ND[a]	
Gin		3	ND	ND[a]	
Fruit spirit		4	41–42	<5–139	
Port		4	18	14–21	
Liqueur		8	129	9–439	
Whisky	1993	6	75.7	26–247	Sen *et al*. (1993)
Rye		1		8	
Bourbon		4		44–208	
Vodka		1		ND	
Gin		1		0.5	
Rum		1		19	
Fruit spirit		3		104–2344	
Apricot spirit		1		11	
Armagnac		2		410–432	
Other brandies		3		25–28	
Spirits	1988–90	22	534	<5–5103	Vahl (1993)
Grappa	2000	6		75–190	Cerutti *et al*. (2000)
Fruit spirit	2006	7	196.7	3.5–689.9	Ha *et al*. (2006)
Whisky		5	20.1	13.9–30.0	
Cheongju		5	20.2	8.4–30.3	
Korean style spirits	2000	10	3.4	ND–15.4	Kim *et al*. (2000)
Stone–fruit spirits	1986–2004	631	1400	10–18 000	Lachenmeier *et al*. (2005b)

ND, not detected; [a] Detection limit at 5 µg/L

Table 1.5 Occurence of ethyl carbamate in bread

Product	Year	No. of samples	Ethyl carbamate (µg/kg)		Reference
			Mean	Range	
Bread	1988	9	ND	ND[a]	Dennis *et al*. (1989)
Bread	1989	30			Canas *et al*. (1989)
White			3.0	ND–8	
Wheat			1.2	ND–4	
Other			0.9	ND–4	
Bread	1993	12	3.1	1.6–4.8	Sen *et al*. (1993)
Light toast	1993	12	4.3	1.3–10.9	
Dark toast	1993	12	15.7	4.9–29.2	
Bread	1988–90	33	3.5	0.8–12	Vahl (1993)
Bread	1994	48	5.2	0.5–27	Groux *et al*. (1994)

ND, not detected; [a] Detection limit at 5 µg/kg

Table 1.6 Occurrence of ethyl carbamate in juices

Product	Year	No. of samples	Ethyl carbamate (µg/L)		Reference
			Mean	Range	
Freshly pressed grape juices	1990	15		19–54	Tegmo-Larsson & Henick-Kling (1990)
Apple and pear juice	1994	6	ND	ND[a]	Groux *et al*. (1994)
Citrus juice		7	0.1	0–0.1	
Grape juice		6	0.1	0–0.2	
Other fruit juices		8	0.1	0–0.2	
Vegetable juice		3	0.1	0–0.1	

ND, not detected; [a] Detection limit at 0.06 ppb = 0.06 µg/L

Table 1.7 Occurrence of ethyl carbamate in miscellaneous fermented foods

Product	Year	No. of samples	Ethyl carbamate (µg/kg)		References
			Mean	Range	
Cheese	1989	16	ND	ND	Canas *et al*. (1989)
Yoghurt		12	0.4	ND–4	
Tea		6	ND	ND	
Yoghurt	1988	9	0–1	<1–<1	
Cheese		19	0.6–5.1	<5–6	
Soya sauce	1993	10		ND–59	Sen *et al*. (1993)
Yoghurt and buttermilk		14		ND–0.4	
Yoghurt and other acidified milk products	1988–90	19	0.2	<0.1–0.3	Vahl (1993)
Kimchi	2000	20	3.5	ND–16.2	Kim *et al*. (2000)
Soy sauce					
Regular		5	14.6	ND–19.5	
Traditional type		15	17.1	ND–73.3	
Soybean paste		7	2.3	ND–7.9	
Vinegar		5	1.2	0.3–2.5	
Soju	2006	7	3.0	0.8–10.1	Ha *et al*. (2006)
Takju		7	0.6	0.4–0.9	

ND, not detected

Table 1.8 Precursors of ethyl carbamate in different food matrices and factors that influence its formation

Precursor	Food matrix	Reference
Diethyl dicarbonate (used as food additive)	Orange juice, white wine, beer	Löfroth & Gejvall (1971)
Carbamyl phosphate (produced by yeasts)	Wine, fermented foods, bread	Ough (1976a)
Diethyl dicarbonate (used as food additive)	Wine	Ough (1976b)
Cyanide, vicinal dicarbonyl compounds	Model systems	Baumann & Zimmerli (1986b)
Carbamyl phosphate and ethyl alcohol, light	Wine	Christoph *et al*. (1987)
Cyanide, benzaldehyde, light	Distilled products	Christoph *et al*. (1988)
Light	Distilled products	Baumann & Zimmerli (1988)
Urea	Wine	Ough & Trioli (1988)
Urea, citrulline, *N*-carbamyl α-amino acids, *N*-carbamyl β-amino acid, allantoin, carbamyl phosphate	White and red wines	Ough *et al*. (1988a)
Amino acids, urea, ammonia	Chardonnay juice fermentation	Ough *et al*. (1988b)
Urea, copper, carbamyl phosphate, citrulline	Wine	Sponholz *et al*. (1991)
Cyanate, cyanide, cyanohydrin, copper cyanide complexes	Grain whisky	Aylott *et al*. (1990)
Cyanide related species (cyanide, copper cyanide complex, lactonitrile, cyanate, thiocyanate)	Scotch grain whisky	MacKenzie *et al*. (1990)
Cyanide	Grain-based spirits	Cook *et al*. (1990)
Cyanide	Grain-based spirits	McGill & Morley (1990)
Temperature, light	Wine	Tegmo-Larsson & Spittler (1990)
Cyanate	Alcoholic beverages	Taki *et al*. (1992)
Yeast strain, arginine, urea	Fortified wine	Daudt *et al*. (1992)
Isocyanate	Wine distillates	Boulton (1992)
Cyanide, copper, light,	Stone-fruit distillates	Kaufmann *et al*. (1993)
Manufacturing conditions	Soya bean tempe	Nout *et al*. (1993)
Urea	Wine	An & Ough (1993)

Table 1.8 (continued)

Precursor	Food matrix	Reference
Urea, citrulline	Wine	Stevens & Ough (1993)
Urea	Wine	Kodama *et al*. (1994)
Citrulline, arginine degradation	Wine	Liu *et al*. (1994)
Yeast arginase activity	Port	Watkins *et al*. (1996)
Azodicarbonamide (used as food additive)	Bread, beer	Dennis *et al*. (1997)
Citrulline	Wine	Mira de Orduña *et al*. (2000)
Citrulline	Model fortified wines	Azevedo *et al*. (2002)
Arginine	Wine	Arena *et al*. (2002)
Arginine	Korean soy sauce	Koh *et al*. (2003)
Storage time, temperature	Wine	Hasnip *et al*. (2004)
Arginine, citrulline	Wine	Arena & Manca de Nadra (2005)
Cyanide	Stone-fruit spirits	Lachenmeier *et al*. (2005b)
Fruit types, fermentation conditions	Fruit mashes	Balcerek & Szopa (2006)
Selected yeasts, different conditions (temperature, pH)	Red wine	Uthurry *et al*. (2006)
Yeast strain, arginine	Stone-fruit distillates	Schehl *et al*. (2007)

Table 1.9 Procedures for reducing ethyl carbamate concentration in different food matrices

Procedure	Food matrix	Reference
Modification of vineyard procedures Use of commercial yeast strains Urease treatment	Wine	Butzke & Bisson (1997)
Use of non-arginine-degrading oenococci	Wine	Mira de Orduña *et al*. (2001)
Metabolic engineering of *Saccharomyces cerevisiae*	Wine	Coulon *et al*. (2006)
Malolactic fermentation with pure cultures at low pH values (<3.5)	Wine	Terrade & Mira de Orduña (2006)
Removal of urea with an acid urease	Sake	Kobashi *et al*. (1988)
Genetic engineering of yeast	Sake	Kitamoto *et al*. (1991)
Non-urea producing yeast	Sake	Kitamoto *et al*. (1993)
Non-urea producing yeast	Sake	Yoshiuchi *et al*. (2000)
Application of acid urease	Takju	Kim *et al*. (1995)
Application of acid urease	Sherry	Kodama & Yotsuzuka (1996)
Precipitation of cyanide (steam washer)	Stone-fruit distillates	Nusser *et al*. (2001)
Application of cyanide catalyst Optimization of distillation conditions	Stone-fruit distillates	Pieper *et al*. (1992a,b)
Dark storage Separation of cyanide	Stone-fruit distillates	Christoph & Bauer-Christoph (1998, 1999)
Complete prevention of ethyl carbamate by state-of-the-art production technology	Stone-fruit distillates	Lachenmeier *et al*. (2005b)
De-stoning of the fruits	Stone-fruit distillates	Schehl *et al*. (2005)
Automatic rinsing of the stills, copper catalysts, separation of tailings, no re-distillation of tailings	Stone-fruit distillates	Weltring *et al*. (2006)
Yeast with reduced arginase activity	Stone-fruit distillates	Schehl *et al*. (2007)

Research on ethyl carbamate in food has led to a significant reduction in its content during the past 20 years. The use of additives that might be precursors of ethyl carbamate has been forbidden in most countries. For stone-fruit spirits — the most problematic food group — the few large distilleries that produce for the mass market have all introduced the good manufacturing practices described above and produce stone-fruit distillates that have only traces of ethyl carbamate. The current problem of ethyl carbamate encompasses in particular small distilleries that have not introduced improved technologies (Lachenmeier *et al*., 2005b).

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2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Previous evaluation

Ethyl carbamate was evaluated by an IARC Working Group in February 1974 (IARC, 1974). It was also the subject of a very extensive review (Salmon & Zeise, 1991). Both reviews evaluated bioassays in which mice, rats and hamsters were exposed to ethyl carbamate by oral, dermal, subcutaneous and/or intraperitoneal routes.

Mice treated orally with ethyl carbamate had an increased incidence of lung adenomas, carcinomas and squamous-cell tumours, lymphomas (mainly lymphosarcomas), mammary gland adenocarcinomas and carcinomas, leukaemia and Harderian gland adenomas and angiomas. When oral administration was accompanied by topical application of the tumour promoter 12-*O*-tetradecanoylphorbol-13 acetate (TPA), the incidence of skin papillomas and squamous-cell carcinomas was significantly increased. Rats treated orally with ethyl carbamate had an increased incidence of Zymbal gland and mammary gland carcinomas. Hamsters treated orally with ethyl carbamate showed an increased incidence of skin melanotic tumours, forestomach papillomas, mammary gland adenocarcinomas, liver hepatomas, liver and spleen haemangiomas and carcinomas of the thyroid, ovary and vagina.

Topical application of ethyl carbamate to mice resulted in a significant increase in the incidence of lung adenomas and mammary gland carcinomas.

Subcutaneous administration of ethyl carbamate induced a significant increase in the incidence of lung adenomas in adult mice and hepatomas in newborn mice. When the treatment was followed by topical application of croton oil, a significant increase in the incidence of skin papillomas was observed.

Intraperitoneal administration of ethyl carbamate to adult mice resulted in a significant increase in the incidence of lung adenomas, hepatomas and skin papillomas. Similar treatment in newborn mice induced lymphomas, lung adenomas, hepatomas, Harderian gland tumours and stromal and epithelial tumours of the ovary.

Mice exposed transplacentally to ethyl carbamate developed an increased incidence of lung tumours, hepatomas and ovarian tumours.

Subsequent bioassays are summarized below.

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male B6C3F$_1$ mice, 6 weeks of age, were given 0, 0.6, 3, 6, 60 or 600 ppm ethyl carbamate (> 99% pure) in the drinking-water for 70 weeks. Mice that survived more than 23 weeks were included in the analysis of tumours (i.e. effective number of mice). The effective number of mice was 49, 49, 48, 50, 50 and 44 for the 0-, 0.6-, 3-, 6-, 60- and 600-ppm ethyl carbamate dose groups, respectively. The mean survival of the 600-ppm dose group was significantly shorter than that of the control group (39.2 weeks versus 69.5 weeks, respectively; $P < 0.01$, Student's *t*-test). The other groups had mean survival times of ≥ 65.5 weeks. All mice were autopsied and histological examinations were conducted. Ethyl carbamate caused dose-related increases in the incidence of lung alveolar/bronchiolar adenomas and carcinomas, liver haemangiomas and angioasarcomas and heart haemangiomas. The incidence of lung alveolar/bronchiolar adenoma was 9/49 (18%), 4/49 (8%), 7/48 (15%), 8/50 (16%), 34/50 (68%) and 42/44 (95%) for the 0-, 0.6-, 3-, 6-, 60- and 600-ppm ethyl carbamate-treated groups, respectively; the increase at 60 and 600 ppm ethyl carbamate was significant ($P < 0.01$) compared with the control group. Lung alveolar/bronchiolar carcinoma was only observed in the 600-ppm ethyl carbamate-treated group (6/44; 14%), an incidence that was significant. Liver haemangioma occurred in the 60- and 600-ppm ethyl carbamate-treated groups (2/50 (4%) and 20/44 (45%), respectively), and the increase in the 600-ppm group was significant ($P < 0.01$). Liver angiosarcoma developed in the 6-, 60- and 600-ppm ethyl carbamate-treated groups at incidences of 2/50 (4%), 2/50 (4%) and 11/44 (25%), respectively; the latter was a significant increase compared with the control group ($P < 0.01$). Heart haemangioma occurred only in the mice treated with 600 ppm ethyl carbamate (4/44; 9%), an incidence that was significant ($P < 0.05$) (Inai *et al*., 1991).

Groups of 48 male and 48 female B6C3F$_1$ mice, 4 weeks of age, were given 0, 10, 30 or 90 ppm ethyl carbamate (> 99% pure) in the drinking-water for 104 weeks. The administration of ethyl carbamate caused a dose-dependent decrease in survival in both male and female mice, and the effect was significant at 30 and 90 ppm ethyl carbamate. Complete necropsies were performed on all mice and histological examinations were conducted. The incidence of tumours in males treated with 0-, 10-, 30- and 90-ppm, respectively, was: lung alveolar/bronchiolar adenomas or carcinomas, 5/48 (10%), 18/48 (37%), 29/47 (62%) and 37/48 (77%) (the increases at 10, 30 and 90 ppm ethyl carbamate were significant; $P < 0.05$); hepatocellular adenomas or carcinomas, 12/46 (26%), 18/47 (38%), 24/46 (52%) and 23/44 (52%) (the increases at 30 and

90 ppm ethyl carbamate were significant; $P < 0.05$); liver haemangiosarcomas, 1/46 (2%), 2/47 (4%), 5/46 (11%) and 13/44 (29%) (the increase at 90 ppm ethyl carbamate was significant; $P < 0.05$); Harderian gland adenomas or carcinomas, 3/47 (6%), 12/47 (25%), 30/47 (64%) and 38/47 (81%) (the increases at all three doses were significant; $P < 0.05$); skin squamous-cell papillomas or carcinomas, 0/47, 1/48 (2%), 3/47 (6%) and 6/48 (12%) (the increase at 90 ppm ethyl carbamate was significant; ($P < 0.05$); forestomach squamous-cell papillomas, 0/46, 2/47 (14%), 3/44 (7%) and 5/45 (11%) (the increase at 90 ppm ethyl carbamate was significant; $P < 0.05$); and heart haemangiosarcomas, 0/48, 0/48, 1/47 (2%) and 5/48 (10%) (the increase at 90 ppm ethyl carbamate was significant; $P < 0.05$). The incidence of tumours in female mice treated with 0-, 10-, 30- and 90-ppm, respectively, was: lung alveolar/bronchiolar adenomas or carcinomas, 6/48 (12%), 8/48 (17%), 28/48 (53%) and 39/47 (83%) (increases at 30 and 90 ppm ethyl carbamate were significant; $P < 0.05$); hepatocellular adenomas or carcinomas, 5/48 (10%), 11/47 (23%), 20/47 (43%) and 19/47 (40%) (the increases at 30 and 90 ppm ethyl carbamate were significant; $P < 0.05$); liver haemangiosarcoma, 0/48, 0/47, 1/47 (2%) and 7/47 (15%) (the increase at 90 ppm ethyl carbamate was significant; $P < 0.05$); mammary gland adenocarcinomas, 4/47 (8%), 3/46 (6%), 3/46 (6%) and 11/48 (23%) (the increase at 90 ppm ethyl carbamate was significant; $P < 0.05$); mammary gland adenoacanthomas, 0/47, 1/46 (2%), 1/46 (2%) and 11/48 (23%) (the increase at 90 ppm ethyl carbamate was significant; $P < 0.05$); Harderian gland adenomas or carcinomas, 3/48 (6%), 11/48 (23%), 19/48 (40%) and 30/48 (62%) (the increases at all three doses were significant; $P < 0.05$); and ovary granulosa-cell tumours, 0/48, 0/46, 2/46 (4%) and 5/39 (13%) (the increase at 90 ppm ethyl carbamate was significant; $P < 0.05$) (National Toxicology Program, 2004; Beland *et al*., 2005).

A study was conducted to compare the carcinogenicity of ethyl carbamate in mice that are proficient and deficient in cytochrome-P450 (CYP) 2E1. Groups of 28–30 male *Cyp2e1*+/+ and *Cyp2e1*–/– mice, 5–6 weeks of age, were administered by gavage 0, 1, 10 or 100 mg/kg body weight (bw) ethyl carbamate (purity, > 98%) once a day on 5 days per week for 6 weeks. The ethyl carbamate was dissolved in water and administered in a volume of 10 mL/kg bw. Twenty-four hours after the last treatment, 14–15 mice per group were killed. The remaining 14–15 mice per group were held for 7 months. Complete gross necropsy and microscopic examination were performed on all mice. Seven months after the end of treatment, liver tumours (haemangiomas and haemangiosarcomas) were observed in male *Cyp2e1*+/+ mice treated with 100 mg/kg bw ethyl carbamate (5/15 (33%) and 8/15 (53%) compared with 0/14 and 0/14, respectively, in control male *Cyp2e1*+/+ mice). The increased incidence was significant ($P < 0.05$ and < 0.01, respectively). Liver haemangioma was detected in a single *Cyp2e1*–/– mouse (1/15; 7%) treated with 100 mg/kg bw ethyl carbamate. The difference in the incidence of liver haemangiosarcomas was significant when *Cyp2e1*+/+ mice were compared with *Cyp2e1*–/– mice treated with 100 mg/kg bw ethyl carbamate (8/15 (53%) versus 0/15; $P = 0.0011$); the difference in the incidence of liver haemangioma was marginally significant (5/15 (33%) versus 1/15 (7%); $P = 0.0843$). In male *Cyp2e1*+/+ mice,

the incidence of bronchioalveolar adenoma was 0/14, 3/14 (21%), 14/14 (100%) and 14/15 (93%) in the control, low-dose, mid-dose and high-dose groups, and tumour multiplicities were 0, 1.0, 2.5 and 15.4 tumours/lung, respectively. The incidence of bronchioalveolar adenoma was significantly increased with doses of 10 and 100 mg/kg bw ethyl carbamate ($P < 0.01$) and there was a significant variation in the tumour multiplicity across doses ($P < 0.0001$). In the respective groups of male *Cyp2e1–/–* mice, the incidence of bronchioalveolar adenoma was 0/15, 0/15, 4/14 (29%) and 9/15 (60%), and tumour multiplicities were 0, 0, 1.0 and 2.4 tumours/lung. The incidence of bronchioalveolar adenoma was significantly increased with doses of 10 and 100 mg/kg bw ethyl carbamate ($P < 0.05$ and < 0.01; respectively). The difference in the incidence of bronchioalveolar adenoma was significant when *Cyp2e1+/+* mice were compared with *Cyp2e1–/–* mice treated with 10 and 100 mg/kg bw ethyl carbamate ($P = 0.0001$ and 0.04, respectively). The difference in the multiplicity of bronchioalveolar adenoma was also significant when *Cyp2e1+/+* mice were compared with *Cyp2e1–/–* mice treated with 10 and 100 mg/kg bw ethyl carbamate ($P = 0.0145$ and < 0.0001, respectively). A single case of bronchioalveolar carcinoma was detected in a *Cyp2e1+/+* mouse treated with 100 mg/kg bw ethyl carbamate. In male *Cyp2e1+/+* mice, the incidence of Harderian gland adenoma was 1/14 (7%), 4/14 (29%), 14/14 (100%) and 13/15 (87%) in control, low-dose, mid-dose and high-dose groups, respectively, and was significantly increased at 10 and 100 mg/kg bw ethyl carbamate ($P < 0.01$). That in male *Cyp2e1–/–* mice was 0/15, 1/15 (7%), 2/14 (14%) and 12/15 (80%), respectively and was significantly increased with the dose of 100 mg/kg bw ethyl carbamate ($P < 0.01$). The difference in the incidence of Harderian gland adenoma was significant when *Cyp2e1+/+* mice were compared with *Cyp2e1–/–* mice treated with 10 mg/kg bw ethyl carbamate ($P < 0.0001$) (Ghanayem, 2007).

3.1.2 *Monkey*

A group of neonatal cynomologus, rhesus and/or African green monkeys [sex, number and distribution not specified] was administered 250 mg/kg bw ethyl carbamate [purity not specified] orally in sterile water [volume not specified] on 5 days per week for 5 years. Thirty-two monkeys survived the first 6 months of treatment, at which time they typically were weaned. Some of the monkeys also received 7–10 weekly courses of whole-body radiation (50 rad per course). None of the monkeys survived after 5 years of treatment. Complete necropsies were performed on all animals. Six of the 32 (19%) monkeys developed one or more primary tumours. The tumours included adenocarcinoma of the lung, pancreas, bile ducts and small intestine, hepatocellular adenoma and carcinoma, haemangiosarcoma of the liver, ependymoma, pheochromocytoma, endocervical adenofibroma and squamous papilloma of the pouch. The specific incidences were not reported. Only two of the six (33%) monkeys that had malignant tumours had been irradiated. A concurrent control group did not appear to be included. Autopsy records were available for 373 breeders and ‘normal controls’.

Nineteen of these monkeys developed malignant and/or benign tumours. While some tumours occurred in both untreated and ethyl carbamate-treated monkeys (e.g. adenocarcinoma of the pancreas and intestine), hepatocellular adenoma and carcinoma and adenocarcinoma of the lung were only found in ethyl carbamate-treated monkeys (Thorgeirsson *et al*., 1994). [The Working Group noted the poor design and reporting of the study.]

3.2 Skin application

Mouse

A study was conducted to determine whether or not ethyl carbamate would act as an enhancer of skin carcinogenesis induced by 7,12-dimethylbenz[*a*]anthracene (DMBA). A group of 16 male and 16 female hairless *hr/hr* Oslo mice [age not specified] was treated topically once with 51.2 μg DMBA [purity not specified] in 100 μL acetone and were observed for 60 weeks. An additional group of the same number of mice was treated identically with DMBA and then, after a 2-week period, were treated topically twice a week for 50 weeks with 100 μL of a solution of 10% ethyl carbamate [purity not specified] in acetone. An additional group of the same number of mice was not treated with DMBA, but was treated with ethyl carbamate for a period of 60 weeks. Gross necropsies and histology were performed. Tumour rates (the percentage of tumour-bearing mice in relation to the number of mice alive at the appearance of the first tumour related to time) and yields (the cumulative occurrence of all skin tumours related to time) were analysed statistically. Mice treated with DMBA alone had a total of 21 skin tumours (primarily papillomas, but also carcinomas and atypical keratoacanthomas) in 11 mice and no lung adenomas; mice treated with ethyl carbamate alone had a total of eight skin tumours in five mice and 79 lung adenomas in 22 mice; and mice treated with DMBA and ethyl carbamate had a total of 60 skin tumours in 16 mice and 121 lung adenomas in 23 mice. Treatment with DMBA and ethyl carbamate induced a significantly higher number of skin tumours than treatment with DMBA alone (Iversen, 1991).

3.3 Inhalation exposure

Mouse

Groups of female JCL:ICR mice [number per group not specified], 28 days of age, were exposed to air containing 0.25 μg/mL ethyl carbamate [purity not specified] for 1, 3, 5 or 10 days or air containing 1.29 μg/mL ethyl carbamate for 0.25, 1, 2, 4 or 5 days. Groups of male JCL:ICR mice, 28 days of age, were exposed to air containing 0.25 μg/mL ethyl carbamate for 10 days (50 mice) or air containing 1.29 μg/mL

ethyl carbamate for 4 days (47 mice). Concurrent controls were exposed to air only. Female mice were killed 5 months after the exposure period and male mice were killed 12 months after the exposure period. Histological analyses were performed. Female mice exposed by inhalation to 0.25 μg/mL ethyl carbamate had a lung tumour incidence [tumour type not specified] and tumour multiplicity (tumours per lung) of 27/51 (53%) and 1.08 ± 0.39 (mean ± 95% confidence interval [CI]) after exposure for 1 day, 44/51 (86%) and 5.29 ± 1.28 after exposure for 3 days, 46/53 (87%) and 7.56 ± 2.05 after exposure for 5 days and 9/11 (82%) and 17.8 ± 4.6 after exposure for 10 days. In each of the exposed groups, the lung tumour incidence [$P < 0.0001$; one-tailed Fisher's exact test] and tumour multiplicity ($P < 0.05$) were significantly increased compared with the concurrent control group, which had values of 2/51 (4%) and 0.04, respectively. Female mice exposed by inhalation to 1.29 μg/mL ethyl carbamate had a lung tumour incidence [tumour type not specified] and tumour multiplicity of 38/79 (48%) and 0.67 ± 0.20 after exposure for 0.25 days, 37/40 (92%) and 10.7 ± 2.9 after exposure for 1 day, 66/70 (94%) and 18.6 ± 3.8 after exposure for 2 days, 81/86 (94%) and 10.6 ± 2.6 after exposure for 4 days and 18/18 (100%) and 12.2 ± 3.9 after exposure for 5 days. In each of the exposed groups, the lung tumour incidence [$P < 0.0001$; one-tailed Fisher's exact test] and tumour multiplicity ($P < 0.05$) were significantly increased compared with the concurrent control group, which had values of 2/51 (4%) and 0.04, respectively. Male mice exposed by inhalation to 0.25 μg/mL ethyl carbamate for 10 days had a lung adenocarcinoma incidence of 40/50 (80%), of which 11 (22%) showed signs of invasion or metastasis. Male mice exposed by inhalation to 1.29 μg/mL ethyl carbamate for 4 days had a lung adenocarcinoma incidence of 14/40 (35%). This group was composed of 47 mice, of which seven died within 7 days of being treated. In each of the exposed groups, the lung adenocarcinoma incidence was significantly increased ($P < 0.01$) compared with the control group, which had an incidence of 1/51 (2%). [The Working Group questioned the high incidence of adenocarcinomas associated with high survival.] The incidence of leukaemia in female mice exposed by inhalation to 0.25 μg/mL ethyl carbamate was 3/51 (6%) after exposure for 1 day, 2/51 (4%) after exposure for 3 days, 5/53 (9%) after exposure for 5 days and 0/11 after exposure for 10 days. The incidence of leukaemia in mice exposed for 5 days was significantly greater [$P = 0.0312$; one-tailed Fisher's exact test] than that in concurrent controls, which had an incidence of 0/51. Female mice exposed by inhalation to 1.29 μg/mL ethyl carbamate had an incidence of leukaemia of 2/79 (2%) after exposure for 0.25 days, 1/40 (2%) after exposure for 1 day, 12/70 (17%) after exposure for 2 days, 18/86 (21%) after exposure for 4 days and 3/18 (17%) after exposure for 5 days. The incidence in mice in each of the groups exposed for 2 or more days was significantly greater [$P \leq 0.0156$; one-tailed Fisher's exact test] than that in the concurrent control group, which had an incidence of 0/51. The incidence of leukaemia in male mice exposed by inhalation to 0.25 μg/mL ethyl carbamate for 10 days was 5/50 (10%). Male mice exposed by inhalation to 1.29 μg/mL ethyl carbamate for 4 days had an incidence of 8/40 (20%). In each of the exposed groups, the incidence of leukaemia was significantly increased [$P \leq 0.0264$;

one-tailed Fisher's exact test] compared with the control group, which had an incidence of 0/51. The incidence of uterine haemangioma in female mice exposed by inhalation to 1.29 μg/mL ethyl carbamate was 0/79 after exposure for 0.25 days, 1/40 (2%) after exposure for 1 day, 2/70 (3%) after exposure for 2 days, 8/86 (9%) after exposure for 4 days and 0/18 after exposure for 5 days. The incidence of uterine haemangioma in mice exposed for 4 days was significantly greater [$P = 0.0212$; one-tailed Fisher's exact test] than that in the concurrent control group, which had an incidence of 0/51. A single uterine haemangioma 1/51 (2%) was also observed in female mice exposed to 0.25 μg/mL ethyl carbamate for 3 days. The incidence of hepatoma in male mice exposed by inhalation to 0.25 μg/mL ethyl carbamate for 10 days was 6/50 (12%). In male mice exposed by inhalation to 1.29 μg/mL ethyl carbamate for 4 days, the incidence of hepatoma was 3/40 (7%). The incidence of hepatoma in the mice exposed to 0.25 μg/mL ethyl carbamate was marginally increased [$P = 0.0529$; one-tailed Fisher's exact test] compared with the control group, which had an incidence of 1/51 (2%) (Nomura *et al*., 1990).

3.4 Other exposures

3.4.1 *Pre-conception*

Mouse

A study was conducted to investigate whether pre-conception exposure of sperm cells to ethyl carbamate resulted in an increased risk for cancer in either untreated progeny or progeny treated with ethyl carbamate. Groups of 45 male CBA/JNCrj mice, 9 weeks of age, received two subcutaneous injections of 10 μL/g bw saline or 10 μL/g bw saline that contained 500 μg/kg bw ethyl carbamate (purity, > 99%) at a 24-hour interval. At 1, 3 and 9 weeks after treatment (i.e. at different stages of spermatogenesis), each male mouse was mated for 4 days with three untreated virgin 12-week-old female CBA/JNCrj mice. When the progeny were 6 weeks of age, one half was treated once with a subcutaneous injection of 10 μL/g bw saline and the other half was treated with 10 μL/g bw saline that contained 100 μg/kg bw ethyl carbamate. The mice were then kept for lifetime. The mean lifetime for the male mice, including the parental males, was 80–91 weeks, and that for the female mice, including the parental females, was 87–94 weeks. Statistical analyses indicated only sporadic differences in survival when ethyl carbamate-treated groups were compared with their appropriate control groups. Complete necropsies and histological examinations were conducted on all animals. Paternal treatment with ethyl carbamate caused a significant increase (98%) in the incidence of lung tumours (bronchioloalveolar adenomas and adenocarcinomas) in parental male mice compared with 22% in the 45 controls. Male F_1 mice treated with saline had a lung tumour incidence of 17–24% (71–135 mice per group); those treated with ethyl carbamate had a lung tumour incidence of 43–60% (83–124 mice per group). Paternal treatment had no consistent effect on lung-tumour incidence in

male F_1 mice. Male F_1 mice treated with ethyl carbamate had a significantly increased incidence of lung tumours [$P \leq 0.0004$; one-tailed Fisher's exact test], irrespective of the paternal treatment. Female F_1 mice treated with saline had a lung tumour incidence of 11–24% (59–111 mice per group) compared with 32–43% (81–104 mice per group) in those treated with ethyl carbamate. Paternal treatment with ethyl carbamate had no effect on the incidence of lung tumours in female F_1 mice. Female F_1 mice treated with ethyl carbamate had a significantly increased lung-tumour incidence [$P \leq 0.0168$; one-tailed Fisher's exact test], irrespective of the paternal treatment, with the exception of mice resulting from the 3-week mating of ethyl carbamate-treated F_0 male mice, which may be a spurious result. Paternal treatment with ethyl carbamate caused a significant increase (76%) in the incidence of liver tumours (hepatocellular adenomas and adenocarcinomas) in the parental male mice, compared with 53% in the 45 controls. Male F_1 mice treated with saline had a liver-tumour incidence of 54–66% compared with those treated with ethyl carbamate (56–70%). Paternal treatment with ethyl carbamate had no effect on the liver-tumour incidence in male F_1 mice. The incidence of liver tumours in male F_1 mice treated with ethyl carbamate did not differ from that in mice treated with saline, irrespective of the paternal treatment. Female F_1 mice treated with saline had a liver-tumour incidence of 2–7%; those treated with ethyl carbamate had a lung tumour incidence of 2–12%. Paternal treatment with ethyl carbamate had no consistent effect on lung-tumour incidence in female F_1 mice. Treatment of female F_1 mice with ethyl carbamate had no consistent effect on the incidence of liver tumours. Lymphomas and histocytic sarcomas occurred in both F_0 male mice (7%) and their F_1 offspring (5–14% in males; 11–20% in females). The haematopoietic tumour incidence was not affected by treatment with ethyl carbamate in either the F_0 male mice or their F_1 offspring of either sex (Mohr *et al*., 1999).

Male Swiss Cr:NIH(S) mice, 6 weeks of age [number not specified], received a single intraperitoneal injection of distilled water [volume not specified] or distilled water that contained 1.5 g/kg bw ethyl carbamate [purity not specified]. Two weeks later, each male mouse was housed with five 8-week-old female mice for an unspecified period of time. This timing was selected to ensure that the sperm used in fertilization would have been exposed postmeiotically, a stage of high sensitivity to pre-conception carcinogenic effects. Three weeks later, female mice that were visibly pregnant were housed individually and allowed to give birth. The offspring were weaned at 4 weeks. The experiment lasted until the last animal died, which was approximately 157 weeks after birth. Seventy-one per cent of the female mice placed with control male mice became pregnant. For the carcinogenesis study, 71 female offspring, arising from 23 litters, and 48 male offspring, arising from 14 litters, were used. These litters were the product of 11 sires. Sixty-six percent of the female mice placed with ethyl carbamate-treated male mice became pregnant. For the carcinogenesis study, 78 female offspring, arising from 20 litters, and 54 male offspring, arising from 20 litters, were used. These litters were the product of 12 sires. Paternal treatment with ethyl carbamate resulted in the induction of adrenal gland tumours in both the male and female offspring. The

incidence was 6/132 (5%), of which five were pheochromocytomas and one was a cortical adenoma. These tumours were not detected in the offspring (0/119) of control male mice that had been treated with distilled water. The increase in the incidence of both pheochromocytomas ($P = 0.039$) and total adrenal gland tumours [$P = 0.020$; one-tailed Fisher's exact test] was significant. Treatment with ethyl carbamate resulted in the induction of glandular stomach tumours in the male offspring. In the 54 male experimental mice, 10 (18%) glandular stomach lesions developed, of which three (6%) were adenomas, three were carcinomas and four (7%) were atypical hyperplasias. In the 48 male control mice, two (4%) adenomas developed. The increase in the incidence of combined neoplastic and non-neoplastic lesions was significant ($P = 0.024$) (Yu *et al*., 1999).

3.4.2 *Transplacental exposure*

Mouse

A group of 25 pregnant Swiss Webster mice, 10 weeks of age, received a single intravenous injection of 3.3 mmol/kg bw ethyl carbamate [purity not specified] in 250 μL phosphate-buffered saline on gestational day 14. A control group of 22 pregnant female mice of the same age received two injections (250 and 100 μL) of the phosphate-buffered saline only. An additional group of 30 virgin female mice was treated with 3.3 mmol/kg bw ethyl carbamate in phosphate-buffered saline and a further group of 29 virgin female mice was injected with phosphate-buffered saline alone. All injections were followed by a 'chaser' injection of 100 μL phosphate-buffered saline. Six months after the pregnant mice gave birth, the dams, their offspring and the virgin female mice were killed to determine lung-tumour incidence by gross analysis of the lungs. One control dam died before the scheduled killing. Survival in the offspring was not indicated. The incidence of lung adenomas in 21 control dams was 28.6%, with a tumour multiplicity of 0.33 tumours per mouse. The comparable values in the 96 male and 72 female offspring were 10.4% and 0.12 tumour per mouse and 16.6% and 0.19 tumour per mouse, respectively. The incidence of lung adenomas in 20 dams treated with ethyl carbamate was 95.0%, with a tumour multiplicity of 10.5 tumours per mouse. The comparable values in the 90 male and 70 female offspring were 45.0% and 0.96 tumour per mouse and 57.1% and 1.3 tumours per mouse, respectively. The incidence of lung adenomas in 29 control virgin females was 44.8%, with a tumour multiplicity of 0.75 tumour per mouse. The comparable values for 30 virgin females treated with ethyl carbamate were 100% and 6.2 tumours per mouse (Neeper-Bradley & Conner, 1992).

3.5 Metabolites of ethyl carbamate

Previous evaluation

During the review of ethyl carbamate by a previous IARC Working Group (IARC, 1974), the carcinogenicity of ethyl carbamate metabolites was considered briefly. The Working Group concluded that ethyl carbamate needed metabolism to exert its carcinogenicity. Bioassays have been conducted on several oxidized metabolites of ethyl carbamate, and these are summarized below.

3.5.1 *Oral administration*

Mouse

Groups of 20 or 25 male and 20 or 25 female Swiss mice, 2–3 months of age, were given a single oral dose of 25 mg ethyl carbamate [purity not specified] or 25 mg *N*-hydroxyethyl carbamate [purity not specified] in distilled water [volume not specified]. A control group of 46 mice remained untreated. Four days after the initial treatment, all groups received twice-weekly dermal applications of 5% croton oil in liquid paraffin [volume not specified]. The incidence and multiplicity of skin tumours were assessed after 20 and 40 weeks of croton-oil application; those of lung tumours were assessed after 40 weeks of croton-oil application. Histopathology was conducted on the lungs. Survival was ≥ 90% after 20 weeks and ≥ 80% after 40 weeks of croton oil application. After 20 weeks, the incidence and multiplicity (± standard deviation [SD]) of skin tumours were 16/18 (89%) and 1.5 ± 0.2 for mice treated with 25 mg ethyl carbamate and 12/25 (48%) and 0.7 ± 0.2 for mice treated with 25 mg *N*-hydroxyethyl carbamate versus 3/45 (7%) and 0.07 ± 0.05 for mice treated with croton oil only. The skin tumour incidence [$P \leq 0.0001$; one-tailed Fisher's exact test] and tumour multiplicity [$P < 0.001$; one-way ANOVA followed by SNK test] in each of the treatment groups were significantly increased compared with the croton oil control mice. The skin tumour incidence [$P = 0.0088$; two-tailed Fisher's exact test] and tumour multiplicity [$P < 0.001$; one-way ANOVA followed by SNK test] in mice treated with 25 mg ethyl carbamate were significantly greater than those in mice treated with the approximately equimolar amount of 25 mg *N*-hydroxyethyl carbamate. After 40 weeks of croton oil application, the incidence and multiplicity (± SD) of skin tumours were 16/18 (89%) and 1.6 ± 0.3 for mice treated with 25 mg ethyl carbamate and 19/20 (95%) and 1.5 ± 0.3 for mice treated with 25 mg *N*-hydroxyethyl carbamate versus 11/44 and 0.4 ± 0.1 for mice treated with croton oil only. The skin-tumour incidence [$P < 0.0001$; one-tailed Fisher's exact test] and tumour multiplicity [$P < 0.001$; one-way ANOVA followed by SNK test] in each of the treatment groups were significantly increased compared with the croton-oil control mice. After 40 weeks of croton-oil application, the incidence and multiplicity (± standard deviation) of lung tumours were 12/18 (67%) and 3.4 ± 1.3 for mice treated with 25 mg ethyl carbamate and 9/20 (45%) and 0.75 ± 0.3 for mice

treated with 25 mg *N*-hydroxyethyl carbamate versus 2/42 (5%) and 0.05 ± 0.03 for mice treated with croton oil only. The lung-tumour incidence [$P \leq 0.0003$; one-tailed Fisher's exact test] and tumour multiplicity [$P < 0.001$; one-way ANOVA followed by SNK test] in each of the treatment groups were significantly increased compared with the croton-oil control mice. The tumour multiplicity in mice treated with 25 mg ethyl carbamate was significantly greater than that in mice treated with the approximately equimolar amount of 25 mg *N*-hydroxyethyl carbamate [$P < 0.001$; two-tailed Fisher's exact test] (Berenblum *et al*., 1959).

3.5.2 *Dermal application*

Mouse

Groups of 40 female CD-1 mice, 6–8 weeks of age, were pretreated topically on the shaved back with 1.2 mg croton oil in 200 μL redistilled acetone. Eighteen to 24 hours later, each mouse was treated topically with 5 or 60 mg ethyl carbamate (> 99% pure by gas chromatography) or 5 mg vinyl carbamate (melting-point, 53–54°C; purity verified by elemental analysis, MS, infrared and nuclear magnetic resonance spectroscopy) in 200 μL acetone or the solvent alone. The application of the carbamate compounds or solvent was repeated 1 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. The lungs were fixed in formalin, and adenomas on the surface (≥ 1 mm in diameter) were counted. Representative tumours were fixed, sectioned and stained with haematoxylin and eosin. The incidence of skin papillomas and the average number of papillomas per mouse at 29 weeks were 1/40 (2%) and 0 for mice treated with the solvent, 10/40 (25%) and 0.3 for mice treated with a total of 10 mg ethyl carbamate, 19/40 (47%) and 3.4 for mice treated with a total of 120 mg ethyl carbamate and 23/35 (66%) and 4.5 for mice treated with a total of 10 mg vinyl carbamate. The incidence of skin papillomas in each of the treated groups was significantly greater than that in the control group [$P \leq 0.0035$; one-tailed Fisher's exact test]. The incidence of skin papillomas in the 10-mg vinyl carbamate-treated group was significantly greater than that in the approximately equimolar 10-mg ethyl carbamate-treated group [$P = 0.0004$; one-tailed Fisher's exact test]. The incidence of lung adenomas and the average number of lung adenomas per mouse at 32 weeks were 7/40 (17%) and 0.4 for mice treated with the solvent, 17/40 (42%) and 1.0 for mice treated with a total of 10 mg ethyl carbamate, 33/40 (82%) and 8.8 for mice treated with a total of 120 mg ethyl carbamate and 34/35 (97%) and 18.9 for mice treated with a total of 10 mg vinyl carbamate. The incidence of lung adenomas in each of the treated groups was significantly greater than that in the control group [$P \leq 0.0135$; one-tailed Fisher's exact test]. The incidence of lung adenomas in the 10-mg vinyl carbamate-treated

group was significantly greater than that in the approximately equimolar 10-mg ethyl carbamate-treated group [$P < 0.0001$; one-tailed Fisher's exact test] (Dahl *et al*., 1978).

In a second experiment, groups of 30–33 female CD-1 mice, 6–8 weeks of age, were treated topically on the shaved back with 1.2 mg croton oil in 200 μL redistilled acetone. Eighteen to 24 hour later, each mouse was treated topically with 2.5, 5 or 60 mg ethyl carbamate or 2.5 or 5 mg vinyl carbamate in 200 μL acetone or the solvent alone. The application of the carbamate compounds or solvent was repeated 1 week later. One week after the second application, all mice were treated twice weekly with 900 μg croton oil in 150 μL acetone. The experiment lasted 35 weeks, at which time ≥ 90% of the mice were still alive. The incidence of skin papillomas and the average number of papillomas per mouse at 32 weeks were 0/30 and 0 for mice treated with the solvent, 3/30 (10%) and 0.1 for mice treated with a total of 5 mg ethyl carbamate, 4/30 (13%) and 0.2 for mice treated with a total of 10 mg ethyl carbamate, 11/29 and 1.8 for mice treated with a total of 120 mg ethyl carbamate, 14/30 (38%) and 1.8 for mice treated with a total of 5 mg vinyl carbamate and 12/32 (37%) and 2.0 for mice treated with a total of 10 mg vinyl carbamate. The incidence of skin papillomas in the 120-mg ethyl carbamate-treated group and each of the vinyl carbamate-treated groups was significantly greater than that in the control group [$P \leq 0.0001$; one-tailed Fisher's exact test]. The incidence of skin papillomas in each of the vinyl carbamate-treated groups was significantly greater than that in the approximately equimolar ethyl carbamate-treated groups [$P \leq 0.0055$; one-tailed Fisher's exact test]. The incidence of lung adenomas and the average number of lung adenomas per mouse at 35 weeks were 15/27 (55%) and 0.9 for mice treated with the solvent, 13/28 (46%) and 0.9 for mice treated with a total of 5 mg ethyl carbamate, 16/30 (53%) and 1.0 for mice treated with a total of 10 mg ethyl carbamate, 24/29 (83%) and 7.3 for mice treated with a total of 120 mg ethyl carbamate, 27/30 (90%) and 4.5 for mice treated with a total of 5 mg vinyl carbamate and 32/32 (100%) and 12.0 for mice treated with a total of 10 mg vinyl carbamate. The incidence of lung adenomas in the 120-mg ethyl carbamate-treated group and each of the vinyl carbamate-treated groups was significantly greater than that in the control group [$P \leq 0.0268$; one-tailed Fisher's exact test]. The incidence of lung adenomas in each of the vinyl carbamate-treated groups was significantly greater than that in the approximately equimolar ethyl carbamate-treated groups [$P \leq 0.0004$; one-tailed Fisher's exact test] (Dahl *et al*., 1978).

Groups of 30 female CD-1 mice, 6–8 weeks of age, were treated topically on the shaved back with 2.5 μg TPA [purity not specified] in 100 μL acetone. Eighteen to 24 hours later, the mice received 5.8 or 11.5 μmol vinyl carbamate [purity not specified] or 5.8 or 11.5 μmol vinyl carbamate epoxide [purity not specified] in 200 μL acetone that contained 15% dimethyl sulfoxide (DMSO). The application of the vinyl carbamate and vinyl carbamate epoxide was repeated at weekly intervals for a total of five applications. This was then followed by twice weekly topical applications of 2.5 μg TPA in 100 μL acetone. Control mice were administered the solvent and TPA only. The experiment was terminated 22 weeks after the first application of vinyl carbamate

and vinyl carbamate epoxide. At this time, 95–100% of the mice were still alive. The average number of papillomas per mouse (± SD), as determined by gross examination, was 6.5 ± 5.2 for 5.8 μmol vinyl carbamate-treated, 10.5 ± 8.4 for 11.5 μmol vinyl carbamate-treated, 13.3 ± 9.2 for 5.8 μmol vinyl carbamate epoxide-treated, 13.8 ± 9.0 for 11.5 μmol vinyl carbamate epoxide-treated and 0.1 ± 0.3 for the solvent control animals. The average number of papillomas per mouse was significantly greater in each of the treated groups compared with the control group [$P < 0.001$; one-way ANOVA followed by SNK test] and significantly greater in the 5.8-μmol vinyl carbamate epoxide-treated group compared with the 5.8-μmol vinyl carbamate-treated group [$P < 0.001$; one-way ANOVA followed by SNK test] (Park *et al*., 1993).

In a second experiment, groups of 30 female CD-1 mice, 6–8 weeks of age, were treated topically on the shaved back with 2.5 μg TPA in 100 μL acetone. Eighteen to 24 hours later, the mice received applications of 1.15 or 11.5 μmol vinyl carbamate or 1.15 or 11.5 μmol vinyl carbamate epoxide in 200 μL acetone that contained 15% DMSO. Beginning 1 week after the treatment with vinyl carbamate or vinyl carbamate epoxide, the mice received twice-weekly topical applications of 2.5 μg TPA in 100 μL acetone. Control mice were given the solvent or TPA only. The experiment ended 22 weeks after the first application of vinyl carbamate and vinyl carbamate epoxide. At this time, 97–100% of the mice were still alive. The incidence of papillomas and the average number of papillomas per mouse (± SD), as determined by gross examination, were 56% and 0.9 ± 1.1 for 1.15 μmol vinyl carbamate-treated, 98% and 7.8 ± 5.1 for 11.5 μmol vinyl carbamate-treated, 93% and 5.2 ± 3.5 for 1.15 μmol vinyl carbamate epoxide-treated, 100% and 9.8 ± 4.7 for 11.5 μmol vinyl carbamate epoxide-treated and 7% and 0.07 ± 0.2 for the solvent control animals. The incidence of papillomas was significantly greater in each of the treated groups compared with the controls [$P < 0.0001$; one-tailed Fisher's exact test] and significantly greater in the 1.15-μmol vinyl carbamate epoxide-treated group compared with the 1.15-μmol vinyl carbamate-treated group [$P = 0.0011$; one-tailed Fisher's exact test]. With the exception of the group treated with 1.15 μmol vinyl carbamate, the average number of papillomas per mouse was significantly greater in each of the treated groups compared with the controls [$P < 0.05$; one-way ANOVA followed by SNK test]. The average number of papillomas per mouse was significantly greater in the 1.15- and 11.5-μmol vinyl carbamate epoxide-treated groups compared with the 1.15- and 11.5-μmol vinyl carbamate-treated groups, respectively [$P \leq 0.027$; one-way ANOVA followed by SNK test] (Park *et al*., 1993).

In a third experiment, groups of 30 female CD-1 mice [age not specified] were treated topically on the shaved back once a week with vinyl carbamate or vinyl carbamate epoxide in 200 μL acetone that contained 15% DMSO at the following doses: 11.5 μmol at weeks 1 and 2, 5.7 μmol at weeks 3 and 4 and 3.8 μmol from weeks 5 to 32. The mice were kept for an additional 10 weeks after the last treatment. Control mice were given the solvent only. Survival was not indicated. Thirty-two weeks after the first application of vinyl carbamate and vinyl carbamate epoxide, the incidence of papillomas and the average number of papillomas per mouse (± SD), as determined by gross

examination, were 4% and 0.03 ± 0.2 for vinyl carbamate-treated, 96% and 4.6 ± 2.6 for vinyl carbamate epoxide-treated and 0% and 0.0 ± 0.0 for the solvent control animals. The incidence of papillomas [$P < 0.0001$; one-tailed Fisher's exact test] and the average number of papillomas per mouse [$P < 0.001$; one-way ANOVA followed by SNK] in the vinyl carbamate epoxide-treated group were significantly greater than those in both the vinyl carbamate-treated and control groups. Twelve mice that received vinyl carbamate epoxide also had epidermoid carcinomas compared with none in the vinyl carbamate-treated or solvent control groups, a difference that was significant [$P = 0.0001$; one-tailed Fisher's exact test]. Forty-two weeks after the first application of vinyl carbamate and vinyl carbamate epoxide, malignant tumours were detected in both groups (two mammary adenocarcinomas, one lymphoblastic lymphoma, one haemangioma and one epidermoid carcinoma in mice treated with vinyl carbamate and 18 epidermoid carcinomas, four keratoacanthomas, three squamous-cell fibrosarcomas and one thymic lymphoma in mice treated with vinyl carbamate epoxide). None of the control mice had malignant tumours (Park *et al*., 1993).

3.5.3 *Subcutaneous or intramuscular administration*

(a) Mouse

Weanling female albino mice [number not specified] were given a subcutaneous injection of 100 μL water containing 12 mg ethyl carbamate [purity not specified] or equimolar amounts of *N*-hydroxyethyl carbamate [purity not specified]. The treatment was repeated 4 days later. The treatment of the control group was not specified and the effect of treatment upon survival was not indicated. Five months after treatment, the mice were killed and adenomas on the surface of the lung were counted. The number sof lung adenomas observed grossly was 434 in 28 mice treated with ethyl carbamate, 159 in 35 mice administered *N*-hydroxyethyl carbamate and six in 30 control mice (Miller *et al*., 1960).

In a second experiment, weanling female albino mice [number not specified] were treated in a manner identical to that described for the previous experiment. Four and a half months after treatment, the mice were killed and adenomas on the surface of the lung were counted. The number of lung adenomas was 90 in 18 mice treated with ethyl carbamate, 30 in 20 mice administered *N*-hydroxyethyl carbamate and two in an unspecified number of control mice (Miller *et al*., 1960).

Newborn SWR/J mice [age, sex and number not specified], weighing 1.1–1.7 g, were given a single subcutaneous injection of 2 μmol/g bw ethyl carbamate [purity not specified] or *N*-hydroxyethyl carbamate (purified by redistillation) in 50 μL/g bw distilled water. The experiment lasted 10 weeks, at which time the incidence of lung adenomas was assessed. Histology was conducted on questionable tumours. No differences in body weights were observed. Survival was not specified and there was no control group. The mean number of adenomas per mouse (95% CI) was 2.3 (1.8–2.7) in

mice treated with 2 μmol/g bw ethyl carbamate and 0.4 (0.0–0.9) in mice treated with 2 μmol/g bw *N*-hydroxyethyl carbamate (Kaye & Trainin, 1966).

(b) Rat

Groups of 12 female Sprague-Dawley rats [age not specified] were given 10 weekly intramuscular injections in the left hind leg of 250 μL trioctanoin or 250 μL trioctanoin that contained 1.15 or 2.30 μmol vinyl carbamate [purity not specified] or vinyl carbamate epoxide [purity not specified]. At 17–18 months, the incidence of injection-site sarcomas and mammary gland tumours was determined. The incidence of injection-site sarcomas was 0/12 for the 1.15-μmol vinyl carbamate-treated group, 1/11 (9%) for the 1.15-μmol vinyl carbamate epoxide-treated group, 0/12 for the 2.30-μmol vinyl carbamate-treated group, 4/11 (36%) for the 2.30-μmol vinyl carbamate epoxide-treated group and 0/11 for the control group. The incidence of injection-site sarcomas was significantly increased in the 2.30-μmol vinyl carbamate epoxide-treated group compared with the 2.30-μmol vinyl carbamate-treated group and the control group [$P < 0.045$; one-tailed Fisher's exact test]. The incidence and total number of mammary gland tumours were 3/12 (25%) and six for the 1.15-μmol vinyl carbamate-treated group, 1/11 (9%) and three for the 1.15-μmol vinyl carbamate epoxide-treated group, 3/11 (27%) and eight for the 2.30-μmol vinyl carbamate-treated group, 6/11 (54%) and 16 for the 2.30-μmol vinyl carbamate epoxide-treated group and 3/11 (27%) and seven for the control group (Park *et al*., 1993).

3.5.4 *Intraperitoneal administration*

(a) Mouse

Groups of 18–30 male or female Swiss mice, 2–3 months of age, were administered a single intraperitoneal injection of 10 mg ethyl carbamate [0.11 mmol] or 11.8 mg *N*-hydroxyethyl carbamate [0.11 mmol] in saline [volume not specified], or 5 or 25 mg *N*-hydroxyethyl carbamate in distilled water [volume not specified]. A control group of 46 mice remained untreated. Four days after the initial treatment, all groups received twice weekly dermal applications of 5% croton oil in liquid paraffin [volume not specified]. The incidence and multiplicity of skin tumours were assessed after 20 and 40 weeks of croton oil application; those of lung tumours were assessed after 40 weeks of croton oil application. Histopathology was conducted on the lungs. Survival was ≥ 97% after 20 weeks of croton oil application and ≥ 80% after 40 weeks of croton oil application. After 20 weeks of croton oil application, the incidence and multiplicity (± SD) of skin tumours were 14/30 (47%) and 0.6 ± 0.1 for mice treated with 10 mg ethyl carbamate, 3/29 (10%) and 0.1 ± 0.05 for mice treated with 11.8 mg *N*-hydroxyethyl carbamate, 14/20 (70%) and 1.0 ± 0.2 for mice treated with 25 mg *N*-hydroxyethyl carbamate and 4/18 (22%) and 0.3 ± 0.1 for mice treated with 5 mg *N*-hydroxyethyl carbamate versus 3/45 (7%) and 0.07 ± 0.05 for mice treated with

croton oil only. The skin tumour incidence was significantly increased in mice treated with 10 mg ethyl carbamate or 25 mg *N*-hydroxyethyl carbamate compared with the croton oil control mice [$P \leq 0.0001$; one-tailed Fisher's exact test]. The tumour multiplicity was significantly increased in all treatment groups [$P < 0.001$; one-way ANOVA followed by SNK test], with the exception of the mice treated with 11.8 mg *N*-hydroxyethyl carbamate. The incidence [$P = 0.0034$; two-tailed Fisher's exact test] and multiplicity [$P < 0.001$; one-way ANOVA followed by SNK test] of skin tumours in mice treated with 10 mg ethyl carbamate were significantly greater than those in mice treated with 11.8 mg *N*-hydroxyethyl carbamate. After 40 weeks of croton oil application, the incidence and multiplicity (± SD) of skin tumours were 18/30 (60%) and 0.9 ± 0.2 for mice treated with 10 mg ethyl carbamate, 6/28 (21%) and 0.2 ± 0.1 for mice treated with 11.8 mg *N*-hydroxyethyl carbamate, 17/18 (95%) and 1.9 ± 0.2 for mice treated with 25 mg *N*-hydroxyethyl carbamate and 8/18 (44%) and 0.25 ± 0.05 for mice treated with 5 mg *N*-hydroxyethyl carbamate versus 11/44 (25%) and 0.4 ± 0.1 for mice treated with croton oil only. The incidence [$P \leq 0.0026$; one-tailed Fisher's exact test] and multiplicity [$P < 0.001$; one-way ANOVA followed by SNK test] of skin tumours were significantly increased in mice treated with 10 mg ethyl carbamate or 25 mg *N*-hydroxyethyl carbamate compared with the croton oil control mice. The incidence [$P = 0.0037$; two-tailed Fisher's exact test] and multiplicity [$P < 0.001$; one-way ANOVA followed by SNK test] of skin tumours in mice treated with 10 mg ethyl carbamate were significantly greater than those in mice treated with 11.8 mg *N*-hydroxyethyl carbamate. After 40 weeks of croton oil application, the incidence and multiplicity (± SD) of lung tumours were 23/26 (88%) and 2.8 ± 0.5 for mice treated with 10 mg ethyl carbamate, 5/26 (19%) and 0.3 ± 0.1 for mice treated with 11.8 mg *N*-hydroxyethyl carbamate, 11/18 (6%) and 0.8 ± 0.2 for mice treated with 25 mg *N*-hydroxyethyl carbamate and 5/18 (28%) and 0.4 ± 0.1 for mice treated with 5 mg *N*-hydroxyethyl carbamate versus 2/42 (5%) and 0.05 ± 0.03 for mice treated with croton oil only. The lung-tumour incidence was significantly increased in mice treated with 10 mg ethyl carbamate or 25 mg *N*-hydroxyethyl carbamate compared with the croton-oil control mice [$P < 0.0001$; one-tailed Fisher's exact test]. Lung tumour multiplicity was significantly increased in all treatment groups [$P < 0.001$; one-way ANOVA followed by SNK test]. The incidence [$P < 0.0001$; two-tailed Fisher's exact test] and multiplicity [$P < 0.001$; one-way ANOVA followed by SNK test] of lung tumours in mice treated with 10 mg ethyl carbamate were significantly greater than those in mice treated with 11.8 mg *N*-hydroxyethyl carbamate (Berenblum *et al*., 1959).

Groups of 20 female Holtzman mice, 10 weeks of age, received an intraperitoneal injection of 200 µL water that contained 15 mg ethyl carbamate [0.17 mmol; purity not specified] or 17.7 mg *N*-hydroxyethyl carbamate [0.17 mmol; purity not specified]. A second, identical injection was given 4 hours later. After 1 week, the backs of the mice were shaved and 300 µL acetone that contained 0.3% croton oil was applied topically once a week for 18 weeks. There was no control group. After 18 weeks, 12/19 (63%) surviving mice treated with ethyl carbamate had a total of 33 skin papillomas and 9/18

(50%) surviving mice treated with *N*-hydroxyethyl carbamate had a total of 25 skin papillomas. [The incidence did not differ between the groups; $P = 0.3175$, one-tailed Fisher's exact test.] The mice were killed after 22 weeks, at which time 11/19 (58%) surviving mice treated with ethyl carbamate had a total of 57 lung adenomas and eight of 18 surviving mice treated with *N*-hydroxyethyl carbamate had a total of 29 lung adenomas. [The incidence did not differ between the groups; $P = 0.3127$, one-tailed Fisher's exact test] (Miller *et al*., 1960).

Groups of 22–25 female weanling SWR/J mice, 9–10 weeks of age, were given a single intraperitoneal injection of 5 or 10 μmol/g bw ethyl carbamate [purity not stated] or *N*-hydroxyethyl carbamate (purified by redistillation) in distilled water. The ethyl carbamate was administered as a 5 or 10% or 5-mM solution; the *N*-hydroxyethyl carbamate was given as a 5-mM solution. Additional groups that received 10 μmol/g bw ethyl carbamate or *N*-hydroxyethyl carbamate were also given 50 μg/g bw 2-diethylaminoethyl-2,2-diphenylpentanoate hydrochloride (SKF-525A) [purity not specified] dissolved in distilled water at a concentration of 5 mg/mL. Controls received injections of the same volume of 0.9% saline. SKF-525A inhibits the conversion of *N*-hydroxyethyl carbamate to ethyl carbamate. The experiment lasted 10 weeks, at which time the incidence of lung adenomas was assessed. Histology was conducted on questionable tumours. There were no differences in body weights, and survival was ≥ 88%. The incidence of adenomas and the mean number of adenomas per survivor (95% CI) were 57% and 1.0 (0.5–1.6) in mice treated with 5 μmol/g bw ethyl carbamate, 27% and 0.4 (0.1–0.7) in mice treated with 5 μmol/g bw *N*-hydroxyethyl carbamate, 100% and 4.0 (2.9–5.1) in mice treated with 10 μmol/g bw ethyl carbamate, 75% and 1.9 (1.2–2.5) in mice treated with 10 μmol/g bw *N*-hydroxyethyl carbamate, 96% and 4.1 (3.0–5.1) in mice treated with 10 μmol/g bw ethyl carbamate and 50 μg/g bw SKF-525A and 62% and 0.6 (0.4–0.9) in mice treated with 10 μmol/g bw *N*-hydroxyethyl carbamate and 50 μg/g bw SKF-525A. The incidence of adenomas [$P = 0.0127$; two-tailed Fisher's exact test] and mean number of adenomas per survivor in mice treated with 10 μmol/g bw *N*-hydroxyethyl carbamate were significantly lower than those in mice treated with 10 μmol/g bw ethyl carbamate. The mean number of adenomas per survivor in mice treated with 10 μmol/g bw *N*-hydroxyethyl carbamate and 50 μg/g bw SKF-525A was significantly lower than that in mice treated with 10 μmol/g bw *N*-hydroxyethyl carbamate alone (Kaye & Trainin, 1966).

Groups of 40–42 female CD-1 mice, 6–8 weeks of age, were treated topically on the shaved back with 1.2 mg croton oil in 200 μL redistilled acetone. Eighteen to 24 hours later, each mouse received a single intraperitoneal injection of 65 μg/g bw ethyl carbamate (> 99% pure by gas chromatography) or vinyl carbamate (melting point, 53–54°C, purity verified by elemental analysis, MS, infrared and nuclear magnetic resonance spectroscopy) in 5 μL/g bw 0.87% saline or the solvent alone. An additional group received two intraperitoneal injections of 1.0 mg/g bw ethyl carbamate in 5 μL/g bw 0.9% saline at a 1-week interval. One week after the last application, all mice were treated topically twice a week with 900 μg croton oil in 150 μL acetone.

The experiment lasted 28 weeks, at which time ≥ 63% of the mice were still alive. All animals were subjected to gross necropsy. The lungs were fixed in formalin and adenomas on the surface (≥ 1 mm in diameter) were counted. Representative tumours were fixed, sectioned and stained with haematoxylin and eosin. The incidence and the average number of skin papillomas per mouse at 25 weeks were 1/41 (2%) and 0 for mice treated with the solvent, 5/41 (12%) and 0.2 for mice treated with 65 μg/g bw ethyl carbamate, 24/37 (65%) and 5.4 for mice treated with a total of 2 mg/g bw ethyl carbamate and 15/26 (58%) and 3.9 for mice treated with 65 μg/g bw vinyl carbamate. The incidence of skin papillomas in the 2-mg/g bw ethyl carbamate-treated group and the 65-μg/g bw vinyl carbamate-treated group was significantly greater than that in the control group [$P < 0.0001$; one-tailed Fisher's exact test]. The incidence of skin papillomas in the 65-μg/g bw vinyl carbamate-treated group was significantly greater than that in the approximately equimolar 65-μg/g bw ethyl carbamate-treated group [$P = 0.0001$; one-tailed Fisher's exact test]. The incidence and the average number of lung adenomas per mouse at 28 weeks were 4/41 (10%) and 0.2 for mice treated with the solvent, 14/39 (36%) and 0.6 for mice treated with 65 μg/g bw ethyl carbamate, 30/32 (94%) and 28.3 for mice treated with a total of 2 mg/g bw ethyl carbamate and 24/26 (93%) and 19.2 for mice treated with 65 μg/g bw vinyl carbamate. The incidence of lung adenomas in each of the treated groups was significantly greater than that in the control group [$P \leq 0.0051$; one-tailed Fisher's exact test]. The incidence of lung adenomas in the 65-μg/g bw vinyl carbamate-treated group was significantly greater than that in the approximately equimolar 65-μg/g bw ethyl carbamate-treated group [$P < 0.0001$; one-tailed Fisher's exact test] (Dahl *et al*., 1978).

In a second experiment, groups of 20 or 33 female A/Jax mice, 6–8 weeks of age, were given a single intraperitoneal injection of 32 or 65 μg/g bw ethyl carbamate or vinyl carbamate in 5 μL/g bw 0.9% saline or 500 μg/g bw ethyl carbamate in 5 μL 0.9% saline or the solvent alone. The experiment lasted 22 weeks. At this time, survival was ≥ 95% in all groups except for the 65-μg/g bw vinyl carbamate-treated group, in which survival was 65%. The incidence of lung adenomas and the average number of lung adenomas per mouse were 3/20 (15%) and 0.2 for mice treated with the solvent, 15/20 (75%) and 0.8 for mice treated with 32 μg/g bw ethyl carbamate, 17/20 (85%) and 1.7 for mice treated with 65 μg/g bw ethyl carbamate, 19/19 (100%) and 17.4 for mice treated with 500 μg/g bw ethyl carbamate, 33/33 (100%) and 42.3 for mice treated with 32 μg/g bw vinyl carbamate and 13/13 (100%) and 19.1 for mice treated with 65 μg/g bw vinyl carbamate. The incidence of lung adenomas in each of the treated groups was significantly greater than that in the control group [$P \leq 0.0002$; one-tailed Fisher's exact test]. The incidence of lung adenomas in the 32-μg/g bw vinyl carbamate-treated group was significantly greater than that in the approximately equimolar 32-μg/g bw ethyl carbamate-treated group [$P = 0.0054$; one-tailed Fisher's exact test] (Dahl *et al*., 1978).

In a third experiment, groups of 20 or 30 female A/Jax mice, 6–8 weeks of age, received a single intraperitoneal injection of 16, 32 or 65 μg/g bw vinyl carbamate in 5 μL/g bw 0.9% saline or the solvent alone. The experiment lasted 28 weeks. At this time,

survival was ≥ 85% in all groups except for the 65-μg/g bw vinyl carbamate-treated, in which survival was 27%. The incidence of lung adenomas and the average number of lung adenomas per mouse were 5/17 (29%) and 0.4 for mice treated with the solvent, 20/20 (100%) and 20.0 for mice treated with 16 μg/g bw vinyl carbamate, 19/19 (100%) and 35.2 for mice treated with 32 μg/g bw vinyl carbamate and 8/8 (100%) and 21.4 for mice treated with 65 μg/g bw vinyl carbamate. The incidence of lung adenomas in each of the treated groups was significantly greater than that in the control group [$P \leq 0.0012$; one-tailed Fisher's exact test] (Dahl *et al*., 1978).

In a fourth experiment, groups of nine to 20 female A/Jax mice, 6–8 weeks of age, were given five intraperitoneal injections of 10 μg/g bw ethyl carbamate, a single intraperitoneal injection of 500 μg/g bw ethyl carbamate, 10 intraperitoneal injections of 5 μg/g bw vinyl carbamate, five intraperitoneal injections of 10 μg/g bw vinyl carbamate or a single intraperitoneal injection of 16 μg/g bw vinyl carbamate. Multiple injections were given at weekly intervals. The compounds were dissolved in 5 μL/g bw 0.9% saline. The control group received 10 weekly injections of the solvent alone. The experiment lasted 20 weeks and all animals survived. The incidence and the average number of lung adenomas per mouse were 3/14 (21%) and 0.4 for mice treated with the solvent, 15/20 (75%) and 1.2 for mice treated with five injections of 10 μg/g bw ethyl carbamate, 9/9 (100%) and 19.3 for mice treated with a single injection of 500 μg/g bw ethyl carbamate, 19/19 (100%) and 25.2 for mice treated with 10 injections of 5 μg/g bw vinyl carbamate, 20/20 (100%) and 53.2 for mice treated with five injections of 10 μg/g bw vinyl carbamate and 20/20 (100%) and 25.2 for mice treated with a single injection of 16 μg/g bw vinyl carbamate. The incidence of lung adenomas in each of the treated groups was significantly greater than in the control group [$P \leq 0.0028$; one-tailed Fisher's exact test]. The incidence of lung adenomas in the mice that received five injections of 10 μg/g bw vinyl carbamate was significantly greater than that in mice that received five injections of approximately equimolar 10 μg/g bw ethyl carbamate [$P = 0.0236$; one-tailed Fisher's exact test] (Dahl *et al*., 1978).

Male and female C57BL/6J × C3H/HeJ F_1 mice (B6C3F_1 mice) [initial number not specified], 1 day of age, were administered eight twice-weekly intraperitoneal injections of 46, 91, 136 or 5625 nmol/g bw ethyl carbamate [purity not specified], 46, 91 or 136 nmol/g bw vinyl carbamate [purity not specified but assessed by melting-point, infrared spectroscopy, MS, high-performance liquid chromatography and GC] or the solvent (5 μL/g bw 0.9% saline). Most (> 90%) of the mice survived the treatment, and 18–25 mice of each sex from each group were weaned. The study was terminated when the mice were 15–16 months old. All animals were subjected to gross necropsy. All tumours were fixed, sectioned and stained with haematoxylin and eosin. The incidence and multiplicity (± SD) of liver tumours (hepatomas) in male and female mice were, respectively: 6/25 (24%) and 0.2 ± 0.4 and 0/24 and 0.0 ± 0.0 for mice that received the solvent; 14/25 (56%) and 0.8 ± 0.9 and 2/23 (9%) and 0.1 ± 0.3 for mice that received 46 nmol/g bw ethyl carbamate; 22/25 (88%) and 2.5 ± 1.4 and 6/22 (27%) and 0.4 ± 0.9 for mice that received 91 nmol/g bw ethyl carbamate; 22/25 (88%) and 2.5 ± 1.9 and

8/23 (35%) and 0.8 ± 1.6 for mice that received 136 nmol/g bw ethyl carbamate; 9/9 (100%) and 3.1 ± 1.4 and 7/10 (70%) and 4.8 ± 5.1 for mice that received 5625 nmol/g bw ethyl carbamate; 15/19 (79%) and 3.6 ± 3.2 and 16/19 (84%) and 5.9 ± 3.9 for mice that received 46 nmol/g bw vinyl carbamate; 13/14 (93%) and 7.9 ± 9.6 and 17/19 (89%) and 2.5 ± 1.6 for mice that received 91 nmol/g bw vinyl carbamate; and 14/18 (78%) and 6.6 ± 5.8 and 10/12 (83%) and 5.6 ± 6.0 for mice that received 136 nmol/g bw vinyl carbamate. All groups, except for female mice treated with 46 nmol/g bw ethyl carbamate, had an increased multiplicity of hepatomas compared with their respective control groups. Also, equimolar doses of vinyl carbamate increased tumour multiplicity compared with equimolar doses of ethyl carbamate. Thymic lymphomas were only observed with 5625 nmol/g bw ethyl carbamate and 91 and 136 nmol/g bw vinyl carbamate. The incidence in male and female mice was, respectively, 5/17 (29%) and 9/20 (45%) for mice that received 5625 nmol/g bw ethyl carbamate, 3/19 (16%) and 4/21 (19%) for mice that received 91 nmol/g bw vinyl carbamate and 9/23 (39%) and 6/19 (32%) for mice that received 136 nmol/g bw vinyl carbamate. The increased incidence of thymic lymphomas compared with the respective control groups was significant in each of these groups, with the exception of male mice treated with 91 nmol/g bw vinyl carbamate. The incidence of thymic lymphomas in male and female mice treated with 136 nmol/g bw vinyl carbamate and female mice treated with 91 nmol/g bw vinyl carbamate was also significantly greater than that in the respective groups treated with an equimolar dose of ethyl carbamate. The incidence of lung adenomas in male and female mice was, respectively: 1/25 (4%) and 0/25 for mice that received the solvent; 0/25 and 2/24 (8%) for mice that received 46 nmol/g bw ethyl carbamate; 4/25 (16%) and 4/22 (22%) for mice that received 91 nmol/g bw ethyl carbamate; 2/25 (8%) and 6/23 (26%) for mice that received 136 nmol/g bw ethyl carbamate; 5/17 (29%) and 9/20 (45%) for mice that received 5625 nmol/g bw ethyl carbamate; 10/19 (53%) and 15/19 (79%) for mice that received 46 nmol/g bw vinyl carbamate; 15/19 (79%) and 16/21 (76%) for mice that received 91 nmol/g bw vinyl carbamate; and 10/23 (43%) and 10/19 (53%) for mice that received 136 nmol/g bw vinyl carbamate. All groups treated with vinyl carbamate (males and females combined) and the group treated with 5625 nmol/g bw ethyl carbamate had an increased incidence of lung adenomas compared with the control group. Also, equimolar doses of vinyl carbamate increased lung tumour incidence compared with equimolar doses of ethyl carbamate. The incidence of Harderian gland tumours in male and female mice was, respectively: 0/25 and 0/25 for mice that received the solvent; 0/25 and 1/24 (4%) for mice that received 46 nmol/g bw ethyl carbamate; 0/25 and 0/22 for mice that received 91 nmol/g bw ethyl carbamate; 2/25 (8%) and 3/23 (9%) for mice that received 136 nmol/g bw ethyl carbamate; 3/17 (18%) and 3/20 (15%) for mice that received 5625 nmol/g bw ethyl carbamate; 4/19 (21%) and 6/19 (32%) for mice that received 46 nmol/g bw vinyl carbamate; 0/19 and 5/21 (24%) for mice that received 91 nmol/g bw vinyl carbamate; and 1/23 (4%) and 4/19 (21%) for mice that received 136 nmol/g bw vinyl carbamate. Only female mice treated with vinyl carbamate and the male mice treated with 46 nmol/g bw vinyl carbamate had an

increased incidence of Harderian gland tumours compared with their respective control groups. Also, male and female mice treated with 46 nmol/g bw vinyl carbamate and female mice treated with 91 nmol/g bw vinyl carbamate had an increased Harderian gland tumour incidence compared with the respective groups treated with equimolar doses of ethyl carbamate (Dahl *et al*., 1980).

In a second experiment, groups of 30 female A/J mice, 6–8 weeks of age, received a single intraperitoneal injection of 3 or 6 μmol/g bw [ethyl-$^{1}H_5$]ethyl carbamate or [ethyl-$^{2}H_5$]ethyl carbamate (melting-point, 46–47 °C, satisfactory elemental analysis, mass spectrum) or the solvent (5 μL/g bw 0.9% saline). The experiment ended 5 months later, at which time most (≥ 87%) of the mice were still alive. The incidence and multiplicity (± SD) of lung adenomas were 8/30 (27%) and 0.3 ± 0.1 for mice that received the solvent; 30/30 (100%) and 5.3 ± 2.4 for mice that received 3 μmol/g bw [ethyl-$^{1}H_5$] ethyl carbamate; 26/26 (100%) and 4.7 ± 2.6 for mice that received 3 μmol/g bw [ethyl-$^{2}H_5$]ethyl carbamate; 29/29 (100%) and 10.9 ± 6.8 for mice that received 6 μmol/g bw [ethyl-$^{1}H_5$]ethyl carbamate; and 30/30 (100%) and 9.6 ± 4.4 for mice that received 6 μmol/g bw [ethyl-$^{2}H_5$]ethyl carbamate. The tumour multiplicity in mice that received [ethyl-$^{1}H_5$]ethyl carbamate did not differ statistically from that observed in mice that received equimolar doses of [ethyl-$^{2}H_5$]ethyl carbamate (Dahl *et al*., 1980).

In a third experiment, a group of 17–20 female A/J mice, 6–8 weeks of age, were administered a single intraperitoneal injection of 4000 nmol/g bw ethyl carbamate, 4000 nmol/g bw *N*-hydroxyethyl carbamate [purity not specified], 150 nmol/g bw vinyl carbamate or the solvent (5 μL/g bw 0.9% saline). Additional groups were pretreated immediately before injection with the carbamate test compounds with intraperitoneal injections of 40 nmol/g bw 2-(2,4-dichloro-6-phenyl)phenoxyethylamine (DPEA), an inhibitor of cytochrome-P450 (CYP). Mice in some of the DPEA-treated groups received seven additional intraperitoneal injections of DPEA at 2-hour intervals. The experiment was terminated 7 months later, at which time most of the mice were still alive. The incidence and multiplicity (± SD) of lung adenomas were 2/19 (10%) and 0.1 ± 0.3 for mice that received the solvent, 18/18 (100%) and 7.1 ± 3.7 for mice that received 4000 nmol/g bw ethyl carbamate, 17/19 (89%) and 4.0 ± 2.3 for mice that received 4000 nmol/g bw *N*-hydroxyethyl carbamate, and 15/15 (100%) and 11.3 ± 3.4 for mice that received 150 nmol/g bw vinyl carbamate. Treatment with a total dose of 320 nmol/g bw DPEA significantly decreased the tumour multiplicity in mice that received 4000 nmol/g bw *N*-hydroxyethyl carbamate (2.4 ± 1.6 versus 4.0 ± 2.3) (Dahl *et al*., 1980).

In a fourth experiment, groups of 10, 15 or 20 female A/J mice, 6–8 weeks of age, received a single intraperitoneal injection of 1120 or 5620 nmol/g bw ethyl carbamate, 950 or 4760 nmol/g bw *N*-hydroxyethyl carbamate, 57 or 115 nmol/g bw vinyl carbamate or the solvent (5 μL/g bw 0.9% saline). The experiment was terminated 6.5 months later, at which time most (> 90%) of the mice were still alive. The incidence and multiplicity (± SD) of lung adenomas were 7/15 (47%) and 0.7 ± 0.1 for mice that received the solvent, 14/15 (93%) and 3.7 ± 2.4 for mice that received 1120 nmol/g

bw ethyl carbamate, 15/15 (100%) and 17.9 ± 4.3 for mice that received 5620 nmol/g bw ethyl carbamate, 12/15 (80%) and 1.5 ± 1.0 for mice that received 950 nmol/g bw *N*-hydroxyethyl carbamate, 14/14 (100%) and 7.8 ± 3.8 for mice that received 4760 nmol/g bw *N*-hydroxyethyl carbamate, 9/10 (90%) and 3.7 ± 3.6 for mice that received 57 nmol/g bw vinyl carbamate, and 14/15 (93%) and 6.4 ± 3.1 for mice that received 115 nmol/g bw vinyl carbamate (Dahl *et al*., 1980).

In a fifth experiment, groups of 13–20 female A/J mice, 6–8 weeks of age, were given a single intraperitoneal injection of 2000 or 4000 nmol/g bw ethyl carbamate or *N*-hydroxyethyl carbamate, 75 or 150 nmol/g bw vinyl carbamate or the solvent (5 μL/g bw 0.9% saline). The experiment was terminated 6.5 months later, at which time most (> 80%) of the mice were still alive. The incidence and multiplicity (± SD) of lung adenomas were 7/16 (44%) and 0.7 ± 0.1 for mice that received the solvent, 15/15 (100%) and 4.3 ± 2.1 for mice that received 2000 nmol/g bw ethyl carbamate, 14/14 (100%) and 9.5 ± 3.6 for mice that received 4000 nmol/g bw ethyl carbamate, 10/15 (67%) and 1.1 ± 1.1 for mice that received 2000 nmol/g bw *N*-hydroxyethyl carbamate, 18/19 (95%) and 3.2 ± 2.2 for mice that received 4000 nmol/g bw *N*-hydroxyethyl carbamate, 19/19 (100%) and 3.8 ± 2.2 for mice that received 75 nmol/g bw vinyl carbamate, and 19/19 (100%) and 12.1 ± 4.0 for mice that received 150 nmol/g bw vinyl carbamate. Tumour multiplicity in mice treated with ethyl carbamate was significantly higher than that in mice treated with equimolar doses of *N*-hydroxyethyl carbamate [$P \leq 0.002$; one-way ANOVA followed by SNK test] (Dahl *et al*., 1980).

A study was conducted to determine whether vinyl carbamate showed the same strain-specific tumorigenicity patterns as ethyl carbamate. Specifically, groups of male and female A/J, C3HeB/FeJ (C3H) and C57BL/6J mice, 3–5 months of age, received single intraperitoneal injections of 100 μL 0.9% saline solution that contained 30, 100, 300 and 1000 mg/kg bw ethyl carbamate (≥ 99% pure) or 1, 3, 10, 30 and 60 mg/kg bw vinyl carbamate (≥ 99% pure). Two control groups, one untreated and the other injected with 100 μL 0.9% saline were available. The groups comprised 32 mice (16 males and 16 females), except for the C3H and C57BL/6J groups treated with 60 mg/kg bw vinyl carbamate, which comprised 16 mice (eight males and eight females). All animals were killed 24 weeks after the injection. At the end of the experiment, 26–32 mice were alive in each of the groups (14 and 16, respectively, in the C3H and C57BL/6J groups treated with 60 mg/kg bw vinyl carbamate). Only mice that survived to the end of the experiment were used to assess the extent of tumorigenicity. The incidence of lung tumours was determined by gross examination of the lungs using a dissecting microscope. The incidence and multiplicity (± SD) of lung tumours in A/J mice were: untreated control, 25% and 0.3 ± 0.54 tumours/mouse; 0.9% saline control, 28% and 0.4 ± 0.71 tumours/mouse; 30-mg/kg ethyl carbamate-treated, 71% and 0.9 ± 0.75 tumours/mouse; 100-mg/kg ethyl carbamate-treated, 94% and 1.7 ± 0.96 tumours/mouse; 300-mg/kg ethyl carbamate-treated, 100% and 7.3 ± 2.86 tumours/mouse; 1000-mg/kg ethyl carbamate-treated, 100% and 29.5 ± 7.67 tumours/mouse; 1-mg/kg vinyl carbamate-treated, 33% and 0.4 ± 0.68 tumours/mouse; 3-mg/kg vinyl carbamate-treated, 81% and 1.4 ± 1.08

tumours/mouse; 10-mg/kg vinyl carbamate-treated, 100% and 7.2 ± 4.16 tumours/mouse; 30-mg/kg vinyl carbamate-treated, 100% and 43.0 ± 12.33 tumours/mouse; and 60-mg/kg vinyl carbamate-treated, 100% and 40.2 ± 14.07 tumours/mouse. The incidence and multiplicity (± SD) of lung tumours in C3H mice were: untreated control, 3% and 0.0 ± 0.19 tumours/mouse; 0.9% saline control, 3% and 0.0 ± 0.17 tumours/mouse; 30-mg/kg ethyl carbamate-treated, 3% and 0.0 ± 0.19 tumours/mouse; 100-mg/kg ethyl carbamate-treated, 6% and 0.1 ± 0.25 tumours/mouse; 300-mg/kg ethyl carbamate-treated, 14% and 0.2 ± 0.47 tumours/mouse; 1000-mg/kg ethyl carbamate-treated, 23% and 0.3 ± 0.70 tumours/mouse; 1-mg/kg vinyl carbamate-treated, 0% and 0.0 ± 0.00 tumours/mouse; 3-mg/kg vinyl carbamate-treated, 0% and 0.0 ± 0.00 tumours/mouse; 10-mg/kg vinyl carbamate-treated, 20% and 0.4 ± 1.00 tumours/mouse; 30-mg/kg vinyl carbamate-treated, 47% and 0.8 ± 1.06 tumours/mouse; and 60-mg/kg vinyl carbamate-treated, 43% and 0.6 ± 0.76 tumours/mouse). The incidence and multiplicity (± SD) for lung tumours in C57BL/6J mice were: untreated control, 6% and 0.1 ± 0.25 tumours/mouse; 0.9% saline control, 3% and 0.0 ± 0.18 tumours/mouse; 30-mg/kg ethyl carbamate-treated, 13% and 0.1 ± 0.34 tumours/mouse; 100-mg/kg ethyl carbamate-treated, 13% and 0.1 ± 0.34 tumours/mouse; 300-mg/kg ethyl carbamate-treated, 23% and 0.3 ± 0.71 tumours/mouse; 1000-mg/kg ethyl carbamate-treated, 66% and 1.2 ± 1.39 tumours/mouse; 1-mg/kg vinyl carbamate-treated, 7% and 0.1 ± 0.40 tumours/mouse; 3-mg/kg vinyl carbamate-treated, 13% and 0.1 ± 0.34 tumours/mouse; 10-mg/kg vinyl carbamate-treated, 9% and 0.1 ± 0.42 tumours/mouse; 30-mg/kg vinyl carbamate-treated, 78% and 1.7 ± 1.53 tumours/mouse; and 60-mg/kg vinyl carbamate-treated, 100% and 6.1 ± 2.91 tumours/mouse. Lung-tumour incidence was significantly greater than that in the 0.9% saline control group in A/J mice with all doses of ethyl carbamate and ≥ 3 mg/kg vinyl carbamate, in C3H mice with doses of 1000 mg/kg ethyl carbamate and ≥ 10 mg/kg vinyl carbamate and in C57BL/6J mice with doses of ≥ 300 mg/kg ethyl carbamate and ≥ 30 mg/kg vinyl carbamate [$P \leq 0.04$; one-tailed Fisher's exact test]. In all three strains, lung tumour incidence with 30 mg/kg vinyl carbamate was significantly greater than that with the approximately equimolar dose of 30 mg/kg ethyl carbamate [$P \leq 0.001$; one-tailed Fisher's exact test]. Lung tumour multiplicity was significantly greater than that in the 0.9% saline control group in A/J mice with doses of ≥ 300 mg/kg ethyl carbamate and ≥ 10 mg/kg vinyl carbamate, in C3H mice with doses of ≥ 10 mg/kg vinyl carbamate and in C57BL/6 mice with doses of 1000 mg/kg ethyl carbamate and ≥ 30 mg/kg vinyl carbamate [$P < 0.05$; one-way ANOVA, followed by Dunnett's test, respectively]. In all three strains, lung tumour multiplicity with 30 mg/kg vinyl carbamate was significantly greater than that with the approximately equimolar dose of 30 mg/kg ethyl carbamate [$P < 0.0001$; one-way ANOVA followed by SNK test] (Allen *et al*., 1986).

Groups of male A/J mice [number not specified], 6 weeks of age, were administered a single intraperitoneal injection of 60 mg/kg bw vinyl carbamate [purity not specified] in 100 μL tricaprylin or the solvent alone. Interim killings were performed at 7, 8, 10, 12 and 14 months of age. The overall survival was not specified. Lungs were fixed

and examined histologically. The number of mice examined and the mean number of lung lesions (hyperplasias, adenomas and/or carcinomas) per mouse (± standard error [SE]) were four and 0.00 ± 0.00 for control mice and nine and 36.89 ± 4.46 for vinyl carbamate-treated mice killed at 7 months of age, five and 0.00 ± 0.00 for control and 12 and 31.25 ± 2.90 for vinyl carbamate-treated mice killed at 8 months of age, 11 and 36.73 ± 1.93 for vinyl carbamate-treated mice killed at 10 months of age (no control mice were sacrified at 10 months), 19 and 0.58 ± 0.14 for control and eight and 39.50 ± 3.58 for vinyl carbamate-treated mice killed at 12 months of age, 10 and 0.80 ± 0.33 for control and 44 and 37.34 ± 1.06 for vinyl carbamate-treated mice killed at 14 months of age. At each time-point (for which control animals were available), the number of lesions per mouse was significantly greater in the vinyl carbamate-treated animals [$P < 0.001$; Student's *t*-test] compared with the control group. At 7, 8, 10, 12 and 14 months, hyperplasias accounted for 32%, 8%, 2%, 2% and ~0%, respectively, of the lesions in the vinyl carbamate-treated mice, the relative contribution of adenomas was 66%, ~90%, ~82%, ~52% and 45%, respectively, and the relative contribution of carcinomas was 2%, 2%, ~16%, ~46% and 55%, respectively (Foley *et al*., 1991).

A group of 55 male and 50 female C57Bl/10J mice, 4–6 weeks of age, received intraperitoneal injections of 6 mg/kg bw vinyl carbamate (purity, > 99%) in 10 µL/g bw sterile physiological saline once a week for 35 weeks. A group of 10 male and 10 female control mice remained untreated. Five vinyl carbamate-treated mice of each sex were killed at 5 weeks; the remaining mice formed the main body of the study. Male mice treated with vinyl carbamate weighed significantly less than control males beginning at week 14, and weighed 76% of the control males by 57 weeks. The body weight of the female mice was not affected by treatment with vinyl carbamate. There were few unscheduled early deaths during the 35-week treatment period; however, ~70% of the mice either died or were removed due to morbidity by the time the experiment was terminated at week 59. Gross necropsy was performed and histopathology was conducted. Treatment with vinyl carbamate resulted in the formation of hepatocellular adenomas (2/49 (4%) males and 1/45 (2%) females), hepatocellular carcinomas (8/49 (16%) males and 9/45 (20%) females), liver haemangiosarcomas (30/49 (6%) males and 25/45 (56%) females), liver haemangiomas (31/49 (63%) males and 24/45 (53%) females) and liver histiocytic sarcomas (6/49 (12%) males and 1/45 (2%) females). The incidence of liver haemangiosarcoma and liver hemangioma was significantly increased in both sexes compared with the control group [$P \leq 0.0015$; one-tailed Fisher's exact test] (Wright *et al*., 1991).

Groups of 30–50 female A/Jax mice, 6–8 weeks of age, received a single intraperitoneal injection of 5 µL/g bw trioctanoin or 5 µL/g bw trioctanoin that contained 34 or 68 nmol/g bw vinyl carbamate [purity not specified] or vinyl carbamate epoxide [purity not specified]. At 6 months, the mice were killed, the lungs were fixed in buffered formalin and the number of adenomas (> 1 mm in diameter) was determined. The number of mice that survived to the end of the experiment was 30/30 for the 34-nmol/g bw vinyl carbamate-treated group, 19/30 for the 34-nmol/g bw vinyl

carbamate epoxide-treated group, 30/30 for the 68-nmol/g bw vinyl carbamate-treated group, 15/50 for the 68-nmol/g bw vinyl carbamate epoxide-treated and 28/30 for the solvent-treated control group. The incidence of lung adenomas and the average number of lung adenomas per mouse (± SD) were 26/30 (87%) and 2.0 ± 1.4 for the 34-nmol/g bw vinyl carbamate-treated group, 16/19 (84%) and 1.4 ± 1.9 for the 34-nmol/g bw vinyl carbamate epoxide-treated group, 30/30 (100%) and 4.4 ± 2.5 for the 68-nmol/g bw vinyl carbamate-treated group, 13/15 (87%) and 3.8 ± 2.8 for the 68-nmol/g bw vinyl carbamate epoxide-treated group and 9/28 (32%) and 0.3 ± 0.5 for the solvent-treated control group. The incidence of lung adenomas in each of the treated groups was significantly greater than that in the control group [$P \leq 0.0007$; one-tailed Fisher's exact test]. The average number of lung adenomas per mouse was greater in the groups treated with 68 nmol/g bw vinyl carbamate and vinyl carbamate epoxide than in the control group [$P \leq 0.001$; one-way ANOVA followed by SNK test] (Park *et al*., 1993).

In a second study, groups of 26–29 male B6C3F1 mice, 12 days of age, received a single intraperitoneal injection of 10 µL trioctanoin or 10 µL/g bw trioctanoin that contained 1400 nmol/g bw ethyl carbamate, 29 nmol/g bw vinyl carbamate or 4.8, 12 or 24 nmol/g bw vinyl carbamate epoxide. At 9 months of age, the mice were killed and the number of hepatomas (> 2 mm in diameter and visible on the surface) were determined. The number of mice that survived to the end of the experiment was 28/28 for the 1400-nmol/g bw ethyl carbamate-treated, 29/29 for the 29 nmol/g bw vinyl carbamate-treated, 29/29 for the 4.8-nmol/g bw vinyl carbamate epoxide-treated, 5/27 for the 12-nmol/g bw vinyl carbamate epoxide-treated, 4/26 for the 24-nmol/g bw vinyl carbamate epoxide-treated and 29/29 for the solvent-treated control animals. The incidence of hepatomas and the average number of hepatomas per mouse (± SD) were 100% and 12.1 ± 3.5 for the 1400-nmol/g bw ethyl carbamate-treated group, 96% and 11.3 ± 5.0 for the 29-nmol/g bw vinyl carbamate-treated group, 28% and 0.4 ± 0.9 for the 4.8-nmol/g bw vinyl carbamate epoxide-treated group, 60% and 8.8 ± 9.1 for the 12-nmol/g bw vinyl carbamate epoxide-treated group, 100% and 49.0 ± 5.4 for the 24-nmol/g bw vinyl carbamate epoxide-treated group and 10% and 0.1 ± 0.3 for the solvent-treated control group. With the exception of the 4.8-nmol/g bw vinyl carbamate epoxide-treated group, the incidence of hepatomas [$P \leq 0.03$; one-tailed Fisher's exact test] and the average number of hepatomas per mouse [$P < 0.05$; one-way ANOVA followed by Dunnett's test] were greater in each of the treatment groups compared with the control group (Park *et al*., 1993).

Groups of 25 male NIH strain A mice, 6 weeks of age, were given single intraperitoneal injections of 10 mL/kg bw isotonic saline alone or containing 1.12, 4.6 or 11.2 mmol/kg bw 2-hydroxyethyl carbamate (purity not stated but assessed by melting-point, GC, nuclear magnetic resonance spectroscopy and MS) or 1.12 or 4.6 mmol/kg bw ethyl carbamate [purity not stated]. The mice were maintained for 16 weeks after the injection, at which time the incidence and multiplicity of lung adenomas was assessed. The incidence of lung adenomas (> 1 mm) was determined by gross examination using a dissecting microscope; representative tumours were sectioned

and examined histologically. With the exception of one mouse in the 4.6-mmol ethyl carbamate-treated group, all mice survived to the end of the experiment. No tumours were observed grossly outside of the lungs. The incidence and multiplicity (± SE) of lung adenomas were: 4/25 (16%) and 0.16 ± 0.07 tumours/mouse for the 1.12-mmol/kg bw 2-hydroxyethyl carbamate-treated group; 7/25 (28%) and 0.32 ± 0.11 tumours/mouse for the 4.6-mmol/kg bw 2-hydroxyethyl carbamate-treated group; 7/25 (28%) and 0.32 ± 0.11 tumours/mouse for the 11.2-mmol/kg bw 2-hydroxyethyl carbamate-treated group; 23/25 (92%) and 3.3 ± 0.3 tumours/mouse for the 1.12-mmol/kg bw ethyl carbamate-treated group; and 24/24 (100%) and 13.5 ± 0.8 tumours/mouse for the 4.6-mmol/kg bw ethyl carbamate-treated group; versus 1/25 (4%) and 0.04 ± 0.04 tumours/mouse for the control group. The incidence in each of the treated groups was significantly greater than that in the control group. The tumour multiplicity in the groups treated with ethyl carbamate was significantly greater than that in the control group. The incidence [$P < 0.0001$; two-tailed Fisher's exact test] and multiplicity [$P < 0.001$; one-way ANOVA followed by SNK test] in the ethyl carbamate-treated groups were significantly greater than those in the respective 2-hydroxyethyl carbamate-treated groups (Mirvish *et al*., 1994).

Male and female C57BL/6J × BALB/cJ mice ($B6CF_1$) [number not specified], 15 days of age, were administered a single intraperitoneal injection of 30 nmol/kg bw vinyl carbamate [purity not specified] in saline [volume not specified]. Subgroups of mice were killed at selected intervals from 30 to 122 weeks of age. Overall survival was not specified. Lungs were examined histologically. In those killed at 6–12 months of age, the number of mice examined, the percentage incidence of lung tumours (alveolar/bronchiolar adenomas or carcinomas) and number of tumours per mouse were: three, 0% and none for male control mice; three, 0% and none for female control mice; six, 0% and none for male vinyl carbamate-treated mice; and three, 0% and none for female vinyl carbamate-treated mice. For those killed at 12–18 months of age, the values were: 10, 30% and 0.40 for male control mice, 10, 10% and 0.20 for female control mice; 15, 33% and 0.40 for male vinyl carbamate-treated mice; and 15, 40% and 0.67 for female vinyl carbamate-treated mice. For those killed at 18–24 months of age, the values were: 27, 22% and 0.30 for male control mice; 47, 13% and 0.13 for female control mice; 65, 46% and 0.71 for male vinyl carbamate-treated mice; and 111, 45% and 0.76 for female vinyl carbamate-treated mice. The incidence of lung tumours was significantly greater in male and female vinyl carbamate-treated mice than in male and female control mice [$P = 0.0264$ and 0.0001, respectively; one-tailed Fisher's exact test]. For those killed at > 24 months of age, the values were: 42, 50% and 0.64 for male control mice; 45, 27% and 0.47 for female control mice; and 20, 45% and 1.0 for male vinyl carbamate-treated mice. For the entire experiment, the values were: 82, 37% and 0.48 for male control mice; 105, 18% and 0.28 for female control mice; 106, 41% and 0.68 for male vinyl carbamate-treated mice; and 129, 43% and 0.73 for female vinyl carbamate-treated mice. The incidence of lung tumours was significantly greater in female vinyl carbamate-

treated mice compared with female control mice [$P = 0.0001$; one-tailed Fisher's exact test] (Massey *et al*., 1995).

An experiment was conducted with $CB6F_1$-Tg *HRAS2* mice (*HRAS2* mice), a hemizygous transgenic mouse strain that carries the human prototype *c-Ha-RAS* gene, and their non-transgenic (non-Tg) littermates. Groups of 31 male and 29 female *HRAS2* and 31 male and 31 female non-Tg mice, 7 weeks of age, received a single intraperitoneal injection of 60 mg/kg bw vinyl carbamate [purity not specified] in 10 mL/kg bw sterile 0.9% saline. Control groups consisting of 10 male and 10 female *HRAS2* and 10 male and 10 female non-Tg mice received a single injection of the solvent. The experiment lasted 16 weeks. Nine male and nine female *HRAS2* mice that were treated with vinyl carbamate died before the end of the experiment. Mean body weights of both sexes of non-Tg mice treated with vinyl carbamate were significantly lower than their respective control non-Tg mice. Complete necropsy was performed. Target tissues (forestomach, lung and spleen) and any gross lesions were examined histopathologically. Statistical comparisons of differences in incidence and multiplicity between *HRAS2* and non-Tg mice were conducted using the one-tailed Fisher's exact test and Student's *t*-test, respectively. The percentage of mice killed 16 weeks after treatment with lung adenomas and the mean number of adenomas (± SD)/mouse were 100% and 14.76 ± 5.36 for male vinyl carbamate-treated *HRAS2* mice, 10.0% and 0.10 ± 0.32 for male solvent-treated *HRAS2* mice, 88.5% and 2.92 ± 2.10 for male vinyl carbamate-treated non-Tg mice, 0% and 0.0 ± 0.0 for male solvent-treated non-Tg mice, 100% and 20.53 ± 7.54 for female vinyl carbamate-treated *HRAS2* mice, 0% and 0.0 ± 0.0 for female solvent-treated *HRAS2* mice, 96.2% and 3.19 ± 1.55 for female vinyl carbamate-treated non-Tg mice and 0% and 0.0 ± 0.0 for female solvent-treated non-Tg mice. In both male and female *HRAS2* and non-Tg mice, the incidence of lung adenomas [$P < 0.0001$] and the mean number of adenomas/mouse [$P < 0.001$] were significantly greater in the mice treated with vinyl carbamate than in their respective control groups. In both male and female *HRAS2* mice treated with vinyl carbamate, the mean number of adenomas/mouse was significantly greater than that in male and female non-Tg mice treated with vinyl carbamate. The percentage of mice with lung carcinomas and the mean number of carcinomas (± SD)/mouse were 47.1% and 0.65 ± 0.79 for male vinyl carbamate-treated *HRAS2* mice, 0% and 0.0 ± 0.0 for male solvent-treated *HRAS2* mice, 3.9% and 0.04 ± 0.20 for male vinyl carbamate-treated non-Tg mice, 0% and 0.0 ± 0.0 for male solvent-treated non-Tg mice, 53.3% and 0.67 ± 0.72 for female vinyl carbamate-treated *HRAS2* mice, 0% and 0.0 ± 0.0 for female solvent-treated *HRAS2* mice, 0% and 0.0 ± 0.0 for female vinyl carbamate-treated non-Tg mice and 0% and 0.0 ± 0.0 for female solvent-treated non-Tg mice. In both male and female *HRAS2* mice, the incidence of lung carcinomas [$P \leq 0.01$] and the mean number of carcinomas/mouse [$P \leq 0.015$] were significantly greater in the mice treated with vinyl carbamate than in their respective control groups. In both male and female *HRAS2* mice treated with vinyl carbamate, the incidence of carcinomas and the mean number of carcinomas/mouse were significantly greater than those in male and

female non-Tg mice treated with vinyl carbamate. The percentage of mice with lung adenomas and carcinomas, and the mean number of adenomas and carcinomas (± SD)/mouse were 100% and 15.41 ± 5.43 for male vinyl carbamate-treated *HRAS2* mice, 10.0% and 0.10 ± 0.32 for male solvent-treated *HRAS2* mice, 88.5% and 2.96 ± 2.18 for male vinyl carbamate-treated non-Tg mice, 0% and 0.0 ± 0.0 for male solvent-treated non-Tg mice, 100% and 21.20 ± 7.59 for female vinyl carbamate-treated *HRAS2* mice, 0% and 0.0 ± 0.0 for female solvent-treated *HRAS2* mice, 96.2% and 3.19 ± 1.55 for female vinyl carbamate-treated non-Tg mice and 0% and 0.0 ± 0.0 for female solvent-treated non-Tg mice. In both male and female *HRAS2* and non-Tg mice, the incidence of adenomas and carcinomas [$P < 0.0001$] and the mean number of adenomas and carcinomas/mouse [$P < 0.001$] were significantly greater in the mice treated with vinyl carbamate compared with their respective controls. In both male and female *HRAS2* mice treated with vinyl carbamate, the mean number of adenomas and carcinomas/mouse was significantly greater than that in the male and female non-Tg mice treated with vinyl carbamate. The percentage of mice with spleen haemangiosarcomas and the mean number of spleen haemangiosarcomas (± SD)/mouse were 91% and 2.88 ± 1.50 for male vinyl carbamate-treated *HRAS2* mice, 10% and 0.10 ± 0.32 for male solvent-treated *HRAS2* mice, 0% and 0.0 ± 0.0 for male vinyl carbamate-treated non-Tg mice, 0% and 0.0 ± 0.0 for male solvent-treated non-Tg mice, 86% and 2.13 ± 1.46 for female vinyl carbamate-treated *HRAS2* mice, 10% and 0.10 ± 0.32 for female solvent-treated *HRAS2* mice, 0% and 0.0 ± 0.0 for female vinyl carbamate-treated non-Tg mice and 0% and 0.0 ± 0.0 for female solvent-treated non-Tg mice. In both male and female *HRAS2* mice, the incidence of spleen haemangiosarcomas [$P < 0.0001$] and mean number of spleen haemangiosarcomas/mouse [$P < 0.001$] were significantly greater in the mice treated with vinyl carbamate than in their respective control groups. In both male and female *HRAS2* mice treated with vinyl carbamate, the mean number of spleen haemangiosarcomas/mouse and incidence of spleen haemangiosarcomas were significantly greater than those in male and female non-Tg mice treated with vinyl carbamate. The percentage of mice with lung haemangiosarcomas was 11.8% for male vinyl carbamate-treated *HRAS2* mice, 0% for male solvent-treated *HRAS2* mice, 0% for male vinyl carbamate-treated non-Tg mice, 0% for male solvent-treated non-Tg mice, 20.0% for female vinyl carbamate-treated *HRAS2* mice, 0% for female solvent-treated *HRAS2* mice, 0% for female vinyl carbamate-treated non-Tg mice and 0% for female solvent-treated non-Tg mice. In female *HRAS2* mice treated with vinyl carbamate, the incidence of lung haemangiosarcomas was significantly greater than that in female non-Tg mice treated with vinyl carbamate. Male *HRAS2* mice treated with vinyl carbamate had a 5% incidence of forestomach papillomas and a 14% incidence of forestomach squamous-cell carcinomas. Female *HRAS2* mice treated with vinyl carbamate had a 5% incidence of forestomach squamous-cell carcinomas. These were not significantly elevated compared with the other treatment groups, in which papillomas and squamous-cell carcinomas were not detected. A low incidence of haemangiosarcomas of

the submandibular gland, epididymis and omentum (5%) was also detected in male vinyl carbamate-treated *HRAS2* mice only (Mitsumori *et al*., 1997).

A study was conducted to compare the prevalence of liver neoplasms among five strains of mice. Groups of male mice, 15 days of age, received a single intraperitoneal injection of either 100 µL saline or 100 µL saline that contained vinyl carbamate [stated as pure]. The strains of mice (amount of vinyl carbamate administered and number of mice examined) were B6D2F$_1$ (control, 64 mice; 30 nmol vinyl carbamate, 130 mice), B6C3F$_1$ (control, 138 mice; 30 nmol vinyl carbamate, 70 mice; 150 nmol vinyl carbamate, 128 mice), C3H (control, 73 mice; 30 nmol vinyl carbamate, 181 mice; 150 nmol vinyl carbamate, 139 mice), B6CF$_1$ (control, 97 mice; 30 nmol vinyl carbamate, 114 mice) and C57BL/6 (control, 166 mice; 30 nmol vinyl carbamate, 107 mice; 150 nmol vinyl carbamate, 231 mice). Three to five mice per group were killed at 3–5-week intervals. The first killing of B6C3F$_1$, C57BL/6 and C3H mice was performed at 36 days of age; that of B6D2F$_1$ and B6CF$_1$ mice was performed at 190 days of age. The final killing was conducted when six or fewer mice per group remained; this ranged between 448 and 869 days of age. Overall survival was not indicated. Representative sections from liver masses and lung metastases were examined histologically. The incidence of mice with hepatocellular adenoma, hepatocellular carcinoma and hepatocellular adenoma or carcinoma were: B6D2F$_1$ (control, 6.3%, 7.8% and 14.1%; 30-nmol vinyl carbamate-treated, 37.7%, 38.5% and 59.2%), B6C3F$_1$ (control, 8.0%, 5.1% and 12.3%; 30-nmol vinyl carbamate-treated, 70.0%, 34.3% and 72.9%; 150-nmol vinyl carbamate-treated, 45.3%, 28.1% and 45.3%), C3H (control, 2.7%, 5.5% and 8.2%; 30-nmol vinyl carbamate-treated, 47.5%, 21.5% and 48.6%; 150-nmol vinyl carbamate-treated, 56.1%, 33.8% and 59.7%); B6CF$_1$ (control, 5.2%, 3.1% and 7.2%; 30-nmol vinyl carbamate-treated, 15.8%, 10.5% and 22.8%) and C57BL/6 (control, 1.8%, 0.6% and 2.4%; 30-nmol vinyl carbamate-treated, 34.6%, 18.7% and 43.9%; 150-nmol vinyl carbamate-treated, 43.3%, 22.5% and 46.8%). The incidence of hepatocellular adenoma, hepatocellular carcinoma and hepatocellular adenoma or carcinoma in each of the groups treated with vinyl carbamate was significantly greater than that in the respective control groups [$P \leq 0.03$; one-tailed Fisher's exact test] (Takahashi *et al*., 2002).

Groups of 9–10 male C57BL/6 mice, 6–8 weeks of age, were injected intraperitoneally once or twice with 60 µg/g bw vinyl carbamate [purity not specified] dissolved in saline [volume not specified]. Mice injected once were killed 12 months later; mice that received two injections were dosed at a 1-week interval and killed 6 months after the second injection. No control mice were available. Lung tumours were evaluated histologically. In mice that received a single injection of vinyl carbamate, the incidence of lung adenomas was 5/10 (50%), with a multiplicity (± SE) of 0.50 ± 0.17 tumours/mouse. Lymphoid nodules, which were indistinguishable from epithelial adenomas, were also observed at an incidence of 2/10 (20%) and a multiplicity of 0.20 ± 0.13 tumours/mouse. In mice that received two injections of vinyl carbamate, the incidence of lung adenomas and lymphoid nodules was 1/9 (11%) and 1/9 (11%), with multiplicities of 0.11 ± 0.21 and 0.11 ± 0.21 tumours/mouse, respectively (Miller *et al*., 2003).

(b) Rat

Groups of male and female Fischer rats [initial number not specified], 1 day of age, were given 10 twice-weekly intraperitoneal injections of 92 or 3370 nmol/g bw ethyl carbamate [purity not specified] or five weekly or 10 twice-weekly intraperitoneal injections of 92 nmol/g bw vinyl carbamate (purity not specified but assessed by melting-point, infrared spectroscopy, MS, high-performance liquid chromatography and GC) or 10 twice-weekly intraperitoneal injections of the solvent (10 μL/g bw 0.9% saline). Most of the rats survived the treatment and 17–20 of each sex from each group were weaned. An additional group received five weekly intraperitoneal injections of 380 nmol/g bw vinyl carbamate. Most of these rats died within 3 weeks of being treated, but those remaining were allocated to the experiment. The study was terminated when the rats were 22–23 months old. All animals were subjected to gross necropsy. All tumours were fixed, sectioned and stained with haematoxylin and eosin. The incidence of hepatic carcinomas (mostly mixed hepatocellular-cholangiocellular carcinomas, with a few hepatocellular or cholangiocellular carcinomas) in the male and female rats, respectively, was 0/20 and 0/19 for 10 injections of the solvent, 3/20 (15%) and 0/20 for 10 injections of 92 nmol/g bw ethyl carbamate, 3/18 (17%) and 6/17 (35%) for 10 injections of 3370 nmol/g bw ethyl carbamate, 6/19 (32%) and 4/19 (21%) for five injections of 92 nmol/g bw vinyl carbamate, 6/18 (33%) and 10/20 (50%) for 10 injections of 92 nmol/g bw vinyl carbamate and 8/10 (80%) and 2/3 (67%) for five injections of 380 nmol/g bw vinyl carbamate, and that in all treated groups (males and females combined) was significantly increased compared with the control group, with the exception of rats that received 10 injections of 92 nmol/g bw ethyl carbamate, and that in the group that received 10 injections of 92 nmol/g bw vinyl carbamate was significantly greater than the incidence in the group that received 10 injections of 92 nmol/g bw ethyl carbamate. The incidence of ear duct carcinomas in male and female rats, respectively, was 1/20 (5%) and 0/19 for 10 injections of the solvent, 2/20 (10%) and 0/20 for 10 injections of 92 nmol/g bw ethyl carbamate, 4/18 (22%) and 1/17 (6%) for 10 injections of 3370 nmol/g bw ethyl carbamate, 1/19 (5%) and 2/19 (10%) for five injections of 92 nmol/g bw vinyl carbamate, 4/18 (22%) and 2/20 (10%) for 10 injections of 92 nmol/g bw vinyl carbamate and 4/10 (40%) and 1/3 (33%) for five injections of 380 nmol/g bw vinyl carbamate. The incidence of ear duct carcinomas (males and females combined) was significantly increased in the groups that received 10 injections of 92 nmol/g bw vinyl carbamate and five injections of 380 nmol/g bw vinyl carbamate compared with controls. The incidence of neurofibrosarcomas of the ear lobe in male and female rats, respectively, was 0/20 and 0/19 for 10 injections of the solvent, 0/20 and 0/20 for 10 injections of 92 nmol/g bw ethyl carbamate, 1/18 (5%) and 0/17 for 10 injections of 3370 nmol/g bw ethyl carbamate, 5/19 (26%) and 2/19 (10%) for five injections of 92 nmol/g bw vinyl carbamate, 4/18 (22%) and 1/20 (5%) for 10 injections of 92 nmol/g bw vinyl carbamate and 0/10 and 1/3 for five injections of 380 nmol/g bw vinyl carbamate. The incidence of neurofibrosarcomas of the ear lobe (males and females

combined) was significantly increased in the groups that received five and 10 injections of 92 nmol/g bw vinyl carbamate compared with controls. In addition, the incidence was increased in rats that received 10 injections of 92 nmol/g bw vinyl carbamate compared with rats that received 10 injections of 92 nmol/g bw ethyl carbamate. A low incidence of a variety of other tumours was also observed (Dahl *et al*., 1980).

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4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Data on the absorption, distribution, metabolism and excretion of ethyl carbamate in experimental animals have been reviewed (National Toxicology Program, 2004). Ethyl carbamate is rapidly distributed in body water after administration; it accumulates somewhat more slowly in adipose tissue than in other organs. Earlier studies with labelled ethyl carbamate indicated that it was largely oxidized to carbon dioxide. Its metabolism was suggested to proceed via an esterase reaction that released ethanol, carbon dioxide and ammonia. The rate of elimination was reported to be lower in newborn than in adult mice, which was attributed to the lack of microsomal esterase.

Human CYP2E1 was shown to be a major catalyst of the oxidation of both ethyl carbamate and vinyl carbamate in experiments with human liver microsomes (Guengerich & Kim, 1991; Guengerich *et al.*, 1991). Furthermore, when human liver microsomes were incubated with nicotinamide adenine dinucleotide phosphate (NADPH) and ethyl carbamate, the products vinyl carbamate, 2-hydroxyethyl carbamate and ethyl *N*-hydroxycarbamate were detected (Guengerich & Kim, 1991). The formation of 1,N^6-ethenoadenosine from adenosine in the presence of ethyl carbamate and vinyl carbamate was demonstrated in these studies and it was noted that this reaction was considerably slower with ethyl carbamate. In a separate study, Forkert *et al.* (2001) showed that the metabolism of vinyl carbamate in human lung microsomes is mediated by lung microsomal CYP2E1. Together, these studies suggest that, in human liver, ethyl carbamate can be converted to its proximate DNA-reactive metabolites, a mechanism similar to that suggested to play a role in carcinogenesis in rodents. [The Working

Group noted that (i) experimental evidence suggests great similarities between rodents and humans in the metabolic activation pathways of ethyl carbamate in target tissues (liver and lung); and (ii) the formation of the same proximate carcinogens that are DNA-reactive and thought to play a major role in ethyl carbamate-induced carcinogenesis in rodents probably also occurs in human cells.]

Ethyl carbamate is metabolized by CYP2E1. *N*-Hydroxylation products have carcinogenic properties, but are less potent than ethyl carbamate itself, and *N*-hydroxyethyl carbamate can be converted to ethyl carbamate (Dahl *et al.*, 1978, 1980; National Toxicology Program, 2004). *N*-Hydroxy derivatives are excreted in the urine as glucuronide and other conjugates. Oxidation of ethyl carbamate to vinyl carbamate, and thence to vinyl carbamate epoxide is thought to account for its carcinogenic properties (National Toxicology Program, 2004).

Yamamoto *et al.* (1988) reported that co-administration of ethanol with ethyl carbamate resulted in delayed clearance of ethyl carbamate and its metabolism to carbon dioxide in male mice; ethanol inhibited the metabolism of ethyl carbamate by liver homogenates. Carlson (1994) also found that ethanol inhibited the metabolism of ethyl carbamate, and that the CYP2E1 inhibitor, diethyldithiocarbamate, substantially reduced the metabolism of ethyl carbamate to carbon dioxide in rats.

Hoffler *et al.* (2003) examined the metabolism of ethyl carbamate in *CYP2E1*-knockout mice and in mice that had been treated with the CYP inhibitor, 1-aminobenzotriazole, and concluded that 96% of the metabolism of radiolabelled ethyl carbamate was mediated by CYP2E1. 1-Aminobenzotriazole also markedly inhibited the metabolism of ethyl carbamate in wild-type mice, and inhibited the residual metabolism in knockout mice. It was suggested that both the oxidation of ethyl carbamate to vinyl carbamate and the subsequent generation of the epoxide are catalyzed by CYP2E1.

Hoffler *et al.* (2005) studied the effects of administration of ethyl carbamate to *CYP2E1*-knockout mice for 6 weeks. The appearance of micronucleated erythrocytes was reduced in the knockout mice. Cell proliferation demonstrated by the appearance of K_i-67, was increased in the lung and liver of ethyl carbamate-treated wild-type mice, but not in the knockout animals. It was concluded that metabolism of ethyl carbamate via CYP2E1 was required for its genotoxicity.

These reports suggest that there are important interactions between ethanol and ethyl carbamate. The ability of ethanol to inhibit the clearance of ethyl carbamate suggests that it does so by competing for metabolic conversion by CYP2E1. Since chronic use of ethanol induces CYP2E1, prior chronic ethanol consumption could be predicted to increase the carcinogenicity of ethyl carbamate (as reported for mice treated with ethanol for 3 days; National Toxicology Program, 2004). Simultaneous exposure to ethanol and ethyl carbamate was reported in several studies to reduce the carcinogenicity of the latter (National Toxicology Program, 2004). However, in a 2-year toxicity study (National Toxicology Program, 2004), there was only a weak interaction between ethanol (0, 2.5 and 5% ethanol) and ethyl carbamate when the two compounds

were co-administered *ad libitum* in the drinking-water to mice (see Section 4.4.2(*b*)(i) of the monograph on Alcoholic beverage consumption.).

4.2 Toxic effects

4.2.1 *Humans*

A clinical trial of ethyl carbamate in patients with leukaemia (32 cases) and other types of somatic cancer (13 cases) involved oral administration of doses of 1–6 g per day for 5 to 109 days (Paterson *et al.*, 1946). The total dose varied by patient from 26 to 390 g. Nausea, vomiting and diarrhoea were reported as common side-ffects. Leukopoenia was observed in patients with somatic tumours, while the observed sharp fall in white cell counts was considered to be a beneficial effect in patients with leukaemia. These health effects were reversible when treatment with ethyl carbamate was discontinued. Similar side-effects were observed by Hirschboeck *et al.* (1948) in patients who took 0.5–2 g ethyl carbamate orally in capsules. When administered intramuscularly (2–4 mL of a 50% solution [1–2 g]), dizziness and drowsiness were also reported. No reports of the possible adverse health effects of ethyl carbamate when it was used as a co-solvent in Japanese patients (doses estimated to be 10–50 mg/kg bw; Nomura, 1975a) are available.

4.2.2 *Experimental systems*

Ethyl carbamate is known to induce acute toxic reactions in rodents. In female C57BL/6J mice that received subcutaneous injections of 4000 mg/kg bw ethyl carbamate for 12 days, spleen and thymus weights and circulating leukocyte levels were reduced (Luebke *et al.*, 1987). The immunocompetence of treated mice was also severely compromised, as measured by the delayed hypersensitivity reaction.

Female B6C3F_1 mice that received a total dose of 4000 mg/kg bw ethyl carbamate by intraperitoneal injection over 14 days also had lower spleen and thymus weights than the controls, but peripheral blood cell counts were not affected (Luster *et al.*, 1982). The presence of micronuclei in peripheral blood cells of mice following administration of ethyl carbamate supports the possibility that blood-forming organs are targets for the toxicity of ethyl carbamate (Bruce & Heddle, 1979). The hypnotic and anaesthetic properties of ethyl carbamate suggest neuropharmacological effects, which may become significant when the chemical is co-administered with ethanol (Salmon & Zeise, 1991).

Various toxic effects were reported in studies of ethyl carbamate administered for 13 weeks in the drinking-water or in 5% ethanol to rats and mice (National Toxicology Program, 1996). Increased lethality was observed in rats that received more than ~300 mg/kg bw ethyl carbamate. Ethyl carbamate was much more toxic in mice; all mice that received more than 1000 mg/kg bw and many that were given ~300 mg/kg died

before the end of the study. Animals in the high-dose groups had lower body weights, reduced water consumption and exhibited thinness, abnormal posture and ruffled fur. Leukopoenia (primarily lymphocytopoenia) was also observed in rats and mice that received doses of ethyl carbamate of ~20 mg/kg bw and ~300 mg/kg bw, respectively.

In separate 4-week and 2-year studies in which male and female B6C3F_1 mice were administered 10–90 mg/kg bw ethyl carbamate in the drinking-water or in 5% ethanol (National Toxicology Program, 2004), no adverse effects on body weight or water consumption were noted at 4 weeks, but increased lethality and decreases in body weight were observed in high-dose groups in the 2-year study.

In a study of ethyl carbamate in the drinking-water conducted by Inai *et al.* (1991), survival of male B6C3F_1 mice exposed to 100 mg/kg bw ethyl carbamate for 70 weeks was decreased, but not that of mice exposed to less than 10 mg/kg bw. A similar decrease in survival of NMRI mice exposed to concentrations of up to 12.5 mg/kg bw ethyl carbamate per day in the drinking-water began at approximately 85 weeks into the study (Schmähl *et al.*, 1977).

Acute oral administration of 1000 mg/kg bw ethyl carbamate in water to Swiss albino mice led to loss of consciousness for up to 5 hours (Abraham *et al.*, 1998).

Atrophy of the spleen and thymus was reported in BALB/c mice that received intraperitoneal injections of 200 and 400 mg/kg bw ethyl carbamate for 7 days (Cha *et al.*, 2000, 2001).

4.3 Reproductive toxicity and teratogenicity

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

(a) Teratogenic effects

(i) *Prenatal and transplacental (gestational) exposures*

Takaori *et al.* (1966) investigated the teratogenic response of Wistar rats to 1000 mg/kg bw ethyl carbamate given orally at different times during gestation: during 7 successive days of the first, second or third trimester, on 2 successive days during organogenesis or as a single dose on the 8th or 9th day of gestation. Fetal body weight was decreased in all treated groups compared with that of controls. The mean number of resorbed fetuses was increased in the animals treated during the first and second trimesters; a smaller increase occurred in animals treated during the third trimester. No gross malformations were apparent in the fetuses of dams treated in either the first or third trimester, but dams treated on days 8–13 of gestation produced offspring without tails and one with exencephaly. Offspring of animals treated during either trimester had increased incidences of skeletal malformations, which were most pronounced

when treatment occurred during days 6–12. In rats treated with two consecutive doses of ethyl carbamate (1000 mg/kg bw), similar observations were reported. The most pronounced effects were a decrease in placental weight, a decrease in the number of live fetuses, an increase in the number of resorbed fetuses and an increased incidence of skeletal malformations.

Ferm and Hanover (1966) injected ethyl carbamate once intraperitoneally or intravenously into female hamsters on gestation day 8 and the fetuses were taken 1–3 days later. An intravenous dose of 200 mg/kg bw led to abnormalities in 33% of the fetuses examined. Higher doses of 400, 800 or 1200 mg/kg bw given by either route 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.

Single intraperitoneal doses of 500–3000 mg/kg bw ethyl carbamate were injected into pregnant Syrian hamsters on day 8 of gestation, and fetuses were examined for malformations on day 13 of gestation (DiPaolo & Elis, 1967). Ethyl carbamate was lethal to pregnant dams at the 3000-mg/kg bw dose. At lower doses, a dose-dependent increase in the number of dead or resorbed fetuses was observed. Malformations (exencephaly, microcephaly, encephalocele) were detected in up to 10% of fetuses, although no dose-dependent effect was found.

Sinclair (1950) observed that female mice became infertile when injected subcutaneously with ethyl carbamate at a dose of 1500 mg/kg bw. Injection of 750 mg/kg bw ethyl carbamate into pregnant mice on day 7 of gestation caused abortions and lethal central nervous system defects in fetuses. Failure of the brain to close and degeneration of the brain and spinal cord were also seen in fetuses produced by mothers that were treated with the same dose on day 8 of gestation.

Nishimura and Kuginuki (1958) reported that intraperitoneal injection of 1500 mg/kg bw ethyl carbamate into pregnant mice during gestation days 3–9 led to fetal toxicity, but not malformations. Injection on days 7–8 caused resorption of all fetuses. After injection on days 9–12, fetal malformations (short tails and skeletal malformations) were found.

A single injection of 1500 mg/kg bw ethyl carbamate to CBA and C3HeB mice on day 8.5 of gestation induced exencephaly in both CBA and C3HeB fetuses, although marked strain differences were noted (Tutikawa & Harada, 1972).

Fetal malformations developed in the offspring of female ICR/Jcl mice administered ethyl carbamate by subcutaneous injection as early as day 5 of gestation with a high dose of 1500 mg/kg bw and on day 10 with lower doses of 500–1000 mg/kg bw (Nomura, 1974). An increased incidence of preimplantation loss and of early and late deaths was also reported in this study, but only with the high dose (1500 mg/kg bw) of ethyl carbamate.

Subcutaneous administration of 1000 mg/kg bw ethyl carbamate to pregnant ICR/Jcl mice on day 17 of gestation caused embryonic deaths and malformations (skeletal defects and cleft palate) in the offspring (Nomura, 1975b). Three subcutaneous

injections of 150 mg/kg bw ethyl carbamate to pregnant ICR/Jcl mice on days 9, 10 and 11 led to a significant increase in fetal malformations (Nomura, 1975a).

Nomura (1977) gave a single subcutaneous injection of 1000 mg/kg bw ethyl carbamate to pregnant ICR/Jcl mice on day 9, 10, or 11 of gestation. Cleft palates were the only anomaly seen in the offspring of animals treated on day 9. Polydactyly, cleft palates, tail anomalies and open eyelids were seen after treatment on day 10. Syndactyly, tail anomalies and cleft palates occurred after treatment on day 11. In a separate study, a single subcutaneous injection of 1000 mg/kg bw ethyl carbamate to pregnant ICR/Jcl mice on gestational day 10 led to fetal malformations such as cleft palates, tail anomalies and polydactyly (Nomura, 1983).

Nakane and Kameyama (1986) studied the teratogenicity of ethyl carbamate in CL/Fr mice, a strain that is characterized by a 30% incidence of spontaneous cleft lip with associated cleft palate in the offspring. Pregnant CL/Fr mice were treated with various doses of ethyl carbamate on different days of pregnancy. In the groups treated with 250, 500 and 750 mg/kg bw ethyl carbamate on day 9 of pregnancy, the frequency of cleft lip/palate decreased to 18%, 14% and 11% of term fetuses, respectively. In the group treated with 1000 mg/kg bw ethyl carbamate on day 9, the frequency of cleft lip/palate decreased to 6%, but isolated cleft palate was observed in 23% of term fetuses. Most fetuses in the same group had severe tail anomalies and showed marked loss in body weight.

Treatment of NMRI mice with a single intraperitoneal injection of 800 mg/kg bw ethyl carbamate on day 14 of gestation caused an increased incidence of polydactylism, cleft palate and microphthalmia in fetuses (Burkhard & Fritz-Niggli, 1987).

Treatment of ICR mice with a single subcutaneous injection of 1500 mg/kg bw ethyl carbamate on gestation day 10 resulted in cleft palate in approximately two-thirds of fetuses evaluated at gestation day 14 (Sharova *et al.*, 2003). The fetal weight:placental weight ratio was not changed by treatment with ethyl carbamate in this study; however, treatment resulted in lower weight of both clefted and morphologically normal fetuses.

(ii) *Parental exposures*

Maternal exposures

Nomura (1975b) observed that, when female ICR/Jcl mice received 1500 mg/kg bw ethyl carbamate and were subsequently mated with untreated males at 1–10-week intervals, dominant lethality was higher than that in controls at 2–3-week intervals. Malformed fetuses (open eyelids, kinky tails, cleft palates and dwarfism) were observed at a significantly higher incidence than in controls, and a higher incidence of malformations was observed in the offspring of ethyl carbamate-exposed females than in those of ethyl carbamate-exposed males.

Nomura (1982) administered a single subcutaneous injection of 1000 or 1500 mg/kg bw ethyl carbamate to female ICR mice; the mice were subsequently mated with untreated males (9 weeks). A significant increase in developmental anomalies was

detected in both 19-day-old fetuses and 7-day-old offspring at both doses with no clear dose–response.

In a subsequent study, Nomura (1988) reported a single subcutaneous injection of 1000–2000 mg/kg bw ethyl carbamate to female mice led to a dose-dependent increase in the incidence of phenotypic anomalies (cleft palate, dwarfism, tail anomalies, open eyelid) in the progeny from subsequent matings. It was noted that immature oocytes of 21-day-old females (mated 10 weeks after exposure) were more sensitive than mature oocytes, but no differences were observed in the anomalies detected after birth.

Paternal exposures

Jackson *et al.* (1959) injected male Wistar rats intraperitoneally with five daily doses of 250 mg/kg bw ethyl carbamate and reported no reduction in litter size following mating with unexposed females for up to 6 weeks after treatment.

Bateman (1967) injected male mice intraperitoneally with 1500 mg/kg bw ethyl carbamate and allowed them to mate with unexposed females. Females in the cage were changed each week for up to 9 weeks after treatment of the males. No significant effect on the number of implants, or early or late deaths was observed at any of the time-points. The study also attempted to increase exposure to ethyl carbamate through injections of 1500 mg/kg bw on 3 successive days. However, most males did not survive beyond 2 weeks after treatment. Nevertheless, no significant effect on the number of implants or early or late deaths was observed in embryos from pregnancies that occurred up to 3 weeks after treatment of the males.

Kennedy *et al.* (1973) administered a single intraperitoneal injection of 50 or 100 mg/kg bw ethyl carbamate to male mice and mated them with untreated virgin females that were changed weekly for 6 weeks. Females were sacrificed 1 week after removal from the breeding cage, and their uterine contents were evaluated for numbers of embryos, implantations and resorptions (early and late). The authors reported that genetic damage, as manifested by dominant lethal mutations, did not occur.

Nomura (1975b) administered a single subcutaneous injection of 1500 mg/kg bw ethyl carbamate to male mice, 9 weeks of age, and subsequently mated the mice with untreated females (9 weeks). Dominant lethality was significantly different from that in controls at all experimental stages. A significantly higher incidence of malformed fetuses (open eyelids, kinky tails, cleft palates and dwarfism) was observed after treatment than in controls.

Nomura (1982) administered a single subcutaneous injection of 1500–2000 mg/kg bw ethyl carbamate to male ICR mice and subsequently mated the mice with untreated females (9 weeks). No dominant lethality was detected at any stage of embryonic development. A significant increase in developmental anomalies was detected in both 19-day-old fetuses and 7-day-old offspring after both doses with no clear dose–response.

Russell *et al.* (1987) administered a single intraperitoneal injection of 1750 mg/kg bw ethyl carbamate to male (101 × C3H)F_1 mice that were then mated with unexposed females. Litter sizes from successive conceptions made in any of the first 7 weeks gave no indication of induced dominant lethality.

Nomura (1988) reported that paternal exposure to a single subcutaneous injection of 1000–2000 mg/kg bw ethyl carbamate led to a nonlinear dose-dependent increase in the incidence of phenotypic anomalies (cleft palate, dwarfism, tail anomalies, open eyelid) in F_1 progeny. It was noted that anomalies were induced more effectively in the F_1 fetuses by treatment at the stage of spermatozoa rather than at that of spermatogonia.

Edwards *et al.* (1999) treated male CD-1 mice with ethyl carbamate, either acutely by intraperitoneal injection of 1250 and 1750 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. One week after the end of each treatment, male mice were mated with untreated females. No genetic effect of acute treatment with ethyl carbamate on male germ cells, as indicated by dominant lethality, was observed. No skeletal or other malformations were observed following acute paternal exposure. A significant increase in post-implantation deaths was observed only after acute administration of ethyl carbamate (1750 mg/kg) and the authors suggested that this was possibly due to perinatal mortality, since no such increase occurred in the dominant lethal part of the study. No effects were observed in offspring of males treated subchronically with ethyl carbamate in the drinking-water.

(iii) *Postnatal exposures*

Increased tumour incidence is the most frequently reported effect of perinatal exposure to ethyl carbamate. These studies are described in detail in Section 3.

(b) Effects on male and female reproductive systems

Russell *et al.* (1987) administered a single intraperitoneal injection of 1750 mg/kg bw ethyl carbamate to male (101 × C3H)F_1 mice. Cytotoxic effects on male reproduction were evident from a slight reduction in the numbers of certain types of spermatogonia in seminiferous tubule cross-sections and a borderline decrease in the number of litters conceived during the 8th and 9th weeks after treatment.

Nomura (1988) reported that a single subcutaneous injection of 1000 or 1500 mg/kg bw ethyl carbamate to male mice did not decrease their fertility for up to 180 days after exposure.

Yu *et al.* (1999) reported no significant decreases in the total number of litters or the average number of offspring born per litter when male NIH Swiss mice were exposed intraperitoneally to 1500 mg/kg bw ethyl carbamate and mated with unexposed females 2 weeks later.

A 13-week study of ethyl carbamate administered in the drinking-water to Fischer 344/N rats (National Toxicology Program, 1996) reported that the only parameter affected in the reproductive system in males was lowered epididymal spermatozoal motility and concentration in the 78- and 287-mg/kg bw groups. When ethyl carbamate was administered in a 5% ethanol vehicle, the responses were similar to those with the drinking-water vehicle. The length of the estrous cycle of female rats that received 201 mg/kg bw ethyl carbamate in 5% ethanol was longer than that of the controls. This

effect was not observed when ethyl carbamate was added to the drinking-water at a dose of 332 mg/kg bw, but was observed with a dose of 525 mg/kg bw.

A 13-week study of ethyl carbamate administered in the drinking-water to B6C3F_1 mice (National Toxicology Program, 1996) reported that minimal to mild degeneration occurred in the testes of males administered ~1500 mg/kg bw. Degeneration of the seminiferous tubules, characterized by loss of germ cells and the presence of a few to numerous spermatid giant cells within tubule lumens, was observed in five males that received ~1500 mg/kg bw. The histopathological changes in the testis were considered to be secondary to the debilitated condition of the mice, as these changes occurred only in mice that died early. Epididymal spermatozoal concentration was generally lower in exposed males than in the controls, and the difference was significant in the 30- and 191-mg/kg bw groups. Spermatozoal motility was also lower in males in the 191-mg/kg bw group than in controls. In females, minimal to mild degeneration occurred in the ovaries at doses above 1500 mg/kg bw. The degenerative changes in the ovarian follicles consisted of greater amounts of cell debris within developing follicles than that observed in control females. The histopathological changes in the ovaries were considered to be secondary to the debilitated condition of the mice, as these changes occurred only in mice that died early. In seven females in the 511-mg/kg bw group, the ovaries were smaller than those of the controls as a result of decreased numbers of follicles and *corpora lutea* and the flattening of interstitial cells and females in this group had effectively ceased to have an estrous cycle. In nine females, no cyclicity was demonstrated, while in the remaining female, the percentage of diestrous smears was doubled.

In the same study (National Toxicology Program, 1996), when ethyl carbamate was administered in 5% ethanol, the effects on epididymal spermatozoal concentration in male mice did not appear to be enhanced. Spermatozoal motility was lower in males in the 370-mg/kg bw group. It was noted that, if 5% ethanol had any effect on the toxicity of ethyl carbamate in the male reproductive system in mice, this may have been masked due to the lower fluid (and therefore ethyl carbamate) consumption in that study. In females, the 5% ethanol vehicle appeared to enhance ethyl carbamate-induced ovarian atrophy. Other effects produced with the water vehicle were also observed when 5% ethanol was used as a vehicle.

Non-neoplastic lesions of the reproductive system in female B6C3F_1 mice were assessed in a 2-year study (National Toxicology Program, 2004). In the uterus of females exposed to increasing concentrations of ethyl carbamate in drinking-water that contained 0% or 2.5% ethanol, the incidence of angiectasis (dilated vascular spaces lined by a single layer of essentially normal endothelial cells) and thrombosis had a positive trend, and was significantly increased in females exposed to ~3 and 12 mg/kg bw ethyl carbamate. In female mice that received ethyl carbamate in 5% ethanol vehicle, no significant effect on these parameters was observed. Haemorrhage from large areas of uterine angiectasis was the cause of death in five females (one exposed to ~3 mg/kg bw and four exposed to ~10 mg/kg bw ethyl carbamate). No significant effects of ethyl carbamate on the male reproductive system were reported in this study.

In the study by Edwards *et al.* (1999), some of the male mice treated acutely with an intraperitoneal injection of 1750 mg/kg bw ethyl carbamate exhibited partial infertility; however, none of the mice treated with 1250 mg/kg bw had adverse effects on reproductive ability. Similarly, no effects on fertility were noted when male mice were treated with ethyl carbamate in the drinking-water at 190 mg/kg bw for 10 weeks or 370 mg/kg bw for 9 weeks.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems (see Table 4.1 for details and references)*

Ethyl carbamate is a weak mutagen in prokaryotes (*Bacillus subtilis, Escherichia coli* and *Salmonella typhimurium*). It appears to be a weak mutagen in fungi, and its mutagenicity and genotoxicity vary greatly in different tester strains. Ethyl carbamate is clearly mutagenic *in vivo* in *Drosophila* and induces sex-linked recessive lethal mutations and reciprocal translocations in germ cells.

The results of in-vitro clastogenicity tests with ethyl carbamate in mammalian systems vary among assays; the infrequent positive responses appeared most often with high doses and with exogenous metabolic activation in specific cell types under stringent conditions. Most of the data indicate that ethyl carbamate is inefficient in causing point mutations in mammalian cells *in vitro*.

A limited number of studies was performed to assess the clastogenicity of ethyl carbamate in human cells *in vitro*, and showed that ethyl carbamate induces sister chromatid exchange in human lymphocytes and causes DNA damage (measured as unscheduled DNA synthesis) in human fibroblasts *in vitro*. However, it was reported that ethyl carbamate does not induce micronucleus formation in human lymphocytes or cause chromosomal aberrations in human germ cells *in vitro*. Furthermore, no effect of ethyl carbamate on gene mutations was observed in a human lymphoblastoid cell line.

Results from in-vivo somatic cell assays with ethyl carbamate in mammalian species were generally positive. Chromosomal aberrations, sister chromatid exchange, gene mutation, DNA damage and micronucleus formation were induced with a wide range of doses and in a large number of experimental model organisms (mice, rats and hamsters) and tissues (liver, bone marrow and lungs). Classical clastogenic effects such as chromosomal aberrations were less dose-dependent than sister chromatid exchange. In studies that also assessed the ability of ethyl carbamate to induce cancer, a poor correlation was found between its carcinogenicity and clastogenicity. Ethyl carbamate also induced point mutations in somatic cells *in vivo*.

Table 4.1 Genetic and related effects of ethyl carbamate

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Bacillus subtillis, M45 *rec*$^-$, differential toxicity	(+)[c]	(+)[c]	2000	Ashby & Kilbey (1981)
Escherichia coli polA$^-$, differential toxicity	–	–	2500	Ashby & Kilbey (1981)
Escherichia coli WP2-WP100, differential toxicity	NT	–	2000	Mamber *et al*. (1983)
Escherichia coli recA$^-$, differential toxicity	(+)[d]	(+)[d]	2000	Ashby & Kilbey (1981)
Escherichia coli gal operon, reverse mutation	NT	–	2000	Ashby & Kilbey (1981)
Escherichia coli PQ37 SOS, reverse mutation	–	–	1000	Dayan *et al*. (1987)
Escherichia coli WP2 *uvrA*, reverse mutation	NT	(+)[e]	25	Bridges *et al*. (1981)
Escherichia coli WP2 *uvrA*, reverse mutation	–	NT	5340	Pai *et al*. (1985)
Escherichia coli K12 *uvrB/recA*, DNA repair host-mediated assay	–	–	50163	Hellmér & Bolcsfoldi (1992)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	10000	McCann *et al*. (1975)
Salmonella typhimurium TA100, TA1535, reverse mutation	–	–	400	Dahl *et al*. (1978)
Salmonella typhimurium TA100, TA1535, TA1536, TA1537, TA98, reverse mutation	–	–	125	Simmon (1979a)
Salmonella typhimurium TA100, reverse mutation	–	–	13	Dahl *et al*. (1980)
Salmonella typhimurium TA100, reverse mutation	NT	(+)[e]	25	Bridges *et al*. (1981)
Salmonella typhimurium TA100, TA1537, TA98, TA97, reverse mutation	–	–	10000	National Toxicology Program (1996)
Salmonella typhimurium TA100, TA102, TA98, reverse mutation	–	+	5000	Hübner *et al*. (1997)

Table 4.1 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA1535, reverse mutation	–	+	6666	National Toxicology Program (1996)
Salmonella typhimurium YG7108pin3ERb5, reverse mutation	–	NT	10000	Emmert *et al*. (2006)
Salmonella typhimurium TA98, frame shift mutation	–	–	400	Dahl *et al*. (1978)
Salmonella typhimurium TA98, frame shift mutation	NT	(+)[e]	25	Bridges *et al*. (1981)
Salmonella typhimurium TA98, frame shift mutation	(+)[f]	NT	500	Flückiger-Isler *et al*. (2004)
Salmonella typhimurium RS112, DEL recombination	+	+	20000	Galli & Schiestl (1998)
Saccharomyces cerevisiae D3, mitotic recombination	–	–	50000	Simmon (1979b)
Saccharomyces cerevisiae D4, mitotic recombination	–	–	333 µg/plate	Jagannath *et al*. (1981)
Saccharomyces cerevisiae T1, T2, mitotic recombination	–	–	1000	Kassinova *et al*. (1981)
Saccharomyces cerevisiae JD1, mitotic recombination	+	+	150	Sharp & Parry (1981)
Saccharomyces cerevisiae D7, mitotic recombination	–	–	4800	Zimmermann & Scheel (1981)
Saccharomyces cerevisiae XV185-14C, reversion	–	(+)	889	Mehta & von Borstel (1981)
Saccharomyces cerevisiae D6, aneuploidy	–	–	600	Parry & Sharp (1981)
Saccharomyces cerevisiae YB110, chromosomal translocation	+	NT	75000	Hübner *et al*. (1997)
Schizosaccharamyces pombe P1, forward mutation	–	–	4.6	Loprieno (1981)
Aspergillus nidulans, aneuploidy	+	NT	20000	Crebelli *et al*. (1986)
Aspergillus nidulans, forward mutation	–	NT	40000	Crebelli *et al*. (1986)
Neurospora crassa, aneuploidy	–	NT	100	Griffiths *et al*. (1986)
Drosophila melanogaster, sex-linked recessive lethal mutation	+		267000	Vogt (1948)
Drosophila melanogaster, sex-linked recessive lethal mutation	+		222750	Oster (1958)

Table 4.1 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Drosophila melanogaster, sex-linked recessive lethal mutation	+		10000	Knaap & Kramers (1982)
Drosophila melanogaster, somatic mutation and recombination	+		445	Frölich & Würgler (1990)
Drosophila melanogaster, somatic mutation and recombination	+		1800	Graf & van Schaik (1992)
Drosophila melanogaster, somatic mutation and recombination	+		890	Osaba *et al*. (1999)
Drosophila melanogaster, somatic mutation and recombination	+		445	Dogan *et al*. (2005)
Drosophila melanogaster, genetic crossing-over or recombination	+		225	Nivard & Vogel (1999)
DNA strand breaks, rat hepatocytes *in vitro*	+	NT	8900	Sina *et al*. (1983)
Unscheduled DNA synthesis, Holtzman rat hepatocytes *in vitro*	–	NT	890	Sirica *et al*. (1980)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	3000	Jotz & Mitchell (1981)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	NT	–	11000	Amacher & Turner (1982)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	5000	Sofuni *et al*. (1996)
Sister chromatid exchange, Chinese hamster DON cells *in vitro*	+	NT	890	Abe & Sasaki (1977)
Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	–	–	25	Popescu *et al*. (1977)
Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	–	–	20000	Allen *et al*. (1982)
Sister chromatid exchange, Chinese hamster ovary (CHO) cells *in vitro*	–	–	1000	Evans & Mitchell (1981)
Sister chromatid exchange, Chinese hamster ovary (CHO) cells *in vitro*	–	–	100	Perry & Thompson (1981)
Sister chromatid exchange, Chinese hamster ovary (CHO) cells *in vitro*	+	+	500	National Toxicology Program (1996)
Sister chromatid exchange, mouse embryo cells *in vitro*	–	NT	8900	Itoh & Matsumoto (1984)
Micronucleus formation, Chinese hamster ovary (CHO) cells *in vitro*	–	NT	5000	Aardema *et al*. (2006)

Table 4.1 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, Chinese hamster lung (CHL) cells *in vitro*	–	NT	5000	Wakata *et al*. (2006)
Chromosomal aberrations, Chinese hamster ovary (CHO) cells *in vitro*	–	–	5000	National Toxicology Program (1996)
Chromosomal aberrations, mouse embryo cells *in vitro*	+	NT	8.9	Itoh & Matsumoto (1984)
Cell transformation, mouse fibroblast C3H2K cells	–	NT	100	Yoshikura & Matsushima (1981)
Cell transformation, C3H 10T1/2 mouse cells	–	–	25000	Allen *et al*. (1982)
Cell transformation, baby hamster kidney (BHK21) cells	–	+	5750	Daniel & Dehnel (1981)
Cell transformation, baby hamster kidney (BHK21) cells	NT	+	200	Styles (1981)
Unscheduled DNA synthesis, HeLa cells *in vitro*	NT	+	0.9	Martin *et al*. (1978)
Unscheduled DNA synthesis, human fibroblasts *in vitro*	+	+	0.8	Agrelo & Amos (1981)
Unscheduled DNA synthesis, HeLa cells *in vitro*	+	+	100	Martin & McDermid (1981)
Gene mutation, human lymphoblastoid TK6 cells, *HGPRT* and *TK* loci *in vitro*	–	–	12500	Hübner *et al*. (1997)
Sister chromatid exchange, human lymphocytes *in vitro*	+	–	8.9	Csukás *et al*. (1979)
Sister chromatid exchange, human lymphocytes *in vitro*	+	–	890	Csukás *et al*. (1981)
Micronucleus formation, human lymphocytes *in vitro*	–	NT	5000	Clare *et al*. (2006)
Chromosomal aberrations, human germ cells *in vitro*	–	NT	1000	Kamiguchi & Tateno (2002)
DNA strand breaks, Sprague-Dawley rat hepatocytes *in vivo*	–		500 ip	Petzold & Swenberg (1978)
DNA strand breaks, Sprague-Dawley rat brain cells *in vivo*	+		25 ip	Petzold & Swenberg (1978)
Unscheduled DNA synthesis, mouse germline cells *in vivo*	+		750 ip	Sotomayor *et al*. (1994)

Table 4.1 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Gene mutation, Chinese hamster lung cells, *in vivo*	+		1000 ip	Dean & Hodson-Walker (1979)
Gene mutation, mouse germline cells *in vivo*	–		1750 ip	Russell *et al*. (1987)
Gene mutation, mouse lung, liver and spleen cells *lacZ* operon *in vivo*	+		900 ip	Williams *et al*. (1998)
Sister chromatid exchange, mouse somatic cells *in vivo*	+		50 ip	Roberts & Allen (1980)
Sister chromatid exchange, mouse germline cells *in vivo*	+		400 ip	Roberts & Allen (1980)
Sister chromatid exchange, mouse somatic cells *in vivo*	+		193 inh and iv	Cheng *et al*. (1981a)
Sister chromatid exchange, mouse somatic cells *in vivo*	+		392 ip	Cheng *et al*. (1981b)
Sister chromatid exchange, mouse bone-marrow cells *in vivo*	+		400 ip	Allen *et al*. (1982)
Sister chromatid exchange, mouse somatic cells, alveolar macrophages and bone-marrow *in vivo*	+		300 ip	Conner & Cheng (1983)
Sister chromatid exchange, mouse bone-marrow cells *in vivo*	+		150 ip	Dragani *et al*. (1983)
Sister chromatid exchange, mouse somatic cells, lymphocytes, alveolar macrophages and bone-marrow *in vivo*	+		300 ip	Goon & Conner (1984)
Sister chromatid exchange, mouse bone-marrow cells *in vivo*	+		400 ip	Sharief *et al*. (1984)
Sister chromatid exchange, mouse lymphocytes *in vivo*	+		200 ip	Neft *et al*. (1985)
Sister chromatid exchange, mouse bone-marrow cells *in vivo*	+		300 ip	Sozzi *et al*. (1985)
Sister chromatid exchange, mouse lung cells *in vivo*	+		1000 ip	Allen *et al*. (1986)
Sister chromatid exchange, mouse fetal liver and bone-marrow cells *in vivo*	+		100 iv	Neeper-Bradley & Conner (1989, 1990)
Sister chromatid exchange, mouse somatic cells, skin and bone-marrow *in vivo*	+		0.6 ip or skin	Barale *et al*. (1992)

Table 4.1 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, rat bone-marrow cells *in vivo*	+		400 ip	Sharief *et al.* (1984)
Sister chromatid exchange, Chinese and Syrian golden hamster bone-marrow cells *in vivo*	+		400 ip	Sharief *et al*. (1984)
Micronucleus formation, mouse polychromatic erythrocytes *in vivo*	+		178 ip	Wild (1978)
Micronucleus formation, mouse polychromatic erythrocytes *in vivo*	+		615 ip	Salamone *et al*. (1981)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		200 ip	Tsuchimoto & Matter (1981)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		1000 sc	Aldovini & Ronchese (1983)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		900 po	Ashby *et al*. (1990)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		400 ip	Holmstrom (1990)
Micronucleus formation, mouse skin cells *in vivo*	+		2 ip	He *et al.* (1991)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		400 po × 3	Westmoreland *et al*. (1991)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		500 ip	Balansky *et al*. (1992)
Micronucleus formation, mouse bone-marrow polychromatic and normochromatic erythrocytes *in vivo*	+		990 ip	Sanderson & Clark (1993)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		500 ip	Balansky (1995)

Table 4.1 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		1000 ip	Choy *et al*. (1995, 1996)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		25 ip	Adler *et al*. (1996)
Micronucleus formation, mouse polychromatic and normochromatic erythrocytes *in vivo*	+		200 po	National Toxicology Program (1996)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		1000 po	Abraham *et al*. (1998)
Micronucleus formation, mouse peripheral blood normochromatic erythrocytes *in vivo*	+		400 ip	Balansky & De Flora (1998)
Micronucleus formation, mouse peripheral blood normochromatic erythrocytes *in vivo*	+		600 po, 12 wk	Director *et al*. (1998)
Micronucleus formation, mouse bone-marrow polychromatic erythrocytes *in vivo*	+		900 ip	Williams *et al*. (1998)
Micronucleus formation, mouse peripheral blood reticulocytes *in vivo*	+		500 ip	Kim *et al*. (1999)
Micronucleus formation, mouse peripheral blood polychromatic and normochromatic erythrocytes *in vivo*	+[g]		10 po 5 d/wk, 6 wk	Hoffler *et al.* (2005)
Micronucleus formation, rat polychromatic erythrocytes *in vivo*	–		2500 ip	Trzos *et al*. (1978)
Micronucleus formation, rat polychromatic erythrocytes *in vivo*	+		600 po	Westmoreland *et al*. (1991)
Micronucleus formation, rat germline spermatid cells *in vivo*	–		500 ip	Adler *et al*. (1996)
Chromosomal aberrations, mouse somatic bone-marrow, thymus and spleen cells *in vivo*	+		1000 sc	Kurita *et al*. (1969)

Table 4.1 (continued)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, mouse bone-marrow and spleen cells *in vivo*	+		500 sc	Miyashita *et al*. (1987)
Chromosomal aberrations, mouse somatic skin and bone-marrow cells *in vivo*	+		600 skin	Barale *et al*. (1992)
Chromosomal aberrations, mouse blood and bone-marrow somatic cells *in vivo*	–		600 po, 12 wk	Director *et al*. (1998)
Chromosomal aberrations, rat bone-marrow cells *in vivo*	+		100 ip	Topaktaş *et al*. (1996)
Dominant lethal mutation, rats	–		250 ip × 5	Jackson *et al*. (1959)
Dominant lethal mutation, mice	–		1500 ip	Bateman (1967)
Dominant lethal mutation, mice	–		1200 ip	Epstein *et al*. (1972)
Dominant lethal mutation, mice	–		100 ip	Kennedy *et al*. (1973)
Dominant lethal mutation, mice	–		2250 ip	Nomura (1982)
Dominant lethal mutation, mice	–		1750 ip	Adler *et al*. (1996)
Sperm morphology, mice	–		1000 ip × 5	Wyrobek & Bruce (1975)
Sperm morphology, mice	–		1000 ip × 5	Topham (1981)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro formations, µg/mL; in-vivo formations, mg/kg bw/day; d, day; inh, inhalation; ip, intraperitoneal; iv, intravenous; po, oral; sc, subcutaneous; wk, week

[c] Two of 7 formations weakly positive without exogenous metabolic system (S9) and 1 of 7 formations weakly positive with S9

[d] Four of 7 formations negative without exogenous metablic system (S9) and two of 7 formations weakly positive with S9

[e] Positive in some, but not all formations performed at different laboratories

[f] One of 5 formations strongly positive

[g] Effect largely absent in*CYP2E1*-null mice

Some reports have indicated that ethyl carbamate can cause DNA damage in mammalian cells *in vitro* and *in vivo*. Ethyl carbamate and/or its metabolites can bind to nucleic acids *in vivo*. Boyland and Williams (1969) showed that, after intraperitoneal injection of radiolabelled ethyl or carboxyethyl carbamate to mice, liver and lung RNA were labelled. It was also noted that the ability of ethyl carbamate to bind to nucleic acids correlates with its organ-, sex- and strain-specific carcinogenic potency (Fossa *et al*., 1985). Sotomayor *et al.* (1994) administered 10–1000 mg/kg bw [^{3}H]ethyl carbamate to male mice intraperitoneally and measured DNA binding and unscheduled DNA synthesis in the liver and testis 12 hours later. A linear increase in the binding of labelled ethyl carbamate to DNA was detected in both organs, although the binding increased more rapidly in the liver at lower doses. Unscheduled DNA synthesis was elevated in early spermatids only with the 750-mg/kg bw dose.

Ribovich *et al.* (1982) demonstrated that 1,N^{6}-ethenoadenosine and 3,N^{4}-ethenocytidine were formed in the RNA of liver after intraperitoneal administration of radiolabelled [ethyl-1,2-^{3}H]ethyl carbamate to mice. Following single and multiple intraperitoneal injections of ethyl carbamate or its metabolites, vinyl carbamate or vinyl carbamate oxide, the formation of 1,N^{6}-ethenodeoxyadenosine and 3,N^{4}-ethenodeoxycytidine was increased in the liver and lung DNA of several mouse strains (Fernando *et al*., 1996). Vinyl carbamate was about threefold more potent in inducing etheno-DNA adducts in either the liver or lung. Recently, Beland *et al*. (2005) reported that the levels of 1,N^{6}-ethenodeoxyadenosine in hepatic DNA were increased by exposure to ethyl carbamate (90 ppm [90 µg/mL], 4 weeks in the drinking-water) but were lower when 5% ethanol served as the vehicle. In the same study, neither ethyl carbamate nor ethanol affected the levels of 1,N^{6}-ethenodeoxyadenosine or 3,N^{4}-ethenodeoxycytidine in lung DNA.

It was also suggested that *N*-7-(2-oxoethyl)guanine may be a key DNA adduct formed after exposure to ethyl carbamate (Scherer *et al*., 1986). These authors also showed that vinyl carbamate is a much more potent inducer of this adduct than ethyl carbamate. Svensson (1988) reported the formation of 2-oxoethyl haemoglobin and the DNA adduct *N*-7-(2-oxoethyl)guanine in mice treated with ethyl carbamate and the number of protein adducts increased linearly with dose. The *N*-7-(2-oxoethyl)guanine adduct is not considered to be pro-mutagenic but it was suggested that it may lead to cross-linking in DNA (Conner & Cheng, 1983), a mechanism that may be involved in the sister chromatid exchange induced by ethyl carbamate in multiple test systems.

4.5 Mechanistic considerations

The following are potential mechanisms that are not mutually exclusive.

4.5.1 *Genotoxicity*

The carcinogenicity of ethyl carbamate is thought to be mediated via a bioactivation pathway in which it is oxidized sequentially by CYP2E1 to vinyl carbamate and vinyl carbamate epoxide (Dahl *et al.*, 1978). Vinyl carbamate epoxide is a DNA-reactive species that can yield promutagenic etheno-DNA adducts. In support of this hypothesis, vinyl carbamate has been shown to induce more hepatocellular carcinomas than ethyl carbamate (Dahl *et al.*, 1980) and vinyl carbamate epoxide is more hepatocarcinogenic than vinyl carbamate (Park *et al.*, 1993). DNA adducts indicative of exposure to vinyl carbamate epoxide have been detected in the liver DNA of mice treated with ethyl carbamate (Beland *et al.*, 2005), vinyl carbamate and vinyl carbamate epoxide (Fernando *et al.*, 1996). In addition, hepatocellular adenomas and carcinomas induced in $B6C3F_1$ mice by ethyl and vinyl carbamate have a characteristic increase in CAA to CTA mutations at codon 61 of the H-*Ras* oncogene compared with CAA to AAA mutations that are typically found in spontaneous tumours (Wiseman *et al.*, 1986; Dragani *et al.*, 1991). Such mutations are consistent with the formation of 1,N^6-ethenodeoxyadenosine, which has been shown to lead to A→T transversion mutations (Levine *et al.*, 2000). In addition, it has been suggested that a potential DNA-cross-linking alkylating adduct, *N*-7-(2-oxoethyl)guanine, may be formed after exposure to ethyl carbamate *in vivo* (Scherer *et al.*, 1986).

4.5.2 *Cell proliferation*

Treatment with ethyl carbamate has been shown to induce cell proliferation in mouse lung (Yano *et al.*, 1997, 2000) and liver (Beland *et al.*, 2005). Cell proliferation can occur either as a regenerative response to cytotoxicity or via the induction of other molecular pathways. In the mouse lung, ethyl carbamate has been purported to act via the induction of ornithine decarboxylase and subsequent polyamine accumulation (Yano *et al.*, 1997), which are events that are thought to be involved in stimulation of the cell cycle. The proliferative effects of ethyl carbamate in the liver seem to be sex-specific, since hepatocellular proliferation was observed only in female mice (Beland *et al.*, 2005). The fact that female mice in the same study had a greater relative increase in the incidence of hepatocellular tumours following administration of ethyl carbamate may suggest that formation of the genotoxic metabolites of ethyl carbamate (see above), coupled with a greater rate of cell replication, contributes to the tumour response. It was shown that ethyl carbamate-induced increases in cell proliferation in the liver and lung are dependent on CYP2E1 because no effect was observed in *CYP2E1*-null mice (Hoffler *et al.*, 2005).

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5. Summary of Data Reported

5.1 Exposure data

Ethyl carbamate may be formed naturally as a result of fermentation and has been detected in a variety of fermented foods and alcoholic beverages. Ethyl carbamate can also be made commercially by various reactions with ethanol. It was formerly used in medical practice as a hypnotic agent, for the treatment of cancer, in particular multiple myeloma, or in analgesics. There is no evidence that ethyl carbamate is currently used in human medicine. It is used as an anaesthetic in veterinary medicine.

The levels of ethyl carbamate in wine and beer are usually below 100 µg/L, whereas higher levels (in the milligram per litre range) have been found in some stone-fruit spirits. Levels in foods have been regulated and significantly reduced during the past 20 years.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

In many studies, mice treated orally with ethyl carbamate demonstrated an increased incidence of lung adenomas, carcinomas and squamous-cell tumours, lymphomas (mainly lymphosarcomas), mammary gland adenocarcinomas, carcinomas and adenoacanthomas, leukaemias, forestomach squamous-cell papillomas or carcinomas, heart haemangiosarcomas, liver haemangiomas and haemangiosarcomas, Harderian gland adenomas or carcinomas and angiomas. Subcutaneous administration of ethyl carbamate to adult and newborn mice induced significant increases in the incidence of lung adenomas and hepatomas, respectively. Topical application of ethyl carbamate to mice resulted in a significant increase in the incidence of lung adenomas

and mammary gland carcinomas. Mice exposed by inhalation to ethyl carbamate had an increased incidence of lung adenocarcinomas, leukaemias and uterine haemangiomas. Intraperitoneal administration of ethyl carbamate to adult mice resulted in a significant increase in the incidence of lung adenomas, hepatomas and skin papillomas. Similar treatment in newborn mice induced lymphomas, lung adenomas, hepatomas, Harderian gland tumours and stromal and epithelial tumours of the ovary. Mice exposed transplacentally to ethyl carbamate developed an increased incidence of lung tumours, hepatomas and ovarian tumours. Mice born after pre-conception exposure of the sires to ethyl carbamate had an increased incidence of pheochromocytomas and adrenal gland tumours.

In one study, oral administration of ethyl carbamate to mice deficient in CYP2E1 resulted in a lower incidence of liver haemangiomas and haemangiosarcomas, lung bronchioalveolar adenomas and carcinomas, and Harderian gland adenomas than that in mice proficient in CYP2E1. In other studies, when the administration of ethyl carbamate was accompanied by topical application of the tumour promoter, 12-*O*-tetradecanoylphorbol-13-acetate, the incidence of skin papillomas and squamous-cell carcinomas was significantly increased. When the treatment with ethyl carbamate was followed by topical application of croton oil, a significant increase in the incidence of skin papillomas resulted. Topical application of ethyl carbamate to mice previously treated with 7,12-dimethylbenz[*a*]anthracene resulted in a significant increase in the incidence of skin tumours.

Rats treated orally with ethyl carbamate had an increased incidence of Zymbal gland carcinomas and mammary gland carcinomas.

Hamsters treated orally with ethyl carbamate showed an increased incidence of skin melanotic tumours, forestomach papillomas, mammary gland adenocarcinomas, liver hepatomas, liver and spleen haemangiomas, and thyroid, ovarian and vaginal carcinomas.

In one study, hepatocellular adenomas and carcinomas and adenocarcinomas of the lung were observed in monkeys treated orally with ethyl carbamate.

The carcinogenicity of ethyl carbamate has been compared with that of *N*-hydroxyethyl carbamate, 2-hydroxyethyl carbamate, vinyl carbamate and/or vinyl carbamate epoxide in mice and rats after oral, dermal, subcutaneous, intramuscular and/or intraperitoneal administration.

Oral administration of ethyl carbamate or *N*-hydroxyethyl carbamate, followed by topical application of croton oil, induced skin and lung tumours in male and female mice; ethyl carbamate was significantly more potent than *N*-hydroxyethyl carbamate.

Topical application of ethyl carbamate or vinyl carbamate, followed by promotion with croton oil, induced skin and lung tumours in female mice; vinyl carbamate was significantly more active than ethyl carbamate. Topical application of vinyl carbamate or vinyl carbamate epoxide, with or without promotion by 12-*O*-tetradecanoylphorbol-13-acetate, induced skin papillomas in female mice; vinyl carbamate epoxide was significantly more active than vinyl carbamate.

Subcubcutaneous injection of ethyl carbamate or *N*-hydroxyethyl carbamate induced lung adenomas in two strains of mice; ethyl carbamate demonstrated greater activity.

Intramuscular injection of vinyl carbamate or vinyl carbamate epoxide into female rats caused sarcomas at the injection site; vinyl carbamate epoxide was more potent. Intraperitoneal injection of ethyl carbamate or *N*-hydroxyethyl carbamate into three different strains of mice, with or without promotion by topical application of croton oil, induced skin and/or lung tumours; ethyl carbamate had similar or greater activity than *N*-hydroxyethyl carbamate.

Intraperitoneal injection of ethyl carbamate or vinyl carbamate, with or without promotion by topical application of croton oil, induced skin papillomas, lung adenomas and/or carcinomas, liver tumours (hepatomas), thymic lymphomas and/or Harderian gland tumours in CD-1, A/J, B6C3F_1, C3H, C57BL, B6CF_1, CB6F_1-Tg *HRas2*, B6D2F_1 and/or B6CF_1 mice; vinyl carbamate was typically more potent.

Intraperitoneal injection of vinyl carbamate or vinyl carbamate epoxide induced lung adenomas in female A/J mice and liver tumours (hepatomas) in male B6C3F_1 mice; vinyl carbamate epoxide was more active than vinyl carbamate. Intraperitoneal injection of ethyl carbamate or 2-hydroxyethyl carbamate induced lung adenomas in male strain A mice; ethyl carbamate was more potent than 2-hydroxyethyl carbamate.

Intraperitoneal injection of ethyl carbamate or vinyl carbamate into male and female rats induced liver and ear-duct carcinomas and neurofibrosarcomas of the ear lobe; vinyl carbamate showed more activity than ethyl carbamate.

These data indicate that, although *N*-hydroxyethyl carbamate and 2-hydroxyethyl-carbamate are carcinogenic, they probably do not make a significant contribution to the carcinogenicity of ethyl carbamate. The data are also consistent with a metabolic activation pathway in which ethyl carbamate is oxidized to vinyl carbamate, which is subsequently oxidized to vinyl carbamate epoxide.

5.4 Mechanistic and other relevant data

Ethyl carbamate is metabolized predominantly by CYP2E1, which generates metabolites (vinyl carbamate and vinyl carbamate epoxide) that are probably proximate carcinogens. The pathways for the metabolism of ethyl carbamate are similar in rodents and humans. Interactions between ethanol and ethyl carbamate are complex.

The data are too scant to make a comprehensive evaluation of the toxic effects of ethyl carbamate in humans.

At high doses, ethyl carbamate exhibits toxic effects on the central nervous system, the gastrointestinal tract, the spleen and the thymus in experimental animals. Lower doses lead to long-term effects on the spleen and the thymus.

There is strong evidence in experimental animals for the teratogenicity of ethyl carbamate when administered during gestation. The teratogenic effects are evident in the offspring when either male or female rodents are exposed before mating or pregnancy.

The effects of ethyl carbamate on the reproductive system in mice and rats are minimal and occur only at high doses.

Ethyl carbamate is genotoxic, mutagenic and clastogenic, especially in the presence of metabolic activation.

Possible mechanisms for the carcinogenicity of ethyl carbamate are induction of DNA damage by its metabolites and an increase in cell proliferation in target tissues.

6. Evaluation and Rationale

6.1 Carcinogenicity in humans

There is *inadequate evidence* in humans for the carcinogenicity of ethyl carbamate.

6.2 Carcinogenicity in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of ethyl carbamate.

There is *sufficient evidence* in experimental animals for the carcinogenicity of vinyl carbamate.

There is *sufficient evidence* in experimental animals for the carcinogenicity of vinyl carbamate epoxide.

Overall evaluation

Ethyl carbamate is *probably carcinogenic to humans (Group 2A).*

Rationale

The Working Group noted that (i) experimental evidence suggests great similarities in the metabolic pathways of the activation of ethyl carbamate in rodents and humans; and (ii) the formation of proximate carcinogens that are DNA-reactive and are thought to play a major role in ethyl carbamate-induced carcinogenesis in rodents probably also occurs in human cells.