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Application of the margin of exposure (MoE) approach to substances in food that are genotoxic and carcinogenic
Example: Ethyl carbamate (CAS 51-79-6)

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ABSTRACT

Ethyl carbamate is mutagenic and produces DNA-adducts in vivo, and is carcinogenic in rodent bioassays. Dose-response modelling of the data for alveolar and bronchiolar adenoma or carcinoma in male and female mice combined gave a $BMDL_{10}$ of 0.25 mg/kg-bw/day. The dietary exposure from consumption of foods and non-alcoholic beverage was estimated to be 1 μg/person/day (15 ng/kg-bw/day), while the exposure of a high-percentile consumer of alcoholic beverages was estimated to be 5 μg/person per day (80 ng/kg-bw/day). The corresponding calculated MOEs were 16600 and 3125, respectively.

1. Introduction

In the 1940s, ethyl carbamate (urethan[e]; $NH_2COOCH_2CH_3$, CAS 51-79-6) was used as a hypnotic in man at doses of 1 g per person per day and as an anaesthetic for laboratory animals. In 1943, it was discovered that ethyl carbamate has a carcinogenic effect in animals and three years later, its activity against leukaemia in man was described. Since 1948, it has been known that ethyl carbamate is mutagenic (see below). Later, the industrial, medical and veterinary uses of ethyl carbamate was discontinued and today the major route of exposure to ethyl carbamate in the human population is through consumption of fermented foods and beverages in which it may be present — for example, as a consequence of its unintentional formation during the fermentation process or during storage.

2. Toxicological data

2.1. Genotoxicity

Ethyl carbamate has been tested in a large number of studies of genotoxicity *in vitro* and *in vivo* (JECFA, 2005). Assays assessing point mutations in mammalian cells were uniformly negative for mouse lymphoma cells, while assays in bacterial, yeast and other types of mammalian cells produced variable results. Results of *in vivo* somatic cell assays, including assessment of induction of chromosomal aberrations, micronuclei and sister chromatid exchange, were almost uniformly positive, with the mouse micronucleus assay showing the strongest response. Co-administration of ethanol delayed rather than prevented the genotoxicity of ethyl carbamate in this assay (JECFA, 2005).

While there was no evidence of *in vivo* mammalian germ cell genotoxicity in dominant lethal and specific locus test, increased tumour incidence was noted in the offspring of male or female mice that had been treated with a single high dose of ethyl carbamate ($\geqslant$1000 mg/kg-bw) prior to mating. Since ethyl carbamate is clastogenic in the mouse micronucleus and *in vivo* chromosomal assays at a dose range resulting in increased malformation rates of the offspring of treated dams, it appears the teratogenic effects are secondary to deleterious effects on embryonic DNA. No multi-generation studies, which meet currently accepted standard protocols for such studies, are available.

2.2. Carcinogenicity

Ethyl carbamate is a multi-site carcinogen with a short latency period. Oral doses of 100–2000 mg/kg-bw have been shown to induce tumours in mice, rats, hamsters and non-human primates. The upper range of these doses overlaps the standard anaesthetic dose (1000 mg/kg-bw) and the rodent LD_{50}s. A lifetime study of carcinogenicity in mouse and rat served as the basis of a previously

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conducted quantitative risk assessment of ethyl carbamate (Schlatter and Lutz, 1990). There were, however, significant problems with these bioassays. The results of one newer lifetime study (NTP, 2004) assessing carcinogenicity in the male and female B6C3F1 mouse have become available since the risk assessments conducted in 1989–1990. Treatment with ethyl carbamate resulted in increased incidences in a number of different tumour types. Treatment of female mice with single or multiple doses of ethyl carbamate during the gestation or lactation period were found to increase the incidence/multiplicity of tumours in the offspring. It appears from this data that the sensitivity of the term foetus and neonate may be attributable to undeveloped enzymatic systems for carcinogen detoxification and clearance, resulting in a greater internal exposure.

In the NTP study (NTP, 2004), groups of 48 male and 48 female B6C3F1 mice, five weeks of age, received ethyl carbamate (purity $\geqslant$99%) at concentrations of 0, 10, 30 or 90 mg/l *ad libitum* in the drinking water for two years (equivalent to 0, 1.2/0.9, 3.3/2.8 and 10.1/8.2 mg/kg-bw/day in males/females. Clinical observations, body weights, water consumption and food consumption were recorded throughout the study. Complete necropsies were performed on all mice. The weights of liver and lung were taken, and all organs and tissues were examined for grossly visible lesions. Histopathological examination was made of all major organs. Significant treatment-related decreases in survival were observed in both sexes as function of ethyl carbamate concentration. The mean body weights of both sexes of mice receiving ethyl carbamate at 90 mg/l were lower than respective controls and ethyl carbamate caused treatment-related decrease in the terminal body weights of mice. Increasing concentrations of ethyl carbamate did not affect water or feed consumption.

In males, there were dose-dependently increased incidences of hepatocellular adenoma/carcinoma, alveolar/bronchiolar adenoma/carcinoma, Harderian gland adenoma/carcinoma, squamous cell papilloma/carcinoma of the forestomach and the skin, and haemangiosarcoma, primarily of the liver and the heart. In females, there were dose-dependently increased incidences of hepatocellular adenoma/carcinoma, alveolar/bronchiolar adenoma/carcinoma, Harderian gland adenoma/carcinoma, mammary gland adenocanthoma/adenocarcinoma, ovarian granulosa cell tumour (benign and malignant) and haemangiosarcoma, primarily of the liver and spleen. The most prominent dose–response effect was seen with increased incidences of alveolar/bronchiolar adenoma/carcinoma and Harderian gland adenoma/carcinoma. These incidences are given in the Table 1, together with the incidences of all organs, and benign and malignant tumours combined.

2.3. Mode of action

In rats and mice, ethyl carbamate undergoes CYP2E1-mediated metabolic activation to vinyl carbamate epoxide, which binds covalently to nucleic acids and proteins, resulting in the formation of adducts, including those that have been shown to induce base-pair substitutions in DNA from tumour tissue. Studies to chemically characterise the DNA-adducts in liver and lung showed the formation of etheno-adducts (1,N^6-ethenoadenosine, 3,N^4-ethenocytidineand as well as 7-(2-oxoethyl)guanine), the same adducts are also seen with vinyl carbamate. The *in vitro* formation of 1,N^6-ethenoadenosine adducts from ethyl carbamate in liver samples from humans showed a close correlation with the formation of adducts from vinyl carbamate. The rate of formation of the adduct was 500 times faster for vinyl carbamate than for ethyl carbamate. CYP2E1 activity is responsible for about 95% of the metabolism of ethyl carbamate to carbon dioxide, the final metabolic product of hydrolysis and side-chain oxidation. Elimination is rapid, with >90% being eliminated as carbon dioxide within six hours in mice (JECFA, 2005).

2.4. Epidemiological data

There are no data from epidemiological studies.

2.5. Dose–response relationships

Table 1 shows dose–response relationships of tumour incidences from the NTP (2004) two-year carcinogenicity study in B6C3F1 mice administered 0, 10, 30 or 90 mg ethyl carbamate/l in drinking water.

2.5.1. Data quality, uncertainties and limitations

The carcinogenicity data to be used for dose–response modelling comes from a recent NTP study (NTP, 2004), which meets the current standards.

3. Human dietary exposure analysis

3.1. Sub-populations of interest

Foetuses and neonate babies may have an increased susceptibility to ethyl carbamate carcinogenicity.

3.2. Concentrations in food

Ethyl carbamate is formed naturally during the fermentation of foods. Levels of ethyl carbamate in a number of foods commonly consumed as part of the diet are available and are given in Table 2 (taken from JECFA, 2005). The highest (albeit still low) levels of ethyl carbamate are typically found in yeast-fermented foods, with low to non-quantifiable levels found in foods fermented by lactic acid bacteria, acetic acid bacteria or moulds. Additionally, the level of ethyl carbamate in a fermented food or drink can increase when ethanol and appropriate precursors remain in contact for extended periods, such

Table 1
Tumour incidences from a two-year carcinogenicity study in B6C3F1 mice administered 0, 10, 30 or 90 mg ethyl carbamate/l in drinking water (NTP, 2004).

	Male B6C3F1 mice				Female B6C3F1 mice			
Ethyl carbamate concentration in drinking water (mg/L)	0	10	30	90	0	10	30	90
Equal to ethyl carbamate dose[a] (mg/kg-bw/day)	0	1.2	3.3	10.1	0	0.9	2.8	8.2
Lung, alveolar/bronchiolar adenoma or carcinoma[b]	5/48	18/48**	29/47**	37/48**	6/48	8/48	28/48**	39/47**
Harderian gland, adenoma or carcinoma[c]	3/47	12/47**	30/47**	38/47**	3/48	11/48*	19/48**	30/48**
All organs, benign and malignant tumours	33/48	39/48*	46/47**	47/48**	37/48	35/48	45/48*	47/48**

* Significantly different ($P < 0.05$) from the control group by the poly-3 (neoplasms) or Williams' (non-neoplastic lesions) test.
** Significantly different ($P < 0.01$) from the control group by the poly-3 (neoplasms) or Williams' (non-neoplastic lesions) test.
[a] Calculated from mean water consumption and mean bw over mean lifespan.
[b] Historical incidence for males: 82/473 (17.3%), range 11–31%; for females 25/515 (4.9%), range 2–11%.
[c] Historical incidence for males: 25/325 (7.7%), range 2–11%; for females: 23/368 (6.3%), range 4–9%.