Review Section

THE CARCINOGENIC POTENTIAL OF ETHYL CARBAMATE (URETHANE): RISK ASSESSMENT AT HUMAN DIETARY EXPOSURE LEVELS

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Summary—Ethyl carbamate is found in fermented foods: bread contains 3–15 ng/g, stone-fruit brandies 200–20,000 ng/g, and about one-third of table-wine samples analysed contained more than 10 ng/g. In animals, ethyl carbamate is degraded to CO₂, H₂O and NH₃, with intermediate formation of ethanol. This degradation has been shown to be inhibited (postponed) in the mouse by ethanol concentrations in the blood of about 0.15% and higher. A quantitatively minor pathway involves a two-step oxidation of the ethyl group to vinyl carbamate and epoxycarbamate, the postulated electrophilic moiety that reacts with DNA. This reaction is probably the mode of the mutagenic action observed in many cellular and animal systems. The fact that only vinyl carbamate, but not ethyl carbamate, is mutagenic in a standard Ames test is probably because there is insufficient production of the intermediate oxidation product in the standard test. Consistent with this metabolism is the carcinogenic activity of ethyl carbamate in various animal species and in different organs; this activity can be seen even after a single high dose in early life. Quantitative analysis of the total tumour incidences after chronic exposure of rats and mice to 0.1–12.5 mg ethyl carbamate/kg body weight/day in the drinking-water showed a dose-related increase. The main target organs were the mammary gland (female rats and mice having similar susceptibilities) and the lung (mice only). On the basis of sex- and organ-specific tumour data and with a linear extrapolation to a negligible increase of the lifetime tumour incidence by 0.0001% (one additional tumour in one million individuals exposed for life), a “virtually safe dose” of 20 to 80 ng/kg body weight/day was estimated. The daily burden reached under normal dietary habits without alcoholic beverages is in the range of about 20 ng/kg body weight/day. Regular table-wine consumption would increase the risk by a factor of up to five. Regular drinking of 20 to 40 ml stone-fruit brandy per day could raise the calculated lifetime tumour risk to near 0.01%.

Introduction

Ethyl carbamate (urethan[e]; NH₂COOCH₂CH₃) has a wide spectrum of biological activities (for reviews see Boyland, 1968; Field and Lang, 1988). In the 1940s, ethyl carbamate was used as a hypnotic in man at doses of 1 g/person/day and as an anaesthetic for laboratory animals. In 1943, it was discovered that ethyl carbamate has a carcinogenic effect in animals. Three years later, its activity against leukaemia in man was described, and since 1948 it has been known that ethyl carbamate is mutagenic in Drosophila melanogaster.

Today, humans are exposed to ethyl carbamate in food and alcoholic beverages. Under normal dietary habits, excluding alcohol, Zimmerli et al. (1986) estimated a daily ethyl carbamate intake for adults of the order of 20 ng/kg body weight, with bread assumed to be the main source (mean concentration 7 ng/g, range 3 to 15 ng/g. Three years ago it was reported that stone-fruit brandies can contain large amounts of ethyl carbamate (average concentration 1500 ng/g, range 200 to 20,200 ng/g; Anonymous, 1986). Regular daily consumption of as little as 7 ml stone-fruit brandy can lead to a ten-fold increase in ethyl carbamate intake based on normal dietary habits.

The mechanism of formation of ethyl carbamate in such spirits has been investigated (Battaglia et al., 1986; Baumann and Zimmerli, 1987 and 1988). From these studies it can be deduced that ethyl carbamate is formed from natural components, most probably by reaction of ethanol with isocyanic acid, an oxidation product of cyanide that is a well known constituent of stone fruits. This formation pathway shows a strong dependence on exposure to (sun)light (Baumann and Zimmerli, 1988).

Recent analytical data have demonstrated that ethyl carbamate is also formed in table wine. Preliminary analytical data show that levels above 10 ng/g are found in about one-third of the analysed samples (Mitchell and Jacobson, 1987; B. Zimmerli, personal communication, 1989). Here, the mechanism of formation of ethyl carbamate probably involves...
urea (Ough et al., 1988), a degradation product of arginine, either directly or after temperature-dependent deamination to isocyanic acid.

To determine whether ethyl carbamate represents a possible health hazard at low exposure levels, it is important to characterize the toxic potential of this compound, including its mechanism of action. In this context, the acute toxicity of ethyl carbamate can be neglected since it is relatively low, of the order of 2500 mg/kg body weight (LD<sub>50</sub> rat, oral administration; NIOSH, 1985).

**Pharmacokinetics and metabolism**

In rats and mice, ethyl carbamate is rapidly distributed throughout the body irrespective of the route of administration. In mice, following injection of [carbonyl-14C] labelled or [methylene-14C]-labelled ethyl carbamate, about 90% of the compound is completely degraded to CO<sub>2</sub>, H<sub>2</sub>O and NH<sub>3</sub> within 24 hr as detected by exhaled radio labelled CO<sub>2</sub>. About equal amounts, of up to 6%, are either excreted unchanged in the urine or ejected as a percentage of the administered dose are: carbamate, epoxyethyl carbamate, when incubated at the ethyl group. The vinyl carbamate and its epoxy derivative generated may represent the proximate and ultimate electrophilic metabolites responsible for genotoxicity and carcinogenicity.

The observed organ specificity in vitro noted above is not consistent with the susceptibility to tumour induction: while the lung is the main target organ for tumours in mice, it is not in rats (see below). The possibility of more active detoxication pathways in vivo (e.g. involving epoxide hydrolase; Vogel-Bindel et al., 1982) may provide an explanation for this inconsistency.

As human exposure to higher levels of ethyl carbamate results from consumption of alcoholic beverages, the interaction of ethyl carbamate with ethanol may be important. The effects of a single high dose of ethanol (5 g/kg body weight, initial blood ethanol level of about 0.48%) on the metabolism of [ethyl-1-14C]ethyl carbamate (11.1 mg/kg body weight) has recently been investigated in the mouse (Waddell et al., 1987; Yamamoto et al., 1988). Under these conditions, a high, constant blood level of ethyl carbamate persisted for 8 hr after oral administration and declined only thereafter. This was paralleled by a large decrease in the rate of 14CO<sub>2</sub> expiration during these 8 hr followed by an increase thereafter. This suggests an inhibition of metabolism of ethyl carbamate by higher blood ethanol levels (>0.15%). Without ethanol, ethyl carbamate (radioactivity) almost completely disappeared from the blood within 4 hours. The authors concluded... "that coadministration of ethanol or other alcohols may inhibit the carcinogenic action of urethane and other compounds" (Yamamoto et al., 1988). However, the cited investigation only shows that acute administration of high doses of ethanol may postpone the metabolism of ethyl carbamate, possibly by blocking metabolizing enzymes, including the group of cytochromes P-450. This may indeed influence the carcinogenic action of ethyl carbamate (see below) but it may also lead to a shift in organotropy to tissues where the P-450 isoenzymes are not inhibited to the same degree. This phenomenon is well known for the interaction of ethanol with the activation of other carcinogens such as nitrosamines (IARC 1988; Swann, 1984). In addition, very little ethyl carbamate is excreted during the inhibition of metabolism by ethanol. The toxic effects might, therefore, merely be postponed. It should also be kept in mind that chronic administration of ethanol, in contrast to the acute situation, may lead to induction of metabolizing enzyme systems such as P-450 (Lieber et al., 1987a,b) and thus modulate the carcinogenicity of ethyl carbamate. It is interesting to note that ethanol is formed as a cleavage product of the ester bond of ethyl carbamate itself. Mirvish (1968) reported that degradation of ethyl carbamate was inhibited up to 90% by blocking esterase activity, which indicates that ethanol may be formed in near equimolar amounts to the administered ethyl carbamate dose. It remains to be shown whether the ethanol thus formed can modulate the further metabolism of ethyl carbamate.

**Mutagenicity**

Vogt (1948) described ethyl carbamate as a chromosome-breaking agent able to induce the same types of mutations in Drosophila as are induced by different types of irradiation or mustard gas. Many studies have been published concerning the mutagenicity
of ethyl carbamate in a wide range of organisms, including plants (for an overview see Bateman, 1976; deSerres and Ashby, 1981; Field and Lang, 1988; Kada and Ishidate, 1980). In bacterial test systems the results were mainly negative. An explanation for this may be that in the standard Salmonella/microsome assay, using rat-liver S-9, there is insufficient oxidation of ethyl carbamate to vinyl carbamate (the first step in the metabolic activation) to give positive results. The fact that vinyl carbamate gives positive results in the Ames test (Dahl et al., 1980) strongly supports this hypothesis.

In tests with eukaryotic cells, positive and negative findings are about equal in frequency. It seems that positive results have been obtained only under conditions of appropriate metabolic activation. At this Institute, ethyl carbamate was tested for mutagenicity in the somatic mutation and recombination test in Drosophila melanogaster (number and shape of wing hairs after treatment of larvae), using a standard strain and a strain in which genetic control of the cytochrome P-450-dependent enzyme systems has been altered by substituting the first and second choice monooxygenase of a wild type, DDT-resistant strain with constitutively increased P-450 enzyme activities (Frölich, 1989; Frölich and Würgler, 1988). A dose-dependent increase in the genotoxic activity of ethyl carbamate was observed. The frequency of induction of mutations in the modified strain with increased P-450 enzyme activities was increased by about one order of magnitude compared with the standard strain. This further suggests that the P-450 enzyme system is involved in the activation of ethyl carbamate.

**DNA-adduct formation**

It is often extremely difficult or even impossible to extrapolate the data from *in vitro* mutagenicity testing (e.g. in systems like the Ames test) to a genotoxic potency *in vivo* in a quantitative manner. In contrast, DNA-adduct formation determined *in vivo* in the target tissue appears to represent a good measure of the biologically effective dose (deSerres, 1988; Lutz, 1979). With ethyl carbamate, more than ten publications give quantitative data on adduct formation, mainly in mice but also in rats. All tissues investigated showed DNA adducts. The liver almost always had the highest values, a few hours after single-dose administration (Fossa et al., 1985; Lutz, 1979; Scherer et al., 1986; Svensson, 1988). For the lung, DNA-binding potency appeared to be about one order of magnitude below the value determined for the liver (Fossa et al., 1985). The level of DNA-adduct formation for the dose administered places ethyl carbamate amongst the moderately potent genotoxic (hepato) carcinogens, a factor of 100 to 1000 below the carcinogenic potency of aflatoxin B1 and a factor of ten below the potency of vinyl chloride.

Several authors proposed a metabolic pathway that leads to the formation of vinyl carbamate and, after epoxidation, to DNA and RNA adducts (Miller and Miller, 1983; Ribovich et al., 1982). Recently, this hypothesis has been supported by the study of Gupta and Dani (1989), who identified N-hydroxy vinyl carbamate and an epoxy derivative of ethyl carbamate as metabolites. Furthermore, 7-(2-oxoethyl)guanine was identified as a DNA adduct in mouse and rat liver after injection of labelled ethyl carbamate (Miller and Miller, 1983; Scherer et al., 1986). In addition, vinyl carbamate was shown to lead to the same DNA adducts as ethyl carbamate, although the potency of the former was much higher (Scherer et al., 1986).

Since 7-(2-oxoethyl)guanine is also formed by vinyl chloride (Laib et al., 1981), Svensson (1988) postulated a common ultimate carcinogen for the two compounds and compared their potency to form DNA-adducts with their respective carcinogenic potencies. This quantitative comparison revealed a good correlation of the ethyl carbamate data with the vinyl chloride data.

**Carcinogenicity**

The data discussed above indicate that ethyl carbamate is genotoxic *in vitro* and *in vivo*. It is therefore not surprising that this compound is also reported to be carcinogenic. There is a vast literature on ethyl carbamate carcinogenicity (IARC, 1974; Mirvish, 1968; National Cancer Institute, 1978 and 1980). The following list is a summary derived from the data reviewed in IARC (1974): Doses of 100–2000 mg ethyl carbamate/kg body weight, typically a single dose of 1000 mg/kg body weight, have been shown to induce tumours in rats, mice and hamsters after administration by inhalation or by oral, dermal, subcutaneous or intraperitoneal routes. A tumour incidence of 40–100% is typical after administration of 100–1000 mg ethyl carbamate/kg body weight in the drinking-water, depending on the duration of treatment. A high tumour incidence (80–100% lung tumours) was also found in newborn mice after a single “standard” dose of 1000 mg ethyl carbamate/kg body weight given by oral gavage. When pregnant mice were given a single intraperitoneal injection of “this standard” dose one day before delivery, 100% of the offspring developed lung tumours within 6 months (9–10 tumours per mouse). Tumour induction in offspring was also demonstrated when ethyl carbamate was administered to lactating mice.

At present, only two experimental carcinogenicity studies with doses in the lower mg/kg body weight range are available in the literature (a further carcinogenicity study is currently being conducted in the National Toxicology Program, Food Chemical News, 1988). In the first study (Dahl et al., 1980), newborn mice were injected intraperitoneally twice a week with 0, 4.1, 8.2, 12.1 or 501.2 mg ethyl carbamate/kg body weight for 4 weeks and rats were similarly injected twice a week with 0, 8.2 or 300 mg ethyl carbamate/kg body weight for 5 weeks. A dose-dependent increase in tumour frequency was found in the lung (mice only) and the liver (thymoma and Harderian gland tumours were also found). Liver tumour incidences were, in male mice, 24, 56, 88, 100% and in female mice, 0, 9, 27, 35 and 70%, for groups given 0, 4.1, 8.2, 12.1 and 501.2 mg/kg, respectively. In male rats the liver tumour incidences were 0, 15 and 28%, and in female rats 0, 9 and 47% for the groups given 0, 8.2 and 300 mg/kg, respectively. As ethyl carbamate administration was
restricted to eight or ten injections, this study is unsuitable for risk assessment in humans.

In the second study (Port et al., 1976, Schmähl et al., 1977), 8-wk-old Sprague–Dawley rats and NMRI mice were exposed to 0, 0.1, 0.5, 2.5 or 12.5 mg ethyl carbamate/kg body weight/day in the drinking-water for up to 2 years. Again, dose-dependent increases in tumour incidence were found in certain organs. However, the quantitative data were reported as tumour incidences pooled for all organs. The sex-specific and target organ-specific data have now been compiled from original data kindly provided by Dr R. Port (German Cancer Research Center, Heidelberg, personal communication, 1989). In the rat, females exposed to the higher doses showed increased tumour incidences in the mammary gland (Table 1). At 12.5 mg/kg body weight/day the incidence of mammary tumours was 24% (9/38) compared with 0% (0/36) in the controls. There was no organ-specific increase in tumour incidence in male rats. In female mice a dose-dependent increase in the incidence of benign and malignant mammary tumours was also seen (6/33 at 12.5 mg/kg compared with 0/38 in the control group). In addition, both male and female mice showed an increased incidence of benign and malignant lung tumours (Table 1); at 12.5 mg/kg males and females showed incidences of 5/32 and 10/33, respectively, while there were no lung tumours in the control groups. At 2.5 mg/kg, only the males had an increased lung-tumour incidence (4/28).

Two Russian studies reported by IARC (1988) investigated the interaction of ethyl carbamate and ethanol in mice. In the first study, mice were treated twice a week for five treatments with 2 mg ethyl carbamate/mouse (about 100 mg/kg body weight) by gastric intubation either in water or in 0.2 ml 40% ethanol. In the second study, mice were injected intraperitoneally with 10 mg ethyl carbamate/mouse (500 mg/kg body weight), again either in water or in 0.2 ml 40% ethanol twice a week for 5 wk. In both studies, the presence of ethanol as a solvent enhanced pulmonary adenoma development, after 6 or 4 months, respectively (the number of pulmonary adenomas per mouse after 12 weeks was 13 [in water] and 30 [in ethanol] in the second study).

Ethyl carbamate carcinogenesis was also shown to be influenced by the co-administration of other chemicals (N-acetylcysteine; deFlora et al., 1986) and by physical agents such as X-rays where, depending on the dose of X-rays co-administered, enhancement or reduction has been observed (see Mirvish, 1968).

Various organs besides the mammary gland and the lung are susceptible to ethyl carbamate tumour induction: lymphomas, vascular tumours, skin tumours, and hepatomas have been found. A comparison of tumour susceptibility among various organs of foetal, young and adult mice showed a high tumour susceptibility in rapidly proliferating and undifferentiated cells (Nomura, 1976).

In almost all carcinogenicity studies, ethyl carbamate doses tested were well above the saturation dose of the metabolizing enzyme system. The data published by Yamamoto et al. (1988) showed that the inhibition of ethyl carbamate metabolism by acute ethanol administration did not open alternative metabolic pathways or markedly increase urinary
excretion. It seems reasonable, therefore, to assume that saturation of metabolism at high levels of ethyl carbamate does not lead to a higher proportion of detoxication. Therefore, a single large dose could well give rise to DNA damage for a number of days and become equivalent to a subacute dosing regimen.

**Low-dose extrapolation and risk assessment**

The data summarized above clearly indicate that ethyl carbamate is a pluripotent carcinogen with respect to tumour induction in different species, organs, and stages of development of the animals.

Animal studies require rather high dose levels for a tumour rate to become significantly increased. The lowest tumour incidence that can be assessed depends on the background tumour rate and on the number of animals used: to establish a 10% increase in tumour rate with a confidence limit of 95% in a population with a spontaneous rate of 1%, 40 animals are needed. To assess an increase in tumour rate of 1% or 0.1%, 1100 or 80,000 animals per dose group would be needed. From this it is clear that the dose range examined for ethyl carbamate in food and beverages (some 10 to 100 ng/kg body weight/day, which is four to five orders of magnitude below the doses needed for animal experimentation) is experimentally inaccessible and a corresponding tumour risk must be estimated.

The FDA in the USA proposed the use of a single, very simple method for the risk assessment of carcinogens: a point on the dose-response curve is chosen where, in the judgement of the FDA, the data are still reliable, and a straight line is drawn from that point through the origin ("linear-at-low-dose, no-threshold model"; Crump, 1984; Lorentzen, 1984). Using the linear-at-low-dose, no-threshold model, a lifetime risk level of 1/10^4 is often considered to provide adequate protection (Rodricks, 1988).

This risk level is considered to be negligible or insignificant and the corresponding dose level may be called a "virtually safe dose".

A linear extrapolation will be used below to calculate such a virtually safe dose for ethyl carbamate. In view of the possibility that DNA-adduct formation represents the mechanism of tumour induction, a linear low dose-response curve appears to be appropriate (Lutz et al., 1990).

Treatment of female rats with 12.5 mg ethyl carbamate/kg body weight/day resulted in an increased incidence of breast tumours of 24% (Table 1). With linear extrapolation to an incidence of one in a million, the dose required for this minute "acceptable" lifetime excess risk can be calculated to be 53 ng/kg body weight/day. In female mice, the corresponding figure is 69 ng/kg body weight/day.

In mice, an additional major target organ is the lung. Using the incidence induced by 12.5 mg/kg body weight (Table 1) and a linear extrapolation, a daily dose of 80 ng/kg body weight/day would result in one additional lung tumour in one million males exposed for lifetime. At 2.5 mg/kg body weight/day four lung tumours were induced in male mice. Using this incidence for a linear extrapolation, a daily dose of 50 ng/kg body weight results in females body weight. In females body weight, the corresponding dose is calculated to be 41 ng/kg body weight/day. It follows that the breasts and lungs of NMRI mice are about equally susceptible to the carcinogenic activity of ethyl carbamate.

The virtually safe dose levels for two organs and two animal species span a factor of only four to five. We therefore use these data with some confidence for a risk estimation in adult humans. Here, a daily burden of about 20 ng/kg body weight/day is reached under normal dietary habits, excluding alcoholic beverages. Individual habits may greatly increase the risk above the "widely accepted" level: if 500 ml table wine are consumed daily, the risk is increased by a factor of up to five, relative to the above-mentioned risk under normal dietary habits. Regular drinking of 20-40 ml stone-fruit brandies per day would raise the calculated tumour risk to near 1/10^4.

Ethyl carbamate is quite carcinogenic even after one application. If administered during early development, typical single doses of 1 g/kg body weight give a high tumour incidence. With chronic administration starting at the age of 8 weeks, a total administered dose of about 1 g/kg body weight resulted in much lower tumour induction (see Table 1). Therefore, exposure to ethyl carbamate during foetal development and early growth appears to be more critical by about one order of magnitude. Consumption of stone-fruit brandy during pregnancy and breastfeeding could, therefore, represent an unacceptable risk to the progeny not only because of the ethanol but also because of ethyl carbamate.

We are aware of all the uncertainties in the risk assessment presented above. The combined knowledge of mechanism, genotoxicity and carcinogenicity under many conditions indicates that ethyl carbamate has to be considered a potentially important human dietary carcinogen and its content in foodstuffs and beverages should be as low as possible. Since ethyl carbamate is formed by different processes in a variety of food items, the pertinent conditions of reaction and formation should be further investigated.

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