Detection and Quantitation of Trace Levels of Ethyl Carbamate in Alcoholic Beverages by Selected Ion Monitoring

Benjamin S. Clegg* and R. Frank

Ethyl carbamate (urethane), a known carcinogen, has been identified in various fermented beverages and distilled products of Ontario. A specific, sensitive gas chromatographic procedure is described for the determination of ethyl carbamate in alcoholic beverages. Initially samples are pretreated with sodium chloride and then the ethyl carbamate is extracted with dichloromethane and the solvent evaporated to incipient dryness. The residue is then redissolved in methanol or ethyl acetate followed by examination without cleanup by capillary gas chromatography using an ion trap detector (ITD). The method proved to be suitable for the determination of trace levels of ethyl carbamate in various matrices. Recoveries were better than 80% in all products analyzed at fortification levels ranging from 10 to 400 $\mu g\ L^{-1}$.

Ethyl carbamate, carbamic acid ethyl ester or urethane as it is more commonly known, has been identified in various fermented beverages and distilled products of Ontario. Urethane is recognized as a carcinogen (Nettleship et al., 1943; Mirvish, 1968; *Food and Chemical News*, 1972), and several agencies have decided to limit levels present in alcoholic beverages (Kwinter, 1984; *Food and Chemical News*, 1986). More specifically, the Canadian Federal Government established guidelines limiting amounts of ethyl carbamate in alcoholic beverages. The levels were as follows: wines, 30 $\mu g\ L^{-1}$; fortified wines (sherries and ports), 100 $\mu g\ L^{-1}$; distilled spirits, 150 $\mu g\ L^{-1}$; fruit brandies and liqueurs, 400 $\mu g\ L^{-1}$ (Kwinter, 1984).

Ethyl carbamate has previously been identified in wines (Walker et al., 1974; Joe et al., 1976) and other fermented products such as beer, ale, sake, olives, soy sauce, and yogurt (Ough, 1976a,b). Various methods have been published for the determination of ethyl carbamate. A radioisotopic dilution technique was described by Lofroth and Gejvall (1971), and later a method was reported (Walker et al., 1974) where ethyl carbamate was extracted with chloroform and subjected to Florisil column cleanup and residues were determined by gas chromatography using a Coulson conductivity detector (N-mode). Confirmation of ethyl carbamate was made by identification of the trifluoroacetic acid anhydride derivative on an alkali flame ionization detector. Shortly after, Ough (1976a) described a method sensitive to 10 $\mu g\ L^{-1}$ in which residues of ethyl carbamate were identified after cleanup using a Coulson nitrogen detector. Another method (Joe et al., 1976) described the use of an alkali flame ionization detector (AFID) or flame ionization detector (FID) for the detection of urethane with confirmation of ethyl carbamate by GLC–MS. Both EI (electron ionization) and CI (chemical ionization) modes of mass spectrometry have been demonstrated (FDA, 1972; Joe et al., 1976).

The present paper describes a sensitive method suitable for the determination of ethyl carbamate in various alcoholic beverages without extensive cleanup procedures. Samples are treated with saturated sodium chloride and extracted with dichloromethane and the solvent concentrated followed by injection and quantitation on a capillary gas chromatograph equipped with an ion trap detector (ITD). Selected ion monitoring with the ITD allowed positive identification of ethyl carbamate in complex matrices at a minimum detectable level of 5 $\mu g\ L^{-1}$.

Provincial Pesticide Residue Testing Laboratory, Ontario Ministry of Agriculture and Food, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

EXPERIMENTAL SECTION

Chemicals. All solvents used were pesticide-grade Caledon Laboratories Ltd. (Georgetown, Ontario, Canada). Ethyl carbamate used for fortification and quantitation was listed as 98% pure and obtained from Aldrich Chemical Co (Milwaukee, WI).

Sample Extraction. A representative sample (50 mL of wine, 25 mL of port/sherry, 15 mL of distilled spirits, 15 mL of brandy/liqueur) was added to a 250-mL glass centrifuge bottle containing 30 g of sodium chloride. Total volumes were then made up to 50 mL with saturated sodium chloride, and a volume of dichloromethane was added to the centrifuge bottle (70 mL for wines, 40 mL for all others). The bottle was stoppered, shaken manually for 1 min, and then centrifuged at 1500 rpm for 10 min. The contents of each centrifuge bottle were then carefully decanted into a 250-mL separatory funnel, ensuring that no solid sodium chloride was transferred during the process. An additional 70 mL of dichloromethane for wines or 40 mL for other beverages was added to the centrifuge bottle and the process repeated. Prior to the second centrifugation step the initial separatory funnel separations were completed. The methylene chloride extract was drained through cotton prewashed with dichloromethane into a 250-mL receiver. The contents of the centrifuge bottles were again decanted into the separatory funnels, and the second separatory funnel separation was repeated. A third and final volume of dichloromethane (70 mL for wines, 40 mL for others) was added to the centrifuge bottle, and the process was repeated once more. To the receiver containing the dichloromethane (ca. 210 mL for wines, ca. 120 mL for others) a volume of 2.0 mL of ethyl acetate or methanol was added, and the extracts were concentrated on a rotary evaporator (27 °C) to incipient dryness (<2.0 mL). Care was taken to ensure that evaporation did not go to dryness as the ethyl carbamate residues could be readily lost by sublimation. The sample residue was quantitatively transferred to a 15-mL glass-stoppered centrifuge tube. The flask was then rinsed once more with an additional 2.0 mL of ethyl acetate, which was transferred to the centrifuge tube and the final volume adjusted to 5.0 mL with ethyl acetate prior to GC analysis.

For beverages that present emulsion problems, the following procedures were used.

Cream-Base Liqueurs and Mashes. A 20-mL aliquot of the liqueur was added to 40 mL of acetonitrile and the resultant mixture mixed well. A 30-mL aliquot (10-mL sample equivalent) was then transferred to the centrifuge

0021-8561/88/1436-0502$01.50/0

Table I. Percent Recovery of Ethyl Carbamate from Fortified Samples

matrix	% recovery[a] at fortification level, μg L^{-1} ppb						
	10	25	50	100	150	200	400
wines	98 (6.3, 6)	96 (9.5, 6)	97 (7.0, 12)	97 (5.6, 6)		96 (11, 4)	
fortified wines (sherries and ports)			81 (11, 12)	87 (14, 6)	84 (7.5, 6)	82 (13, 4)	
distilled spirits				94 (6.5, 6)	91 (7.6, 12)	93 (9.8, 12)	
fruit brandies/liqueurs					84 (12, 12)	78 (9.0, 4)	
cream-base liqueurs				93 (2.7, 6)			87 (7.0, 6)
mashes				84 (6.9, 4)	92 (9.7, 8)		

[a] Standard deviation and number of replicates in brackets (SD, replicates).

bottle as in other extractions, and 100 mL of saturated sodium chloride solution was added. The extraction was then processed in a manner similar to that for wines (i.e., 3 × 70 mL of dichloromethane, etc.).

A 20-mL sample of the mash sample was added to 40 mL of acetonitrile and the resultant solution mixed well. A 30-mL aliquot (10-mL sample equivalent) was then removed and processed in a manner similar to that for the cream-based liqueurs.

Gas Chromatography–Mass Spectrometry. A Perkin-Elmer Model 8320B capillary gas chromatograph, equipped with an ion trap detector (ITD), was used for detection, quantitation, and confirmation of ethyl carbamate. The capillary column used was a Supelcowax 10 fused silica column, 30 m × 0.25 mm, with 0.25-μm coating (Supelco Canada Ltd., Oakville, Ontario, Canada) coupled directly to the ion trap detector. The chromatographic conditions were as follows: injector, 225 °C; transfer line, 225 °C; column oven, initial 60 °C, hold 1 min, programmed at 5 °C min^{-1} to 150 °C, and then 30 °C min^{-1} to 220 °C with a hold time of 2 min to allow elution of late compounds; helium carrier gas (UHP) with a head pressure of 15 psi, 30 cm s^{-1} linear velocity. The ion trap was optimized with the disk software under AUTO-TUNE condition with PFTBA (perfluorotributylamine) calibration. Mass spectra were acquired over the 30–175-amu range at 1 scan s^{-1}. Quantitation of ethyl carbamate was performed with use of reconstructed ion chromatograms of stored data. The most suitable ion for quantitation was the m/z 62 ion since most extracts presented interferences that made quantitation difficult when more abundant ions such as m/z 44 and 74 were used. The molecular ion of ethyl carbamate was protonated in the ITD and appeared at m/z 90, confirming the molecular weight of 89.

A total ion chromatogram and characteristic reconstructed ion chromatograms for ethyl carbamate are given in Figure 1. The retention time for ethyl carbamate is 16.95 min, which corresponds to scan number 1017. Figure 2 (top) shows the mass spectrum of standard ethyl carbamate along with results of an NBS library search. The NBS library spectrum shows the molecular ion at m/z 89 whereas the standard presents the protonated molecular ion at m/z 90. Figure 2 (bottom) shows the reconstructed mass chromatogram of the m/z 62 ion used for quantitation.

Fortification. Recoveries were determined by fortifying various products prior to extraction with ethyl carbamate at levels that bracketed the guideline levels established by Health and Welfare Canada. Recovery data for the various matrices are given in Table I. The minimum detectable level of ethyl carbamate was 5 μg L^{-1} based upon the complexity of the matrices analyzed.

RESULTS AND DISCUSSION

An ion trap detector was used to detect the presence of ethyl carbamate in a wide variety of alcoholic beverages and products. The detection system proved to be extremely precise and was able to make positive identification of ethyl carbamate in these very complex matrices.

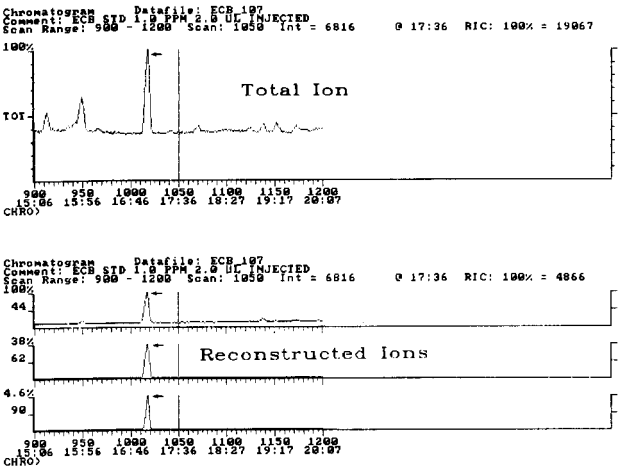

Figure 1. Total ion chromatogram (top) and reconstructed ion chromatograms (bottom) for ethyl carbamate.

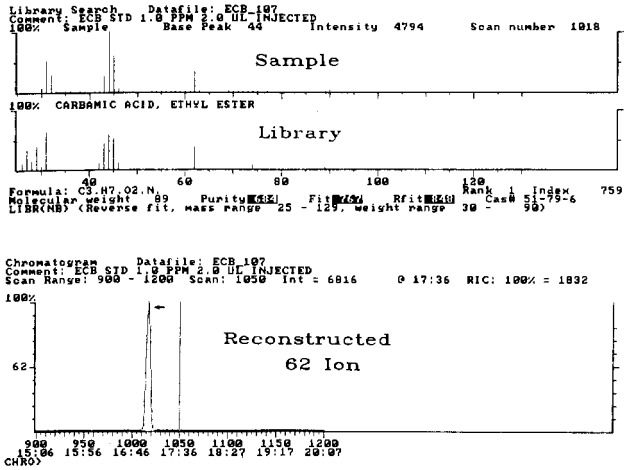

Figure 2. Mass spectrum of ethyl carbamate (top) and reconstructed m/z 62 ion (bottom) used for quantitation.

The recovery for this method was greater than 80% in all products as indicated in Table I, with an overall mean recovery for all substrates tested of 89%. These results are slightly better than the results obtained in a method evaluation/certification program initiated by Health and Welfare Canada in 1986. The percent recovery and corresponding standard deviations of our laboratory in this program were as follows: wine, 96.3 (7.6); sherry, 77.9 (5.0); rye, 82.7 (12.8); brandy, 73.2 (11.3). The mean recovery for beverages analyzed was 83% at fortification levels ranging from 20 to 500 μg L^{-1}.

The final dissolving solvent was ethyl acetate or methanol. We found no significant variation in results when these two solvents were compared. Methanol, the original choice of solvent, was chosen because the solubility of ethyl carbamate was greater than that in ethyl acetate. However, since the boiling point of methanol was somewhat

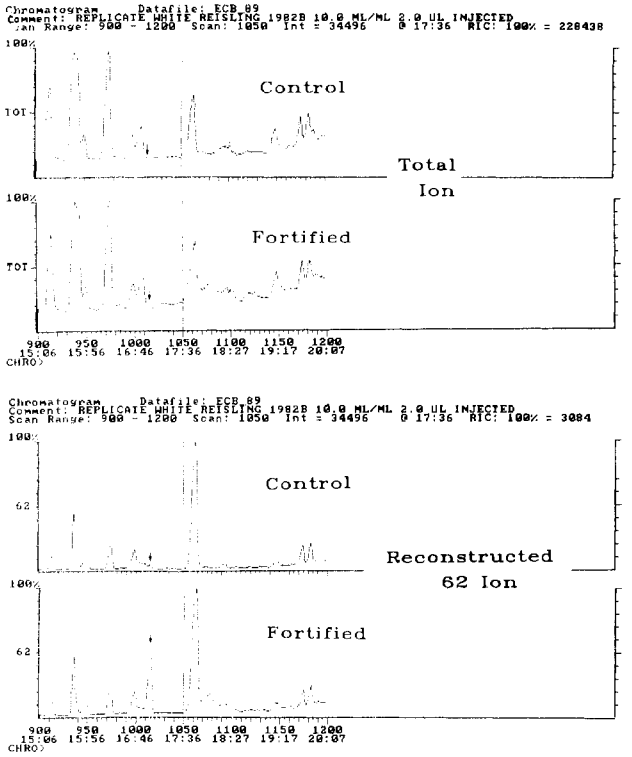

Figure 3. Total ion chromatograms (top) and reconstructed m/z 62 ion (bottom) for a control wine and wine fortified at 50 μg L^{-1}.

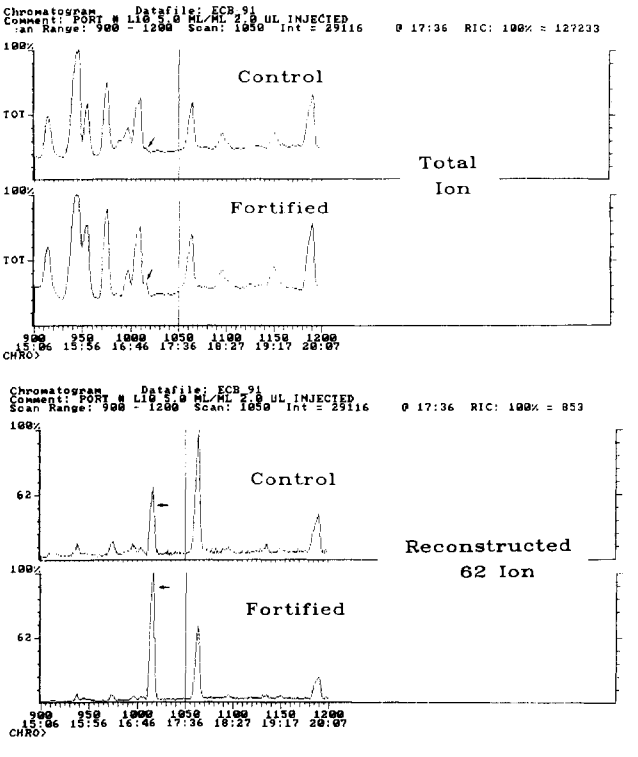

Figure 4. Total ion chromatograms (top) and reconstructed m/z 62 ion (bottom) for a control port and port fortified at 200 μg L^{-1}.

lower than ethyl acetate (i.e. 64.96 vs. 77.08 °C), more care had to be taken in the evaporation step to prevent extracts from going to complete dryness. There was also some suggestion that, in the presence of slightly acidic conditions, a transesterification reaction could occur between methanol and ethyl carbamate resulting in low recoveries (McAlees, 1986; Page, 1986). This was not observed in this study. However, for consistent results that could be suitably compared as inter-laboratory data, the final dissolving solvent used was ethyl acetate as recommended by Health and Welfare Canada (1986).

Ethyl carbamate was determined by examining the reconstructed ion chromatograms of the mass spectral data acquired over the 30–175-amu range. In particular, the reconstructed m/z 62 ion was the most suitable for quantitation rather than the other characteristic ions of m/z 44 and 74. Under the conditions used in this study, an ion at m/z 90 was observed (Figure 2) that is representative of the protonated molecular ion of ethyl carbamate (m/z 89). This result is in agreement with that published earlier (Joe et al., 1977). In many cases the other characteristic ions of ethyl carbamate (i.e., m/z 44 and 74) were unsuitable because the different matrices often presented interferences at the same retention time of ethyl carbamate which made quantitation difficult.

Figure 3 shows the reconstructed ion chromatograms (total ion and m/z 62) for a blank wine and a wine fortified at 50 μg L^{-1}. In wine extracts a peak elutes just prior to ethyl carbamate that makes quantitation difficult using the m/z 74 ion. This compound was identified as diethyl butanedioic acid (diethyl succinate). A variety of wines (16) were analyzed, and only two were found to contain ethyl carbamate above the 30 μg L^{-1} guideline (levels of 770 and 1800 μg L^{-1}). It is interesting to note that these two wines were known to be fermented and aged in the presence of urea, a yeast nutrient source believed to contribute to the formation of ethyl carbamate (Ough, 1976b). Analysis of white and red wine grape juice concentrates showed no detectable residues of ethyl carbamate.

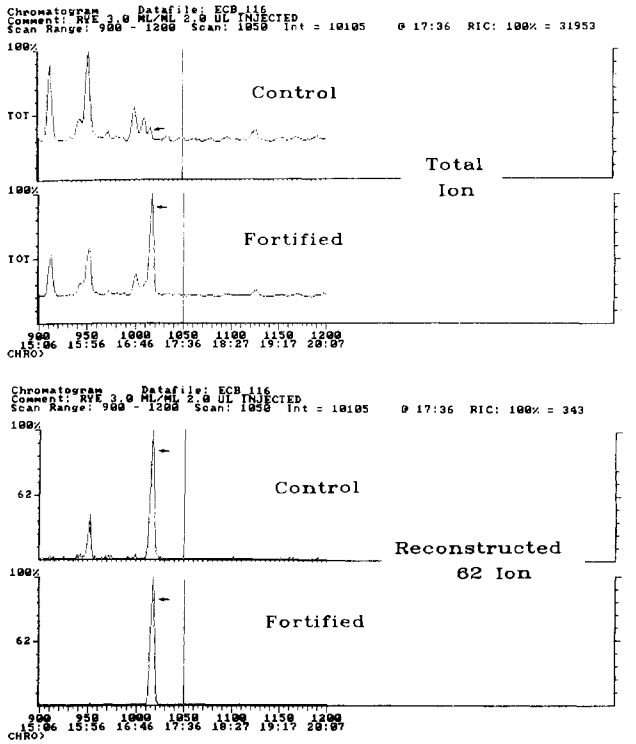

Figure 5. Total ion chromatograms (top) and reconstructed m/z 62 ion (bottom) for a control rye and rye fortified at 60 μg L^{-1}.

Reconstructed ion chromatograms (total ion and m/z 62) for a control port and port fortified at 200 μg L^{-1} are given in Figure 4. Diethyl succinate was identified on the total ion chromatograms as the peak eluting prior to ethyl carbamate. Analysis of eight fortified wines (two sherries and six ports) showed residues of ethyl carbamate ranging from 55 to 830 μg L^{-1}. Only one port showed residues below the 100 μg L^{-1} guideline (i.e., 55 μg L^{-1}), while two sherries were marginally above tolerance (110, and 140 μg L^{-1}). The remaining five ports analyzed were found to be

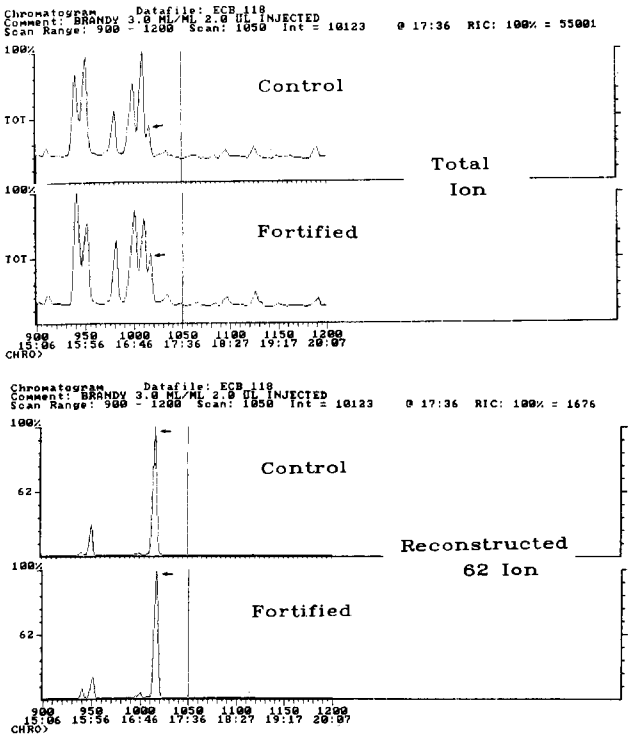

Figure 6. Total ion chromatograms (top) and reconstructed m/z 62 ion (bottom) for a control brandy and brandy fortified at 1000 $\mu g\ L^{-1}$.

above tolerance with residues from 170 $\mu g\ L^{-1}$ (tank-aged port) to 830 $\mu g\ L^{-1}$ (barrel-aged port). The results for tank- and barrel-aged ports show the potential for increased levels of ethyl carbamate following barrel aging.

Representative ion chromatograms (total ion and m/z 62) for a control rye and rye fortified at 60 $\mu g\ L^{-1}$ are given in Figure 5. The total ion chromatograms indicate that the distilled products, in general, do not demonstrate the complex matrices seen in wines, sherries, and ports. Distilled spirits (21) including various blending components were analyzed for ethyl carbamate. No distilled spirits showed residues above tolerance (100 $\mu g\ L^{-1}$) with levels ranging from 5 to 70 $\mu g\ L^{-1}$; however, one blending component showed residues of 320 $\mu g\ L^{-1}$.

Figure 6 shows the reconstructed ion chromatograms (total ion and m/z 62) for a control brandy and a brandy fortified at 1000 $\mu g\ L^{-1}$. Fruit brandies (14) and liqueurs (7) were analyzed for ethyl carbamate with levels determined for these products ranging from 10 to 1500 $\mu g\ L^{-1}$. Individual levels were 10–1500 and 19–63 $\mu g\ L^{-1}$ for fruit brandies and liqueurs, respectively. No liqueurs exceeded tolerance while eight fruit brandies contained >1000 $\mu g\ L^{-1}$.

The method described is currently being used to monitor a large variety of products with the purpose of identifying more specifically those products where increased levels of ethyl carbamate are observed. Further work is also in progress to identify more adequately the sources of ethyl carbamate, i.e. potential precursors, and/or specific production conditions that contribute to the levels of ethyl carbamate found in various alcoholic beverages.

ACKNOWLEDGMENT

We acknowledge Young Ja Paik for her tireless technical assistance and the cooperation of G. W. B. Hostetter; H. Gras and D. Nichol (Brights Wines Ltd., Niagara Falls, Ontario); and S. A. Wright and M. Sobolov (Hiram Walker and Sons Ltd., Walkerville, Ontario) for supplying many of the samples required to complete this study.

Registry No. Urethane, 51-79-6.

LITERATURE CITED

Food and Chemical News **1986**, Dec 1, 21–38.
Food and Chemical News **1972**, *14*, 32.
Food and Drug Administration *Food Additives Analytical Manual* **1972**, 320–333.
Health and Welfare Canada *1986 Laboratory Procedure* January 7, 1986.
Joe, F. L.; Kline, D. A.; Miletta, E. M.; Roach, J. A. G.; Roseboro, E. L.; Fazio, T. "Determination of Urethane in Wines by Gas-Liquid Chromatography and Its Confirmation by Mass Spectrometry". *J. Assoc. Off. Anal. Chem.* **1976**, *60*(3), 509–516.
Kwinter, M. Communication to House of Commons, Dec 1984.
Lofroth, G.; Gejvall, T. "Diethyl Pyrocarbonate: Formation of Urethan in Treated Beverages". *Science (Washington, D.C.)* **1971**, *174*, 1248–1250.
McAlees, A., personal communication, 1986.
Mirvish, S. S. "The Carcinogenic Action and Metabolism of Eurethan and N-Hydroxyurethan". *Adv. Cancer Res.* **1968**, *11*, 1.
Nettleship, A.; Henshaw, P. S.; Meyer, H. L. "Induction of Pulmonary Tumors in Mice with Ethyl Carbamate (Urethane)". *J. Natl. Cancer Inst.* **1943**, *4*, 309.
Ough, C. S. "Ethyl Carbamate in Fermented Beverages and Foods. 1. Naturally Occurring Ethyl Carbamate". *J. Agric. Food Chem.* **1976a**, *24*, 323–328.
Ough, C. S. "Ethyl Carbamate in Fermented Beverages and Foods. 2. Possible Formation of Ethyl Carbamate from Diethyl Dicarbonate Addition to Wine". *J. Agric. Food Chem.* **1976b**, *24*, 328–331.
Page, D., Health and Welfare Canada, personal communication, 1986.
Walker, G.; Winterlin, W.; Fonda, H.; Seiber, J. "Gas Chromatographic Analysis of Urethan (Ethyl Carbamate) in Wine". *J. Agric. Food Chem.* **1974**, *22*, 944–947.

Received for review February 18, 1987. Accepted October 7, 1987.