Occurrence of Ethyl Carbamate (Urethane) in U.S. and Canadian Breads: Measurements by Gas Chromatography-Mass Spectrometry

WILLIAM F. HADDON,[1,2] MICHAEL L. MANCINI,[3] MARGARET McLAREN,[4] ADAN EFFIO,[4] LESLIE A. HARDEN,[1] RICHARD L. DEGRE,[5] and JEFFREY L. BRADFORD[1]

Cereal Chem. 71(2):207–215

ABSTRACT

In a survey of U.S. and Canadian breads, urethane, a chemical shown to be carcinogenic in animals, was detected at low levels in 22 batches of commercial breads and rolls that were sampled immediately after baking. The mean level of urethane was 2.06 ± 0.2 ppb for measurements on composite samples of 12 loaves prepared using established procedures for trace nutrient analysis. The mean value of urethane was less than one third the 7-ppb value used by others to estimate probable nonalcohol human dietary exposure to urethane. Based on the newly determined mean level for urethane and bread consumption data, the per capita dietary intake per year in the United States is 47 ± 4 μg for untoasted bread. Chemically specific gas-chromatographic detectors employing either high-resolution mass spectrometry or methane chemical-ionization mass spectrometry were used for all measurements, yielding a quantification precision of ±0.2 ppb at the 1-ppb level. The average recovery of urethane from white bread was 110% in the 3–7 ppb range.

Urethane (ethyl carbamate, EC) is one of a large number of chemical compounds in the food supply that are carcinogenic in animals (Ames 1990, Gold et al 1992). Regulatory interest focused on urethane in the 1970s when it was reported that use of the sterilant diethyl pyrocarbonate (DEPC) produced elevated levels of urethane in alcoholic beverages (Loforth and Gejvall 1971), ostensibly by its reaction with residual ammonia. However, urethane could still be detected in wines after DEPC was banned as a food additive in 1972, and it was later shown to be produced intrinsically in fermented foods and beverages, occasionally at levels exceeding 1,000 ppb (Ough 1976a,b). EC is, in fact, widely distributed in the food supply. It occurs, usually at levels below 10 ppb, in virtually all fermented food products, including bread (Dennis et al 1989, Battaglia et al 1990). Although there have been few definitive studies on the source of intrinsic urethane, recent reports (Ough et al 1988, Monteiro et al 1989) suggest that the major pathway for its production in wine is the reaction of urea, an end product of arginine metabolism, with alcohol produced during fermentation. Isocyanic acid may be the direct biosynthetic precursor of urethane, rather than urea itself. As expected, levels of urethane in alcoholic beverages, where several thousand parts per billion have been measured, can be much higher than those in nonalcoholic foods, where mean levels are usually below 10 ppb. The exceptionally high levels of up to 10,000 ppb occurring in stone fruit brandies may be due to additional biosynthetic pathways of urethane synthesis, possibly involving cyanide precursors (MacKenzie et al 1990).

The carcinogenic potential of urethane has been recognized for about 50 years. It is a pluripotent carcinogen; studies performed at high-dose rates in several animal species showed tumors were produced in different organs and at different stages of animal development (Schlatter and Lutz 1990). Accurate information on the dietary exposure level has particular importance because the linear-at-low-dose, no-threshold model for risk assessment, when applied to urethane carcinogenesis data, specifies a daily exposure limit of 50–80 ng/kg of body weight per day, which is uncomfortably close to the presumed dietary exposure from bread: ~20 ng/kg, based on a 7-ppb mean urethane level (Schlatter and Lutz 1990). While this risk assessment model has been seriously questioned (Ames 1990), it is likely to be applied for some time in regulatory decisions. Long-term animal feeding studies now underway in the National Toxicology Program should produce improved risk assessment information for EC.

When 7 ppb is used for the mean urethane level, bread must be considered as an important source of urethane intake in the human diet. However, a more recent sampling of retail bread from grocery stores in the Washington D.C. area showed that urethane was present above a 2-ppb limit of quantification in only 13 of 30 samples (Canas et al 1989). It was suggested that urethane levels are correlated with bread type, varying from 3.0

[1]Western Regional Research Center, USDA, Albany, CA.
[2]Author to whom correspondence should be addressed.
[3]Agriculture Canada, St. Hyacinthe, PQ, and Biotechnology Research Center, Montreal. Present address: L.A.B. Bio-Research International, Inc., Senneville, PQ, Canada.
[4]Diversified Research Laboratories Ltd., Toronto, ON, Canada.
[5]Lallemand Inc., Montreal, PQ, Canada.

ppb in white bread to 1.2 ppb in wheat bread, and 0.9 ppb in specialty breads, including bagel, sour dough, rye, and others.

In view of the disparity in reported levels, we undertook a more comprehensive study of the occurrence of urethane in U.S. and Canadian breads. The principal objectives of the study were to develop reliable sampling and analytical procedures for detecting urethane in bread, and to conduct a survey to establish a reliable measure of dietary intake from bread. In this regard, the sampling of bread was geographically diverse and involved a distribution of types of breads that match the dietary consumption pattern reasonably closely. To address the problem of quantification, high-resolution and chemical-ionization mass-spectrometry detection methods were developed to provide the sensitivity and specificity necessary for the low levels of occurrence of urethane in bread.

Analytical methodology for measurement of urethane in food and beverages has been recently reviewed (Battaglia et al 1990). Addition of a fat-removal step to extraction procedures developed for beverage analysis provides an extract from bread and other nonalcoholic foods of substantially reduced complexity. However, chemically specific detection methods, in conjunction with chromatographic analysis, are required to avoid interferences. Nitrogen-specific detectors (Aylott et al 1987, Canas et al 1988) and several different techniques based on selected ion-monitoring mass spectrometry (Aylott et al 1987, Conacher et al 1987, Pierce et al 1988, Dennis et al 1989, Hurst et al 1990) have been utilized to reduce interferences to the analysis of urethane. Nitrogen-specific detection, however, shows reduced selectivity for bread extracts compared with other foods and beverages, giving a quantification limit of only about 2 ppb. Limited data suggests that bread extracts are significantly more complex than, for example, soya sauce or beer (Canas et al 1989). When using mass spectrometry as the detector, the selectivity of low-resolution electron ionization is insufficient for analysis of endogenous urethane in some matrices (Lau et al 1987, Clegg and Frank 1988). High-resolution chemical ionization (Lau et al 1987) and tandem mass spectrometry techniques (Brumley et al 1988) provide increased selectivity for measurements in alcoholic beverages, suggesting they might be used for measuring EC in bread.

The mass-spectrometric measurement methods for urethane have an additional advantage over N-specific gas-chromatography detectors, in that ^{13}C,^{15}N-labeled ethyl carbamate (LEC) is detected separately and can thus be added early in the extraction-separation sequence as a coextractant. Use of isotopic internal standards avoids the correction for differential recovery that is necessary when chemically related standards such as propyl carbamate (Aylott et al 1987) are used. Among the possible substitutes for mass spectrometry in urethane measurements, Fourier transform-matrix isolation infrared spectrometry appears promising because it can differentiate the isotopically labeled compound from intrinsic urethane at levels around 10 ppb (Mossoba et al 1988).

MATERIALS AND METHODS

Sampling of Breads

Breads were sampled immediately after baking, using standard procedures for trace nutrient analysis according to 21 Code of Federal Regulations (CFR) 101.9 e (1) and (2). To prepare composite samples, we selected 12 loaves randomly from a single bake and combined the heel and first slice of loaf 1, first and second slice of loaf 2, second and third slice of loaf 3, and so on, until two composite loaves were obtained. After the test loaves were weighed, they were shipped, unfrozen, for analysis. On receipt, the loaves were frozen, undried, or processed immediately. If loaves were dried before freezing or analysis, they had to be reconstituted with water. Each composite loaf contained portions of each of the 12 randomly sampled loaves.

Extraction Procedures

For gas-chromatography high-resolution mass spectrometry (GC-HRMS), bread was prepared for extraction by tearing each slice of one complete loaf, including crusts and heels, into small (5 mm^2) pieces. After these pieces were chopped in a blender, they were thoroughly mixed and stored frozen in zip-lock bags. Measurement of samples by GC-HRMS followed established procedures for extraction (Canas et al 1988, 1989; Dennis et al 1989). The extraction procedure involves weighing 10 g of bread in a Sorvall cup, adding distilled water, homogenizing, adding Celite, and extracting on an alumina column. Then fat is removed using a Sep-Pak Florisil cartridge. For quantification by mass spectrometry, 1 ml of dilute standard LEC solution (about 100 ng of LEC, equivalent to 10 ppb of EC) was added to the mixture of bread crumb and water before homogenization. For dried bread, it was necessary to reconstitute with an additional 15 ml of water per 10 g of dried bread before homogenization.

For GC chemical-ionization mass spectrometry (GC-CIMS), we scaled the procedure to 30 g of bread, fresh weight, by adding 45 ml of distilled water instead of 15 ml, and eluting from the alumina column (10 g of deactivated alumina capped with 40 g of sodium sulfate) with 350 ml of methylene chloride instead of the 150 ml used for 10-g samples. As with the 10-g samples, it is important to concentrate the elutant to 1–2 ml, but not to dryness, before fat removal with the Florisil cartridge. For quantification, LEC standard solution was added to about 6 ppb of LEC (~200 ng of LEC). As an alternative to increasing the sample size, it should be possible to concentrate the final extract threefold by evaporating the solvent.

To obtain blanks, all dilutions and extractions are performed as described, but without use of bread. In all cases, the final extracts are 1-ml evaporates of the final 7% MeOH in methylene chloride solution and are stored in screw-cap vials at −10°C until analysis by GC-MS.

Reagents are Celite 545 (not acid washed, heated to 700°C for 16 hr) (Fisher Scientific, Fair Lawn, NJ); methylene chloride, capillary GC-GC-MS grade (Burdick and Jackson), or Optima grade methylene chloride (Fisher Scientific); methanol, HPLC grade (Burdick and Jackson); alumina (Aldrich Chemical Co., Milwaukee, WI), deactivated by heating to redness in a porcelain crucible.

GC-HRMS Analysis

For high-resolution selected-ion monitoring (HRSIM), we analyzed samples in static mode by monitoring the exact mass of the ions being measured or in dynamic mode by recording the ion current in a repetitive mass scan of several hundred parts per million around the expected masses. The dynamic method has advantages for qualitative confirmation because it shows the profiles of the mass spectral peaks during elution from the GC, thus allowing their elemental composition to be confirmed for each sample (Haddon et al 1977, Tong et al 1991).

In the GC-HRMS methods, the ions to monitor for EC are m/z 62.0242, 74.0242, and, optionally, 89.0477. For LEC they are m/z 64.0242 and 76.0246, along with a suitable lock mass ion. In most of our measurements, the molecular ion of EC, m/z 89.0477, was undetectable at levels below 5 ppb. Measurements were performed in static mode on a ZAB-HF mass spectrometer and 11/250 data system (VG instruments, Manchester, England) using HRSIM with 12,000 mass resolution, and using CF_3^+ from perfluoroalkane reference compound as the lock mass ion. The dwell time was 150 msec per measured mass. The gas chromatograph was a Varian Model 6000—30 m × 0.25 mm i.d. DBWAX column (J&W Scientific, Folsom, CA), 0.25-μm film thickness—interfaced directly to the mass spectrometer with transfer lines heated to 200°C. EC and LEC elute at 11.0 min (130°C) using a GC program at 50°C for 1 min, programmed at 20°C/min to 90°C, 5°C/min to 160°C, and 20°C/min to 200°C, holding at 200°C for 5 min. For most analyses, the elution time was shortened to about 5 min by holding at 60°C for 2 min, then programming at 50°C/min to 140°C, and at 3°C/min to 150°C, then at 50°C/min to 200°C and holding for 5 min. The sample size is 2 μl of extract using splitless injection. The amount of EC injected is 0.04 ng for a 10-g bread sample with EC at the 2-ppb level.

For dynamic HRSIM, we used a VG 7070-HS mass spectrometer and an HP5800 gas chromatograph (Hewlett Packard Instruments, Palo Alto, CA). The mass spectrometer was adapted for HRSIM with a PC data system and a 28-bit precision digital-analog converter (model 202, RC Electronics, Santa Barbara, CA). Typically, m/z 62.0242 from EC and 64.0246 were monitored at 5,000 resolution, using a mass scan window of 800 ppm for quantification or 1,400 ppm for characterizing interferences. The reference lock mass ion was the $C_4H_9^+$ ion, m/z 57.0704, from background or from *n*-hexadecane injected in the batch inlet system. Peak ratios used for quantification were measured on a Kratos Mach3 data system in raw data mode (Kratos Analytical, Manchester, England). A DBWAX chromatographic column was used as described above. To compensate for reduced sensitivity, compared to that of the static mode, final extracts were concentrated threefold before injecting 2.0-μl aliquots.

Both mass spectrometers were operated in positive electron ionization mode (EI) using 70 eV of ionizing energy and source temperature of 180 or 200°C. The chromatographic column extended directly to the ion source through transfer lines maintained at 200°C.

GC-CIMS Analysis

In SIM mode, using methane reagent gas, monitor pairs of ions at m/z 90,92 and 62,64 corresponding to the protonated molecular ion, MH^+, and $(MH\text{-}C_2H_4)^+$. We used an HP5988A quadrupole GC-MS and data system (Hewlett Packard) with a DB-624 (J&W Scientific) or HP-20M capillary GC column (25-m × 0.32-μm film thickness). Spectra were obtained using methane CI reagent gas at 240V of ionizing energy at an ion source temperature of 200°C. Splitless injection was used with a 225°C injector temperature. Transfer lines to the mass spectrometer were at 250°C.

EC Standard Solutions

For standard reference solutions we used reagent grade (+99% purity) EC from Aldrich Chemical Co. and $^{13}C,^{15}N$ LEC from MSD Isotopes (Montreal), now available from C/D/N Isotopes (Montreal), to prepare a concentrated solution and a dilute (working) solution with concentrations in methylene chloride of ~1.0 mg/ml and 10 μg/ml for EC and ~1.0 mg/ml and 4.0 μg/ml for LEC. Calibration standards were prepared from the working solutions by adding 0, 200, 600, 1,800, 2,700, and 4,000 μl of dilute EC solution to 2.50 ml of dilute LEC standard solution. In the course of the work, several sets of standards were prepared with approximately the same amounts and concentrations of EC and LEC. For measurements by CIMS, 30-g bread samples were analyzed with the amount of added LEC increased from 100 to 200 ng.

Quantification

The calibration curve for quantification was obtained by measuring peak areas for m/z 62:64 and m/z 74:76 for EI and m/z 62:64 and 90:92 for methane CI, using five or more standard solutions. The response ratio was plotted as areas of chromatographic peaks for EC versus LEC, against the amount of EC (nanograms) per milliliter of standard solution. The level of EC in the original bread was calculated by determining the slope and intercept of the least-squares line through the calibration data. For example:

$$A_{62}/A_{64} = b + mC_{EC}$$

where m and b are the slope and intercept, respectively, of the standard calibration curve and C_{EC} is the concentration of EC (ng/ml) in each standard solution at constant C_{LEC}. Similiarly, the ppb (ng/g) of EC is given by:

$$(ng/g)_{EC} = W_{LEC}\,(b - A_{62}/A_{64})\,(1/m)\,(1/W_b)$$

where W_{LEC} is the weight, in nanograms, of LEC used as internal standard, and W_b is the weight of bread, in grams, used for extraction. In both equations, A_{62} and A_{64} are the chromatographic peak areas for m/z 62 and 64.

A few measurements were based on measurements from a single calibration solution after verifying linearity and approximately zero intercept of the full calibration curve. In this case, we calculated a single point relative response factor (RRF) from:

$$RRF = (A_{62}/A_{64})(C_{LEC}/C_{EC})$$

Using the measured RRF, the concentration of EC in the bread is calculated for m/z 62:64 data from the equation:

$$(ng/g)_{EC} = W_{LEC}(A_{62}/A_{64})(1/RRF)(1/W_b)$$

Values of RRF averaged about 1.15 for the ZAB-HF mass spectrometer, based on about 30 calibrations and at least four independent weighings of EC and LEC standard. Sometimes, with aging or contamination of the GC column, the RRF values increased to around 1.25. RRF values measured on the VG 7070-HS were higher, averaging about 1.26, partly because of discrimination arising from the lower accelerating voltage of the VG 7070, compared to that of the ZAB mass spectrometer. The relative response ratio tends to increase with GC column usage. Thus, injected samples should be as dilute as possible to be create accurate results. For methane CI measurements on the quadrupole mass spectrometer, the RRF was 1.107 for m/z 62:64. Values slightly above 1.0 are expected because of the isotope effect on fragmentation (Millard 1978).

Spiking-Recovery Measurements

To establish percent of recovery, spiking studies were performed using bread with low endogenous EC. LEC was added to the final extract to correct for endogenous urethane, which was measured separately. The recovery (R) is given by:

$$R(\%) = 100 \times [(C_{EC})_{tot} - (C_{EC})_{end}]/(C_{EC})_{spike}$$

where tot, end, and spike refer, respectively, to the total, endogenous, and spiked amount of EC in parts per billion.

Statistical Analysis

The statistical validity of the 12-loaf sampling procedures, including the compositing of the sample bread into two loaves, are well established for trace nutrient measurements. These procedures were adopted without modification. Measured mean levels of urethane for the four bread varieties were of particular interest in view of prior suggestions of major differences in EC levels (Canas et al 1989). The means were first examined by testing for homogeneity of variance using Bartlett's test. No significant differences in variance between data sets were found. Analysis of variance was performed on the four means corresponding to the four varieties of bread, each sampled from several breadbaking plants. The means were compared using standard F and t tests. Finally, by considering the experiments as a stratified sampling of bread, weighted means were calculated using bread consumption data for 1990. The consumption-adjusted mean urethane level was used to estimate total dietary intake from bread.

RESULTS

Full-Scan GC-MS Analysis of Final Extracts

At urethane levels of several parts per billion, the final purified extracts are still very complex, even after removal of fat. Figure 1A shows a total ion current (TIC) chromatogram for full-scan mass spectra recorded for an extract of a single-loaf sample of white bread containing approximately 6 ppb of EC and 7 ppb of added LEC. Urethane elutes at scans 386–388, as indicated by the reconstructed ion-current profiles for masses 62 and 74 shown in part B of the figure. It yields a mass spectrum (Fig. 1C) that contains many interfering ions from coeluting compounds along with those of EC and LEC. Identification of EC can be made by comparing the reference EI mass spectra for EC and

LEC (Fig. 2A,B), as well as by retention time comparison with authentic EC. Tentative identifications for several other chromatographic peaks, based on library matching of the mass spectra against 160,000 reference spectra, are given in Table I as a general indication of the composition of the final extracts. Dimethylsulfoxide (scan 326) and dimethylsulfone (scan 537), both of which are common solvents, were not used at any stage of the purification or analysis procedures. Another major component, 2-phenylethanol, elutes just after the region of the chromatogram shown in Figure 1A and is the major volatile component of the extract.

The reconstructed ion-current profiles of the m/z 62 and 74 ions in Figure 1B indicate the extent of interferences that may be expected when using unit-mass resolution spectra for analysis. Not unexpectedly, there are many compounds that yield one or both ions in their spectra, suggesting that use of low-resolution mass spectrometery, below the EC level for this sample, would be tenuous.

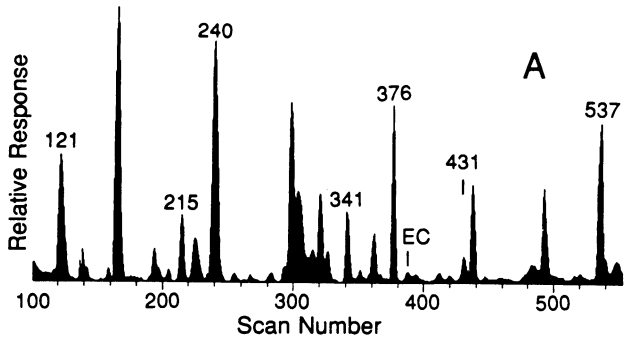

Fig. 1. Gas-chromatography mass spectrometry (GC-MS) analysis of final extracts of bread containing 6 ppb of urethane (ethyl carbamate, EC) and 7 ppb of added $^{13}C,^{15}N$-labeled EC (LEC). **A,** total ion-current chromatogram for full-scan electron-ionization mass spectra. **B,** reconstructed ion-current chromatogram for characteristic EC ions at m/z 62 and 74. **C,** electron-ionization mass spectrum of EC and LEC from scans 387-88 of total ion-current chromatogram. Gas chromatography retention time in minutes is scan number × 0.02850.

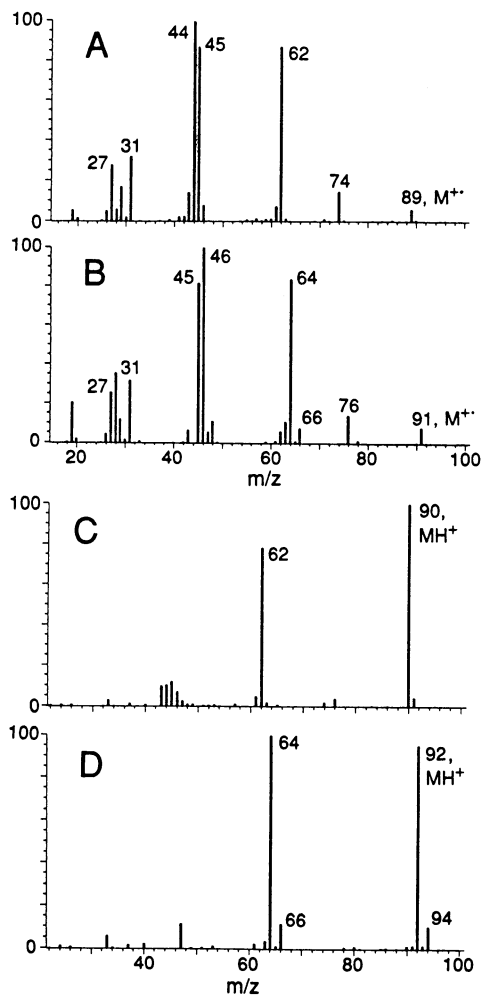

Fig. 2. Reference electron-ionization mass spectra and methane chemical-ionization mass spectra for ethyl carbamate (EC) and $^{13}C,^{15}N$-labeled EC (LEC) at ion source temperature of 145°C. **A,** 70 eV electron-ionization spectrum of EC. **B,** electron-ionization spectrum of LEC with peaks at m/z 78, 66, and 48 attributed to 8% ^{18}O impurity. **C,** methane chemical-ionization spectrum of EC. **D,** methane chemical-ionization spectrum of LEC. Peaks at m/z 94 and 66 attributed to $^{13}CH_4{}^{15}NO^{18}O$.

High-Resolution Selected Ion Monitoring

By restricting the analysis to the mass of the $CH_4NO_2{}^+$ ion at m/z 62.0242 and using high-resolution to separate interfering ions of the same nominal mass but different elemental composition, most interferences can be removed at the desired detection levels. Figure 3 illustrates the increased specificity for urethane in bread extracts containing 6 and 1 ppb of intrinsic EC. Here, the dynamic mode of selected ion monitoring was used with a 5,000 mass resolution. Figure 3A shows the computer-reconstructed chromatogram obtained by integrating the ion-current signal from m/z 61.93 to 62.10, which would be the observed signal for unit-mass resolution selected-ion monitoring for the 6-ppb sample. EC and LEC elute at scan 177 (11 min), but there are additional peaks in this region of the chromatogram. In Figure 3B and C, high-resolution TIC curves derived from the ion current at the center of the mass peaks, attributable to EC at m/z 62.0242, are shown for the same 6-ppb sample and for one containing 1 ppb of EC (Fig. 3C). Figure 3D is the response from $^{13}CH_4{}^{15}NO_2{}^+$ for LEC in the 1-ppb sample. Curves B–D in Figure 3 are equivalent to static HRSIM at 5,000 mass resolution and exhibit greatly increased specificity for EC and LEC compared to that of unit-mass resolution analysis. These data establish the suitability of HRSIM for bread extracts.

However, even at mass resolution of 5,000, a few peaks occur near the retention time of EC (Fig. 3B,C). Their origin can be established by calculating the exact masses of the peaks observed in the mass profile scans that are recorded during the GC-MS analysis. Figure 4 shows some examples for the test sample

TABLE I
Components of Urethane-Containing Extracts from Bread Identified from a Gas-Chromatography Mass Spectroscopy Library Search of B_2F_4 Protein Mutant Lines and Their Kinmaze Parent

Scan Number[a]	Match[b]	Formula	Compound, CAS Number
121	95	$C_6H_{12}O$	Hexanal (caproaldehyde) [66-25-1]
215	92	$C_6H_{12}O_2$	3-Ethoxy-1-propanol [11-35-3]
240	64	$C_6H_{14}O_2$	2-Butoxy ethanol [111-76-2]
326	92	C_6H_6OS	Methyl sulfoxide [67-68-5]
341	70	$C_5H_{12}O_3$	2-(2-Methoxyethoxy) ethanol [111-77-3]
362	93	$C_4H_6O_2$	Dihydro-2(3H) Furanone(γ-Butyrolactone)[96-48-0]
376	99	$C_5H_6O_2$	Furfuryl alcohol [98-00-0]
384	...[c]	$C_3H_7NO_2$	Ethyl carbamate (urethane) [51-79-6]
431	66	$C_4H_{10}OS$	3-(methylthio)-1-propanol [505-10-2]
537	94	C_2H_6OS	Dimethyl sulfone [67-71-0]
540	99	C_7H_8O	Benzyl alcohol [100-51-6]
550	91	$C_{10}H_6O$	E,E-2,4 Decadienal [25152-84-5]

[a] See Fig. 1A. To calculate retention time in minutes, multiply scan number by 0.02852.

[b] Percent probability of correct identification for named compound or isomer from reverse search of Wiley 160,000 spectra library using Kratos MACH3-PBM software.

[c] Verified by injection of standard and comparison with reference spectra of Fig. 2.

containing 1 ppb of EC. At a retention time of 11 min (Fig. 4A), ions a and b from EC and LEC are well defined for the 1-ppb sample. Peak c in this scan was unexpected; it is the loss of ^{13}CO from the molecular ion of LEC and would appear at m/z 61 in the spectrum of EC. For m/z 62, the interfering ion d, $C_5H_2^+$, can be seen as just a shoulder on the low-mass side of $CH_4NO_2^+$ from EC. It, along with $C_5H_4^+$ at m/z 64, is present in background scans recorded before and after the EC and LEC elute, as shown in Figure 4B. This ion has much lower intensity than do ions from EC and LEC, even at 1 ppb of EC, and thus does not interfere with the analysis. Most of the observable m/z 62 responses in the calculated unit-mass resolution chromatogram (Fig. 3A) can be attributed to $C_5H_2^+$. Some additional ions appear at other retention times. For example, $CHNCl^+$ is a minor peak in the mass profile scan shown in Figure 4C.

Highly purified solvents must be used at all stages of the extraction and analysis. Figure 3E–F show the effect of rinsing the syringe with reagent-grade MeCl before injecting the 6-ppb sample. The ion-current trace in Figure 3E is the exact mass chromatogram for $CH_4NO_2^+$, reconstructed as before from mass profile data at 5,000 resolution. $C_2H_3{}^{35}Cl$ ions (Fig. 3F) are prominent in the sample, and account for about 12 different compounds eluting within 1 min of EC, including a compound that coelutes with EC itself. For this sample, the chlorine-containing peaks originate from impurities down to, or below, the 100-ppb level in the MeCl.

As an added safety factor, and to provide increased selectivity for the m/z 74,76 confirmatory ions, all subsequent samples were analyzed at mass resolution of 12,000. In over 100 runs of bread extracts on the ZAB mass spectrometer, no significant interferences were observed in the HRSIM results at m/z 62.

Calibrations based on m/z 62,64 and 74,76 peak area ratios were linear over a 20-fold EC-to-LEC ratio, as shown in Figure 5A for m/z 62,64. The concentration of added LEC in this example was 202 ng/ml. For most samples, measurements were made at 94 ng of added LEC. The average RRF is 1.15 for GC-HRMS calibration and 1.11 for measurements by CI; these were surprisingly high, in view of the chemical identity of EC and LEC. Close examination of the full-scan mass spectra in Figure 2B and D show peaks of about 8% abundance two mass units above the quantification ions at m/z 64 and 92 of LEC. These ions arise from a ^{18}O isotopic impurity in the LEC that originates from the $^{13}CO_2$ used in the synthesis of LEC, and it accounts for the rather high RF. The calibration process compensates for the isotopic impurity.

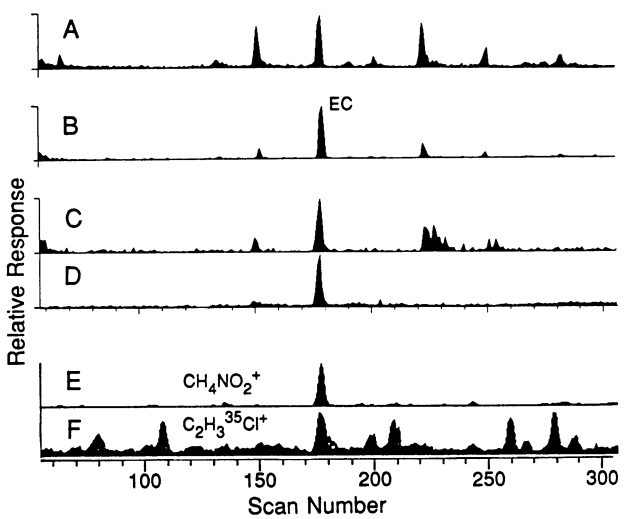

Fig. 3. Partial selected ion-monitoring chromatograms for gas-chromatography high-resolution mass spectrometry (GC-HRMS) analysis of bread extracts containing urethane (ethyl carbamate, EC) at 1 and 6 ppb. **A,** unit mass-resolution chromatogram for m/z 62 in 6-ppb sample obtained by integrating mass-profile data from m/z 61.93 to 62.10. **B,** ion current for $CH_4NO_2^+$ from EC in 6-ppb sample at 5,000 mass resolution. **C,** $CH_4NO_2^+$ in 1-ppb sample at 5,000 resolution. **D,** ion chromatogram for $^{13}CH_4{}^{15}NO_2^+$ from labeled EC standard at 7 ppb in sample containing 1 ppb of EC. **E,** ion chromatogram from $CH_4NO_2^+$ from EC at 6 ppb in sample contaminated with MeCl impurities. **F,** $C_2H_3Cl^+$ ion current from same sample. Each curve is separately normalized.

Chemical-Ionization Selected Ion Monitoring

The suitability of methane chemical-ionization measurements was established by comparing results for test samples of white bread containing EC at levels of 1 and 6 ppb to those for high-resolution analyses of the same sample. Figure 2C is the methane CI spectrum of EC with peaks at m/z 90 (MH^+) and 62 ($MH-C_2H_4$)$^+$. An advantage of the CI method has over the EI method is the availability of a second confirming ion of significant abundance. The SIM results for the 6-ppb sample, obtained on the quadrupole mass spectrometer and shown in Figure 6, illustrate the importance of using a second confirmatory ion for quantification by CI. EC and LEC for this sample elute at 9.9 min, but the compound appearing 14 sec later at 10.12 min also yields peaks at m/z 62 and 90 with significant intensity. Had they coeluted, the different peak ratios for m/z 62,64 versus 90,92 would have indicated a problem with the sample. As with HRSIM measurements, calibrations were linear over the desired range. Figure 5B is an example of GC-CIMS calibration using m/z 62,64.

Table II compares quantitative results on 1.0- and 6.2-ppb samples from single loaves of white bread, using both dynamic and static HRSIM as well as chemical-ionization methods of de-

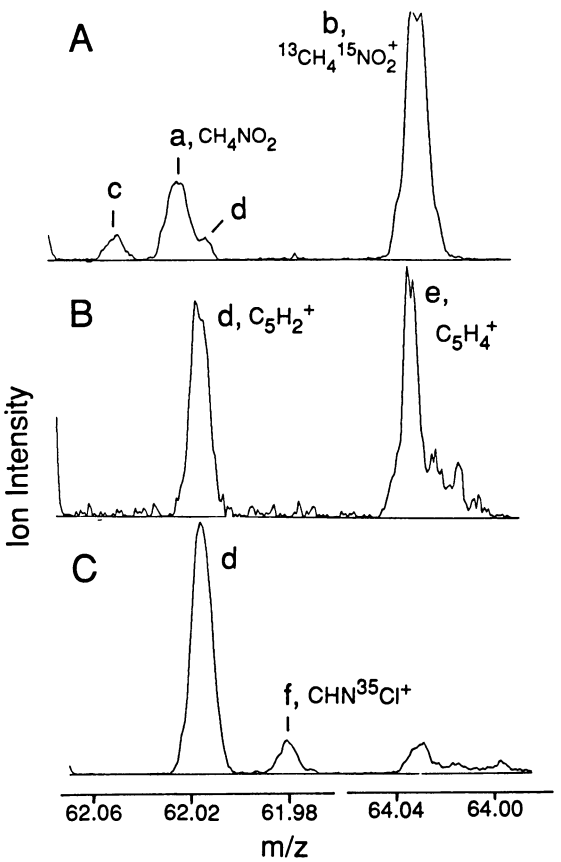

Fig. 4. Mass profile scans at 5,000 mass resolution from gas-chromatography high-resolution mass spectrometry analysis of bread extracts containing urethane (ethyl carbamate, EC). **A,** mass profiles for EC and labeled EC (LEC) in 1-ppb sample. Ion a = $CH_4NO_2^+$ from EC. Ion b = $^{13}CH_4{}^{15}NO_2^+$ from LEC. Ion c = $C_2H_7{}^{15}NO^+$ (measured mass 62.0498, error +8 ppm) from loss of ^{13}CO from LEC molecular ion. Ion d = $C_5H_2^+$ from background. **B,** mass profiles recorded before elution of EC (scans 120–125 of Fig. 3A). Ion e = $C_5H_4^+$. **C,** chlorine-containing component eluting at scans 1–8 of Fig. 3A. Ion f = $CHN^{35}Cl$ (measured mass 61.9791, error +10 ppm). Each curve is separately normalized.

tection. Measurements were made in three separate laboratories on different mass spectrometers, using the same sample extracts and standard solutions so that results would reflect only differences in the detection methods themselves. Measured urethane levels remained constant over a period of three weeks for these test samples within experimental error and were independent of the detection method.

Analytical Precision and Criteria for Confirmation

To establish the precision of the measurement of EC by HRSIM at m/z 62, the same sample was extracted and analyzed five times by high-resolution mass spectrometry, yielding a method standard deviation of 0.6 ppb for a single determination at 3 ppb of EC

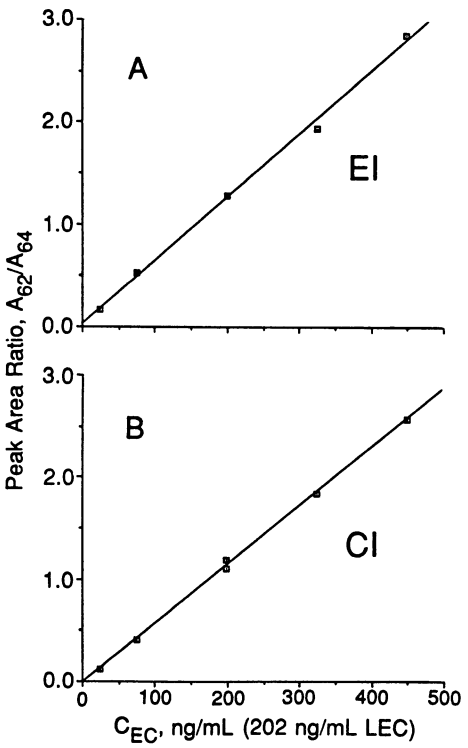

Fig. 5. Calibration curves for gas-chromatography high-resolution mass spectrometry (GC-HRMS) analysis of urethane (ethyl carbamate, EC) using 202 ng/ml of $^{13}C,^{15}N$-labeled ethyl carbamate (LEC) as internal standard. **A**, electron-ionization (EI) calibration for GC/HRMS at 12,000 mass resolution for m/z 62 (EC) and 64 (LEC). **B**, methane chemical-ionization (CI) GC-MS calibration for m/z 62 and 64 on quadrupole mass spectrometer.

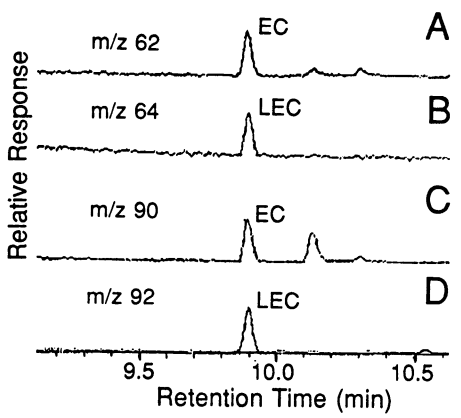

Fig. 6. Selected ion-current monitoring for urethane (ethyl carbamate, EC) and $^{13}C,^{15}N$-labeled EC (LEC) in bread with with 6 ppb of EC and 7 ppb of LEC using gas chromatography chemical-ionization mass spectrometry. **A** and **B** are m/z 62 and 64 ($MH\text{-}C_2H_4^+$). **C** and **D** are m/z 90 and 92 (MH^+).

(Table III). Data for these samples, and for additional measurements based on m/z 74,76 area ratios, were used to estimate the relative standard error as ±20%. The 74:76 mass ratios for GC-HRMS are of poorer precision below the 2-ppb level because of the low intensity of this ion. The criteria for confirmation of EC in subsequent determinations by HRSIM were: 1) the elution of defined chromatographic peaks for the 62,64 and 74,76 pairs of ions at the measured retention time for EC, and 2) agreement in amounts of EC calculated from m/z 62:64 peak area ratios within two standard deviations of calculated EC level based on m/z 74:76.

The errors for the CI method given in Table III are measures of the repeatability of the GC-MS determination only. However, data given in Table II, where CI and HR results on the same sample are compared, yielded about the same standard deviation for both methods (again for GC-MS analysis only), suggesting that the CI and EI methods have similar overall precisions down to the 1-ppb level.

Spiking and Recovery Measurements

Standard spiking and recovery experiments on a bread sample containing the intrinsic level of 0.72 ppb of urethane established the overall accuracy of the extraction-analytical scheme for urethane in bread. The results of 10 determinations at four spiking levels for a single-loaf white pan bread containing 0.72 ppb of intrinsic urethane (fresh weight) are shown in Table IV. The 110% recovery compared favorably with the 108% obtained from independent measurements by GC-CIMS. The overall accuracy was sufficient for the study.

TABLE II
Comparison of Urethane (Ethyl Carbamate, EC) Quantification at Levels of 6 and 1 ppb Using Gas-Chromatography High-Resolution Mass Spectrometry (GC-HRMS) and Chemical-Ionization Mass Spectrometry (GC-CIMS)

		Measured Urethane, ppb[b]				
Method[a]	Sample	Week 1	Week 2	Week 3	Week 4	Average
GC/CIMS[c]	A[d]	5.6	6.5	7.2	6.8	6.5 ± .7
	B[e]	0.95	1.1	1.1	0.95	1.0 ± .10
GC/HRMS	A	6.2	7.6	7.5	7.1	7.1 ± .6
(Static)	B	0.80	1.0	1.1	1.1	1.0 ± .14
GC/HRMS	A	6.4	6.4	7.2	nd[f]	6.7 ± .5
(Dynamic)	B	1.2	1.0	1.3	nd	1.2 ± .2

[a] All values determined from m/z 62 (EC) and 64 labeled EC (LEC).
[b] Based on fresh weight. All measurements are for same extract of bread. Each value is the average of three injections. Reported values for each bread represent a single extraction and sample work-up.
[c] For CI, data for weeks 1 and 4 calculated from regression analysis of full calibration. Week 2 and 3 data are from RRF determined on a 6-ppb calibration sample.
[d] Single loaf of white bread with EC at ~7 ppb.
[e] Single white loaf at ~1 ppb.
[f] Not determined.

TABLE III
Analytical Precision for Extraction and Analysis of Urethane (EC) in Bread

Method	Number of Extracts	m/z	N	Avg. EC (ppb) +/−SD[a]	RSD, %
GC-HRSIM[b]	5	62,64	5	2.9 ± 0.6	20
	4	62,64	4	2.4 ± 0.4	16
	4	74,76	6	3.5 ± 0.6	17
GC-CIMS[c]	1	62,64	12	0.9 ± 0.16	14
	1	90,92	12	0.9 ± 0.12	13
	1	62,64	12	6.1 ± 0.45	8
	1	90,92	12	6.4 ± 0.6	9

[a] Standard error for a single measurement.
[b] Gas chromatography high-resolution selected-ion monitoring. Measurements are for static (nonscanning) mode.
[c] Gas chromatography chemical-ionization mass spectroscopy. Errors are for GC-MS only. Same sample data as in Table II.

Limit of Detection

Because all samples contained significant amounts of EC, a direct evaluation of the limit of detection (LOD) for measurements using the m/z 62 response could not be made. Accurate mass chromatograms for m/z 62 from the 1-ppb test sample shown in Figure 3C have a signal-to-background (S/B) ratio of approximately 30:1 and suggest an LOD of about 0.1 ppb at 3:1 S/B for a mass resolution of 5,000. At 12,000 resolution, the LOD increases to about 0.2 ppb. For m/z 74 data, where the ion abundance is lower (Fig. 2A), the LOD was about 0.5 ppb at 12,000 mass resolution. Comparable LOD values were obtained for CI measurements on 30-g bread samples.

Measurements on Commercial U.S. and Canadian Breads

In total, 22 samples of bread and rolls were selected for analysis based on a distribution of bread types, including white, wheat, whole wheat, rye, and white hamburger buns. Both continuous mix and sponge-and-dough preparations were represented. For each type of bread, the sampling was made in different baking plants over a wide geographical range of the United States and Canada.

The results for all measurements of urethane appear in Table V. In all cases, bread was sampled immediately after baking and was composited as described earlier to achieve a statistically valid sample within a given lot. The indicated urethane levels in the table represent a single extraction and either single or duplicate GC-HRMS analysis, as noted. Quantification was based on regression analysis of the standard calibration curve, which was predetermined for each set of samples. The sample of wheat bread from plant N (Table V) gave area measurements for m/z 74,76 outside the criterion for confirmation and was not included in the calculation of mean.

The mean value of urethane calculated over 21 composited samples, and based on the values measured at m/z 62,64, is 2.0 ppb. Blanks that were measured concurrently with each extraction of bread always gave undetectable urethane. The overall agreement for results at m/z 74, for which the mean is 2.3 ppb, provided an encouraging validation of the data, considering the unexpectedly low values of EC and the general complexity of the analytical matrix.

TABLE V
Urethane Content of Bread (ppb) from 12-Loaf Composite Samples Measured by Gas Chromatography-High Resolution Mass Spectrometry[a]

		Monitor Pairs of Ions	
Bread	**Plant**	***m/z* 62,64**	***m/z* 74,76**
Rye[b]	A	1.3	1.4
	B	2.5	2.0
	C	2.1	2.0
	D	1.3	2.2
	E	2.2	2.9
	Mean, ppb	*1.8*	*2.1*
White[c]	A	2.9	2.8
	E	2.1	2.6
	F	1.5	1.7
	G	2.8	4.1
	H	1.9	0.9
	I	2.2	2.2
	Mean, ppb	*2.3*	*2.4*
Wheat[b,d]	J	1.7	2.6
	K	2.5	2.9
	L	1.8	2.2
	M	2.4	3.5
	N	2.3	4.5[e]
	I	2.5	1.7
	Mean, ppb	*2.2*	*2.6*
Hamburger buns[c]	A	1.3	2.1
	B	1.8	2.1
	C	2.0	3.2
	D	1.8	1.4
	H	1.2	1.6
	Mean, ppb	*1.6*	*2.1*

[a] Each pair of measurements represents a 12 loaf composite sample from a single bake.
[b] Mean of two or three measurements.
[c] Based on single measurements (RSD = ± 20 percent).
[d] Category includes both wheat and whole wheat bread.
[e] Data not used in computing average because of poor agreement between 62:64 and 74:76 values.

Statistical Analysis

Analysis of the plant-to-plant variability for the four bread types represented in the study, using the data in Table V, showed equal variance for the four groups of measurements, thus validating a statistical comparison of their means. Analysis of variance on the four means gave a marginally significant F value of 2.17 ($P = 0.13$). However, t-tests of the six pairs of means for the four bread types revealed significant differences for hamburger buns versus white and wheat bread ($P = 0.04$ and 0.05, respectively). Based on this analysis, weighted means were calculated using the known bread consumption percentages for 1990 (Annual Survey of Manufacturers, U.S. Dept. of Commerce). The weighted means were used to compute a consumption-adjusted mean urethane level of 2.06 ppb ± 0.2 ppb (standard error of the mean).

Urethane Levels in Toasted Bread

In single-loaf samples of white and whole wheat bread, with endogenous urethane levels of 1.3 and 2.3 ppb, respectively, urethane increased with increased toasting time. Figure 7 shows these results as nanograms per slice. A 3-min toasting time yields a light brown toast with urethane levels equivalent to 5 and 9 ppb for white and whole wheat bread, respectively, based on the original fresh weight before toasting. The larger increase for whole wheat bread at equal toasting time is in agreement with earlier data (Canas et al 1989) showing the same effect, although there is less difference between white and whole wheat bread in our study. The data for both breads suggest that increases in urethane content are small preceding an onset time corresponding to very light toasting, especially for white bread, where no increase was observed at 2 min. A previous study of toasting showed that the increases in EC were independent of the initial urethane level of the bread (Canas et al 1989). The higher value

TABLE IV
Spiking and Recovery Measurements for Urethane (EC) from White Bread Measured by Gas Chromatography High-Resolution Mass Spectroscopy

Intrinsic EC, ppb	Added EC, ppb	Recovery %	Average
0.72	3.32	105,120,130	127
0.72	4.63	104,115	110
0.72	6.56	90,103,107	103
0.72	7.66	106,124	115
Avg. ± SD[a]		110 ± 4[b]	(36% RSD)

[a] Standard deviation.
[b] Standard error of mean for n = 10.

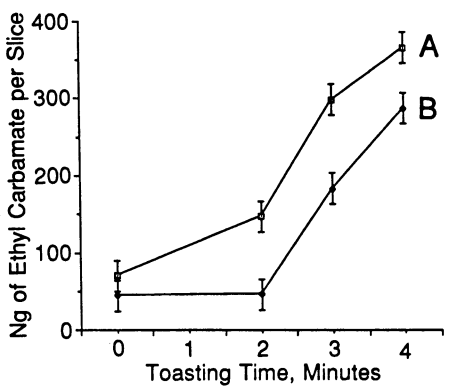

Fig. 7. Changes in urethane level for toasted bread. **A,** whole wheat bread with initial ethyl carbamate level of 2.3 ppb. **B,** white bread with initial ethyl carbamate level of 1.3 ppb.

for whole wheat bread would therefore not be explained by its higher level of EC before toasting.

DISCUSSION

The principal objectives of this study were to determine an accurate figure for the mean level of urethane in commercial breads and to assess the variability among several of the more common types of bread. For these objectives, the compositing procedure was especially useful because it allowed us to sample a very large number of individual loaves. Because all measurements, except on test samples, were made on composited loaves, the loaf-to-loaf variation cannot be calculated from the data.

The group of sampled loaves represents both a geographical distribution and a distribution of bread types that approximates the consumption pattern in the United States and Canada. From this perspective, white bread was undersampled by a factor of two (27% versus 56% of consumption by weight), wheat bread was slightly oversampled (27% versus 20% consumption by weight). Hamburger buns were sampled approximately equally (23% versus 27% of consumption by weight). Rye bread (2% of consumption) was included as an example of a bread product with a reduced percentage of wheat. We calculate a dietary intake of 47 μg of urethane per person per year for untoasted bread (consumption-adjusted mean of 2.06 ppb) by assuming that non-sampled breads have approximately the same urethane level, and by using 50 lb. per year as the average per capita consumption of bread for the United States.

Although the data were obtained from a sampling of different breadbaking plants, there was no intention to invoke processing variables explicitly. The sampling from different plants was undertaken to mimic a market-basket sampling, but on bread with a defined composition and history. Also, we did not address the question of postbaking changes in urethane, except for toasted bread. A comparison between the earlier study of bread sampled from grocery shelves (Canas et al 1989) is probably not meaningful because of the higher measurement error, quantification limit, and rather limited sampling in their study. No measurements of the spatial distribution of urethane within a loaf were made; the compositing procedure should average whatever distribution may exist.

There was no evidence of significant dependence on bread type except for the slightly lower mean level of 1.6 ppb for hamburger buns. In view of the very low mean value of urethane that we have established for bread, the lower value for hamburger buns has no significance in risk analysis. An earlier suggestion (Canas et al 1989) of a 2.5× higher mean for EC in white versus whole wheat bread (3.0 vs. 1.2 ppb) was not substantiated by our data.

The probable accuracy of our result for mean urethane is indicated by the spiking and recovery measurements made as a function of added urethane on a single-loaf sample with a very low (0.72 ppb) intrinsic level of urethane. While there was evidence for a systematic relative error of as much as 10%, yielding a recovery of 110% on added urethane, there was no trend in recovery as a function of spiking amount, suggesting that significant losses did not occur down to the 3-ppb level. The use of the LEC as a coextractant provides good protection against losses during sample workup.

The low detection limits and excellent specificity achieved by use of the mass spectrometric methods were crucial, because our use of compositing to increase the number of sampled loaves has the effect of clustering the EC levels near the mean of 2 ppb, an amount just at the detection limit for N-specific GC detection. The choice of mass spectrometric technique was also significant. Both the high-resolution and the methane chemical-ionization methods performed well at the low urethane levels observed in commercial breads. Should a more extensive study be required, we have established that the chemical-ionization technique provides the requisite specificity and sensitivity for measurements on bread to below the 1-ppb level, so long as the extraction procedure is scaled up slightly to 30 g from the 10 g used for measurements based on EI mass spectrometry. The CI method has the advantage of simplicity for routine measurements. Because our CI method is used with unit-resolution mass spectrometry, the equipment is more widely available and probably has a somewhat lower analysis cost than does the high-resolution techniques. The fact that the majority of our measurements in this study were made using high-resolution mass spectrometry reflects availability of resources rather than technical necessity.

CONCLUSIONS

Our study of urethane in bread products has established the first accurate measure of mean urethane level at 2.06 ± 0.2 ppb. When combined with bread consumption data, the newly determined value indicates a dietary input from bread that is more than three times lower than the value used previously in estimating carcinogenic risk factors. The current study contradicts a previous suggestion that urethane levels are a function of bread type, although statistical analysis of the data support a slightly lower urethane level for hamburger buns (1.6 ppb) compared to that of white or wheat bread, where the measured mean level was 2.3 ppb.

ACKNOWLEDGMENTS

We thank Agriculture Canada, the American Bakers Association, Lallemand Corp., and George Weston North American Bakeries for partial funding. Ira Dorfman and Anne Giesecke of the ABA, Barbara Giuliany of Diversified Research Laboratories, Wilda Martinez of the USDA, and members of the Urethane Task Force provided significant guidance and support for the study. Rella Dwyer of W. E. Long Baking Foods Laboratory and Linda Whitehand of the USDA advised us on nutrient sampling and statistical analysis of data.

LITERATURE CITED

AMES, B. N. 1990. Too many rodent carcinogens: Mitogenesis increases mutagenesis. Science 249:970-971.

AYLOTT, R. I., McNEISH, A. S., and WALKER, D. A. 1987. Determination of ethyl carbamate in distilled spirits using nitrogen specific and mass spectrometric detection. J. Inst. Brew. 93:382-386.

BATTAGLIA, R., CONACHER, H. B. S., and PAGE, B. D. 1990. Ethyl carbamate (urethane) in alcoholic beverages and foods: A review. Food Addit. Contam. 7:477-496.

BRUMLEY, W. C., CANAS, B. J., PERFETTI, G. A., MOSSOBA, M. M., SPHON, J. A., and CORNELIUSSEN, P. E. 1988. Quantitation of ethyl carbamate in whiskey, sherry, port, and wine by gas chromatography/tandem mass spectrometry using a triple quadrupole mass spectrometer. Anal. Chem. 60:975-978.

CANAS, B. J., HAVERY, D. C., and JOE, F. L., Jr. 1988. Rapid gas chromatographic method for determining ethyl carbamate in alcoholic beverages with thermal energy analyzer detection. J. Assoc. Off. Anal. Chem. 71:509-511.

CANAS, B. J., HAVERY, D. C., ROBINSON, L. R., SULLIVAN, M. P., JOE, F. L., Jr., and DIACHENKO, G. W. 1989. Ethyl carbamate levels in selected foods and beverages. J. Assoc. Off. Anal. Chem. 72:873-876.

CLEGG, B. S., and FRANK, R. 1988. Detection and quantitation of trace levels of ethyl carbamate in alcoholic beverages by selected ion monitoring. J. Agric. Food Chem. 36:502-505.

CONACHER, H. B. S., PAGE, B. D., LAU, B. P.-Y., LAWRENCE, J. F., BAILEY, R., CALWAY, P., HANCHAY, J.-P., and MORI, B. 1987. Capillary column gas chromatographic determination of ethyl carbamate in alcoholic beverages with confirmation by gas chromatograph/mass spectrometry. J. Assoc. Off. Anal. Chem. 70:749-751.

DENNIS, M. J., HOWARTH, N., KEY, P. E., POINTER, M., and MASSEY, R. C. 1989. Investigation of ethyl carbamate levels in some fermented foods and alcoholic beverages. Food Addit. Contam. 6:383-389.

GOLD, L. S., SLONE, T. H., STERN, B. R., MANLEY, N. B., and AMES, B. N. 1992. Rodent carcinogens: Setting priorities. Science 258:261-265.

HADDON, W. F., MASRI, M. S., RANDALL, V. G., ELSKEN, R. H., and MEHEGHELLI, B. J. 1977. Mass spectral confirmation of aflatoxins. J. Assoc. Off. Anal. Chem. 60:107-113.

HURST, H. E., KEMPER, R. A., and KURATA, N. 1990. Measurement

of ethyl carbamate in blood by capillary gas chromatography/mass spectrometry using selected ion monitoring. Biomed. Environ. Mass Spectrom. 19:27-31.
LAU, B. P.-Y., WEBER, D., and PAGE, D. 1987. Gas chromatographic-mass spectrometric determination of ethyl carbamate in alcoholic beverages. J. Chromatogr. 402:233-241.
LOFORTH, G., and GEJVALL, T. 1971. Diethyl pyrocarbonate. Formation of urethane in treated beverages. Science 174:1248-1250.
MacKENZIE, W. M., CLYNE, A. H., and MacDONALD, L. S. 1990. Ethyl carbamate formation in grain based spirits. II. The identification and determination of cyanide related species involved in ethyl carbamate formation in scotch grain whisky. J. Inst. Brew. 96:223-232.
MILLARD, B. J. 1978. Quantitative Mass Spectrometry. Heydon & Son Ltd.: London.
MONTEIRO, F. F., TROUSDALE, E. K., and BISSON, L. F. 1989. Ethyl carbamate formation in wine: Use of radioactively labeled precursors to demonstrate the involvement of urea. Am. J. Enol. Vitic. 40:1-8.
MOSSOBA, M. M., CHEN, J. T., BRUMLEY, W. C., and PAGE, S. W. 1988. Application of gas chromatography/matrix isolation/Fourier transform infrared spectrometry to the determination of ethyl carbamate in alcoholic beverages and foods. Anal. Chem. 60:945-948.
OUGH, C. S. 1976a. Ethyl carbamate in fermented foods and beverages. I. Naturally occurring ethyl carbamate. J. Agric. Food Chem. 24:323-328.
OUGH, C. S. 1976b. Ethyl carbamate in fermented foods and beverages. II. Possible formation of ethyl carbamate from diethyl dicarbonate addition to wine. J. Agric. Food Chem. 24:328-331.
OUGH, C. S., CROWELL, E. A., and MOONEY, L. A. 1988. Carbamyl compound reactions with ethanol. Am. J. Enol. Vitic. 39:139-42.
PIERCE, W. M., CLARK, A. O., and HURST, H. E. 1988 Determination of ethyl carbamate in distilled alcoholic beverages by gas chromatography with flame ionization or mass spectrometric detection. J. Assoc. Off. Anal. Chem. 71:781-784.
SCHLATTER, J., and LUTZ, W. K. 1990. The carcinogenic potential of ethyl carbamate (urethane): Risk assessment at human dietary exposure levels. Food Chem. Toxicol. 28:205-211.
TONG, H. Y., GIBLIN, D. E., LAPP, R. L., MONSON, S. J., and GROSS, M. L. 1991. Mass profile monitoring in trace analysis by gas chromatography/mass spectrometry. Anal. Chem. 63:1772-1780.

[Received August 30, 1993. Accepted December 17, 1993.]