methane as the carrier gas. Comparison of the same fractions from the silica gel column by both analytical methods shows large variations in the relative peak intensities. The percentage composition of several components (10, 43, 116, 139, and 165; Table I) was found by EC to be 70–130% of the corresponding values with CI total ion current. Some smaller components (*i.e.*, 1, 133, and 135) appeared to be in >fourfold higher amounts when analyzed by EC than by CI-ms. It is clear that the EC response is not directly proportional to that of CI-ms. The EC response is known to vary with isomeric compounds; for example, the α, β, γ, and δ isomers of hexachlorocyclohexane differ by up to fourfold in their apparent ionization efficiencies (Ishida and Dahm, 1965). Similarly, the CI-ms response will differ with isomeric materials since the total ion current depends on the relative intensities of the ions generated which in turn varies with different isomers. Attempts to quantitate using the FI detector proved fruitless due to its insensitivity with these types of compounds.

The approximate composition values for toxaphene take on additional significance when one multiplies these percentage values by one billion (the number of pounds of toxaphene already used) to obtain the number of pounds of each component introduced into the environment over the past 25 years. It is cautioned, however, that the composition data are only approximate and are directly related to the CI-ms technique used in the quantitation. It should also be noted that one batch of toxaphene may differ from another so the present composition values for individual components may vary over a small range with different batches.

Toxaphene is a very complex mixture which is difficult to analyze as to composition even under idealized conditions. The complexity will be even greater on considering a mixture of these components with their metabolites and photoproducts such as may be the case under environmental conditions. It is fortunate that, at least with toxaphene itself, most of the components undergo rapid metabolism in mammals (Casida *et al.*, 1974; Ohsawa *et al.*, 1974).

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Gas Chromatographic Analysis of Urethan (Ethyl Carbamate) in Wine

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An analytical method is described for the determination of urethan (ethyl carbamate) in wines. The quantitative method involves an extraction with chloroform followed by a cleanup with Florisil and detection by gas–liquid chromatography. A Coulson electrolytic conductivity detector is capable of detecting levels of urethan at <100 ppb. Confirmation of identity is carried out with trifluoroacetic anhydride derivatized urethan, by gas chromatography using an alkali flame ionization detector.

Diethyl pyrocarbonate (DEP) has been widely used as a food additive for controlling microbiological activity in alcoholic and nonalcoholic beverages (Pauli and Genth, 1966; Fischer, 1970; Gejvall and Löfroth, 1971). Recently, however, Löfroth and Gejvall (1971) were able to show, by use of isotope dilution analysis with tritium-labeled DEP, that the DEP can result in the formation of urethan (ethyl carbamate), a known carcinogen (Nettleship *et al.*, 1943; Mirvish, 1968). The experiments performed by Löfroth and Gejvall (1971) showed that, under laboratory conditions, white wine and beer which normally contain about 5–128 mg/l. of ammonia (Muth and Malsch, 1934; Bishop, 1943) can react with DEP added in the amounts of 280–560 mg/l. to form 1.3–2.6 mg, respectively, of urethan. The reaction is pH dependent. As a result of these studies, the beverage industry has withheld any further use of DEP pending studies to see what levels of urethan can be and are produced under actual food processing conditions. Since urethan is considered a carcinogen, any levels detected could fall within the Delaney Clause, preventing the use of DEP in foods.

The purpose of this study was to develop an analytical method for low levels of urethan useful for routine monitoring applications and for quantitatively studying formation of urethan under varying conditions (Ough, 1974).

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EXPERIMENTAL SECTION

Chemicals. All solvents used were double distilled or purchased commercially as pesticide quality or equivalent with the exception of anhydrous ethyl ether, purchased from Mallinckrodt Chemical Co. and used as received. Urethan (ethyl carbamate) was obtained from Eastman Kodak Co., mp 48–50°. Florisil, PR grade, 60–100 mesh, Floridin Co., was used as received. Hodag Antifoam FD-62, Hodag Chemical Corp., was diluted 1:50 with deionized water. Trifluoroacetic anhydride, Aldrich Chemical Co., and BuffAR pH 7 buffer concentrate, Mallinckrodt, were used as received.

Gas Chromatography. A modified Varian Aerograph Model 550 HyFI gas chromatograph was coupled to a Coulson electrolytic conductivity detector (Tracor Inc.). The modification consisted of extending the glass column out one side of the gas chromatograph for coupling directly to the vent block of the Coulson detector system. The gas chromatograph was equipped with a Varian Aerograph Model 328 temperature programmer and a 6 ft × 3 mm i.d. glass column packed with Gas-Chrom Q, 80–100 mesh, coated to 10% with OV-17 and 5% with Carbowax 1540. Appropriate amounts of the liquid phases were mixed in chloroform prior to coating on the Gas-Chrom Q. The column, injector, and pyrolysis temperatures were held constant at 140, 200, and 800°, respectively. The temperature of the transfer block between the column and the pyrolysis unit, and the vent block, was 220°. Helium was the carrier gas and had a flow rate of 50 ml/min. The flow rate of hydrogen was also 50 ml/min.

The derivatization study utilized a Varian Aerograph Model 1700 gas chromatograph equipped with a rubidium sulfate alkali flame ionization detector and a 4 ft × 2 mm i.d. glass column containing 5% OV-225 on acid-washed DMCS-treated Chromosorb G, 100–120 mesh. The column, injector, and detector temperatures were 100, 188, and 210°, respectively. The flow rates of nitrogen carrier gas, hydrogen, and air were 20, 25, and 200 ml/min, respectively. Slight variations were made from time to time to achieve optimum response for 100-ng injections of trifluoroacetylurethan.

Extraction. A 100-ml aliquot of either red or white wine was transferred to a 250-ml separatory funnel. Sodium chloride (15 g) was added, followed by gentle shaking until the salt was completely dissolved. Antifoam solution (0.5 ml) was added and again the contents were mixed by gentle shaking. Chloroform (100 ml) was added to the separatory funnel and the contents were shaken gently for at least 2 min to allow for a complete extraction of the urethan from the beverage. Care was taken to avoid emulsions in this step by providing gentle shaking only. The chloroform was filtered through approximately 30 g of anhydrous sodium sulfate into a 300-ml round-bottomed flask. The extraction was repeated two or more times with 50 ml of chloroform. The sodium sulfate was rinsed with a final 20-ml portion of chloroform and the combined chloroform extract was reduced in volume on a steam bath with a three-ball Snyder column attached to the top of the round-bottomed flask. To prevent bumping, two or three boiling chips were added to the flask prior to placing on the steam bath. The final volume was about 10 ml and never allowed to go to dryness. Following evaporation, the extract was allowed to cool and the Snyder column rinsed five times with 1-ml portions of chloroform.

Florisil Cleanup. A 15 mm o.d. × 10 cm chromatographic column was prepared from bottom to top with a small plug of glass wool, 5 g of Florisil, and 1.5 cm of anhydrous sodium sulfate. The packed column was prewashed with 25 ml of chloroform and the washing discarded. The concentrated extract was quantitatively transferred to the column using three 5-ml rinsings of chloroform followed by a total elution of 200 ml of chloroform. The eluate was collected in a round-bottomed flask. Ethyl acetate (50 ml) was added along with one boiling chip, and the sample evaporated over a steam bath using a three-ball Snyder column wrapped in aluminum foil. The sample was not concentrated beyond 5 ml. The sample was cooled and the Snyder column was washed five times with 1-ml portions of ethyl acetate. The concentrate was then transferred to a 10-ml concentrator tube (Kontes ±K-570050) followed by several washings of the flask with ethyl acetate. The concentrator tube was equipped with a micro-Snyder column (Kontes ±K-569251) and the sample, containing one boiling chip, was concentrated on a steam bath to about 3 ml. Further concentration to 0.5 ml was achieved using a gentle stream of nitrogen. Evaporated extracts and rinsings were transferred using disposable pipets. Extreme care was taken with all evaporation steps as this was found to be the most likely cause for loss.

Preparation of Derivative for Characterization. To a solution of 1 g of urethan in 23 ml of ethyl acetate, contained in a 25-ml volumetric flask, was added 1.25 ml of trifluoroacetic anhydride and 0.25 ml of pyridine. The reaction mixture was shaken briefly and then placed in a water bath maintained at 70° for 30 min. The resulting solution was cooled to room temperature and extracted with four portions of 25 ml each of pH 7 buffer concentrate in a separatory funnel. The ethyl acetate layer was dried (sodium sulfate) and evaporated to dryness in a nitrogen stream to give a clear colorless liquid which crystallized spontaneously. The product was recrystallized twice from ethyl ether–pentane to give 0.7 g of trifluoroacetylurethan as a colorless solid, mp 85–86°.

Microscale Derivatization. To 1 mg of urethan in 1 ml of ethyl acetate, contained in a 10-ml volumetric flask, was added 25 μl of trifluoroacetic anhydride and 10 μl of pyridine. The flask was stoppered and shaken and then placed in a water bath maintained at 70° for 30 min. To the resulting solution 4 ml of pH 7 buffer concentrate was added, the stoppered flask shaken, and phases allowed to separate. The mixture was then transferred to a 30-ml separatory funnel with several 1-ml washings of ethyl acetate. The bottom aqueous phase was withdrawn and again the solution washed with buffer solution. The ethyl acetate phase was filtered through a small plug of anhydrous sodium sulfate into a 10-ml volumetric flask. Several 1-ml washings of ethyl acetate were included, before adjusting to final volume with ethyl acetate.

Beverage extracts were handled in the same manner as the standard urethan. A cleaned-up extract in 1 ml of ethyl acetate, equivalent to 100 ml of beverage, was transferred to a 10-ml volumetric flask and derivatized with 25 μl of trifluoroacetic anhydride. Final volume prior to injection into the gas chromatograph was made in a McNaught and McKay–Shevky–Stafford sedimentation tube by carefully evaporating the solvent with a gentle stream of dry nitrogen.

Gas Chromatographic Analysis. Following cleanup on the Florisil column, samples containing underivatized urethan were injected directly into the gas chromatograph equipped with the Coulson conductivity detector. Appropriate dilutions were made depending on the residue found in the samples. Quantitation of urethan was determined by measuring peak area by means of a planimeter and comparing this area with those found using urethan standards in the range from 5 to 40 ng at attenuation 2×. A 40-ng peak gave a 90% full-scale response at this attenuation.

The TFA-derivatized urethan was injected directly into the gas chromatograph equipped with the alkali flame ionization detector. A standard curve linear for the range from 20 to 160 ng (100% full scale) was achieved at an attenuation of 2×10^{-12} afs. These levels were sufficient for confirming the presence of urethan at the 100-ppb fortifi-

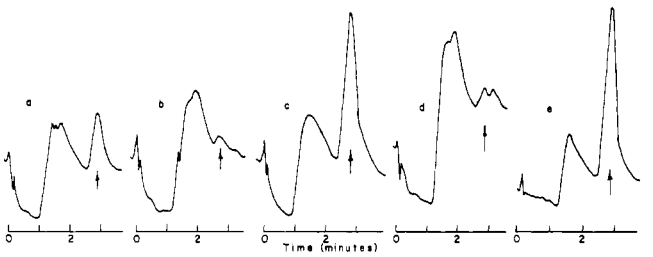

Figure 1. Gas chromatograms using Coulson electrolytic conductivity detector representing (a) an injection of 5 ng of urethan; (b) 1 g of white wine control; (c) white wine fortified at 100 ppb with urethan; (d) 1 g of red wine control; (e) red wine fortified at 100 ppb with urethan.

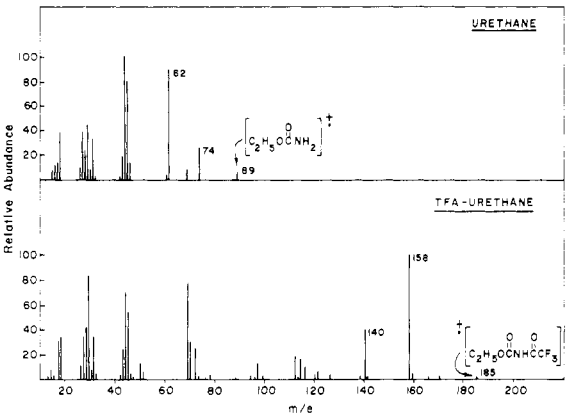

Figure 2. Mass spectra of standards of urethan and the TFA derivative of urethan (Varian Model M-66, solid probe inlet).

cation level; extrapolation of the standard curve allowed estimation of even lower levels.

Electron capture detection gave a linear response for the recrystallized TFA derivative in the range from 1 to 10 ng. Though the limit of detectability was appreciably lower with this detector than with the alkali flame, the background from treated samples was too high for use in this study.

RESULTS AND DISCUSSION

One of the most critical factors governing detectability limits in trace analysis is adequate cleanup. The Florisil chromatographic column gave adequate cleanup for injecting an equivalent of 1 ml of wine on the gas chromatograph. An alternate cleanup procedure, using silicic acid contained in a disposable Pastuer pipet (Kadoum, 1967) and an 8% (v/v) ethyl acetate–benzene eluting mixture, also proved successful. Since the capacity for removing interferences was greater with the Florisil column, it was the column of choice. Both columns were also tried in succession but with no appreciable advantage over the use of one column.

Several glc columns were evaluated for the resolution of urethan from interfering substances present in wines. Column bleed from Reoplex 400, Versamid 900, and AN 600 caused high background with the Coulson conductivity detector. Several other liquid phases (OV-101, DC-200, OV-225, PDEAS, Dexsil 300GC, Apiezons L and N, and commercially prepared SP-2250/SP-2401) resulted in moderate to bad tailing with urethan. A mixed phase consisting of OV-17 and Carbowax 1540 gave adequate resolution and sensitivity with a minimum of tailing.

Several detectors, including the flame ionization, alkali flame ionization, electron capture, and Coulson electrolytic conductivity detectors, were tried. Only the Coulson detector gave satisfactory results, due primarily to its specificity for nitrogen-containing compounds such as urethan and its ease of routine operation (Coulson, 1966; Patchett, 1970). The nonspecific detectors such as electron capture resulted in lower limits of detectability with urethan standards but too high background with wine samples for practical use.

Figures 1b and 1d show typical chromatograms of unfortified wine extracts when the equivalent of 1.0 ml was injected on the gas chromatograph with an attenuation setting at 2×. The second small peak on the control samples was an extractable interference peak which remained after cleanup. The lower limit of detectable urethan was 5 ng at this attenuation which gave a peak height of approximately 10% FSD (Figure 1a). Figures 1c and 1e depict the same wine fortified before extraction with 100 ppb of urethan. Recovery studies with fortified wines at 100 ppb generally ranged between 80 and 100%. All recovery studies were based on a standard curve run at the time of analysis.

The confidence of any analytical procedure is greatly enhanced by alternate detection procedures. Gc-mass spectroscopy was evaluated as a possible means for confirming the presence of urethan in these fortified extracts. However, interfering materials in the cleaned-up extracts precluded its use in this study. A derivatization procedure using trifluoroacetic anhydride was more successful and confirmed the presence of urethan in the fortified wine samples.

Carbamate esters (Seiber, 1972; Shafik and Mongan, 1972; Khalifa and Mumma, 1972) and ureas (Miller, 1971) react with trifluoroacetic anhydride to form *N*-trifluoroacetyl (TFA) derivatives for trace analysis. The derivatives are less polar, have a greater thermal stability, and are more electron capturing than the starting esters or ureas. The greatest advantage of the derivative employed in this study was the resulting change in glc retention. In the present method, the derivative was formed in ethyl acetate at 70° in the presence of pyridine and analyzed by alkali flame ionization glc.

The structure of the TFA derivative of urethan was confirmed by mass spectrometry (Figure 2). Only a small parent peak, *m/e* 185, was observed, but abundant fragments could be assigned in analogy with the reported spectra of ethyl *N*-alkylcarbamates (Lewis, 1964) based on, first, cleavage at the ethyl C–O with proton transfer to give *m/e* 158, followed by loss of water and subsequent fragmentation of the protonated trifluoroacetyl isocyanate cation (*m/e* 140). Comparison of the spectra of urethan and the TFA derivative indicates little cleavage occurs in the latter through loss of the trifluoroacetyl group to regenerate urethan.

The infrared spectra of urethan and the TFA derivative (Figure 3) showed marked differences. Change in N–H absorption, from the broad band at 2.9 μ in urethan to a sharp doublet at 3.05 μ in the derivative, confirmed substitution at the nitrogen position. The ester carbonyl, found at 5.9 μ in urethan, occurred at 5.6 μ in the derivative; this agrees qualitatively with the spectra of urethan and the *N*-chloroacetyl derivative reported by others (Pianka and Polton, 1960). In addition the derivative possessed a band at 6.54 μ, absent in urethan, of nearly equal intensity to the carbonyl absorption. We assign this to the C=N stretch from contribution of the enolate form in the KBr medium in which the spectrum was obtained (Nakanishi, 1962). The infrared spectrum of trifluroroacetylurethan in chloroform, which exhibited a pair of carbonyl absorptions of 5.5 μ (amide) and 5.7 μ (ester) but none in the region around 6.5 μ, indicates little contribution occurs in dilute solution from the enolate form.

The microscale derivatization reaction was essentially complete (98% conversion) within 30 min under the conditions used. In addition, no significant loss of derivative occurred upon washing the reaction solution with aqueous buffer to remove excess pyridine and trifluoroacetic anhydride.

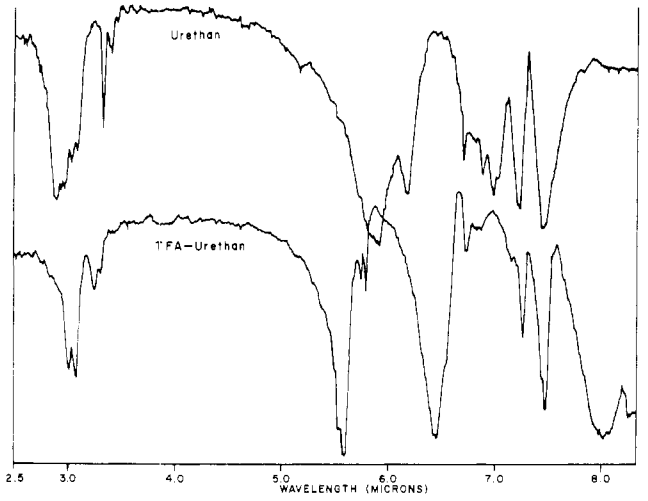

Figure 3. Infrared spectra of urethan and the TFA derivative of urethan in potassium bromide.

Two methods for detecting the derivative were investigated. Electron capture gave a linear response to a lower limit of approximately 1 ng, while alkali flame ionization gave a lower limit of detectability of approximately 20 ng when standards were analyzed. Despite the larger sample requirements, the latter was chosen for residue confirmation because of electron capturing interferences encountered in actual samples.

Results from derivative analysis of wine control samples and samples fortified with 100 ppb of urethan, previously subjected to Florisil cleanup, are given in Figure 4. While accurate quantitation was not possible due to base-line drift from the large solvent response, the peak from trifluoroacetylurethan was distinct and corresponded roughly in intensity with the recoveries calculated from analysis of urethan with the Coulson conductivity glc system.

The foregoing methods allow for quantitative determination and confirmation of urethan in wine at levels down to at least 100 ppb. This is well below the concentration range found previously by isotope dilution analysis of DEP-treated beverages (Löfroth and Gejvall, 1971). The methods, which utilize techniques and instrumentation readily available to residue analysts, have been applied successfully to wine samples. With minor modification, they should be generally applicable to a variety of beverages including beer suspected of urethan contamination.

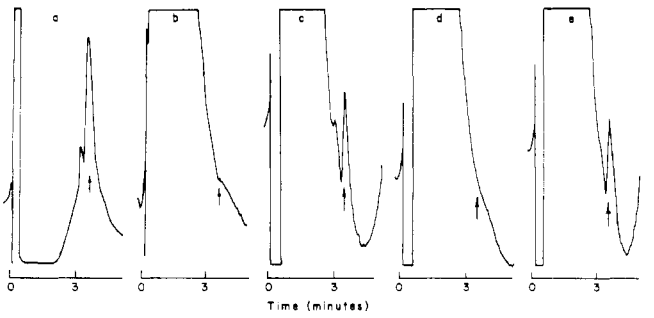

Figure 4. Gas chromatograms using alkali flame ionization detector of trifluoroacetic anhydride derivatized samples representing (a) an injection of 40 ng of urethan; (b) 300 mg of red wine control; (c) red wine fortified at 100 ppb with urethan; (d) 300 mg of white wine control; (e) white wine fortified at 100 ppb with urethan.

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