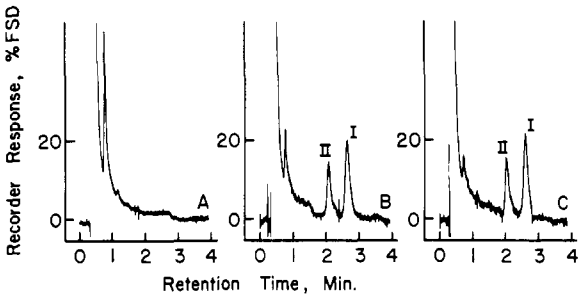

Figure 1. Chromatograms from extracts of whole milk: (A) 25 mg of control milk; (B) 25 mg of control milk fortified (0.01 ppm) with 0.25 ng each of I and II; (C) standard containing 0.25 ng each of I and II.

samples, they are an accurate test of manipulative efficiency. These experiments also demonstrated the absence of interfering co-extractives. Unpublished data (J. B. Miaullis, Stauffer Chemical Co., 1976) on the metabolism of N-2596 in a corn plant indicate that 95% of the N-2596 and 90% of the N-2596 oxygen analogue (both ring-^{14}C labeled) remaining in the plant are removed by benzene extraction. The recovery results from fortifications of 0.01–5.0 ppm are shown in Table II. Each value represents the average of at least two determinations. The analysis precision was ±10%. Figure 1 shows a typical chromatogram for whole milk for the 0.01 ppm fortification level.

All recoveries of I and II were satisfactory for each sample type except beef liver, beef cardiac muscle, and chicken liver. However, the high fat content of cream and beef fat lowered the recoveries from these tissues to near 75%. Using this method, the recoveries of I from field-weathered (1 month) sand and silty clay loam were within 20% of the values obtained upon 24-h Soxhlet extractions of these soils using benzene or acetonitrile.

Table II shows that recovery of II from freshly fortified beef liver was only 5%; a delay of 10 min between fortification and extraction reduced the recovery to zero. Likewise, if there was a delay between fortification and extraction of kidney tissue, recoveries of II were reduced from 99% for tissue extracted immediately after fortification to 45% for a 10-min delay and 16% for a 75-min delay. These results were obtained when the tissue extracts were analyzed immediately after extraction. However, if the kidney extract giving 99% recovery was stored at room temperature for 7 days, the recovery of II was reduced to 67%. The low recoveries of II in these tissues, as well as the low values obtained for beef cardiac muscle and chicken liver may be the result of enzymatic degradation of II. Similar behavior has been reported for ethion dioxon (*S,S'*-methylene *O,O,O',O'*-tetraethyl phosphorothioate) in liver and kidney tissues of turkey and cattle (Ivey and Mann, 1975). The recoveries of I were unaffected by delay between fortification and extraction for any tissue.

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A Rapid Gas–Liquid Chromatography Method for Determining Benomyl Residues in Foods

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A GLC method for determining residues of benomyl as a composite value of MBC and 2-AB acetates was described. Using a high-resolution glass capillary GLC technique, and nitrogen–phosphorus selective and EC detectors, a sensitivity of 0.00001 ppm benomyl for a 10-g sample was achieved. The method was tested for various vegetables and fruits.

Methyl 1-(butylamino)carbonyl-1*H*-benzimidazol-2-ylcarbamate, generally known as benomyl, is a protective and eradicant fungicide, effective against a wide range of fungi affecting fruits, berries, nuts, vegetables, field crops, turf, and ornamentals.

Because of its relatively low toxicity, the recommended maximum amounts of benomyl and its degradation products in foods may be high as several milligram/kilogram in the case of citrus fruits and wine grapes. On the other hand, the recommended maximum amount of benomyl residues in vegetables may be, depending on legislation, as low as 0.1 ppm (Coduro, 1974).

In acidic conditions, benomyl is easily hydrolyzed to methyl benzimidazol-2-ylcarbamate (MBC) and a further hydrolysis leads to 2-aminobenzimidazole (2-AB). Benomyl is known to degradate in animal systems to 5-hydroxybenzimidazol-2-ylcarbamate (5-HBC) and 4-hydroxybenzimidazol-2-ylcarbamate (4-HBC) (Gardiner et al., 1974).

In foods the residues of benomyl and its degradation products can be determined by several different methods, the first steps of the analysis being similar in each case. Benomyl is quantitatively hydrolyzed to MBC, which is then determined, or MBC may be further hydrolyzed to 2-AB, which in turn is determined. The residues of benomyl, MBC and 2-AB are then measured as a composite value.

Aharonson and Ben-Aziz (1973) measured MBC by a direct fluorometric method. Pease and Gardiner (1969) and Pease and Holt (1971) have described a method where 2-AB is determined by fluorometric measurement, or by colorimetric analysis following bromination. Mestres et

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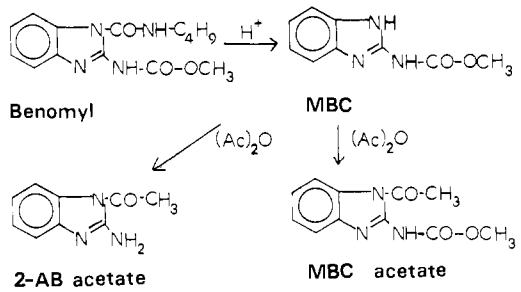

Figure 1. Preparation of 2-AB and MBC acetates.

al. (1971) have described a UV-spectroscopic method for determining the residues of MBC.

Kirkland (1973) and Kirkland et al. (1973) have developed a high-speed liquid chromatographic method for determining MBC and 2-AB residues. Benomyl, when present in the milligram range, can also be determined spectroscopically as a copper–benomyl–2-dimethylamino-2-methyl-1-propanol complex (Miller et al., 1974). In the routine measurement of pesticide residues for control purposes, there are some weaknesses in these methods. Many of them are laborious and demand time-consuming precleaning of both sample and solvents. Small laboratories are not always equipped with a fluorescence spectrometer, and, finally, the sensitivity of the above methods, varying from 0.05 to 0.1 ppm benomyl/50 g of the sample, is insufficient for small samples.

Rouchaud and Decallone (1974) have developed a method whereby benomyl is hydrolyzed to MBC, from which compound MBC trifluoroacetate (MBC-TFA) is prepared. MBC-TFA is then determined by GLC. The sensitivity of this method is dependent on the GLC equipment available, and especially on the sensitivity of the detector. Using a ^3H capture detector and packed columns, the sensitivity of the method is reported to be 0.02 ppm/100 g of the sample. Even if the GLC method is more sensitive than the colorimetric methods, its sensitivity is not sufficient when the sample is very small. Moreover, MBC-TFA seems to be rather unstable and is easily absorbed on to many column materials. It is not known if, in addition to MBC-TFA, 2-AB-TFA is produced through the esterification of MBC.

This paper describes a modification of the method of Rouchaud and Decallone. Using a high-resolution glass capillary column and NPS (nitrogen–phosphorus selective) or EC detectors, benomyl is determined as the composite value of MBC and 2-AB acetates (MBC-Ac and 2-AB-Ac) in GLC. With this method 10^{-12} g of MBC-Ac or 2-AB-Ac can be detected. With high-resolution glass capillary GLC columns, precleaning of the solvents is not necessary and several analyses per day can be made. The method can also be adapted to packed GLC columns, but sensitivity is lower and precleaning of sample and solvents is required.

The method was tested with materials such as apples, red peppers, strawberries, black currants, lettuce, and tomatoes.

EXPERIMENTAL SECTION

Extraction. A sample of 10 g was homogenized in a high-speed blender with 25 mL of benzene saturated with ammonia. The homogenate was filtered and the sediment was rehomogenized with 25 mL of benzene and filtered. The sediment was washed twice with 10 mL of benzene.

Preparation of MBC and 2-AB Acetates. Fifty milliliters of 1 N HCl was added to the combined benzene extracts and the solution shaken vigorously in a separatory funnel for 5 min. The acidic layer was separated, and HCl treatment of the benzene was repeated. The combined

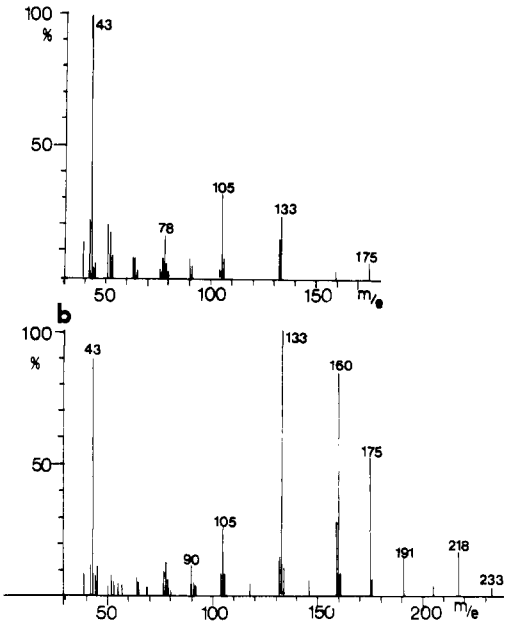

Figure 2. Mass spectra of (a) 2-AB and (b) MBC acetates.

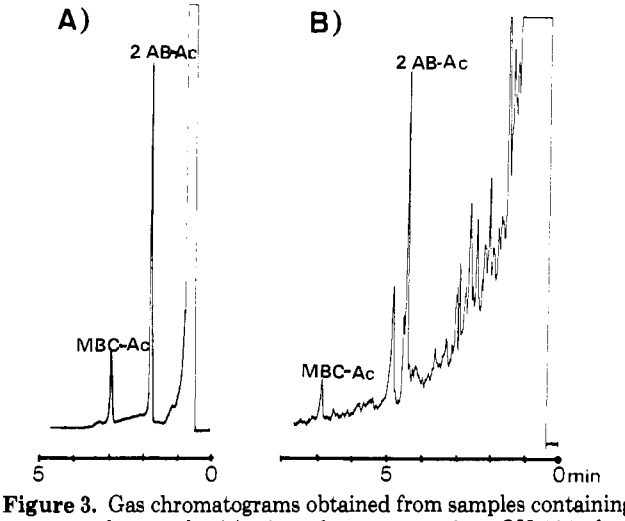

Figure 3. Gas chromatograms obtained from samples containing 0.01 ppm benomyl: (a) 10 g of tomatoes, 15-m OV-101 glass capillary column, NPSD; (b) 50 g of lettuce, 30-m SF-96 glass capillary column, ECD.

acidic fractions were washed three times with 25 mL of chloroform and then neutralized (pH 7.8–8.2) with 1 N and 0.1 N NaOH. According to mass spectrometry, the benomyl is at this point quantitatively converted to MBC. MBC is extracted from the neutral solution with 3 × 25 mL of ethyl acetate (Figure 1).

The ethyl acetate solution was dried with Na_2SO_4, and after filtration the Na_2SO_4 was washed with 2 × 10 mL of dry ethyl acetate. The combined solutions were concentrated at 30–40 °C by nitrogen flow to 2–3 mL. The concentrate was quantitatively transferred into a 10-mL Pyrex glass ampule, and ethyl acetate was added to the amount of 5 mL. Then 0.4 mL of acetic anhydride was added, and the ampule was carefully scaled and submerged for 30 min in boiling water. The cooled ampule was opened, the solution was evaporated to dryness with a nitrogen flow, and 1 mL of ethyl acetate was added. When the concentration of benomyl is low, it is better to add 0.1–0.5 mL of ethyl acetate. With a GLC–MS system (Figure 2), the above esterification procedure was found to yield mainly 2-AB acetate, with MBC acetate as the minor component (Figure 3).

GLC Measurements. The composite amount of 2-AB and MBC acetates in the final ethyl acetate concentrate can be measured with a gas–liquid chromatograph variously equipped. In this work a 50-m i.d. 0.25-mm glass capillary column, coated with OV-101 liquid phase (Grob and Grob, 1976), hydrogen as the carrier gas, and a splitless injection technique was used. The chromatograph was provided with a nitrogen–phosphorus detector (NPSD, Carlo Erba) or with a nickel type EC detector. The chromatograms were recorded at 150 °C. In addition to the OV-101 phase, SF-96 and Silar 5CP phases also were successfully tested. The sensitivity of this method (using signal to noise level 2:1) was 10^{-12} g with the NPSD and 10^{-11} g with the ECD. When 0.1 mL of ethyl acetate is used as the final solvent and 1 μL is injected into the GLC, a sensitivity enabling detection of 0.0001–0.00001 ppm benomyl/10 g is obtained.

The high-resolution glass capillary GLC columns may be replaced by normal packed columns, e.g., 3% OV-17 and OV mixture (1.5% OV-17 and 1.95% OV-210) with the result, however, that the sensitivity is now one-tenth of that above and careful precleaning of the solvents is necessary. When packed columns are used, a temperature of 240 °C is needed to obtain the signal from both 2-AB and MBC acetates. Quantitative determinations were based on peak areas in GLC. Standard solutions were prepared from commercial clean chemicals. To avoid errors caused by the loss of benomyl or incomplete esterification during preparation of the sample, an internal reference was used. The sample was divided into two equal parts, and one of these was contaminated with a known amount of benomyl. Measuring now the peak areas of 2-AB acetate and MBC acetate for the two parts, the amount of benomyl in the original sample could be calculated.

Recovery. The recovery of the method is above all dependent on the initial step of extracting the sample with benzene. The extraction can also be made using ethyl acetate (Pease and Holt, 1971; Pease and Gardiner, 1969; Kirkland et al., 1973; Rouchaud and Decallone, 1974) or by acetone (Aharonson and Ben-Aziz, 1973). Mestres et al. (1971) recommend the use of ethyl acetate saturated with ammonia. Preliminary tests using benzene also indicated that saturation with ammonia increased the recovery considerably. When tomatoes, strawberries, red peppers, black currants, cucumber, or lettuce were analyzed, the recovery varied from 70 to 90%. This corresponds well with the recoveries found by Rouchaud and Decallone (1974).

DISCUSSION

In this work benomyl was quantitatively hydrolyzed to MBC, which in turn gave, upon esterification, 2-AB and MBC acetates. Consequently, the area of two signals is to be determined and the combined value of 2-AB and MBC concentrations obtained. Using pure MBC, 10–30% MBC-Ac and 90–70% 2-AB-Ac were formed during acetylation. However, the relative proportions of these two compounds were not sufficiently constant that it would have been possible to estimate total amounts of 2-AB-Ac and MBC-Ac on the basis of the 2-AB-Ac signal alone.

The proportions of MBC-Ac and 2-AB-Ac may depend not only on acetylation conditions but also on the total amount of MBC. It is also possible that benomyl present in the sample has degenerated to 2-AB as well as to MBC, in which case the amounts of MBC-Ac and 2-AB-Ac formed during acetylation will not be constant even though acetylation conditions were identical in the analysis of the different samples.

If the GLC equipment available is such that the MBC acetate signal is not obtainable, a quantitative hydrolysis of MBC to 2-AB is needed in preparing the GLC sample. This alkali hydrolysis can be made as described by Pease and Gardiner (1969) or by Aharonson and Ben-Aziz (1973). Even if alkali hydrolysis of MBC to 2-AB is simple, however, it lengthens the time required for the pretreatment of the sample and decreases the number of analyses per day.

The most sensitive detector for 2-AB and MBC acetates is NPSD. However, in most cases a high enough sensitivity is also obtained with ECD.

At the present, most laboratories investigating the residues of pesticides work with GLC's provided with packed columns and ECD and NPSD. However, with high-resolution glass capillaries, a higher sensitivity is obtained, and because of the high-resolution capacity, the signals of 2-AB and MBC acetates can be assigned even if nondistilled solvents are used and the sample is not precleaned. A combination of high-resolution glass capillary columns and ECD is in routine use in many laboratories, and sensitive phosphorus–nitrogen detectors which can be easily connected to glass capillaries are rapidly coming into routine use.

With the method described in this paper and suitable equipment, as many as ten analyses/day can be made by a trained technician.

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