Metal addition reactions were performed to B_1S, giving partial exchange of Li and K for the Na. These spectra are shown in Figure 5. Since the reaction product was already a sodium salt, no change in the spectrum was observed on addition of NaCl.

The reaction product of G_1 with sodium bisulfite, G_1S, which would have a molecular weight of 432 amu shows ions at m/z 433 and 455, corresponding to $(M + 1)^+$ and $(M + Na)^+$, respectively (Figure 5). Figure 5 also shows an HPLC chromatogram of a mixture of aflatoxins, extracted from contaminated rice, and with peaks corresponding to aflatoxins B_1 and G_1. The HPLC chromatogram of the sodium bisulfite reaction product obtained from this extract shows peaks corresponding to B_1S and G_1S, in approximately the same ratio. A FAB mass spectrum of the reaction product mixture confirms the presence of B_1S and G_1S since peaks corresponding to $(M + 1)^+$ and $(M + Na)^+$ are found for both compounds.

CONCLUSION

Fast atom bombardment appears to be a useful technique for the identification of aflatoxins and aflatoxin bisulfite reaction products. By use of this technique, molecular weight information was obtained on the bisulfite reaction products that was not accessible by other ionization techniques (EI or CI). The metal addition reaction technique provides a convenient method for molecular weight confirmation. Further studies are in progress to characterize the bisulfite reaction products and to identify the site of bisulfite addition.

Registry No. Aflatoxin B_1, 1162-65-8; aflatoxin G_1, 1165-39-5; aflatoxin B_2, 7220-81-7; aflatoxin G_2, 7241-98-7; aflatoxin B_1 sodium sulfonate, 83219-44-7; aflatoxin G_1 sodium sulfonate, 83219-45-8.

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Use of a Sulfuric Acid Cleanup Step in the Determination of 1,2-Dibromoethane Residues in Lemons, Oranges, and Grapefruits

After isolation of ethylene dibromide (EDB) from citrus rind or whole fruit samples by steam distillation via a benzene–water azeotrope, the dried benzene distillate was cleaned up by addition of silica gel (2 parts) impregnated with fuming sulfuric acid (1 part). This cleanup procedure allowed facile quantification of 5 ppb (nanograms per gram) of EDB by gas chromatography with electron-capture detection. Recovery of EDB from samples of rind and whole fruit fortified with 500, 50, and 5 ppb averaged 87 ± 6%. Hexane could be substituted for benzene if a dimethyl silicone defoaming agent was used. With the use of hexane, recovery of EDB from whole fruit samples fortified at 5 ppb of EDB was 102 ± 3%.

The capture in detection traps of two Mediterranean fruit flies, *Ceratitis capitata*, in northern California and one in southern California on June 5, 1980, created considerable concern in the agricultural community (Hagen et al., 1981). The California citrus industry was faced with the requirement by a number of domestic and foreign markets that fruit be fumigated with ethylene dibromide (1,2-dibromoethane, EDB) prior to its acceptance. In addition, EDB residues had to meet strict legal tolerance requirements. Therefore, a method to analyze for low levels of EDB in all major varieties of citrus fruits was needed. King et al. (1980) reported on a method for the

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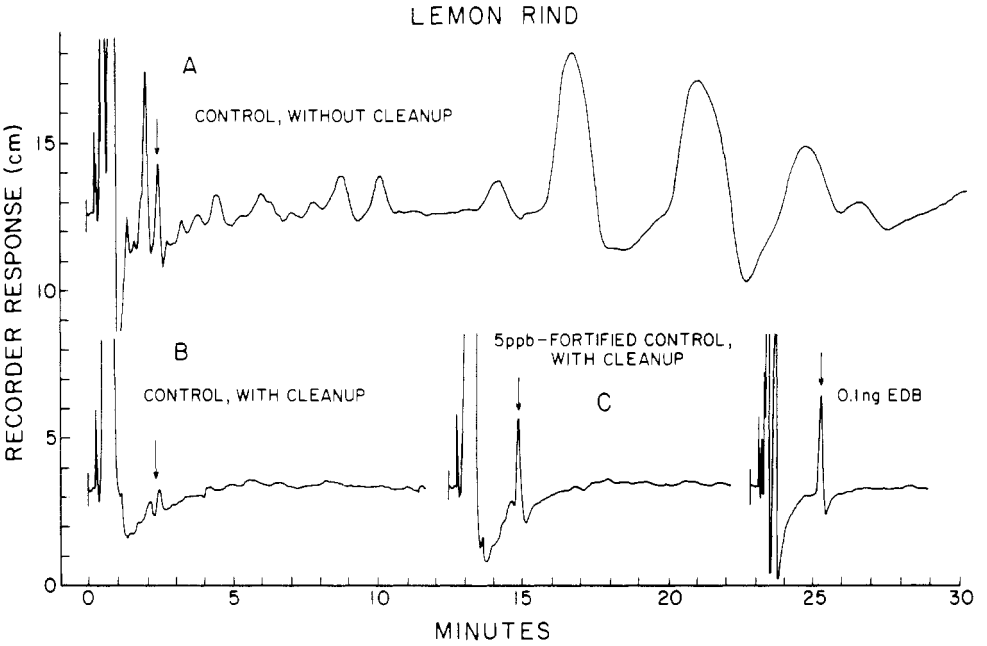

Figure 1. Chromatograms obtained after 4-μL injections of benzene distillates prepared from 100 g of lemon rind. (A) Control sample without cleanup; (B) control sample with cleanup; (C) 5-ppb-fortified control sample with cleanup.

analysis of EDB in Florida grapefruit. The method involved isolation of EDB by steam distillation via a benzene–water azeotrope and quantification by gas chromatography by using electron-capture detection. In our hands, volatile compounds in the fruits which steam distilled along with the EDB produced numerous extraneous peaks on the gas chromatograms. Some peaks eluted from the gas chromatographic column quite close to EDB and others eluted over a 30-min period. These interfering peaks made quantification unreliable at low concentrations and considerably extended analysis time. Reported here is a cleanup step for the method of King et al. (1980). Sulfuric acid sorbed on silica gel was used to remove interfering materials from the benzene distillate prior to gas chromatographic analysis. The procedure is based on the works of Davidow (1950), Stanley and LeFavoure (1965), and Murphy (1972), who have used sulfuric acid to clean up sample extracts containing acid-stable organochlorine insecticides and environmental pollutants such as polychlorinated biphenyls. Analytical methods for EDB residues have been summarized by Newsome and Panopio (1977), King et al. (1980), and Rains and Holder (1981).

EXPERIMENTAL SECTION

Reagents. One part of fuming sulfuric acid was added to 2 parts (w/w) of 60–200-mesh silica gel. The mixture was thoroughly stirred and shaken to produce a freely flowing, acid-impregnated silica gel. Exposure of this mixture to moisture will decrease its effectiveness.

One should be very careful while handling fuming sulfuric acid. It will react violently with water. If a tightly capped bottle is used, the container with the sulfuric acid–silica gel mixture should be vented frequently while being shaken and periodically while being stored. The acid does not contact all the solid immediately, so if there is a release of gas such as carbon dioxide from carbonate, the release occurs over a period of time. Also, exposure to benzene and EDB should be avoided as much as possible since adverse health effects have been ascribed to these chemicals.

Procedure. Samples of citrus rind or whole fruit were processed by using the method described by King et al. (1980) to obtain a benzene distillate. Briefly outlined, a 100-g sample was cut into small pieces and placed in a blender jar. Then, 150 mL of water, 20 mL of benzene, and 0.5 mL of benzene or EDB solution in benzene were added. After the mixture was macerated for 30 s at low speed, the mixture was transferred to a 1000-mL round-bottomed flask with the aid of 100 mL of water. By use of a heating mantel, distillation was conducted until approximately 70 mL of distillate was collected. The distillate was transferred to a separatory funnel and was shaken after addition of 5 g of NaCl. The benzene phase was passed through anhydrous Na_2SO_4 and into a collection tube.

Two grams of acid-impregnated silica gel was added to a 5-mL aliquot of the benzene extract. The combination was vortex mixed and allowed to stand for 10 min. The supernatant liquid was filtered through Whatman No. 1 filter paper. A few milligrams of $NaHCO_3$ was added to the filtrate to remove any traces of acid. The benzene solution was then subjected to gas chromatographic analysis. When hexane was substituted for benzene in the procedure, 0.5 mL of a defoaming agent containing 10% dimethyl silicone fluid emulsion (Foam Fighter, Miller Chemical and Fertilizer Corp., Hanover, PA) was added prior to steam distillation.

Analysis. A 1.8 m × 4 mm i.d. glass column packed with 10% Carbowax 20M on 60–80-mesh Gas-Chrom Q was used with a nitrogen carrier gas flow rate of 80 mL/min. Injector, column, and ^{63}Ni-detector temperatures were 218, 110, and 278 °C, respectively. Quantification was by the use of peak height measurements and by reference to a standard curve. Calculations were based on the assumption that the collected distillate (about 16 mL) was an aliquot of the total amount (20.5 mL) of organic solvent added to the sample prior to distillation.

RESULTS AND DISCUSSION

Figure 1A shows a gas chromatogram obtained for a benzene distillate prepared from 100 g of lemon rind. It clearly illustrates that considerable amounts of unwanted materials can codistill with benzene, water, and EDB. Gas chromatographic peaks produced by these unwanted materials can interfere with EDB quantification, can be mistaken for EDB residues, can extend analysis time

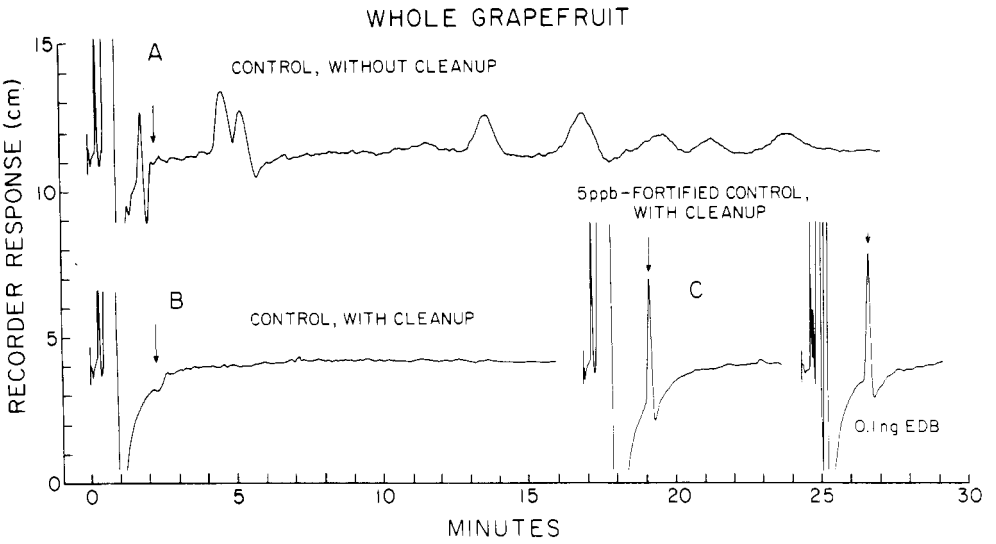

Figure 2. Chromatograms obtained after 4-μL injections of benzene distillates prepared from 100 g of whole grapefruit. (A) Control sample without cleanup; (B) control sample with cleanup; (C) 5-ppb-fortified control sample with cleanup.

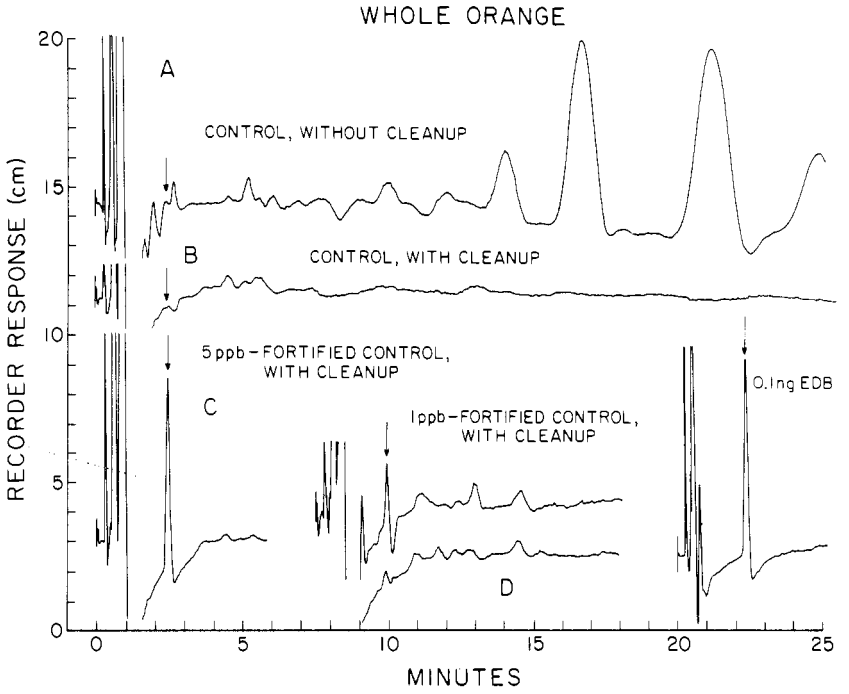

Figure 3. Chromatograms obtained after 4-μL injections of hexane distillates prepared from 100 g of whole orange. (A) Control sample without cleanup; (B) control sample with cleanup; (C) 5-ppb-fortified control sample with cleanup; (D) 1-ppb-fortified control sample with cleanup (8-μL injection; the corresponding control sample is also shown).

considerably due to prolonged elution from the column, and can foul the electron-capture detector and consequently decrease its performance. Figure 1B shows that treatment of the benzene extract with acid-impregnated silica gel removes essentially all interfering materials. Figure 1C shows that 5 ppb (nanograms per gram) of EDB added to lemon rind can be readily detected since EDB is stable to acid treatment. In addition to alleviating the problems outlined earlier, use of this cleanup step gives greater assurance as to the identity of the EDB peak. The cleaned up sample should also be more amenable to further EDB confirmation procedures.

Figure 1A is a worst-case example due to the use of lemon rind with its considerable content of citrus oils. Figure 2A is a more realistic example and shows a gas chromatogram obtained for a benzene distillate prepared from 100 g of whole grapefruit. It too illustrates that interfering peaks can make EDB quantification difficult and can extend analysis time. Figure 2B shows that the cleanup is effective in removing the unwanted materials. Figure 2C illustrates that 5 ppb of EDB added to whole grapefruit can be readily detected. The only precaution found necessary in our laboratory was the need to extract the water with benzene prior to its use. The water contaminants steam distilled and were stable to the acid treatment.

Table I gives the percent recovery values obtained for samples of whole fruit or rind alone fortified with 500, 50, and 5 ppb. Since most of the volatile interferences originate from the rind with its associated citrus oils, rind samples were included to represent worst-case samples. The mean recovery value and standard deviation for all 54 samples listed in Table I were 87 ± 6%. All samples were quantified on the same day that the samples were fortified and steam distilled. When sample solutions which were ready for final quantification were stored overnight

Table I. Recovery of EDB from Fortified Citrus Samples[a]

substrate	fortification, ppb	recovery, % replicate A	B	C	mean ± SD
whole grapefruit	500	84	86	86	85 ± 1
	50	90	97	97	95 ± 4
	5	87	94	88	90 ± 4
grapefruit rind	500	82	84	86	84 ± 2
	50	94	94	100	96 ± 3
	5	74	73	81	76 ± 4
whole orange	500	88	96	90	91 ± 4
	50	90	86	86	87 ± 2
	5	92	84	83	86 ± 5
orange rind	500	98	88	90	92 ± 5
	50	83	89	86	86 ± 3
	5	81	78	75	78 ± 3
whole lemon	500	90	92	94	92 ± 2
	50	81	94	87	87 ± 7
	5	82	91	84	86 ± 5
lemon rind	500	88	96	84	89 ± 6
	50	86	94	86	89 ± 5
	5	79	78	76	78 ± 2

[a] Samples were processed by using benzene. Apparent EDB residues in control samples were too insignificant to be considered.

Table II. Recovery of EDB from Whole Citrus Fruits Fortified at 5 ppb[a]

substrate	recovery, % replicate A	B	C	mean ± SD
grapefruit	102	102	98	101 ± 2
orange	98	106	106	103 ± 5
lemon	102	98	102	101 ± 2

[a] Samples were processed by using hexane. Apparent EDB residues in control samples were too insignificant to be considered.

in a refrigerator, EDB residue levels changed erratically, and interfering background peaks appeared on the gas chromatograms.

Due to the adverse health effects ascribed to benzene, alternate solvents are sought by many laboratories. Use of ethyl acetate in place of benzene yielded a similar background profile as shown in Figure 1A. However, sulfuric acid cannot be placed safely in contact with ethyl acetate. The procedure of King et al. (1980) most likely designated benzene, rather than hexane, because samples, especially whole fruit, foam excessively when hexane is used. A dimethyl silicone defoaming agent was used with hexane with good results. Figure 3A shows a gas chromatogram prepared from 100 g of whole orange and by using a defoaming agent. Prior to use, the hexane needed to be passed through a column of activity grade I basic alumina to remove background interferences. Figure 3B shows the cleanup achieved by addition of acid-impregnated silica gel. Figure 3C shows that 5 ppb of EDB can be readily detected. Figure 3D shows that 1 ppb of EDB can also be detected.

Table II gives the percent recovery values obtained for samples of whole fruit fortified at 5 ppb and by using hexane with the steam distillation. The mean recovery value and standard deviation for all nine samples listed in Table II was 102 ± 3%. All samples were quantified on the same day that the samples were fortified and steam distilled.

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Registry No. EDB, 106-93-4; sulfuric acid, 7664-93-9.

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Lack of Gut Absorption of Solubilized Polystyrene by the Rat

[^{14}C]Polystyrene of a molecular weight range similar to that found in commercial expanded polystyrene containers was synthesized and dissolved in lemon oil. Two microcuries was administered intragastrically to male rats of the Long Evans strain. After 5 days all radiation was recovered from fecal samples. No radiation was detected in blood, urine, major organs, or tissue samples. Ninety-nine percent of ^{14}C label was excreted within 48 h after intubation.

Phillips (1979) indicated the possibility that polystyrene might be a food contaminant by reporting his observation of the deterioration of an expanded polystyrene container by lemon tea. Phillips, however, could not find polystyrene dissolved in tea from the damaged cups. Using a [^{14}C]-polystyrene we were able to explain the observations of Phillips (Monte, 1982) as well as quantitate the solubility of polystyrene in several essential oils and detect traces of the polymer solubilized in some cooking oils with which it made contact (Monte and Landau, 1982).

Oppenheimer et al. (1955) reported polystyrene film as a carcinogen, causing tumors when implanted in rats.

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