Table II. Effect of Some Organophosphorus Insecticides on Aflatoxin Biosynthesis in YEM Medium[a]

Chemical added[b]	Aflatoxin B_1, μg	Dry cell mass, mg	Relative specific productivity, %
Dichlorvos	75	164	13
Parathion	325	159	59
Malathion	350	161	62
Guthion	325	142	66
Diazinon	390	148	76
Atrazine	395	144	79
Ethion	416	147	81
Trition	455	158	83
Phosdrin	465	157	85
Triguvon	545	165	95
Control	540	155	100

[a] Production in 10 ml of medium in 5 days. [b] Each in a 10 ppm concentration.

while not all the organophosphates are inhibitory to aflatoxin biosynthesis, dichlorvos is much more potent than the others. Therefore it is unlikely that the mechanism of inhibition of aflatoxin biosynthesis is similar to that of acetylcholinesterase inhibition.

It was noted that the yield of aflatoxin B_1 in the 100-ml YEM medium (10.8 mg/l., Table I) was considerably higher than the yield in the 10-ml medium (7.5 mg/l., Table II), but the susceptibility to the inhibition of dichlorvos was much higher in the 100-ml medium than in the 10-ml medium. One explanation of this difference may be that in the smaller volume of medium more acids were produced (as shown by the lower pH of the broth) because of a better oxygen transfer, which enhanced conversion of aflatoxin B_1 to its hemiacetal, aflatoxin B_{2a}, and also antagonized the action of dichlorvos.

Dichlorvos has been found also to strongly inhibit biosynthesis of Zearalenone by *Fusarium roseum* 'Graminearum' (Walf *et al.*, 1972). Since Zearalenone, like aflatoxins, is biosynthesized *via* the acetate–polymalonate metabolic system (Mirocha, 1973), dichlorvos may very well be an inhibitor of a key enzyme in the general polyketide pathways and inhibit synthesis of many fungal secondary metabolites. Dichlorvos is widely used as a household insecticide and its residues have been found in stored grains (Rowlands, 1971). As the residual dichlorvos did inhibit aflatoxin production in peanut, rice, and other grains (Rao and Harein, 1972), its role in the control of mycotoxin problems deserves continued attention and investigation.

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Gas–Liquid Chromatographic Detection of Actively Metabolizing *Aspergillus parasiticus* in Peanut Stocks

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A method incorporating air sweeping, trapping, and gas–liquid chromatography of headspace gas over the heated trapping agent was devised for detecting a major volatile metabolite elaborated by *Aspergillus parasiticus* cultured on wet peanuts. The metabolite was identified as acetone by mass spectral analysis. Weight dilutions of the wet moldy peanuts with uninoculated sound peanuts gave a 40-fold increase in the acetone peak for 1:70 dilution and a 4.5-fold increase for a 1:1400 dilution as compared to the control samples. Drying the wet moldy peanuts to a 6% moisture level resulted in a marked decrease of the acetone metabolite, indicating that the technique is applicable only to actively metabolizing mold.

Sporadic and nonselective invasion of many agricultural products, including peanuts, by mycotoxin elaborating strains of *Aspergillus flavus* and *Aspergillus parasiticus* during unfavorable conditions of harvesting and storage is now recognized as a serious agricultural problem (Wogan, 1964). Accordingly, the peanut industry adopted a code of good practice which calls for an extensive monitoring system of peanut stocks using microscopic detection of *A. flavus* and aflatoxin assay to assure that all peanuts entering the edible trade are wholesome (Marketing Agreement for Peanuts No. 146, 1968). However, evidence that aflatoxin in peanut stocks is associated with only a small proportion of the kernels (Cucullu *et al.*, 1966) imposes formidable problems of adequate sampling of raw materials. If the presence of mold-contaminated kernels could be de-

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tected by nondestructive analysis of large subsamples, suspect lots could be more easily diverted into nonedible uses. Since molds frequently impart characteristis odors, it appears that detection of a characteristic volatile metabolite elaborated by *A. flavus* or *A. parasiticus* could serve as a warning of mold contamination to peanut shellers.

Gas–liquid chromatographic (glc) measurements of the volatile constituents of raw uncured peanuts during the maturation period have been reported by Pattee *et al.* (1970). They reported five major and two minor volatile constituents, one of the minor constituents being acetone. While acetone is present in small amounts in raw peanuts, it is one of the five major volatile products of mold metabolism found in the present study (Figure 1). The present report describes a laboratory technique for trapping this metabolite and for its detection by temperature-programmed glc.

MATERIALS AND METHODS

Peanuts. Segregation 1 shelled Spanish peanuts of 6% moisture content served as control sound peanuts. Samples of Spanish peanuts were adjusted to 25% moisture, inoculated with *A. parasiticus* (NRRL A-16, 462) and with *A. flavus* (NRRL A-16, 464), and incubated 4 days at 30° to provide wet moldy peanuts with actively metabolizing mold. Virginia peanuts officially designated as Segregation 1, 2, and 3 containing 5% moisture were used in trapping experiments to detect the presence of mold in commercial peanuts.

Programmed glc. Apparatus, Hewlett Packard 5750; detector type, flame ionization; recorder range; strip chart; speed 0.25 in./min; attenuation 10^4. Column: 6 ft × 0.25 in. o.d. stainless steel packed with Chromosorb WAW 80/100 mesh with 10% Carbowax 20M + TPA conditioned for 64 hr at 200° in a forced stream of He. Temperature (°C): injection port, 180; detector, 200; column, programmed at 4°/min from 60–200°. Flow rate of gases: He, 50; H, 50; air, 400 ml/min. Sample: headspace gas injected with disposable 5-ml plastic syringe with Luer-lok tip. Quantitation: method, peak height.

EXPERIMENTAL PROCEDURES AND RESULTS

Initially, single wet moldy peanuts (30% moisture) containing actively metabolizing *A. parasiticus* or *A. flavus*, and single control uninoculated peanuts were sealed in separate 8-ml serum bottles fitted with silicone rubber stoppers and aluminum retainer rings (Dupuy and Fore, 1970). After heating in an oven for 45 min at 100°, a 5-ml sample of the headspace gas was withdrawn and subjected to programmed glc as outlined above under Materials and Methods. Chromatograms were similar on both types of inoculated peanuts; a typical chromatogram (Figure 1) showed the presence of six major volatile components from a typical heated moldy peanut and lower amounts of the same components from the control peanut. Using co-glc headspace gas analysis with known compounds, three of the volatiles were tentatively identified as acetaldehyde, acetone, and ethanol (Figure 1). There was about a 20-fold increase in both the acetone and the ethanol peak heights from heated moldy peanuts as compared to similar chromatograms made from sound control peanuts.

Because of the similarity of chromatograms from heated peanuts inoculated with either *A. flavus* or *A. parasiticus*, all other experiments were done using only *A. parasiticus*. In one set of experiments single moldy peanuts were mixed with 40, 60, 80, and 100 g of control high-quality peanuts and heated in sealed 600-ml serum bottles for 45 min at 100°, followed by programmed glc of 5 ml of headspace gas. Under these conditions there was a significant increase in the acetone peak and very little increase in other volatile peaks. Apparently the sound peanuts adsorbed the several other volatile components produced from heated moldy peanuts (Figure 1).

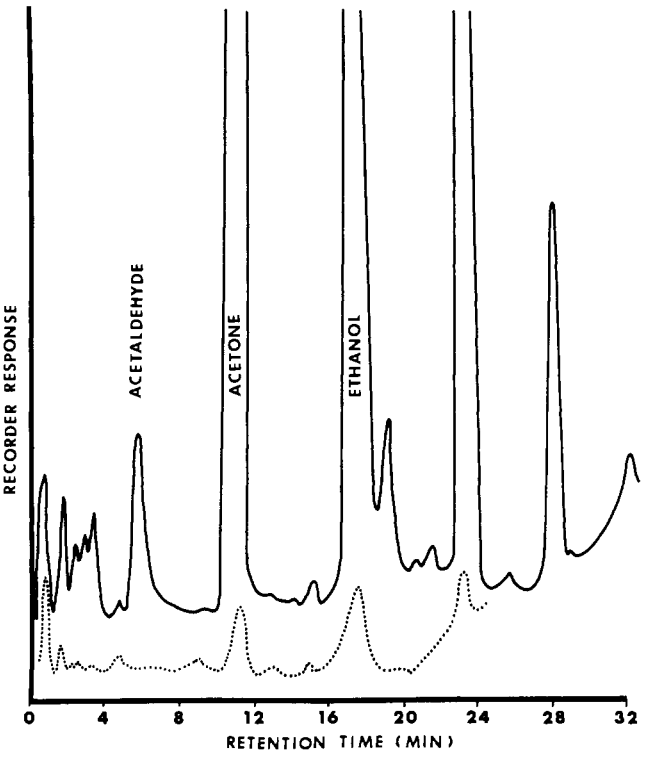

Figure 1. Typical chromatograms from temperature-programmed glc in which the recorder response (cm) is compared for 5 ml of headspace gas over a single heated wet moldy peanut (solid line) and a heated control peanut (broken line).

Since heating of large samples of peanuts is an unwieldy technique, experiments were designed to evaluate the practicability of air sweeping over reasonably large samples of unheated peanuts, trapping the volatiles on an adsorbent, and subsequently analyzing the headspace gas above the heated adsorbent as a means of detecting actively metabolizing mold in peanuts.

Trapping Experiments. *Wet Moldy Peanuts.* One wet moldy peanut mixed with 50 g of sound control peanuts (1:70 dilution) was placed in a glass tube (4-cm diam × 24-cm long) fitted with a small inlet and exit trap (1.5-cm diam × 5-cm long), each filled with 3 g of Chromosorb 101, 60–80 mesh, previously conditioned in an evaporating dish in a forced draft oven for 72 hr at 150°. The inlet trap removed atmospheric contaminants, and the exit trap adsorbed volatiles from the peanuts. A diagram of the equipment is shown in Figure 2. Using a small hand vacuum cleaner, room temperature air at the rate of *ca.* 300 ml/min was swept over the peanuts for 1 min. The exit trapping agent was then transferred to a 120-ml serum bottle, and the bottle capped and heated for 45 min at 100°. A 5-ml headspace sample was immediately withdrawn by syringe and subjected to programmed glc as previously outlined. The recorder response due to air appeared 1 min after injection. Using this peak as a reference, the acetone peak appeared 11 min from injection. A typical chromatogram shown in Figure 3 indicates about a 40-fold increase in the acetone peak as compared to the chromatogram from a similar experiment using only 50 g of sound control peanuts. Moreover, the acetone peak was the only significant difference in the two chromatograms. Variations in sweeping time from 1–5 min showed no appreciable difference in the height of this acetone peak. The volume of the all-glass container approximately equaled the amount of air which swept over the peanuts in 1 min; this suggests that all of the acetone present at the beginning of the trapping experiment was swept into

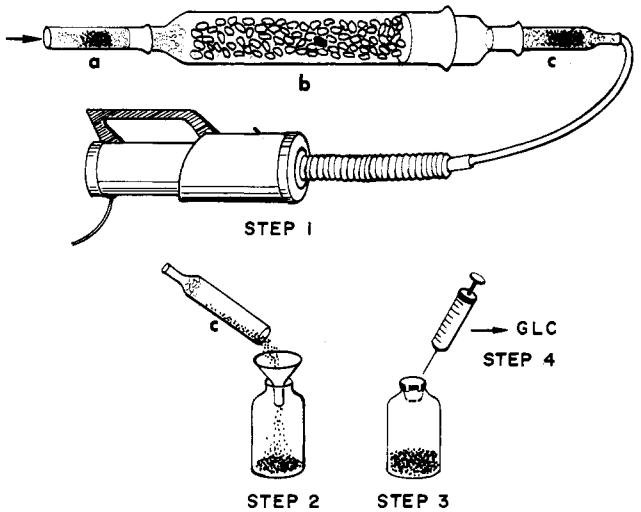

Figure 2. Schematic diagrams of the all-glass system showing the steps in trapping the volatiles from a peanut sample. Step 1: a, air filter; b, peanuts in large Butt-type glass tube 4-cm diam × 24-cm long; c, drying tube containing 3 g of Chromosorb 101 held rigid by two plugs of glass wool. Air flow is provided by a portable vacuum cleaner. Step 2: trap (c) is emptied into a 120-ml serum bottle. Step 3: removal of 5-ml aliquot of headspace gas from heated sealed bottle. Step 4: glc.

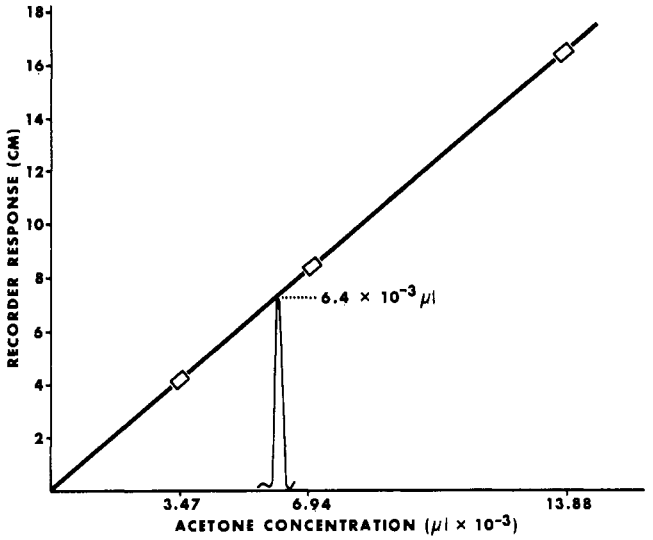

Figure 4. Standard calibration curve for acetone with the glc peak for a 5-ml aliquot of headspace gas above the heated trapping agent from the 1:1400 dilution described for the spiked 20-lb sample in Trapping Experiments superimposed.

the trap in the initial sweeping, and an increase in sweeping time did not necessarily effect an increase in the amount trapped. Using exit traps in series also produced no difference in the height of the acetone peak, indicating that the 3 g of adsorbent was sufficient to trap all of the acetone produced. The identity of the peak shown in Figure 3 as acetone was confirmed by mass spectral analysis of a sample of trapped headspace gas from moldy peanuts using a Perkin-Elmer MS 270 spectrometer.

Larger scale trapping experiments were conducted in which ten wet moldy peanuts mixed with 20 lb of sound peanuts (1:1400 weight dilution) were placed in a stainless steel cylinder container (20-cm diam × 62-cm long) and subjected to the trapping technique outlined above. A control experiment was also conducted on 20 lb of sound peanuts. The acetone peak shown in Figure 4 superimposed on an acetone standard curve is from a chromatogram made from headspace analysis of the heated trapping material from the large scale spiked experiment. To prepare the standard curve, 10 µl of acetone was injected into a sealed 120-ml serum bottle, and after equilibration was further diluted by injecting 10 ml of headspace gas into another 120-ml sealed serum bottle. Aliquots of 0.5, 1.0, and 2.0 ml of the standard which contained 6.94 × 10^{-3} µl of acetone/ml were subjected to the glc program. As shown in Figure 4, the 5 ml of headspace gas over the heated adsorbent was equivalent to *ca.* 6.4 × 10^{-3} µl of acetone; the acetone peak represents a 4.5-fold increase over the acetone peak obtained from 20 lb of sound control peanuts.

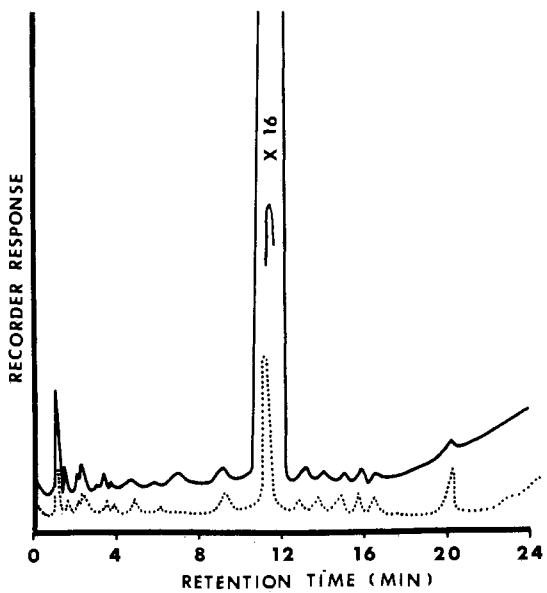

Figure 3. Typical chromatograms from temperature-programmed glc in which the recorder response (cm) is compared for 5 ml of headspace gas over heated trapping material, one moldy peanut in 50 g of control kernels (solid line), and 50 g of control kernels (broken line).

Dry Peanuts. When single moldy peanuts (30% moisture) were mixed with 50 g of sound peanuts (6% moisture) and stored in a closed bottle for 4 days prior to trapping of volatiles as described above, there was no appreciable increase in the acetone peak as compared to the chromatogram from trapping material from the 50 g of control sound peanuts. The moisture content of the moldy peanuts was reduced from 30 to 6% after equilibration with the control peanuts. Similar results were obtained from trapping experiments with 50-g samples of shelled segregation 1, 2, and 3 peanuts; *i.e.*, no significant difference in the acetone peak between segregation 1 (high quality) and segregation 3 (visible *A. flavus*). Moreover, when wet moldy peanuts of *ca.* 30% moisture containing actively metabolizing *A. parasiticus* were subjected to air drying to a 5-6% moisture level, no significant acetone peak could be detected when the dried mold contaminated peanuts were subjected to the trapping technique. All of these results indicate that acetone, a major volatile metabolite of actively growing *A. parasiticus*, is rapidly lost upon air drying and consequently the trapping technique is not applicable to normal dried and cured peanuts. However, the technique should be applicable for detecting the presence of actively metabolizing mold in shelled peanuts. This actively growing state can be expected in a warehouse where wet spots may result from variations in temperatures and humidity due to changes in weather conditions.

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Biological Effects of Sucrose Acetate Isobutyrate in Rodents and Dogs

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Sucrose acetate isobutyrate (SAIB), a suspending agent for essential oils in soft drinks, was studied in rodents and dogs. Oral and ip LD_{50}'s in rats and mice were in excess of 25 g/kg. SAIB produced no skin irritation or contact dermatitis in guinea pigs. Rats fed 5.0% for 95 days had slightly reduced body weights (males) and increased liver weights (females). No micropathology was associated with these changes nor were they reproducible. Subacute feeding to dogs increased liver weights and elevated serum alkaline phosphatase activities at levels of 0.6, 2.0, and 5.0%. Appropriate studies indicated that this increased SAP activity was liver derived. Indocyanine green clearance was unaffected in the rat, but was prolonged in the dog. Liver microsomes were unaffected in both the rat and the dog. It is concluded that SAIB is harmless to rats and causes only adaptive and functional changes in dogs which are reversible when the compound is withdrawn from the diet.

Sucrose acetate isobutyrate (SAIB) is a mixture of sucrose esters, about 95% esterified, having 2 mol of acetate and 6 mol of isobutyrate/mole of sucrose. It is a colorless, sticky, tasteless, highly viscous liquid with a molecular weight of 830–860 (Touey and Davis, 1960).

SAIB is currently being used as a suspending agent for certain oils in soft drinks and is a potential component of food packaging materials. Canadian soft drink manufacturers use 50 ppm of SAIB in combination with 15 ppm of brominated vegetable oil.

The composition of SAIB, an ester of sucrose with fatty acids of wide normal occurrence, suggests that it is an essentially innocuous substance when taken orally. In light of its possible extended use in soft drinks, the safety of SAIB was evaluated using acute toxicity studies in rats, mice, and guinea pigs and subacute feeding studies in rats and dogs, including studies of liver microsomal enzymes and indocyanine green clearance rates.

MATERIALS AND METHODS

Acute Toxicity Studies. A 50% solution of SAIB in corn oil was administered orally or intraperitoneally to determine these LD_{50}'s in rats and mice.

Five to 20 ml of a 20% solution of SAIB in acetone and corn oil (9:1) was held in contact with the depilated skin of guinea pigs by means of a gauze pad and rubber cuff for 24 hr to determine skin irritation.

Delayed sensitivity was tested using SAIB in acetone, dioxane, and guinea pig fat (7:2:1). Ten drops of this solution were applied to the depilated rump area of guinea pigs. Twenty-four and 48 hr later these areas were observed for primary irritation. After three applications in the next 5 days and a 3-week rest period, challenging doses were applied, to the right, and a week later, to the left shoulder area. Reactions were scored on a scale of 0 to 4 for erythema and edema.

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Subacute Toxicity Studies. Two subacute feeding studies were done in rats (no. I and II) and three subacute feeding studies were done in beagle dogs (no. I, II, and III). Dog Study I was carried out by the Food and Drug Research Laboratories (FDRL), Inc., Maspeth, N. Y. The other two dog studies and the rat studies were done in this laboratory. Another study (Rat Study III) was undertaken to determine indocyanine green plasma clearance rates at various intervals up to 36 days in male rats fed 4.0% SAIB in the diet.

RAT STUDIES

Rat Study I. Groups of 50 Sprague–Dawley (Holtzman) rats (25 males and 25 females) randomly assigned to two treatment and one control group were housed, five of each sex per cage, and fed 1.0, 5.0, or 0.0% SAIB in the diet for 95 days. Water was available *ad libitum*. SAIB was solubilized in acetone and incorporated into a basal diet of ground Purina Lab Chow. The mixtures were spread on shallow pans to allow the acetone to evaporate. A control diet, to which only acetone was added, was handled similarly.

Individual body weights and group feed consumption were recorded weekly, and hemoglobins, hematocrits, and white cell and differential counts were done on five animals from each group 3 days prior to feeding and on the 24th, 52nd, and 87th day of feeding.

On day 95, all animals were autopsied. Individual liver and kidney weights were recorded and tissue samples were processed for microscopic examination.

Rat Study II. Three experiments were run concurrently to elucidate and complement data obtained in Rat Study I. Two-hundred-and-eighty (140 males and 140 females) albino rats (Sprague–Dawley, Carworth Farms, Inc.) were randomly divided into 14 treatment groups of each sex with ten animals per group. Five of each sex were housed in wire-bottom cages and fed SAIB at concentrations of 1.0, 2.0, or 4.0% (w/w) in ground Purina Laboratory Chow supplemented with 5.0% Mazola Corn Oil (w/w). The groups of ten rats per sex were fed their respective experi-