Measurement of Residues of Dichlorvos Absorbed by Food Exposed during Disinsection of Aircraft

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Improved methods for extraction, cleanup, and gas chromatographic analysis have been developed for determination of dichlorvos (2,2-dichlorovinyl dimethyl phosphate, DDVP) in foods. The methods have been used to determine the residues absorbed by food exposed to dichlorvos concentrations used in in-flight disinsection of aircraft. Whole meals exposed for 30 min to 0.25 μg/l. of dichlorvos absorbed approximately 0.18 ppm. Approximately 1/10 of this concentration was found in beverages similarly exposed, while concentrations in margarine were three times as high. The method is sensitive to 0.005 ppm of dichlorvos.

A system for disinsection of aircraft in flight by the use of vapors of dichlorvos (2,2-dichlorovinyl dimethyl phosphate, DDVP) has been described by Jensen *et al.* (1965). The system was installed in a commercial DC-6B passenger aircraft and tested on scheduled flights in the Caribbean area in 1963. Complete kill of mosquitoes and houseflies was observed when exposed in flight for 30 min at concentrations of 0.13–0.25 μg/l. of dichlorvos. Since that time the above system with modifications has been installed and tested in commercial 707, 727, 747, and VC-10 passenger aircraft.

Recently the question of absorption of dichlorvos vapors by exposed foodstuffs has been raised. The purpose of this work was to determine the quantity of dichlorvos which would be absorbed by food served on board an aircraft during the in-flight vapor disinsection cycle. This has been accomplished by analysis of restaurant-type meals and beverages after exposure to dichlorvos vapor concentrations simulating those found in actual flight during the disinsection cycle.

Several methods for determination of dichlorvos residues in foods have been reported, but none were satisfactory for analysis of the restaurant-type meals used in the present experiment. The method of Ivey and Claborn (1969) results in poor recoveries when applied to fat and muscle tissues. The method of Schultz *et al.* (1971) was designed for animal tissues and fluids, but does not have the required sensitivity. Elgar *et al.* (1970) reported on a method which was developed for analysis of dichlorvos in crops and tissues with a sensitivity to 0.01 ppm. This method was used by Elgar *et al.* (1972) for determination of dichlorvos residues in meals exposed to vapors from dichlorvos resin strips in houses in England and France. The method was found to be unsatisfactory for use with the meals exposed in this experiment because of their high fat content, which would have required a lengthy steam distillation cleanup procedure. To solve this problem, an improved method of extraction and cleanup was developed which removed 99% of the fat without loss of dichlorvos. The new method and recovery data are presented below. Residues found in precooked meals, beverages, and margarine exposed to air concentrations equivalent to, below, and above those maintained during in-flight disinsection of aircraft are also given.

EXPERIMENTAL SECTION

Materials and Equipment. Analyses were made on a Micro-Tek MT-220 gas chromatograph equipped with a Melpar flame photometric detector with interference filter for spectral isolation of phosphorus emission at 526 mμ. The detector was held at 225°, the inlet and outlet blocks were held at 250°, and the column oven was held at 235°. The column was a 1 ft × 1/4 in. o.d. aluminum tube packed with Chromosorb 101 with no liquid phase added, 100/120 mesh (Johns-Manville, Celite Division). Gas parameters were N_2 = 150 cm³/min at 70 psi, H_2 = 100 cm³/min at 30 psi, and O_2 = 19 cm³/min at 40 psi.

Column cleanup was conducted with a glass column 30 × 150 mm, with a capillary tip 2 mm i.d. × 50 mm long packed with 30 g of silica gel no. 923, 100/200 mesh (Fisher Scientific Co.). The column was packed by tamping the dry silica gel. Glass wool pads were placed at the bottom and top of the column.

The *n*-hexane solvent was prepared by distillation of 4 l. of technical grade hexane over 200 ml of methanol plus 2 g of metallic sodium. The first liter of distillate was discarded.

Cotton, USP, was extracted overnight in a Soxhlet extractor with acetone and then air dried.

A standard solution of dichlorvos was prepared by accurately weighing approximately 20 mg of dichlorvos (99.7%, 2,2-dichlorovinyl dimethyl phosphate, Shell Chemical Co., Modesto, Calif.) into a 50-ml volumetric flask and making to volume with isooctane. A working standard containing approximately 0.40 ng/μl was prepared by serial dilution of the above standard with isooctane.

PROCEDURES

Whole Meals. After exposure to dichlorvos vapors, the restaurant meal, exclusive of drink, was placed in a high speed Waring type blender and ground to form a homogeneous sample. An aliquot containing 100 g of the homogenized sample was weighed into a 500-ml beaker and 40 g of Celite 545-C was added and mixed until a dry fluff was obtained. This was transferred quantitatively to a 1-l. glass-stoppered bottle and 300 ml of ethyl acetate–hexane (1:9) and 1 ml of concentrated HCl were added. The mixture was shaken for 30 min in a wrist-action shaker. After separation of the phases, the liquid phase was filtered through glass wool and a 100-ml aliquot of the extract was taken for analysis. A silica gel column was prepared and wetted by passing 50 ml of *n*-hexane through the column. The 100-ml sample extract was then passed through the column and the eluate was discarded. The column was eluted with 125 ml of acetone–hexane (1:9) and this eluate was discarded. Finally, the column was eluted with 150 ml of acetone–hexane (3:7), which was collected in a 250-ml beaker and evaporated to approximately 5 ml on a steam bath. The residue was quantitatively transferred to a 15-ml graduated centrifuge tube, where it was further evaporated. A melting point tube sealed about 1 cm from the end was placed in the centrifuge tube to prevent bumping during evaporation. Samples with very low dichlorvos content were evaporated down to 1 ml. Injections

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of 5-μl aliquots of the concentrated solution were injected into the gas chromatograph and the concentration of dichlorvos was determined by comparison of peak heights with those obtained with the standard solution.

Beverages. Beverage samples containing 150 ml were acidified with 1 ml of HCl and extracted with 50 ml of *n*-hexane in a 1-l. separatory funnel. The mixture was shaken for 1 min and the phases were allowed to separate. This was repeated two times, after which the aqueous phase was transferred to a second funnel and extracted again in the same manner with another 50 ml of *n*-hexane. The hexane extracts were passed through a cotton plug and combined in a 250-ml beaker. The funnels were rinsed with two 5-ml portions of *n*-hexane and the rinses were passed through the cotton plug and combined with the 50-ml extracts. The solvent was evaporated on a steam bath and analyzed for dichlorvos, as described above for whole meals.

Margarine. A sample of margarine 1 in.^{2} × ¼ in. thick was weighed into a 100-ml beaker and dissolved in 50 ml of ethyl acetate–hexane (1:9). The solution was passed through the silica gel cleanup column, eluted, and analyzed for dichlorvos content by the procedures described above for whole meals.

Analysis of Spiked Samples. The precision of the method was determined by analysis of samples to which known amounts of dichlorvos were added. Banquet meatloaf dinners were cooked as directed on the package and the entire meal was ground in a Waring type blender to form a homogeneous sample. Four 50-g subsamples were weighed into 500-ml beakers and spiked with 0.23, 0.46, 4.6, and 46.0 μg of dichlorvos in acetone. The samples were analyzed according to the above procedures using proportionally smaller quantities of reagents. Results are presented in Table I. Similar experiments were conducted with margarine and beverages to which microgram quantities of dichlorvos were added. Recoveries of 97.5–100% of the added dichlorvos were obtained.

The efficiency of the cleanup steps was determined by extraction and cleanup of a 100-g sample of a homogenized meatloaf dinner according to the above procedures and by weighing the residues of fat obtained in each of the cleanup steps. The total fat extracted was found to be 10.7 g. Of this, 67% was found in the ethyl acetate–hexane (1:9) eluate, 32% in the acetone–hexane eluate, and only 1% in the acetone–hexane eluate (3:7), which contained the dichlorvos residue.

Exposure and Analysis of Experimental Samples. A low speed wind tunnel 40 ft long × 5 ft in diameter, constructed of galvanized sheet steel, was used to simulate the conditions in an aircraft during the disinsection cycle. The tunnel was equipped with a 5-ft fan driven by a 20-hp electric motor. The speed of the fan was adjusted to deliver a stream of air through the tunnel at 5 mph. Dichlorvos vapors were introduced into the tunnel by passing compressed air through an oil filter cartridge impregnated with 1.4 g of dichlorvos, as described by Jensen *et al.* (1965). The concentration of dichlorvos vapors in the tunnel was controlled by appropriate splitting of the stream of compressed air from the cartridge into the wind tunnel. Approximately 6 ft from the exhaust end of the tunnel a rack was constructed to hold the samples during exposure.

The exposed meals consisted of frozen Banquet meatloaf and Ocona turkey TV dinners cooked as directed. Both types of dinners contained potatoes and other vegetables. Coffee was prepared by the addition of 1 teaspoonful of instant coffee in 150 ml of hot water in a 250-ml beaker. Cola drinks from a cold vending machine were used to prepare samples of 150 ml each in 250-ml beakers. Margarine samples 1 in.^{2} × ¼ in. thick were cut from ¼-lb sticks of margarine. The samples were prepared just prior to exposure so the temperature of the food and beverages would be representative of a typical meal served on an aircraft.

Table I. Recovery of Added Dichlorvos from Meatloaf TV Dinners

Dichlorvos added		Dichlorvos found, μg	Average	Standard deviation	Relative standard deviation, %	Recovery %
ppm	μg					
0.0046	0.23	0.219				
		0.205				
		0.250				
		0.250	0.231	0.022	9.81	100.4
0.0092	0.46	0.419				
		0.476				
		0.488				
		0.454	0.459	0.030	6.59	99.8
0.092	4.60	4.34				
		4.84				
		5.44	4.87	0.550	11.30	105.8
0.920	46.0	48.86				
		43.75				
		45.83				
		46.43	46.22	2.10	4.55	100.5

Table II. Residues of Dichlorvos Found in Food and Beverages Exposed to Various Concentrations in the Atmosphere for 30 Min

Concentration of dichlorvos in air, μg/l.	Concentration of dichlorvos in food and beverages, ppm				
	Cola	Coffee	TV dinner, meatloaf	TV dinner, turkey	Margarine
0.035	0.005	0.007	0.040	0.038	0.157
0.256	0.028	0.019	0.165	0.189	0.610
0.323	0.028	0.025	0.230	0.220	0.988
0.440	0.031	0.027	0.349	0.266	1.653
0.440	0.022[a]	0.016[a]	0.161[a]	0.132[a]	0.558[a]
0.577	0.038	0.028	0.460	0.295	1.561

[a] Exposure time, 15 min.

Immediately following the placement of the samples on the rack in the wind tunnel, the fan and dichlorvos dispensing system were started. The concentration of dichlorvos in the air was determined by the method described by Miles *et al.* (1970). The air samplers were placed adjacent to the exposure rack and air was sampled at the rate of 9.5 l./min. Experiments were conducted with air concentrations ranging from 0.035 μg/l. to more than 0.5 μg/l. Exposure times were 15 and 30 min. Duplicate samples of each type of food were exposed to the dichlorvos vapors. Quadruplicate analyses of each sample were performed by the procedure given above. The average concentrations of dichlorvos found are given in Table II.

In addition to the experiments conducted with meals completely exposed to the dichlorvos vapors, another experiment was conducted to determine the absorption of dichlorvos vapors under conditions of partial exposure. The purpose of this experiment was to determine if food stored in storage cabinets aboard the aircraft would absorb the dichlorvos vapors. Metal cabinets similar to those used for food storage aboard aircraft were placed in the wind tunnel and cooked turkey TV dinners were placed in the cabinets. The door to one of the cabinets was closed, while that of the other cabinet was left open. The wind tunnel was operated for 30 min with a dichlorvos concentration of 0.25 μg/l. of air. Upon analysis, the dinner which was exposed in the open cabinet was found to contain 0.063 ppm of dichlorvos. No dichlorvos (<0.005 ppm) was found in the dinner stored in the closed cabinet.

DISCUSSION

An improved method for extraction and cleanup of dichlorvos residues in food products has been useful in the determination of residues which would be absorbed by food if served during the disinsection of an aircraft with dichlorvos vapors. Quantitative recovery of dichlorvos has been achieved with a minimum of labor by shaking the Celite-treated sample with a mixture of ethyl acetate and hexane. Removal of 99% of the fat residue has been achieved by elution with 10% acetone in hexane on a silica gel column similar to that suggested by Kadoum (1967). The gas chromatographic separation of dichlorvos on the column used in this work required a relatively high temperature, but no decomposition was observed and sharp symmetrical peaks were obtained.

The wide range of air concentrations of dichlorvos was chosen to cover all possible exposure conditions and to determine if the absorption is proportional to air concentrations. Results presented in Table II show that the concentrations of dichlorvos in any given medium are generally proportional to the air concentrations as well as to the exposure times. It was noted that the concentration of dichlorvos in margarine exposed to the vapors was about three times as high as that in cooked meals and 30 times as high as that in beverages. Meals stored in open cabinets absorbed approximately one-third as much dichlorvos as those held in freely circulating air. No residues were found in meals stored in closed cabinets.

In recent in-flight experiments conducted in a Boeing 727 aircraft with the current model of the system described by Jensen *et al.* (1965), 19 air samples were taken in the cabin of the craft during six disinsection cycles. The concentrations ranged from 0.084 to 0.38 μg/l. with an average of 0.20 μg/l. (Ross, 1971). If one were to consume a 300-g dinner with 5 g of margarine and two beverages after exposure for 30 min to a concentration of 0.25 μg/l., he would consume approximately 60 μg of dichlorvos. The maximum acceptable daily intake of dichlorvos recommended by FAO/WHO is 0.004 mg/kg, or 280 μg for a 70-kg person (World Health Organization, 1972).

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Minor Peroxide Components as Catalysts and Precursors to Monocarbonyls in the Autoxidation of Methyl Linoleate[1]

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Autoxidized methyl linoleate (PV = 50) was fractionated by column chromatography and thin-layer chromatography. The 9- or 13-monohydroperoxide and minor peroxide fractions were obtained. In hexane with cupric stearate, every fraction of peroxide was equally active for catalysis of the autoxidation of methyl linoleate, while in an aqueous emulsion with Tween 20 and cupric sulfate, the monohydroperoxide was a very poor catalyst and more polar fractions of peroxides were the active catalysts. The monohydroperoxide was not the precursor to monocarbonyl compounds, except for 2-heptenal, and the precursors to 2,4-decadienal and hexanal were contained in the minor peroxide fractions.

In lipid autoxidation, it is accepted as the established theory that hydroperoxides are the initial and predominant products, they act as catalysts for the chain reaction by supplying radicals, and they are the precursors to various off-flavor substances, volatile carbonyl compounds, etc., and to polymerized substances. The major components of the hydroperoxides are those which originate from abstraction of the allylic hydrogen and, often the following rearrangement, *e.g.*, 9- and 13-monohydroperoxides, from linoleate.

This paper presents an important complement or revision to the theory that: (1) in water emulsion, 9- or 13-monohydroperoxide of methyl linoleate showed very low catalytic activity for autoxidation, while the polar fractions of hydroperoxides had high activity; and (2) 9- and 13-monohydroperoxides were not the precursors to any of such carbonyl compounds as hexanal, 2-nonenal, and 2,4-decadienal (Gaddis *et al.*, 1961). Precursor fractions to 2,4-decadienal and hexanal were obtained separately from the monohydroperoxides.

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