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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Table I. Promotive Effect of the Peroxide Fractions on the Oxygen Uptake of Methyl Linoleate[a]

		Peroxide fraction					
		1	2	3	4	5	6
The promotive effect expressed by O_2 uptake (μl/hr)	In hexane	106	98	108		84	104
	In aqueous emulsion	0	21	25		40	46
Concentration of RO_2H added (10^{-3} M)		2.25	2.33	2.22		2.35	2.28
Solvent for the elution of the fraction (hexane–ethyl ether)		90:10 ~85:15	85:15 ~75:25	70:30	60:40	40:60	0:100
Dialkyl peroxide: hydroperoxide		0	0.33	0.73		0.20	0

Warburg manometry: the mixture of 1.5 ml of methyl linoleate, 1.5 ml of hexane, and 10^{-4} M cupric stearate or that of 1.5 ml of methyl linoleate, 1.5 ml of aqueous Tween 20 solution (10%), and 10^{-4} M cupric sulfate was shaken at 37° for 15 min.

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EXPERIMENTAL SECTION

Materials. Methyl linoleate was purchased from Kanto Chemical Co., Ltd. (Japan), and was oxidized by keeping it at room temperature. For the peroxide fractionation, a sample of peroxide value 40–50 was used. Solvents used were negative in peroxide tests.

Methods. Peroxides were chromatographically fractionated from methyl linoleate oxidized (100 ml in 500 ml of hexane) on a silicic acid column (4 cm × 40 cm) by successive elution with hexane, hexane–ethyl ether (90:10, 85:15, 75:25, 70:30, 60:40, and 40:60 v/v), and ethyl ether (Gardner and Weisleder, 1970). Further separation of the fractions obtained above was carried out by column chromatography on silicic acid using solvent systems consisting of hexane–benzene (1:1), benzene, benzene–ethyl acetate (95:5 and 90:10), or hexane–ethyl ether (80:20), and by preparative thin-layer chromatography (tlc) on Kieselgel G (activated at 110°) with a solvent system of benzene–ethyl acetate (80:20, 90:10, or 95:5). Purity of the fractions was checked by tlc with a solvent system of benzene–ethyl acetate (80:20 or 90:10) or hexane–ethyl ether (60:40), duplicate development being done if necessary. Peroxide was detected by spraying a potassium iodide–starch solution. In the case of preparative tlc, the developed patterns were transcribed to filter paper sheets from the wet thin layer and the sheets were sprayed with the color reagent. The peroxide fractions were stored under dark and deaerated conditions. The rate of autoxidation was measured by Warburg manometry at 37°. Methyl linoleate for the substrate was preincubated with an equal volume of hexane and 10^{-4} *M* cupric stearate, or with an equal volume of 10% aqueous Tween 20 solution containing 10^{-4} *M* cupric sulfate under degassed conditions at 50° for 3–4 days in order to decompose peroxides. Peroxide samples to be tested were dried under reduced pressure, dissolved in the above substrate solution or emulsion, and subjected to manometry. Peroxide value was measured as follows: the sample to be tested was dissolved in 10 ml of chloroform and 15 ml of acetic acid and nitrogen was bubbled through. A saturated aqueous solution of potassium iodide (1 ml) was added and 5 min later the liberated iodine was titrated with N/200 sodium thiosulfate, as usual. Dialkyl peroxide was estimated according to Hiatt *et al.* (1968). Peroxide fractions were decomposed in benzene with 10^{-4} *M* cupric stearate under degassed conditions at 80° for 3 hr (Kimoto and Gaddis, 1969). Carbonyl compounds were converted to 2,4-dinitrophenylhydrazones by keeping them with 2,4-dinitrophenylhydrazine in benzene and 2 *N* hydrochloric acid in methanol for 30 min. The benzene solution was washed with water, evaporated to dryness, and subjected to tlc (20 × 20 cm plate). After development with benzene, the area higher than acetaldehyde hydrazone on the thin layer was collected and extracted with chloroform. The hydrazones extracted were separated into classes containing methyl ketone, alkanal, alk-2-enal, and alk-2,4-dienal by preparative paper chromatography (Gaddis and Ellis, 1959) using Toyo filter paper no. 514 (thick type, Toyo Roshi Kaisha, Ltd., Japan) with petroleum ether as the solvent. The samples separated were identified and estimated spectrophotometrically (Jones *et al.*, 1956; Pippen and Nonaka, 1958) and the carbon number in each homolog of the carbonyl compounds was further determined by reversed-phase paper partition chromatography (Gaddis and Ellis, 1959) using paper treated with vaseline and with 80% aqueous methanol as the solvent.

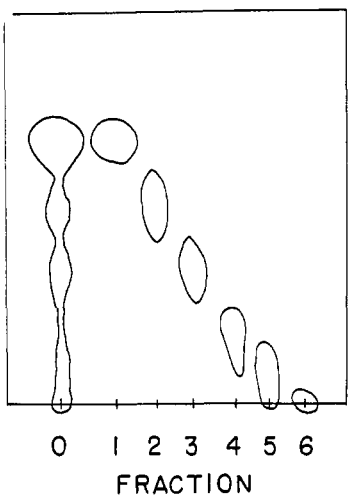

Figure 1. Diagram of thin-layer chromatogram of peroxide fractions. 0: methyl linoleate oxidized. Plate: Kieselgel G activated at 110°. Solvent for development: hexane–ethyl ether (60:40 v/v). Color reagent: potassium iodide–starch solution. Reprinted with permission: *Agr. Biol. Chem.* **36,** 2263 (1972).

RESULTS

A thin-layer chromatogram of the peroxides in autoxidized methyl linoleate (PV = 50) is shown in Figure 1. The methyl linoleate sample was applied to a silicic acid column. After unchanged methyl linoleate was eluted with hexane, a mixture of 9- and 13-monohydroperoxides (fraction 1) was eluted with hexane–ethyl ether (90:10–85:15). Then, successively, fraction 2 was eluted with hexane–ethyl ether (85:15–75:25), fraction 3 with hexane–ethyl ether (70:30), fraction 4 with hexane–ethyl ether (60:40), fraction 5 with hexane–ethyl ether (40:60), and finally fraction 6 with ethyl ether. Thin-layer chromatograms of fractions 1–6 are shown in Figure 1. The relative amounts were about 80% (fraction 1), 15% (fractions 2 and 3), and 5% (fractions 4, 5, and 6) on the basis of the molar ratio of peroxide.

The promotion of the oxidation of methyl linoleate by

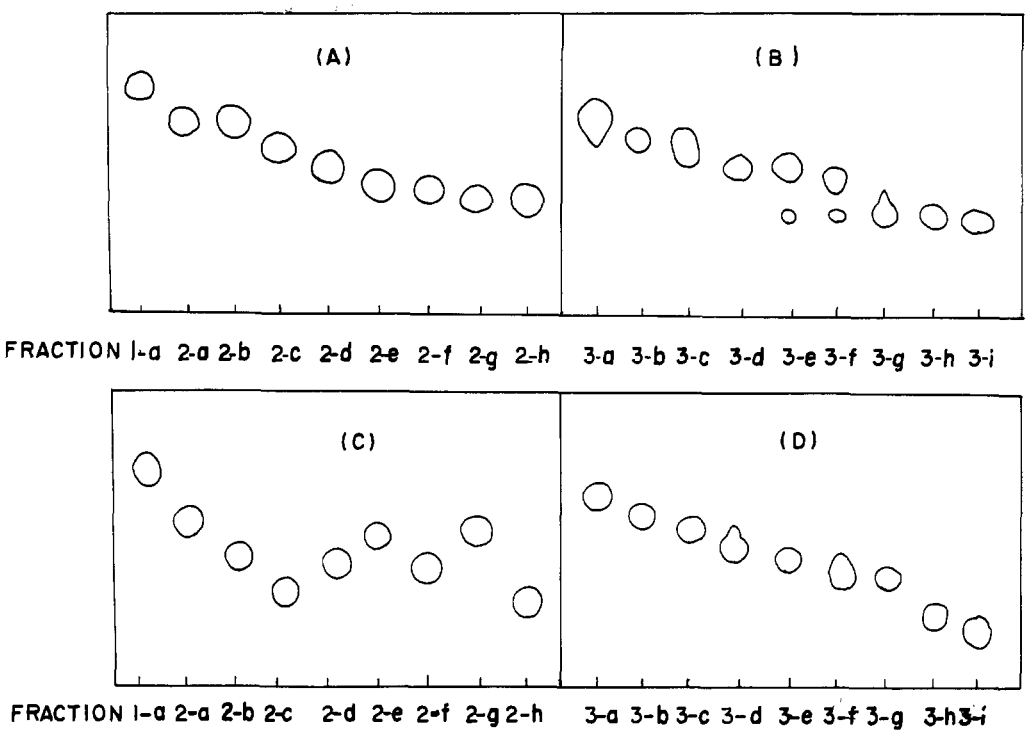

Figure 2. Diagram of thin-layer chromatograms of peroxide fractions further separated. Plate: Kieselgel G activated at 110°. Solvents for development: (A) hexane–ethyl ether (60:40); (B) hexane–ethyl ether (60:40) by duplicate development; (C) benzene–ethyl acetate (90:10); (D) benzene–ethyl acetate (90:10) by duplicate development. Color reagent: potassium iodide–starch solution.

Table II. 2,4-Dinitrophenylhydrazones of Carbonyl Compounds Formed from the Peroxide Fractions

Original peroxide fraction		λ_{max}, mμ	Absorbance	Carbon no.
1	Methyl ketone	364	0.408	
	Alkanal	359	0.727	6
	Alk-2-enal	375	0.525	8, 9
	Alk-2,4-dienal	390	0.149	10
2	Methyl ketone	361	0.634	
	Alkanal	357	1.076	6
	Alk-2-enal	371	0.427	7, 8, 9
	Alk-2,4-dienal	389	1.317	10
3	Methyl ketone	362	0.479	
	Alkanal	357	1.518	6
	Alk-2-enal	369	0.464	7, 8, 9
	Alk-2,4-dienal	390	0.400	9, 10
5	Methyl ketone	361	0.623	
	Alkanal	357	1.343	6
	Alk-2-enal	368	0.471	8
	Alk-2,4-dienal	390	0.285	9, 10
6	Methyl ketone	360	0.463	
	Alkanal	358	1.241	6
	Alk-2-enal	370	0.397	8
	Alk-2,4-dienal	390	0.135	

The peroxides used were 4.75×10^{-5} mol (fr 1), 5.30×10^{-5} mol (fr 2), 4.80×10^{-5} mol (fr 3), 5.50×10^{-5} mol (fr 5), and 5.00×10^{-5} mol (fr 6). Absorbance was measured in 10 ml of chloroform.

these fractions was measured as shown in Table I. In hexane with cupric stearate, the promotive effect of each fraction was almost equal. On the other hand, in an aqueous emulsion with Tween 20 and cupric sulfate, fraction 1 showed very poor promotion and fractions 2, 3, 5, and 6 showed an increasing effect in this order. During such a short reaction time as 15 min, oxygen uptake was not observed without the addition of peroxide fractions, even in the presence of 10^{-4} *M* copper. The substrate methyl linoleate contained no peroxide and, after each experiment, only the peroxide added was observed on tlc.

Fractions 1, 2, 3, 5, and 6 were decomposed in benzene with cupric stearate, and the carbonyl compounds formed were determined as listed in the Table II. Fraction 1 yielded carbonyl compounds in relatively small amounts. Fraction 2 gave 2,4-decadienal. Fraction 1 was further purified to fraction 1a by tlc with a benzene–ethyl acetate solvent (95:5). Fraction 2 was separated to fractions 2a–2h by rechromatography on a silicic acid column with hexane–ethyl ether (80:20) and by tlc with a benzene–ethyl acetate solvent (90:10). Fraction 3 was separated to fractions 3a–3i by rechromatography on a silicic acid column with benzene–ethyl acetate (95:5 and 90:10) and by tlc with a benzene–ethyl acetate solvent (80:20). These fractions on the thin-layer chromatogram are shown in Figure 2. Carbonyl compounds formed by the decomposition of these fractions are listed in Table III. Fraction 1a did not form a carbonyl compound, except for 2-heptenal. Fraction 2f contained the precursor to 2,4-decadienal. Fractions 3a and 3d contained the precursor to hexanal. Hydroperoxide and dialkyl peroxide contents of fraction 2f were 5.0 and 1.5 mequiv/g, respectively. Those of fractions 3a and 3d were not measured because of their low yields.

DISCUSSION

It is accepted that hydroperoxide promotes autoxidation by the formation of radicals which initiate the chain reaction. As hydroperoxides in lipids are relatively stable in the absence of transition metals and the activity of the metal largely influences the autoxidation rate, one-electron transfer between the hydroperoxide and the metal is considered as the main route of radical formation. Although the real environment around the reaction site remains highly speculative, the following viewpoints may be worthy of consideration. Hydroperoxide exists in the oil phase (nonpolar phase) but is a polar component therein. On the other hand, transition metals show affinity for the

polar phase. Hydroperoxide and metal usually come into contact in an environment which is not completely homogeneous and the reaction may be influenced by the polarities of the hydroperoxide and the metal ion involving the metal ion coordination sphere.

From the results in Table I, the promotive activities of hydroperoxides toward autoxidation were not related to their polarities in hexane with cupric stearate, which suggests that the probability of the reaction of hydroperoxide with metal is not influenced by the polarity of the hydroperoxide in such a nonpolar homogeneous environment. In the aqueous emulsion with Tween 20, the reaction of hydroperoxides with metal may occur on the interface between oil and water. Table I presents, in this case, the striking result that 9- or 13-monohydroperoxide showed very low promotive activity for autoxidation and increasing polarity of the hydroperoxide fractions markedly enhanced the activity. This result may be explained by the possibility that the affinity of hydroperoxide for the oil-water interface relates to the promotive activity for autoxidation. In most foods, autoxidation occurs in lipids in contact with aqueous phase, and problems with lipids in the absence of water or moisture are rare. In most foods, the minor and polar fractions of the hydroperoxides may play the main role in autoxidation catalysis rather than the monohydroperoxide. As reviewed by Labuza *et al.* (1971), foods and some model systems containing moisture over the monomolecular layer level undergo oxidation more slowly than dry ones. The reason for this phenomenon may partly be that the monohydroperoxide or less polar hydroperoxide fraction is repelled from the oil-polar phase interface by water in the polar phase, and reaction with metals in the polar phase becomes difficult.

Contrary to expectation, 9- or 13-monohydroperoxide did not give a monocarbonyl compound, except for 2-heptenal. The possibility that the 2-heptenal also comes from a precursor contaminating the monohydroperoxide fraction is not excluded. Thus, the former theory of carbonyl formation, as reviewed by Badings (1959), in which the formation of various carbonyl compounds is explained by various modes of scission of 9- or 13-monohydroperoxide may not be valid. The precursors to various monocarbonyl compounds exist in more polar fractions than in the monohydroperoxide fraction, as shown in Table II. The conditions of decomposition (Kimoto and Gaddis, 1969) strongly suggest that the precursors are compounds of the peroxide type. The precursor fractions to 2,4-decadienal and hexanal were further separated from fractions 2 and 3 and each of them gave a single spot on tlc.

From Table I, dialkyl peroxide groups were estimated in fractions 2 and 3. Dialkyl peroxide groups may induce the scission of carbon-carbon bonds and lead to carbonyl formation, especially by the thermal decomposition of peroxides. Kimoto and Gaddis (1969) reported that 2,4-decadienal was formed in good yield by thermal decomposition in the absence of metal.

Table III. Formation of Carbonyl Compounds from Further Separated Peroxide Fractions

Peroxide fractions to be decomposed		2,4-Dinitrophenylhydrazone of the carbonyl compounds formed					
	Amount ($\times 10^{-5}$	Alkanal		Alk-2-enal		Alk-2,4-dienal	
No.	mol)	λ_{max}	o.d.	λ_{max}	o.d.	λ_{max}	o.d.
1a	2.79			367	0.548[a]		
2a	1.40	359	(0.836)	372	(0.816)	383	(0.536)
2b	2.84			372	0.472	382	0.740
2c	2.84						
2d	2.53	358	0.448				
2e	2.53					380	0.442
2f	2.83			373	0.540	384	1.842[b]
2g	4.60			372	0.624	385	0.500
2h	2.56	358	(0.646)	372	(0.780)	385	(0.934)
3a	1.84	356	(1.500)[c]				
3b	3.33	355	(0.942)				
3c	2.89	355	(0.980)				
3d	4.25	356	(1.400)				
3e	0.98	355	(0.790)				
3f	4.13	358	(1.122)	371	(0.428)	380	(0.508)
3g	1.75	363	(0.608)	373	(0.530)	382	(0.324)
3h	1.95			375	(0.546)		
3i	2.58	359	(0.490)	377	(0.542)		

Absorbance was measured in 10 ml of chloroform. Figures in parentheses are those without subtraction of the blank values.

[a] It was mainly 2-heptenal. [b] It was further identified as 2,4-decadienal (yield: 0.011 mol/peroxide mol). [c] It was further identified as hexanal (yield: 0.019 mol/peroxide mol).

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