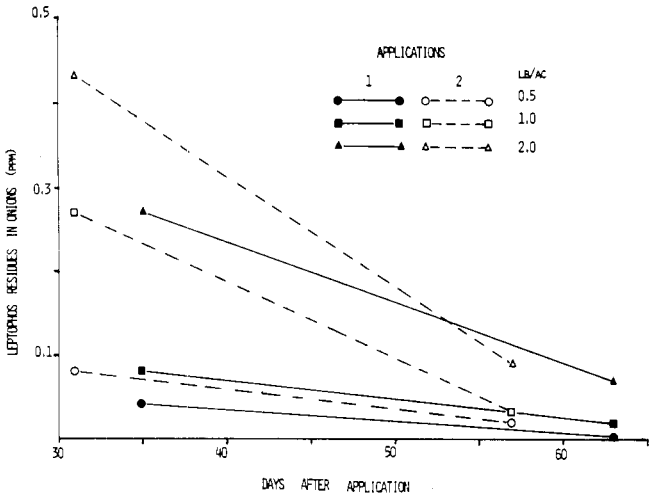

Figure 2. Leptophos residues in onions at intervals after application of two formulations at three dosage rates.

cation and number of applications; however, residues were below 0.03 ppm after 22 days from both 1- and 2-lb/acre applications applied once or twice.

Residues of leptophos in pea vines were high and significant amounts of the leptophos oxon and the phenol were present. Residues of leptophos on the pea vines did not correlate with dosage applied. However, much higher residues were found following two applications and the shorter interval than from the single application.

(*vi*) *Rutabagas.* Residues of leptophos and its metabolites were found in the tops of rutabagas at 29, 37, 45, and 53 days after application. Residues reflected the rate of application and number of applications and declined rapidly (in most instances) between the two harvest dates. Residues in the roots were not as high as those found in the tops. Residues in roots declined rapidly between the first and second harvestings and residues were below 0.02 ppm at 45 and 53 days after application.

(III) Four Applications. (*i*) *Cabbage, Cauliflower, and Broccoli.* When leptophos was applied to these crops once when the plants were very small, little residue was detectable at harvest time (Table II). Where leptophos was applied to these crops in a weekly program, initial residues following the fourth application were high. Residues declined as the interval from last application to harvest increased. In both cabbage and broccoli, levels near 1 ppm were still present 13 days after application (Table IV).

DISCUSSION

Results from the present study indicate that residues of leptophos and/or its metabolites are not present in significant amounts at harvest when this insecticide is used for cutworm control early in the season. Residues do persist, however, for a considerable time and use of leptophos near harvest will result in high residues. Intervals in excess of 13 days must be observed if residues or such cole crops as cabbage, broccoli, and cauliflower are to be less than 1 ppm.

Long persistence of trace amounts in the stover of field corn agrees with findings of Struble and McDonald (1973) with wheat straw. Whether or not such levels (0.3 ppm of corn stover; 0.07 ppm of wheat straw) pose a problem remains to be established.

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LITERATURE CITED

Braun, H. E., *J. Ass. Offic. Anal. Chem.* **57**, 182 (1974).
Struble, D. L., McDonald, S., *J. Econ. Entomol.* **66**, 1321 (1973).

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Bromide Residues in Apples Fumigated with Ethylene Dibromide

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When three varieties of apples were fumigated with 8–24 mg/l. of ethylene dibromide for 4 hr at 13° and held at this temperature the ethylene dibromide in the edible portion (skin and pulp) desorbed to a level below 0.1 ppm in less than 13 days. In the seeds 30 ppm of ethylene dibromide was found immediately after treatment and this did not desorb during 13 days of storage at 13°. In apples stored at 4° after treatment desorption of ethylene dibromide was slower than in apples held at 13°, taking nearly 4 weeks to reach the 0.1-ppm level. The inorganic bromide residue found after the desorption of organic bromide was below 5 ppm even in apples fumigated at twice the required dosage.

Ethylene dibromide has been found effective for controlling certain insects and mites on harvested apples without causing injury to the fruit (Richardson, 1955; Sanford, 1962; Bond *et al.*, 1973). Shipments of apples requiring treatment usually have to be fumigated at cool autumn temperatures (down to 13°) with the apples subsequently being stored at about 0°. At these lower temperatures the sorption of the ethylene dibromide increases and consequently excessive residues may accumulate in the fruit. Normally unchanged ethylene dibromide and other volatile reaction products are thought to desorb to tolerable levels at higher temperatures. However, it has been found that at 13° the fumigant is retained in fruit for several days after the treatment (Dumas, 1973). Furthermore, analyses of residues using a glc method have shown that a former method of determination based on ashing and oxidation to bromate, followed by iodometric titration (Kolthoff and Yutzy, 1937), which was thought to indicate total bromide residue did not represent all of the ethylene dibromide left in the fruit. Experiments were therefore

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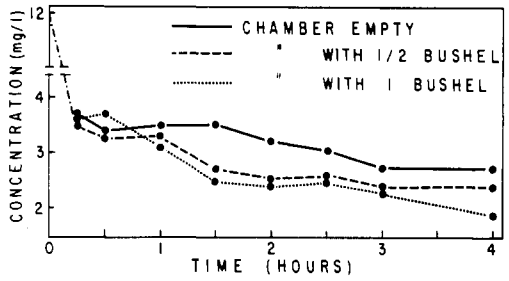

Figure 1. Concentration of ethylene dibromide in the free space of an empty and partly loaded fumigation chamber at 13° when 12 mg/l. of fumigant was applied (note: highest level of fumigant found by analysis of the concentration in the space (*i.e.*, at 0.25 hr) was less than 4 mg/l).

Table I. Residue of Ethylene Dibromide (ppm) in Delicious Apples after 4 hr Exposure When Maintained at the Temperature of Treatment

Days after fumigation	13°		20°		25°	
	12 mg/l.[a]	24 mg/l.	10 mg/l.	20 mg/l.	8 mg/l.	16 mg/l.
1	31 (29)	67	13.5	24	4.3	7.7
2	15 (14)	28	1.7	2.4	0.5	1.4
3	6.5 (10)	20	0.3	2	0.2	2.2
4	6	3.9	0.1	1.3	0.08	0.4
5	2.2	1.8	0.05	0.3		
6	2.9 (3)	4.2				
7	0.6 (2)	0.6				
8	0.4	0.8				
13	(0.1)					

[a] Figures in parentheses refer to results from apples harvested in 1973; all other results are from the 1972 crop.

undertaken to establish residue levels in fruit treated and held at different temperatures, to determine the distribution of residue in the fruit and to measure the rate of disappearance of desorbing ethylene dibromide.

MATERIALS AND METHODS

Red Delicious apples were fumigated in wire baskets containing up to 40 apples with ethylene dibromide in 525-l. chambers for 4 hr at three temperatures (25, 20, and 13°) and at dosages ranging from 8 to 24 mg/l. The method for applying the fumigant was described by Dumas (1973). Loss of fumigant by sorption on the walls of the chambers and by the fruit was determined by measuring depletion of the gas from the free space during the treatment. For this analysis a dosage of 12 mg/l. (the dosage found to give control of mite eggs on apples) was applied at 13° and fumigant concentration was determined at several intervals using glc with a column containing 30% didecyl phthalate liquid phase on Chromosorb W, 60–80 mesh, and a thermal conductivity detector. After treatment, the apples were aerated at the treatment temperature for various periods of time and then analyzed for both inorganic and organic bromide. In one set of tests, apples were aerated for 3 days and then stored at 4° for 1, 2, and 3 weeks to determine desorption under storage conditions. Two other varieties, McIntosh and Spy, were treated with 12 mg/l. of ethylene dibromide at 13° for comparative purposes. Distribution of residues in the apples was studied by making an analysis of edible parts—skin, outer half and inner half of the pulp and the seeds.

Residues of inorganic and organic bromide were investigated separately. Inorganic bromide residue was determined by oxidation (to bromate) and iodometric titration as modified by Dumas (1973) using 50 g of apple. Unchanged ethylene dibromide was extracted by steam distillation (Kennett and Huelin, 1957) followed by glc analysis. A Bendix 2200 gas chromatograph with electron capture (tritium) detector and two 3-mm i.d. stainless steel columns with different packings (to identify the products and verify the results) were used. One of the columns was 4 m long with 5% didecyl phthalate liquid phase on Chromosorb W 60–80 mesh; the other was 2.5 m long and contained 15% Ucon oil liquid phase on Chromosorb W, NAW 60–80 mesh. The first column was run at 85° with 40 cm^3/min of nitrogen carrier gas and the second at 120° with nitrogen at the same flow rate. The sensitivity of this method for full scale deflection on the 1-mV recorder was 2 ng of ethylene dibromide per sample injected into the glc. Recovery of ethylene dibromide from control samples of apples spiked with known amounts of fumigant in the range 0.75–19 mg was 99.9–101%. Size of sample from the treated apples ranged from 10 g when a high level of residue was expected to 200 g when the quantity was low. Simultaneously the amount of benzene solvent used for extraction of ethylene dibromide for the distillate was reduced from 10 ml down to 2 ml. Under these conditions amounts as low as 0.01 ppm could be measured.

Table II. Inorganic Bromide Residue in Delicious Apples after Fumigation with Ethylene Dibromide for 4 hr

Fumigation temp, °C	Concn, mg/l.	Time after treatment, days	Bromide residue, ppm
13	24	21	4.8
13	12	21	2.2
20	20	14	4.1
20	10	14	2.6
25	16	7	3.5
25	8	7	2.0
Control			0.5

RESULTS

Measurement of concentration during treatment showed that when a dosage of 12 mg/l. (at 13°) was vaporized in a chamber of 525 l. the fumigant established in the free space after 15 min was about one-third of the amount applied (Figure 1). However, the concentration subsequently declined slowly during the 4-hr treatment; in the empty chamber it dropped from 3.7 to 2.7 mg/l. during the exposure and when 1 bu of apples was present it dropped from 3.9 to 1.9 mg/l.

Determination of residues of unchanged ethylene dibromide in fruit treated at different temperatures showed that sorption increased as temperature declined and desorption was slower at the lower temperatures (Table I). It is noteworthy that desorption data obtained from apples of the 1973 crop were comparable to those of the previous year.

The variability in residue levels of ethylene dibromide among individual apples was considerable. A maximum of ±10% variability was found when the residue was high on the first day after treatment and levels in individual apples became even more variable as the quantity of residue dropped toward the limits of detectability. Determinations for inorganic bromide in apples were made when the amount of unchanged ethylene dibromide was below 0.01 ppm. Previous results had shown that inorganic bromide levels did not change appreciably during the aeration period (Dumas, 1973) and hence analyses were made after ethylene dibromide had desorbed. The maximum amount

Table III. Residues of Ethylene Dibromide (ppm) in Newly Harvested Delicious Apples Fumigated for 4 hr, Held 3 Days at the Treatment Temperature, and Stored at 4° [a]

Time in storage, weeks	Treatment, mg/l., at 13° 12,a	12,b	12	24	20° 10	20	25° 8	16
0	(12.8)	(7.1)	6.5	20	0.3	2.0	0.2	2.2
1	(1.0)	(1.1)	3.7		0.10	0.6	0.01	0.2
2	(0.3)	(0.1)	0.2	0.4	0.03	0.1		
3	(0.7)	(0.03)	0.01	0.03				
4	(0.02)	(0.007)						

[a] The results in parentheses are for apples held 10 months in storage at 4° and then fumigated with 12 mg/l. of ethylene dibromide at 13°, held (a) 1 and (b) 2 days at that temperature, and analyzed for residue after storage at 4° for periods up to 4 weeks.

of inorganic bromide was found in apples treated with the highest dosage of 24 mg/l. at 13° (Table II) and the quantity of inorganic bromide increased about twofold when the dosage was doubled.

The rate of desorption of ethylene dibromide from newly harvested apples held in cold storage at 4° is shown in Table III. These apples were aerated for 3 days at the treatment temperature before going into storage. The results, which are the average of determinations from two individual apples where the variability ranged up to ±6%, show that desorption continued at 4° but at a considerably reduced rate. The residues of ethylene dibromide found at 25, 20, and 13° fell to comparable low levels at or below 0.1 ppm in 1, 2, and 3 weeks, respectively.

Since preliminary results had suggested some difference in retention of residue between newly harvested and stored apples a bushel of apples was held in cold storage for 10 months and then fumigated and analyzed for residues. The apples were treated with 12 mg/l. of ethylene dibromide at 13°, held for 1 and 2 days at that temperature, transferred to cold storage at 4°, and then analyzed for residues at weekly intervals (Table III). However, these results were similar to those obtained for newly harvested apples; residues fell below 0.1 ppm in 3-4 weeks just as they did in the new apples.

To determine the degree of penetration and distribution of fumigant in the treated apples, samples of skin, outer pulp, and inner pulp were analyzed (Table IV). Analyses were made immediately after treatment and after intervals of 2, 3, and 4 days at 25, 20, and 13°. These data show that the lowest levels of residue occurred in the inner pulp with appreciably more in the outer pulp, and with more than five times as much in the skin of the apples. Desorption of fumigant from these parts of the fruit progressed during the aeration with the greatest reduction, in proportion to content, occurring in the skin. Analysis of ethylene dibromide residue in seeds showed that while the initial residue was similar to the level in the edible portion initially it did not decline on standing and was still at the same level 13 days after treatment. In the meantime residue in the edible portion had dropped below 1 ppm.

To obtain comparative data on uptake and desorption by different varieties of apples, residue levels were measured in Delicious, McIntosh, and Spy apples for 8 days after treatment. The apples were treated simultaneously with 12 mg/l. of ethylene dibromide at 13° for 4 hr and maintained at this temperature until analyzed. The results (Figure 2) show that desorption rate was rapid for all three varieties in the first 2 days after treatment and gradually declined thereafter. Initial residues were about twice as high in McIntosh as in the other two varieties; however, desorption at low levels was slowest in Delicious. The degree of variability (standard deviation) for data on Delicious is shown on the graph for which at least four samples were analyzed for each point. With the other two varieties duplicate samples were averaged; their variability was of a similar order to that found in the Delicious.

Table IV. Distribution of Residual Ethylene Dibromide in Delicious Apples after 4 hr Fumigation and 2, 3, and 4 Days at the Treatment Temperature

Temp, °C	Concn, mg/l.	Fruit part	Ethylene dibromide: Immediately after treatment, ppm	After aeration: Days	After aeration: ppm
13	12	Skin	308		26
		Outer pulp	59	4	5
		Inner pulp	46		6
		Seeds[a]	25		30
20	10	Skin	250		3.6
		Outer pulp	48	3	1.1
		Inner pulp	29		1.3
25	8	Skin	150		2
		Outer pulp	28	2	0.6
		Inner pulp	4		0.8

[a] Level of ethylene dibromide in seeds did not drop below 30 ppm in 13 days after fumigation.

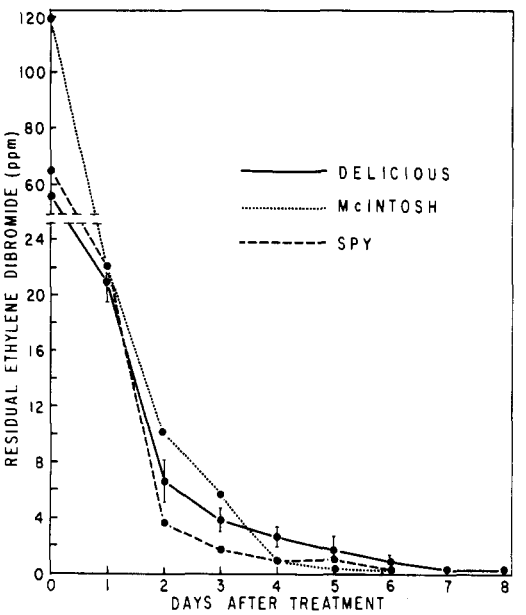

Figure 2. Residues of ethylene dibromide in three varieties of apples fumigated with 12 mg/l. for 4 hr at 13° and retained at this temperature.

CONCLUSION

When ethylene dibromide was vaporized in the fumigation chamber a large proportion appeared to be sorbed rapidly so that only one-third of the dose applied became established as vapor in the free space. Apparently a large proportion of the material applied was rapidly sorbed to surfaces in the chamber and this occurred to a similar degree when apples were present and when the chamber was empty. Fumigant was then absorbed steadily by the fruit during the treatment to leave high residues of organic bromide in the tissues.

The results of residue analyses from the apples showed that unchanged ethylene dibromide will desorb to low levels (below 0.1) in less than 4 weeks when the apples are held at both the treatment temperature and when placed in cold storage. The time required for desorption to a low level is partly dependent on temperature; when apples were treated and held at the lowest temperature likely to be used in field treatments (13°) at least 8 days were needed for desorption to take place. When similarly treated apples were placed in cold storage after a 3-day aeration at the treatment temperature the desorption time was extended to 3 weeks. Fumigant desorbed from different varieties of apples at somewhat similar rates; however, with Delicious, it was retained for a slightly longer time than in the McIntosh and Spy. Levels of inorganic bromide were of a low order even after treatment with fumigant at twice the required dosage.

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LITERATURE CITED

Bond, E. J., Herne, D. H. C., Dumas, T., *Can. Entomol.* **105**, 903 (1973).
Dumas, T., *J. Agr. Food Chem.* **21**, 433 (1973).
Kennett, B. H., Huelin, F. E., *J. Agr. Food Chem.* **5**, 201 (1957).
Kolthoff, I. M., Yutzy, M., *Ind. Eng. Chem. Anal. Ed.* **9**, 75 (1937).
Richardson, H. H., *J. Econ. Entomol.* **48**, 483 (1955).
Sanford, K. H., *J. Econ. Entomol.* **55**, 659 (1962).

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Metabolic Dechlorination of Toxaphene in Rats

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Rats treated orally with [^{36}Cl]toxaphene and each of seven fractions of [^{36}Cl]toxaphene of equal total chlorine content excrete about 50–60% of the ^{36}Cl in urine and 30–40% in feces within 14 days. In each case about half of the dose is excreted as chloride ion determined as phenylmercuric [^{36}Cl]chloride. Similar studies with ^{14}C-labeled preparations of toxaphene and one or both of two components of high mammalian toxicity, toxicants A and B, establish that the feces contain unmetabolized compound and that the metabolites probably include acidic materials, products formed by partial or complete dechlorination and [^{14}C]carbon dioxide. The tissues retain relatively low levels of ^{14}C several days after administration of [^{14}C]toxaphene or ^{14}C-labeled toxicant B. The structural features important for high toxicity to house flies and mice are present in only a few toxaphene components while those conferring biodegradability appear to be shared by most if not all components.

There is extensive knowledge on the metabolic fate of most chlorinated insecticides (Brooks, 1974) but this is not the case for toxaphene. Toxaphene is a very complex mixture obtained on chlorination of camphene. Analysis of toxaphene by gas chromatography (gc)–chemical ionization (CI)–mass spectroscopy (ms) reveals the presence of more than 177 C_{10} polychloro compounds (Holmstead *et al.*, 1974) only one of which has been identified, 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane (Casida *et al.*, 1974; Palmer *et al.*, 1975). This component, referred to as toxicant B, and a $C_{10}H_{10}Cl_8$ material or materials of even higher biological activity, referred to as toxicant A, have only recently been isolated from the technical mixture (Khalifa *et al.*, 1974). Another advance important in metabolic fate studies is the preparation of [^{36}Cl]- and [^{14}C]toxaphene similar in composition to the technical insecticide (Hercules Inc., 1972).

Toxaphene appears to be less persistent in mammals than many other chlorinated insecticides (Guyer *et al.*, 1971). It is stated, without supporting evidence, that toxaphene is believed to be detoxified in the liver in as much as sulfate and glucuronide conjugates are found in the urine (Conley, 1952). The lack of specific information on toxaphene metabolism is not surprising in light of the complexity of the problem.

The present investigation concerns the metabolic fate of [^{36}Cl]- and [^{14}C]toxaphene administered orally to rats with particular attention to the metabolites formed on *in vivo* dechlorination. Seven fractions of [^{36}Cl]toxaphene and ^{14}C-labeled preparations of toxicants A and B are used to gain a preliminary concept of the variations in distribution, metabolism, and persistence among the various toxaphene components.

MATERIALS AND METHODS

Chemicals and Chromatography. Hercules Incorporated (Wilmington, Del.) provided the following samples of toxaphene: unlabeled standard (sample X-16189-49); [^{36}Cl]toxaphene from chlorination of camphene with $^{36}Cl_2$ (sample X-18306-27-1); [^{14}C]toxaphene from chlorination of [8-^{14}C]camphene (sample X-19098-4-2R). Toxicants A and B were isolated from toxaphene by the procedure of Khalifa *et al.* (1974). ^{14}C-Labeled preparations of purified toxicant A (1.5 mg) and pure toxicant B (0.8 mg) were obtained by chromatography of [^{14}C]toxaphene (100 mg) on a silica gel column followed directly by preparative gc; the chromatographic methods are those referred to as adsorption chromatography system I and preparative gc system I by Khalifa *et al.* (1974). Purified ^{14}C-labeled toxicant A consisted of 70% toxicant A, 14% of one impurity, and 16% of a second impurity. These components give gc R_t values of 10.5, 10.0, and 10.5 min, respectively, on the SE-30 column at 170° (conditions described below). Their thin-layer chromatography (tlc) R_f values on silica gel 60

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