Table VI. Results of the Analysis of Pydrin Fenvalerate Formulation Using a High-Performance LC System with Tandem IR and UV Detectors

detector	range, AUFS	Pydrin dilution used, %	fenvalerate, lb/gal
IR, 5.7 μm	0.1	0.5	2.37
		0.5	2.84
	0.025	0.5	2.33
		0.5	2.47
		0.5	2.77
		0.5	2.35
		mean ± SD:	2.5 ± 0.2
UV, 280 nm	0.64	0.5	2.22
		0.5	2.42
		0.5	2.27
		0.5	2.67
		0.5	2.32
		mean ± SD:	2.4 ± 0.2

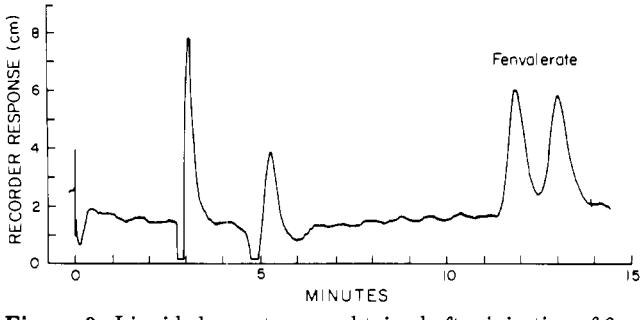

Figure 9. Liquid chromatogram obtained after injection of 6 μg of fenvalerate in 10 μL of CCl_4. The two fenvalerate diastereomers are shown at 11.5- and 12.6-min retention times. A UV detector was operated at 280 nm and 0.08 AUFS. The mobile phase was 80% cyclohexane, 19% CCl_4, and 1% acetonitrile at a flow rate of 1.2 mL/min.

± 0.2 lb/gal for the IR and UV detector analyses, respectively. There was no statistical difference in the two values. The two detectors were comparable in accuracy and reproducibility for the formulation analysis.

Fenvalerate is unstable under gas chromatographic conditions (Mourot et al., 1979). Formulation analysis was reported by Mourot et al. (1979) and involved direct analysis with high-performance LC–UV using both reversed and normal-phase conditions. It was reported that with reversed phase, aside from interference from the alkylbenzene in the formulation, the diastereomers were not separated; with normal phase retention times could not be kept constant but slowly decreased. No quantitative data were present for use in comparison with the system reported herein.

If a UV detector is used, a mobile phase of 80% cyclohexane, 19% CCl_4, and 1% acetonitrile can be used to separate the fenvalerate diastereomers; all other instrument conditions are the same as reported for the other studies reported herein. This system with retention times of 11.5 and 12.6 min for the two diastereomers is comparable to that of Mourot et al. (1979) in terms of analysis time and resolution but yields constant retention times. Figure 9 shows a sample chromatogram.

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An Electron Capture Gas Chromatographic Method for Determination of Residues of 1,2-Dibromoethane in Fumigated Grapefruit

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An analytical procedure was developed for determining residues of 1,2-dibromoethane or ethylene dibromide (EDB) in grapefrruit. The method involves steam distillation from a benzene–water mixture for separation and cleanup. When a gas chromatograph equipped with a nickel-63 electron capture detector was used to determine the concentration of EDB present in fortified samples of grapefruit, residues as low as 0.00038 mg/kg could be detected. The method was used to study the effect of storage time and temperature on the residue of EDB in fumigated grapefruit. Residues in fiberboard carton material were also determined.

The recent Rebuttable Presumption Against Registration (RPAR) for 1,2-dibromoethane [commonly referred to as ethylene dibromide (EDB)] by the Environmental Protection Agency (1977) greatly enhances the need for appropriate residue data. Likewise, the recommendation of the FAO/WHO Joint Meeting (FAO/WHO, 1967) that no residues of EDB be allowed to reach the consumer indicates the need for highly sensitive analytical methodology.

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At the present time fresh grapefruit is the most important citrus export from Florida. The government of Japan, the largest importer, requires that fruit imported from Florida must be fumigated with EDB as a quarantine treatment to eliminate possible infestation by larvae of the Caribbean fruit fly, *Anastrepha suspensa* (Loew).

The method of fumigation used is that prescribed by the Animal and Plant Health Inspection Services of the U.S. Department of Agriculture (APHIS, 1973). The fruit is then shipped to Japan under refrigeration at ~13 °C. The trip requires 3–4 weeks.

Analytical methods for the determination of fumigant residues were thoroughly reviewed by Malone (1971) and Berck (1975). Briefly, Kennett and Huelin (1957) devised a method to separate EDB from fruits by steam distillation and extraction into a benzene layer; iodimetric titration was then used to determine the bromide content. Bielorai and Alumot (1965) used extraction by steam distillation and then analyzed the benzene layer by gas–liquid chromatography (GLC) by using thermal conductivity (TC) and flame ionization detectors (FID) to achieve sensitivities of 40 and 4 mg/kg, respectively. More recently, Hargreaves et al. (1974, 1978) developed an X-ray fluorescence method of determining bromide in EDB steam distilled from various commodities into toluene whereby they achieved a sensitivity of 0.2 mg/kg. However, under long-term storage, as during refrigerated shipment, EDB residues fall below levels that are detectable by all existing methods. Therefore, a more sensitive procedure was required for grapefruit. We report here a method that involves steam distillation via a benzene–water azeotrope for separation and cleanup of the EDP and uses electron capture gas–liquid chromatography for further separation and quantification. Only commonly available equipment is needed. The procedure has been used to measure residues of EDB in tomatoes, mangos, canistels, sapodillas, avocados, polystyrene, and cardboard, as well as in grapefruit (King et al., 1979; Spalding et al., 1978). The sensitivity that can be obtained depends on the substrate.

EXPERIMENTAL SECTION

Reagents. EDB (mp 9–10 °C, No. 1827), sodium chloride (No. 7581), anhydrous sodium sulfate (No. 8024), and nanograde benzene were products of Mallinckrodt, Inc., and were used as received. The EDB exhibited no significant extraneous peaks in assays by GLC with either flame ionization (FID) or electron capture (ec) detection. Bottles of nanograde benzene having exceptionally small amounts of interfering impurities were reserved for samples expected to have very low residues. Norit A decolorizing carbon (No. 7-E344) and Celite 503 (No. 8-E 406) were obtained from J. T. Baker Chemical Co. and were used as received.

Distilled water at this laboratory contained high levels of interfering impurities, so 200-mL portions of water were extracted with 20 mL of nanograde benzene to provide water of sufficient purity. An alternative procedure in which 5 g of carbon and 5 g of Celite were stirred with 1800 mL of water for 10 min and then removed by filtration gave water suitable for most analyses. The suitability of water thus treated was monitored by extracting 100 mL with 10 mL of benzene and testing the benzene for interfering impurities by GLC with ec detection.

Procedures. The grapefruit was cut into wedge-shaped slices such that they included portions of peel and pulp representative of the whole fruit. The peel was removed from some slices to provide separate samples of peel and pulp to determine the distribution of EDB within the fruit. For a typical assay, a 100-g sample of grapefruit was weighed into a 500-mL Eberbach blending container. Twenty milliliters of benzene and 100 mL of water were added. The container was then sealed with a Teflon-lined lid and blended on a two-speed Waring Blendor (701,05) for 30 s at low speed. The blending was repeated if necessary to eliminate large pieces. Then the contents were poured through a 10-cm powder funnel into a 1-L round-bottom flask (Kimble, 25285). Also, an additional 100 mL of water was used to wash the container, lid, and funnel, and this was added to the flask.

Table I. Analyses of EDB Residues in Fortified Samples of Grapefruit

EDB added, mg/kg	EDB recovd ± SD,[a] mg/kg	% recovery ± SD
20	20.33 ± 0.07	101.7 ± 0.4
10	10.27 ± 0.22	102.7 ± 2.2
5	5.12 ± 0.03	102.4 ± 0.6
0.100	0.1015 ± 0.0006	101.5 ± 0.6
0.010	0.00993 ± 0.00035	99.3 ± 3.5
0	0.00019 ± 0.00001	

[a] Four replicates at each level were assayed to obtain the means and standard deviations (SD) listed. The background of 0.00019 mg/kg was subtracted when significant.

The flask was placed in a Glas-Col (TM 108) heating mantle and a 75 °C connecting tube (Kimble, 44920), a 300-mm Liebig condenser (Kimble, 18130), a connecting tube (Kimble, 45005), and a 100-mL round-bottom receiving flask (Kimble, 25285) were connected in the order listed. The 100-mL receiving flask was cooled in an ice bath during distillation. A Statco, Inc. (No. 3pN 1010), variable transformer was used to regulate the heating mantle to achieve a rapid rate of distillation with no carry-over of material. The distillation was continued until ~70 mL (20 mL of benzene, 50 mL of water) of liquid was collected. The benzene–water mixture was transferred to a 125-mL separatory funnel (Kimble, 29048-F), and 5 g of sodium chloride was added. The mixture was shaken for 30 s and allowed to separate. The water was removed, and a portion of the benzene layer was percolated through a plug of sodium sulfate (35 mm diameter × 10 mm high) into a 12-mL (16 × 100) culture tube (Kimble, 445066-A) and sealed with a cap having a Teflon-faced liner.

The concentration of EDB in the benzene was determined by using a Hewlett-Packard 5730A gas chromatograph equipped with a linear nickel-63 electron capture detector. The samples were diluted as necessary so they would be in the linear range of the detector as determined by injecting a series of standard solutions. A 180 cm long glass column, 6 mm o.d., 4 mm i.D., packed with 10% Carbowax 20M on 80–100 mesh Gas-Chrom Q was used with the following conditions: carrier gas, 5% methane in argon at 60 mL/min; injection port, 150 °C; oven temperature, 140 °C; detector temperature 300 °C. Injections of 5 μL were made with a 10-μL Hamilton syringe equipped with a chaney adapter.

RESULTS

Unfumigated grapefruit were fortified with known amounts of EDB by injecting 1 mL of a benzene solution of EDB at the appropriate concentration into the fruit; then the samples were assayed as described except that only 19 mL of benzene was added. The calculated recovery slightly exceeded 100% because the calculations were based on 20 mL of benzene solvent, and presumably more benzene then EDB is lost because of evaporation or solubility in water; the results obtained with unknown samples were corrected accordingly. Also, similar control samples (four replicates) gave a background level of 0.19 ± 0.01

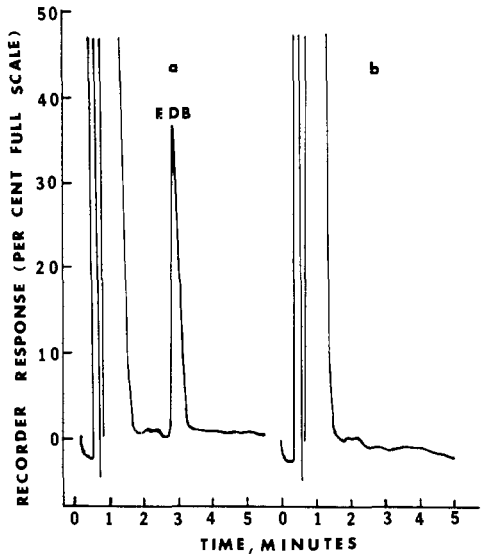

Figure 1. Comparison of typical gas chromatograms obtained from (a) unfumigated grapefruit fortified with 0.010 mg/kg EDB and (b) unfumigated grapefruit control. Five-microliter injections of the benzene phase from the steam distillation cleanup were employed in conjunction with a ^{63}Ni linear electron capture detector at an attenuation of 8×.

Table II. EDB Residues in Grapefruit after Fumigation and Storage at Ambient Temperatures[a]

time, h	mg of residues/kg of sample[b]				
	1	2	3	4	mean ± SD
0	9.1	11.8	18.2	13.9	13.3 ± 3.8
1	15.6	15.6	11.9	16.7	15.0 ± 2.1
2	14.2	11.1	13.4	13.5	13.1 ± 1.3
24	6.1	5.8	5.5	5.1	5.6 ± 0.4
48	2.1	3.1	2.0	2.1	2.3 ± 0.5
72	0.83	1.64	0.79	0.70	0.99 ± 0.4
168	0.023	0.041	0.046	0.039	0.037 ± 0.010

[a] Fumigated with EDB at a dose of 8 g/m³ for 2 h and, except for zero time, aerated for 1 h by using an exhaust fan. [b] Each sample was taken from a separate whole fruit.

μg/kg; therefore twice background sensitivity was 0.38 μg/kg. The results (Table I) showed excellent recovery and precision. Typical chromatograms are shown in Figure 1. Besides Carbowax 20M, other liquid phases tested were OV-101, OV-1, OV-17, OV-225, and Silar-10C. None worked as well as Carbowax 20M in separating the EDB from trace impurities, but an OV101 column was useful in confirming the identity of EDB at high (>10 μg/kg) levels.

Grapefruit in fiberboard cartons were fumigated in a 1.4-m³ chamber for 2 h with EDB at the recommended dosage of 8 g/m³. The chamber was opened immediately after fumigation to take samples at zero time and then closed and aerated with a blower for 1 h to simulate a commercial fumigation. The cartons were then removed and stored at ambient (~25 °C) temperature for the remainder of the test period. Samples of grapefruit and fiberboard were subsequently assayed for EDB at various times after the fumigation.

The results for each of the four fruits assayed are included in Table II to demonstrate the variation in residue levels. (Zero-time samples were plainly unreliable because the level depends on the speed of sample preparation). Even in the small chamber we used here, there was obviously much variation in the exposure of individual fruit to EDB. Thus, the dosage must be sufficiently high so the least-exposed fruit receives enough to ensure the required mortality of fruit fly larvae. The average levels (zero-time residue data omitted) were used to fit the equation $\ln c = mt + b$, where t is the time in hours, m is the slope, $\ln c$ is the natural logarithm of the concentration in ppm, and b is the intercept. This gave values of −0.0356 and 2.624 for m and b, respectively, and a correlation coefficient of −0.9994, which indicated a good fit of the data to the equation. Therefore, the rate of loss of EDB was proportional to the amount present, i.e., $dc/dt = kc$ where $k = 1/m$.

Table III. EDB Residues in Fiberboard after Fumigation and Storage at Ambient Temperatures[a]

time, h	mg of residues/kg of sample[b]			
	1	2	3	mean ± SD
0	453	397	363	404 ± 45
1	390	391	378	386 ± 7
2	322	316	303	314 ± 10
24	91	101	96	96 ± 5
48	90	70	65	75 ± 13
72	46	21	39	35 ± 13
168	17	18	18	18 ± 1

[a] Fumigated with EDB at a dose of 8 g/m³ for 2 h and, except for zero time, aerated for 1 h with an exhaust fan and stored at ~25 °C. [b] Each sample was taken from a separate piece of fiberboard.

Table IV. EDB Residues in Grapefruit Peel and Pulp after Fumigation and Storage at Ambient Temperatures[a]

time, h	residues, mg/kg, ± SD[b]	
	peel	pulp
2	38.3 ± 9.5	1.87 ± 0.64
24	12.3 ± 4.0	2.17 ± 0.22
48	5.7 ± 2.0	1.22 ± 0.22
72	2.2 ± 0.8	0.71 ± 0.13
168	0.072 ± 0.042	0.027 ± 0.005

[a] Fumigated with EDB at a dose of 8 g/m³ for 2 h, aerated for 1 h with an exhaust fan, and stored at ~25 °C. [b] Mean of four replicates ± the standard deviation.

The results of the assays of pieces of fiberboard, 10 × 10 cm, weighing ~8 g each, removed from each of three carton covers at various intervals after storage at ambient temperature and assayed for EDB are shown in Table III. The absorption of EDB and the residue levels during this 7-day test period were much higher for fiberboard than for grapefruit. [Since EDB desorbed from such boxes would contribute to worker exposure when the trucks are unloaded at a warehouse, a more detailed study of the absorptivity of fiberboard was conducted and is reported elsewhere (King et al., 1979). In this case, the rate of loss of EDB from fiberboard was found to be approximately proportional to the square of the concentration, and a plot of $\ln t$ vs. $\ln c$ is linear.]

The distribution of EDB in the fruit was determined by separate assays of samples (50-g total) of peel and pulp (edible portion). The results are shown in table IV. Residue in the peel decreased exponentially (a correlation coefficient of −0.996). However, the residue in the pulp increased during the interval between 2 and 24 h, apparently because of the movement of EDB from the peel into the pulp. Such penetration would be necessary to ensure a high mortality rate of fruit fly larvae.

The effect of the temperature of storage on residue levels at various intervals after fumigated fruit were stored at ambient temperature (25 °C) for 24 h and then refrigerated at 13 °C or not refrigerated was also investigated. (In a typical commercial fumigation, the fruit usually remains at ambient temperature for ~24 h before it is loaded onto

Table V. EDB Residues in Grapefruit after Fumigation and Storage with or without Refrigeration[a]

	residues, mg/kg,[b] after at storage at							
	13 °C				25 °C			
time, h	1	2	3	mean ± SD	1	2	3	mean ± SD
2					9.9	16.8	9.6	12.1 ± 4.1
24					5.5	5.0	5.6	5.4 ± 0.3
48	2.7	4.2	4.2	3.7 ± 0.9	2.5	2.3	2.1	2.3 ± 0.2
72	2.8	2.5	1.9	2.4 ± 0.5	1.0	1.4	0.8	1.1 ± 0.3
168	0.88	0.50	0.68	0.69 ± 0.19	0.046	0.045	0.039	0.043 ± 0.004

[a] Fumigated with EDB at a dose of 8 g/m³ for 2 h, aerated for 1 h with an exhaust fan, and stored for 23 h at 25 °C without mechanical aeration. [b] Each sample was taken from a separate whole fruit.

Table VI. EDB Residues in Grapefruit after Fumigation and Simulated Shipment to Japan[a]

	EDB residues, mg/kg,[b] after storage at							
	21 °C				13 °C[c]			
time, days	sample 1	sample 2	sample 3	mean ± SD	sample 1	sample 2	sample 3	mean ± SD
0[d]	22.6	13.8	13.8	16.7 ± 5.1				
1	6.66	6.17	5.92	6.25 ± 0.38				
3	2.32	1.25	1.36	1.64 ± 0.59	3.38	2.11	2.64	2.71 ± 0.64
6	0.084	0.238	0.050	0.124 ± 0.100	0.931	0.496	0.346	0.591 ± 0.304
8	0.029	0.027	0.048	0.035 ± 0.012				
10					0.128	0.283	0.093	0.168 ± 0.101
14	0.034	0.007	0.017	0.019 ± 0.014	0.020	0.001	0.002	0.008 ± 0.011
16	0.012	0.023	0.014	0.016 ± 0.006				
21	0.003	0.003	0.003	0.003 ± 0.000				
22					0.001	0.024	0.001	0.009 ± 0.013
28	0.003	0.001[e]	0.003	0.002	0.014	0.009	0.019	0.014 ± 0.005
35	0.001[e]	0.001[e]	0.001[e]	0.001[e]	0.016	0.007	0.003	0.009 ± 0.007

[a] Fumigated with EDB at a dose of 8 g/m³ for 2 h. [b] Each sample was taken from a separate whole fruit. [c] Aerated at ambient temperature for the first 24 h. [d] Two-hours postfumigation time. [e] Background of 0.00019 mg/kg prevented determination because these samples were in the 0.0003–0.0005 range.

Table VII. EDB Residues in Grapefruit after Commercial Fumigation in Florida, Shipment to Japan by Ship, and Return to Florida by Air at Ambient Temperature

replicate[a]	EDB residue, mg/kg		
	peel	pulp	composite
1	0.001	0.009	0.006
2	0.001	0.016	0.011
3	0.001	0.021	0.014
mean ± SD:	0.001	0.015 ± 0.006	0.010 ± 0.004

[a] Each replicate was taken from a separate carton.

a ship and cooled for shipment.) The results (Table V) showed that the loss of EDB from the fruit was much slower at the lower temperature and that residues remained higher in fruit that is refrigerated.

Also, seven fiberboard cartons containing size 48 grapefruit were fumigated in a 1.4-m chamber with EDB (dose of 8 g/m) for 2 h, aerated for 1 h with vent fans, and held for 24 h at ambient temperature (~25 °C). Four of the cartons were stored at 13 °C to simulate a commercial shipment to Japan and three were stored at ambient temperature. Fruit were removed and assayed at various intervals for 35 days. (A typical shipment of fruit to Japan requires ~3 weeks.) The data, Table VI, show the variability of residues among the fruit. Residues were higher in fruit held at lower temperatures, but after from 3 to 6 days of storage, residues were less than 1 ppm in fruit held at 13 or 21 °C. They were, however, detectable in fruit stored up to 25 days.

Finally, three boxes of grapefruit from a commercial shipment of Japan were assayed for residues of EDB. The fruit had been fumigated in Florida, shipped by boat to Japan, and then returned via airfreight to Miami, FL. Results of these assays (Table VII) agreed well with the data obtained from the simulated shipment.

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