phate, and *p*-nitrophenol in the total residue increased from the initial sample at both rates of application to the harvest sample although the opposite occurred in the parts per million values obtained in the samplings. Aminoparathion, *S*-ethylparathion, and *S*-phenylparathion either did not increase in percentage of the harvest residues or were not detected as being present.

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The technical assistance of James D. Stokes and Clayton Reece in aspects of this work is gratefully acknowledged.

Supplementary Material Available. Tables V-VIII will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JAFC-74-974.

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Fumigant Residues of Carbon Tetrachloride, Ethylene Dichloride, and Ethylene Dibromide in Wheat, Flour, Bran, Middlings, and Bread

Ben Berck

Dowfume EB-5, consisting of carbon tetrachloride, ethylene dichloride, and ethylene dibromide (CT, EDC, and EDB) in 63:30:7 w/w proportions, was applied to 1000 bu (27.3 metric tons) of wheat stored in a paper laminate bin. The CT-EDC-EDB distribution-persistence patterns were monitored at 16 bin locations over a 14-day period by gc and hydrogen flame ionization detection. CT gas concentrations were greatest at the bottom, the descending order of magnitude being bottom, middle, top, headspace. EDB gas concentrations, and those of EDC to a lesser extent, were greatest in the headspace-top interface, the descending order of magnitude being the reverse of CT. Fumigant residues in wheat, in the flour, bran, and middlings derived from wheat, and in bread baked from the flour, were determined over a 7-week period of fumigant exposure by gc with EC detection. Amounts of unchanged CT and EDB as small as 0.01 ng could be detected. EDC residues could not be satisfactorily removed or determined. CT and EDB residues of the wheat varied, depending on the bin location and contact time, and ranged from 3.2 to 72.6 ppm of CT, and from 0.0 to 3.3 ppm of EDB. CT and EDB residues of bran and middlings were greater than those of flour, and ranged from 0.2 to 2.23 ppm of CT and 0 to 0.4 ppm of EDB. No EDB residues were found in any portion of the bread tested. CT residues in bread ranged from 0 to 0.04 ppm.

Fumigants are gaseous pesticides used to control infestations of insects, mites, rodents, and, to a lesser extent, bacteria, yeasts, and molds in stored foodstuffs. All commercial fumigants are physically or chemically sorbed, the amount depending on the nature and amount of fumigant used, gas-air concentrations, nature of the substrate, temperature, moisture content, period of exposure, etc. (Berck, 1966, 1971).

At least 14 factors (nature of the fumigant; applied dosage; method and conditions of application; nature of the substrate; dockage content (foreign matter); moisture content; absolute humidity; vapor pressure; temperature; diffusion and atmospheric pressure; interstitial atmospheric composition; chemical and physical sorption affinities of the fumigants; chromatographic properties of the cereal substrates; and air movement patterns affected by temperature gradients) influence the distribution and persistence (concentration-space-time) relationships of fumigant gases of volatile liquids applied to grain piles (Lindgren and Vincent, 1962; Berck, 1964, 1965c). The behavior of stored wheat as a chromatographic column toward fumigant gas mixtures applied to the surface of a wheat pile, with concomitant effects on migration patterns of the components, has been described (Berck, 1956, 1965a; Berck and Solomon, 1962). A range of analytical techniques was developed to show comparative sorption and chromatographic behavior of fumigants toward diverse food products, soils, and other substrates (Berck, 1960, 1961, 1962, 1965b, 1968a,b; Berck and Solomon, 1962; Berck *et al.*, 1970; Berck and Gunther, 1970). The scientific literature through April, 1970 on analytical methods for the determination of fumigants was reviewed by Malone (1971). A multidetection scheme for the gas chromatography of solvent extracts of fumigant residues in cereal and other foodstuffs was developed by Heuser and Scudamore (1969). Methods and data pertaining to determination of unchanged residues after application of carbon tetrachloride (CT), ethylene dichloride (EDC), and ethylene dibromide (EDB) in admixture were reported by Lynn and Vorhes (1957), Mapes and Shrader (1957), Conroy *et al.* (1957), Lindgren *et al.* (1968), Malone (1969, 1970), and McMahon (1971).

While data on gas concentrations and sorption affinities of different fumigants toward a wide range of products are available, more facts are needed on the nature and amount of unchanged residues that persist after applica-

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tion of a fumigant or fumigant mixture to foodstuffs under commercial conditions of use. These should include all stages of food processing. Storey *et al.* (1972) found that steaming of dehulled and flaked soybeans meats, followed by commercial hexane extraction to extract the oil, resulted in translocation of EDC-CT residues from the meats to the hexane. Thus, the possibility of build-up of residues in the recycled hexane posed a contamination problem to the soybean processor.

This paper deals with the results of a field experiment in which insect-infested grain was fumigated with Dowfume EB-5, a mixture of CT, EDC, and EDB in 63:30:7 w/w proportions (Dow Chemical Co., Sarnia, Canada). The main objective was to ascertain the correlation between CT-EDC-EDB gas concentrations resulting from the application and residues of unchanged CT, EDC, and EDB that might remain in or on the wheat after the fumigation with Dowfume EB-5. Contingent upon finding residues in the fumigated wheat, the residue levels in some of the wheat fractions (flour, bran, and middlings) after milling would be investigated. Similarly, if residues were found in the flour, then bread baked from the flour would be investigated for residual amounts of unchanged fumigant molecules. A second objective was to ascertain the effect on residue levels of prolonged (7-week) exposure to the fumigant gases, in order to determine the effects on unchanged residue levels of time of sampling after treatment, in the absence of aeration as such beyond normal air movement within a grain bin.

METHODS, MATERIALS, AND APPARATUS

The field experiment was located at a farm area near Oak Bluff, Manitoba. Dowfume EB-5 was applied to stored wheat of 14.3% moisture content and 6% dockage at the commercially recommended dosage of 4 imperial gal/1000 bu (18.2 l./27.3 metric tons). A multilayered laminated paper bin (paper-asphalt-fiberglas laminate), 16 ft (4.8 m) diameter × 6 ft (1.8 m) high, of 1000-bu capacity, supplied by the St. Regis Paper Co., Kansas City, Mo., was used as a temporary bin for this purpose. The work was done in July during Manitoba's brief but very enjoyable summer period.

The fumigant mixture was applied with a pressure sprayer (11.4-l. (2.5 imperial gal) capacity) to the surface of the wheat using a long-handled metal extension pipe. The bin surface was marked with string into sectors, and a portable weight scale (bathroom type) was used to register the progressive change in weight of the spray container as a means of dispensing uniformly the amounts of fumigant calculated for each sector.

Although information on residues was the main objective, a limited field test of biological effectiveness was included in the experiment. Gas sampling lines consisting of 0.25 in. (6 mm) o.d. polyethylene tubing were positioned beforehand on 6 ft × 1 in. dowel rods, to permit air sampling from 16 locations, at the top, middle, and bottom areas of the bin, including one location at the headspace-bin interface. The sampling lines were capped with rubber septums when not in use. Grain temperatures were taken by thermocouples in conjunction with a potentiometer. Samples of grain were taken by a grain sampling probe before and after application of the fumigant. Fifteen samples were taken at each of seven times. The wheat samples were divided into appropriate subsamples for microbiological assessment of the surface bacteria, yeasts, and molds, and to assess the effect of the treatment on per cent germination.

The wheat samples were taken from the top, middle, and bottom levels of the bin, each level comprising five locations (center, north, east, south, and west) during each sampling time. Samples from the five locations were subsequently divided in equal weights and recombined into three composite samples per sampling period for milling and baking purposes.

Gas Sampling. Gas samples were drawn into 70-cm^3 gas flasks, fitted with Teflon stopcocks and silicone rubber septums, by means of a small battery-powered air pump (Casella (London), available from Carleton Instruments Ltd., Ottawa) attached to the gas-sampling lines. Gas concentrations were determined by gas chromatography (gc) using a hydrogen flame ionization (HFI) detector with a Model 2100 Varian Aerograph gas chromatograph (Varian Aerograph Ltd., Walnut Creek, Calif.). Gas concentration standards of CT, EDC, and EDB were prepared from reagent grade chemicals using 6.25-l. all-glass flasks fitted with two stopcocks. By serial dilution, concentrations in the range 0.01–100 μg/cm^3 of gas–air mixture were prepared for calibration purposes. A 10.5 ft × $^3/_{16}$ in. o.d. (3.2 m × 5 mm) stainless steel column packed with 6% XE-60 on Chromosorb W, 80–100 mesh, was used at a column temperature of 125°. Injection port and detector temperatures were 200 and 225°, respectively. The flow rates of nitrogen carrier gas, hydrogen, and air were 45, 20, and 200 cm^3/min, respectively. Peak heights were used to determine gas concentrations in the headspace of the bin and in the interstitial grain atmospheres. Each location was sampled in duplicate.

Aliquots from the gas-sampling flasks were taken from Hamilton gas-tight syringes (Hamilton Co., Whittier, Calif.) for injection of 0.5–5.0-cm^3 air samples, depending on the fumigant gas concentrations. It was thus possible to measure amounts as low as 0.002 μg/cm^3 of air (= 0.002 mg/l. of air) of CT, EDC, or EDB.

Five small openings in the top cover of the bin were used to accommodate gas sampling lines, to permit removal of insect cages, and to provide entry for a grain sampling probe. These were sealed with moisture-proof tape when not in use.

Fumigant Residues in Wheat and Wheat Fractions. The samples of fumigated wheat, and of the flour, bran, and middlings milled therefrom, were placed in tied polyethylene plastic bags and stored in a constant temperature refrigerated room at −7° until required for analysis. The samples were stored at subzero temperatures to reduce volatility and to help hold the fumigant residues at the level they were at during sampling. Before the determination of wheat residues, the chilled samples were rolled at −7° between two mechanical rollers to fracture the kernels coarsely. Fumigant residues of the wheat, and of the flour, bran, and middlings milled therefrom were extracted with Pestigrade quality acetone (Matheson Coleman and Bell Co., Norwood, Ohio), using 10-g samples and 20 ml of acetone in vials fitted with screwcap tops lined with aluminum foil disks. The samples were weighed in duplicate and, after gentle manual oscillation, were placed in a cold room held at −18° for 10 days. During the extraction period, the samples were gently shaken for about 1 min every 2 days. No further increase in residues was found after 7 days of extraction.

The residues were measured by gc, using a Model 2100 Varian Aerograph equipped with a tritium foil electron capture (EC) detector. Each sample was assessed for traces of CT, EDC, and EDB residues at the following times after fumigant application: 3 days, and 1, 2, 3, 5, 6, and 7 weeks. The fumigation period was extended beyond the usual 3–7 days to ascertain the effects on residue levels of prolonged fumigation. Samples of the fumigant-air concentrations were taken just before the grain samples were obtained from the test locations. Aliquots (1 to 10 μl size) of the acetone extracts of the residues and of standard solutions of CT, EDC, and EDB were injected into a 10.5 ft × 0.25 in. o.d. (3.2 m × 6 mm) stainless steel column packed with Porapak Q-S, 80–100 mesh at a column temperature of 160°. The injection and detector temperatures were 185 and 210°, respectively; the flow rate of nitrogen

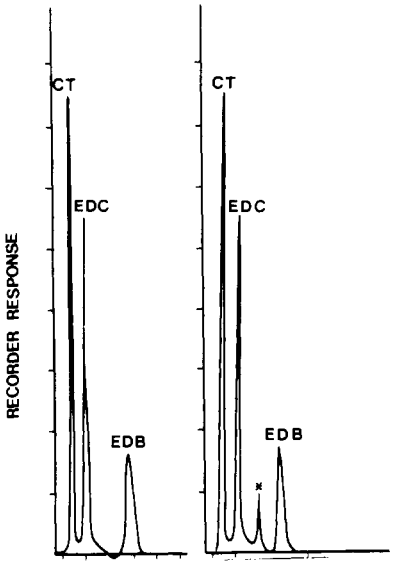

Figure 1. Separation of CT, EDC, and EDB in air by gas chromatography and HFID. Sample taken from grain bin fumigated with Dowfume EB-5. Duplicate determination shown; the asterisk indicates attenuation change.

carrier gas was 45 cm^3/min. Standard curves were made by serial dilution of CT, EDC, and EDB as analytical grade chemicals dissolved in acetone encompassing 19 points in triplicate to accommodate the range 0.002–200 ng. The standard solutions were stored in glass bottles in a refrigerator (4°) until required. A Fisher-Victoreen 4400 series gc apparatus (Fisher Scientific Co., Pittsburgh, Pa.) equipped with a ^{63}Ni EC detector and a vibrating reed electrometer was used to check the operation of the analytical procedure. Peak areas were used for the EC mode, and symmetrical and reproducible peaks were obtained in all cases.

CT residues were determined with particular sensitivity by EC detection. Although 0.001 ng (= 1 pg or 10^{-12} g of CT) was detectable by electron capture, the base-line noise level for that amount was excessive. Accordingly, 0.05 ng was selected as a practical lower working limit for both CT and EDB residues.

Residues in Bread. Samples from the three levels (top, middle, and bottom) and five locations were subsequently composited in equal weights for milling and baking purposes. Each of the 24 loaves of bread baked from the flour of the 21 composite samples of fumigated wheat, and of three control samples from the top, middle, and bottom of the bin taken before fumigation, was divided into three zones, namely: (a) upper crust (outer brown portion), (b) lower crust (lower outer white portion), and (c) crumb (inner portion). The 72 subsamples were cold-extracted with Pestigrade quality acetone for at least 10 days. Each extract was analyzed in duplicate for CT and EDB residues, using a Varian Aerograph Model 2100 gc apparatus filled with a tritium foil (^3H) EC detector. Every seventh sample was cross-checked with the Fisher Victoreen Model 4400 gc apparatus fitted with a ^{63}Ni EC detector and a vibrating reed electrometer sensitive to 10^{-13} a.f.s.

Mixograph tests were made to determine the quality of the bread dough. The mixograph is a recording dough mixer used in cereal research to determine flour quality criteria. The particular model used was designed and constructed by the Engineering Research Services, Canada Agriculture, Research Branch, Ottawa, as an electronic recording mixograph (Voisey *et al.*, 1966).

Loaf volume was determined on each of 24 loaves. Loaf volume is a measure of the total effects of treatment or environmental and genetic history on gluten quality, enzyme activity, fermentation properties, etc. of the flour.

Bioassays. Wheat samples were taken at seven times during the 7-week test period to determine possible effects of the fumigation on per cent germination. Microbiological determinations were also made of the fumigated wheat before and during the 7-week period after fumigation of the 1000-bu (27.3 metric ton) bin. The tests employed duplicate 11-g aliquots from each bin location and included: (a) total bacterial populations as spc (standard plate count), (b) proteolytic bacteria, (c) anaerobic bacteria, and (d) yeast and mold count (Thatcher and Clark, 1968).

RESULTS AND DISCUSSION

Fumigant Gas Profiles. By gc and HFID detection, the three fumigant gases applied in admixture were determined from samples of interstitial grain atmospheres in amounts as low as 0.01 μg. In each of the hundreds of duplicate determinations, peak heights used as an index of gas concentrations agreed within ±10%, and generally were identical. A typical separation by HFID is shown in Figure 1, representing a sample taken 7 days after fumigant application. CT, EDC, and EDB gas concentrations at 16 bin locations and seven different times during the 2-week postfumigation period are shown in Tables I, II, and III, respectively. The vapor phase trends or profiles of CT, EDC, and EDB are shown in Figure 2. The ordinates are the means of the five bin locations indicated in Tables I–III.

Figure 2 shows distinctively different profiles for each of the fumigant components. During the 14-day period, CT gas showed the greatest concentrations at the bottom, with the descending order of magnitude being bottom, middle, top, and headspace; the top and headspace lines

Table I. Concentration of Carbon Tetrachloride (μg of CT/cm^3 of Interstitial Air) of Fumigated Grain after Application of Dowfume EB-5

Location		2 hr	1 day	2 days	3 days	1 week	10 days	2 weeks
Headspace	Center	123.0	54.3	39.5	40.8	23.3	17.7	13.5
Top	Center	147.9	64.5	47.7	48.8	21.1	15.8	13.8
	North	137.9	74.2	48.6	23.0	22.9	15.6	14.9
	East	115.9	64.9	46.6	40.4	18.5	14.2	1.7
	South	128.6	69.4	51.2	37.9	13.1	16.3	11.8
	West	189.9	59.6	51.2	34.0	14.4	12.9	8.2
Middle	Center	136.2	91.5	76.0	73.4	29.3	21.7	9.5
	North	162.7	98.1	74.2	49.0	34.2	22.7	17.8
	East	145.9	91.0	70.8	51.7	28.9	22.6	16.5
	South	134.4	68.9	70.3	58.7	31.3	20.6	13.8
	West	159.2	80.0	69.8	46.8	34.0	20.4	15.8
Bottom	Center	190.0	93.7	59.2	72.5	45.2	16.3	14.6
	North	147.7	111.4	96.4	80.4	45.9	28.4	15.9
	East	169.8	99.9	84.0	78.7	45.5	30.0	16.6
	South	111.4	96.8	91.0	60.5	33.7	23.8	9.0
	West	154.8	113.2	89.7	73.3	41.0	17.2	14.5

Table II. Concentration of Ethylene Dichloride (μg of EDC/cm^3 of Interstitial Air) of Fumigated Grain after Application of Dowfume EB-5

Location		2 hr	1 day	2 days	3 days	1 week	10 days	2 weeks
Headspace	Center	8.92	19.27	14.02	12.93	9.39	7.48	6.14
Top	Center	30.43	16.94	14.05	13.68	8.69	6.60	6.73
	North	46.09	9.21	7.76	1.60	6.23	4.48	4.39
	East	5.92	4.17	4.11	3.90	3.13	2.69	1.38
	South	12.00	7.90	7.81	6.39	4.81	4.59	3.55
	West	12.82	11.16	10.79	9.63	5.53	4.67	3.10
Middle	Center	5.38	5.53	6.46	5.67	5.48	4.71	3.13
	North	25.69	7.53	6.41	4.83	5.43	4.48	4.36
	East	2.69	2.19	2.67	2.71	3.13	3.13	3.20
	South	3.71	2.64	3.85	3.99	4.67	3.90	3.70
	West	6.23	6.13	7.16	5.73	7.81	5.71	5.60
Bottom	Center	6.60	3.24	2.94	4.09	4.34	5.76	2.23
	North	4.45	3.69	3.80	3.94	3.50	2.92	1.83
	East	1.05	1.69	1.94	2.41	2.50	2.29	1.62
	South	1.15	1.75	2.50	2.17	2.36	2.39	1.33
	West	1.83	2.97	3.41	3.71	4.01	2.52	2.30

Table III. Concentration of Ethylene Dibromide (μg of EDB/cm^3 of Interstitial Air) of Fumigated Grain after Application of Dowfume EB-5

Location		2 hr	1 day	2 days	3 days	1 week	10 days	2 weeks
Headspace	Center	1.021	1.053	1.067	1.233	0.987	1.080	0.905
Top	Center	0.330	0.540	0.820	1.187	0.853	0.726	0.935
	North	0.340	0.353	0.407	0.340	0.447	0.373	0.300
	East	0.391	0.440	0.387	0.513	0.333	0.247	0.185
	South	0.292	0.386	0.367	0.353	0.320	0.327	0.240
	West	0.234	0.287	0.387	0.427	0.273	0.327	0.225
Middle	Center	0.173	0.280	0.293	0.407	0.300	0.287	0.275
	North	0.000	0.187	0.227	0.227	0.253	0.200	0.145
	East	0.180	0.260	0.233	0.313	0.207	0.160	0.130
	South	0.187	0.247	0.220	0.280	0.233	0.200	0.140
	West	0.000	0.187	0.233	0.267	0.247	0.213	0.155
Bottom	Center	0.160	0.260	0.220	0.367	0.313	0.707	0.160
	North	0.000	0.167	0.187	0.260	0.233	0.213	0.120
	East	0.000	0.260	0.233	0.313	0.187	0.247	0.135
	South	0.160	0.220	0.193	0.227	0.187	0.267	0.130
	West	0.000	0.213	0.220	0.260	0.213	0.240	0.115

were relatively close and parallel, and showed a crossover at 4 days. EDB gas, on the other hand, showed the greatest concentration in the headspace, with the descending order of magnitude being headspace, top, middle, and bottom, the reverse shown by CT gas. EDC gas showed a trend similar to EDB, except for low EDC concentrations in the headspace area at the 2-hr sampling period.

Tables I–III show that the initial 9:4.2:1 weight ratio of CT–EDC–EDB of Dowfume EB-5 in the liquid state prior to application changed markedly when the liquid mixture volatilized during downward migration. A more quantitative index of change in proportionality is shown in Table IV. Similar changes in composition and concentration of mixed fumigant gases were observed previously (Berck, 1956, 1960, 1961, 1965a; Berck and Solomon, 1962). Such changes cannot be determined by biological assessment alone, and fixed or initial proportions cannot be assumed. Accordingly, whether fumigants are applied singly or in admixture, analytical data obtained under field conditions are prerequisites. They are essential (a) to assess biological effectiveness (dosage–mortality relationships) of the fumigant against insects, mites, nematodes, rodents, etc. under different environmental conditions, and (b) to determine the effects of applications on residue levels and sorption patterns in relation to concentration–space–time data of the fumigant gas.

Earlier research (Berck, 1956, 1961; Berck and Solomon, 1962) showed that EDB, which by itself descends very little when applied to the surface of a grain bin, is assisted in downward migration when EDC and particularly CT are present in admixture. The earlier work is confirmed in Table III, which shows that EDB gas concentrations were relatively low at the 2-hr point, but increased appreciably between 1 and 3 days indicating that the downward movement of EDB was assisted by CT and EDC acting as carrier gases. This is important in control because EDB is much more toxic to insects than either EDC or CT (Brown, 1951; Lindgren and Vincent, 1962). It is also more strongly sorbed by a wide range of substrates, and physically bound EDB residues are not readily removed by aeration at ambient temperatures (Berck, 1964, 1965a–c). In the present experiment, CT, EDC, and EDB gas concentrations above the zero base line persisted up to 14 days (Figure 2), indicating that the laminated paper bin was sufficiently tight to contain the gases.

Residues of the Fumigated Wheat and Wheat Fractions. With the Varian Model 2100, injection in triplicate of 1–10-μl amounts of acetone standard solutions containing CT, EDC, and EDB, respectively, yielded gc peaks that were linear in the range 0.01–10 ng for CT and EDB, and in the range 1–40 ng for EDC, with a reproducibility between peak areas of ±10%. With the Fisher-Victoreen 4400 series apparatus the linearity range was more than doubled in each instance.

In contrast to the ease of measuring EDC in the vapor state by HFID, EDC residues of the fumigated wheat and wheat fractions could not be determined satisfactorily by gc with EC detection. This may be partly due to the higher limits of detectability, namely, 1.5 ng for EDC *vs.* 0.005 and 0.025 ng for CT and EDB.

Zero or trace amounts of EDC were found as residues in the cereal samples. This was not due to inadequate sensitivity of the analytical method. Thus, when acetone extracts of fumigated wheat were fortified with an EDC–

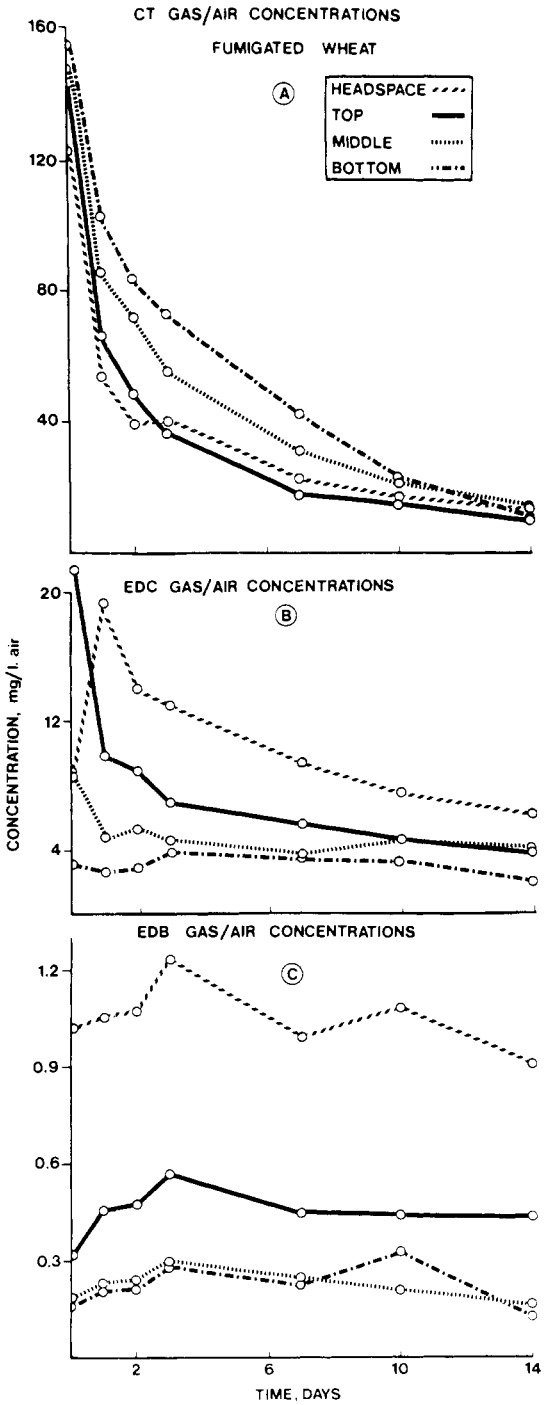

Figure 2. CT, EDC, and EDB gas–air concentrations determined by HFID and gas chromatography, as $\mu g/cm^3$ of interstitial air (= mg/l. of air) at four locations in wheat fumigated with Dowfume EB-5 at 4 imperial gal/metric bu.

acetone standard, the response was sharp and clear, and in exact proportion to the amount of EDC added. It is possible that the EDC component of the fumigant mixture was only partially sorbed by the wheat due to more rapid volatilization of the EDC after the fumigation treatment, or was changed or degraded to a non-EDC residue that did not show as an EDC peak. The low amounts of EDC residues cannot be explained without further experimentation. In the meantime, because of zero and suboptimal peaks that were obtained, it was considered best to postpone attempts to estimate EDC until further research could be undertaken. In contrast, CT and EDB residues were reproducibly determined with considerable sensitivity by EC detection. Sharp, reproducible, symmetrical peaks and clean base lines were obtained, as shown in Figure 3. Data on CT and EDB residues expressed as parts per million of fumigated wheat sampled at seven different intervals and at 15 bin locations after fumigant application are shown in Table V.

CT residues of the fumigated wheat at bottom locations (Table V) ranged from 72.6 ppm 1 week after fumigant application to 3.2 ppm 7 weeks after treatment. In general, CT residues at the bottom, at least for the first week, were higher than those at the top and middle locations. This distribution trend is similar to the gas–air distribution patterns shown in Table I. Nevertheless, there are wide variations in residue levels at different sample locations. Thus, the highest CT residues were obtained at the bottom areas in the center and north locations (68 and 73 ppm) with peak residue levels at the 1-week period. The peak for the east location was 39 ppm which was obtained at the top at the 1-week period. The highest residues for the west location occurred at 2 weeks at the bottom but with somewhat similar CT residue values (33, 31, and 34 ppm) for the top, middle, and bottom locations, respectively. In the south location, however, the middle area had the highest CT residue (54 ppm at 2 weeks) with both top and bottom locations showing 21 ppm of CT residues.

EDB residues were much smaller than those of CT and ranged from 0 to 3.3 ppm. CT residues were highest at the bottom locations, whereas EDB residues were highest at the top locations, particularly the top center after 1 week. Thereafter, these diminished gradually at all locations. In general, with the exception of the west locations, EDB residue profiles at the top locations (Table V) are correlated with the gas–air profiles of Table III. Relationships at the middle and bottom locations between EDB gas concentrations and EDB residues varied.

Table V shows that the highest EDB residue (3.3 ppm) was at the center top location at the 1-week period. The east and south top locations showed the next highest levels (1.3 and 1.4 ppm) at the 2-week period. The north and west locations, however, had the highest EDB residues at the middle areas (0.58 and 0.78 ppm, respectively) at the 2-week period and, after a subsequent downward trend, showed an increase to 0.21 and 0.76 ppm at the 6-week period. An increase was also shown at the west top loca-

Table IV. Vapor State Ratios, w/w, of CT, EDC, and EDB at Four Bin Locations and Four Time Periods after Application of Dowfume EB-5 to 1000 bu of Wheat

Location[a]	2 hr, CT–EDC–EDB	2 days, CT–EDC–EDB	7 days, CT–EDC–EDB	14 days, CT–EDC–EDB
H	121:9:1	37:13:1	24:9:1	15:7:1
T	450:67:1	104:19:1	41:13:1	27:10:1
M	1340:79:1	301:22:1	123:26:1	88:24:1
B	2560:53:1	420:14:1	188:15:1	107:14:1
T–M–B Mean	1450:66:1	275:18:1	117:18:1	74:16:1

[a] Headspace (H) of bin represents top center location immediately above grain surface. Top, middle, and bottom (T, M, and B) ratios are based on mean values for each gas at five areas (center, north, east, south, west) of the T, M, and B locations, based on data of Tables I–III of this report. EDB concentration of a given sampling period was assigned a reference value of 1.

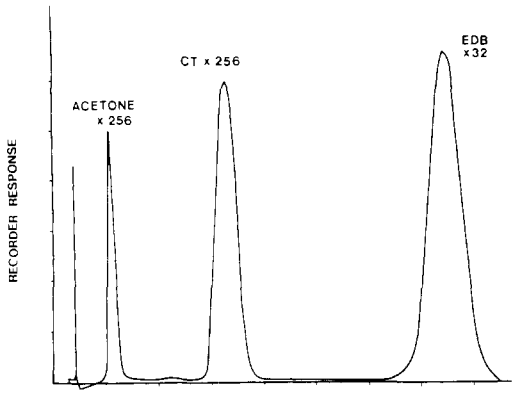

Figure 3. Quantitative separation of CT and EDB residues in acetone extract of wheat sampled 3 days after application of Dowfume EB-5. Gc and tritium foil EC detector used.

tion from 0.22 at 2 weeks to 0.30 ppm at 6 weeks.

The variations in the residue (Table V) and gas–air profiles (Tables I–III) indicate that residue levels are affected both by location and time of sampling. They also may be related to heterogeneous distribution of the fumigant gases in the grain. Differences in the rate of air movement and in various factors that influence the microclimate of stored grain (Berck, 1974) may be conducive to differential sorption of the fumigant gases. Thus, residue data must be correlated with a variety of other contributing facts in order to establish their significance.

Residues in Wheat Fractions. Table VI shows the CT and EDB residues found in extracts of flour, bran, and middlings obtained by milling samples of fumigated wheat taken 3 days to 7 weeks after fumigation. In general, CT and EDB residues were small in the three wheat fractions. In flour, the CT residues ranged from 0.20 to 0.93 ppm and EDB from 0.01 to 0.29 ppm. In bran, residues were higher than in flour, and ranged from 0.43 to 3.53 ppm for CT and from 0 to 0.40 ppm for EDB, depending on time of sampling and location. In middlings, CT residues ranged from 0.20 to 1.65 ppm, and EDB from 0 to 0.30 ppm.

Although the CT residue profiles shown in Table VI for flour, bran, and middlings do not invariably show a greater preponderance at the bottom locations (*cf.* Tables I and V), the EDB residues in the three fractions tend to be higher at the top than at the bottom locations. This trend is indicated by the correlated patterns shown in Tables III and V.

On a weight basis the three fractions may be regarded as different "concentrates" of fumigated wheat kernels, *e.g.*, 1 g of bran is derived from 7 g of cleaned wheat. Apparently appreciable amounts of unchanged CT and lesser amounts of unchanged EDB were lost from the fumigated wheat, presumably by accelerated volatilization that would occur during milling. The lesser loss of EDB residues may be due to stronger retention of EDB and its greater affinity for bran and middlings than for flour or starch (Berck, 1965a–c). It should be noted that appreciable amounts of bran and middlings are used by the animal feed industry.

Residues in Bread Fractions. No EDB residues were found in any of the 72 subsamples from the crusts or crumb of the 24 loaves of bread under any conditions of treatment. The highest levels of CT residues occurred in the bread baked with flour from wheat sampled at the top

Table V. CT and EDB Residues (ppm) of Fumigated Wheat at Seven Time Periods after Application of Dowfume EB-5 at 4 Imperial gal/1000 bu of Wheat

		3 days		1 week		2 weeks		3 weeks		5 weeks		6 weeks		7 weeks	
Sample	Location	CT	EDB	CT	EDB	CT	EDB	CT	EDB	CT	EDB	CT	EDB	CT	EDB
Top	Center	15.1	0.74	19.4	3.26	21.2	3.10	16.9	2.43	8.0	1.15	6.4	1.44	7.6	1.36
	North	39.4	0.15	28.8	0.15	56.6	0.48	17.0	0.15	14.4	0.29	11.2	0.20	14.3	0.97
	East	34.0	0.18	38.7	0.17	35.0	1.32	31.7	0.12	16.7	0.23	20.4	0.17	17.8	0.38
	South	23.5	0.98	8.9	0.87	19.8	1.40	11.4	0.28	8.6	0.20	10.3	0.99	9.7	0.50
	West	27.2	0.08	31.5	0.09	32.6	0.22	12.2	0.01	13.5	0.02	12.1	0.30	14.4	0.12
Middle	Center	38.0	0.07	52.3	0.02	41.7	0.16	20.8	0.09	17.8	0.01	18.9	0.22	20.3	0.08
	North	13.3	0.42	19.8	0.54	23.0	0.58	11.2	0.20	3.4	0.01	6.6	0.25	3.6	0.05
	East	27.2	0.16	31.4	0.07	33.3	0.12	17.5	0.06	15.7	0.01	14.1	0.21	12.6	0.04
	South	45.4	0.16	39.8	0.18	54.5	0.17	19.0	0.09	20.2	0.01	14.9	0.12	12.4	0.01
	West	16.2	0.47	14.8	0.13	31.1	0.78	9.9	0.29	1.5	0.01	7.7	0.76	19.8	0.39
Bottom	Center	27.3	0.14	68.4	0.08	53.7	0.42	15.4	0.05	13.7	0.05	15.6	0.29	16.6	0.22
	North	25.9	0.08	72.6	0.05	29.5	0.27	20.5	0.00	16.4	0.20	19.4	0.13	16.7	0.11
	East	14.2	0.35	8.7	0.55	22.4	1.00	13.5	0.05	12.7	0.43	5.6	0.52	5.0	0.35
	South	23.3	0.06	13.0	0.23	21.2	0.14	16.5	0.05	16.6	0.04	6.6	0.13	5.8	0.16
	West	31.5	0.07	19.3	0.03	33.5	0.07	20.3	0.00	19.3	0.00	19.4	0.14	3.2	0.11

Table VI. Residues of CT and EDB (ppm) at Seven Time Periods in Flour, Bran, and Middlings Derived from Wheat[a] Fumigated with Dowfume EB-5 at 4 Imperial gal/1000 bu of Wheat

		3 days		1 week		2 weeks		3 weeks		5 weeks		6 weeks		7 weeks	
Wheat fraction	Wheat location	CT	EDB	CT	EDB	CT	EDB	CT	EDB	CT	EDB	CT	EDB	CT	EDB
Flour	Top	0.28	0.25	0.30	0.22	0.38	0.17	0.64	0.29	0.25	0.10	0.28	0.03	0.32	0.02
	Middle	0.25	0.22	0.45	0.21	0.20	0.13	0.30	0.18	0.29	0.10	0.30	0.03	0.22	0.02
	Bottom	0.28	0.03	0.30	0.02	0.93	0.02	0.70	0.01	0.62	0.02	0.22	0.02	0.38	0.01
Bran	Top	0.76	0.30	0.65	0.40	0.70	0.20	0.70	0.09	0.99	0.30	2.23	0.30	1.31	0.03
	Middle	0.86	0.18	0.78	0.04	0.58	0.01	0.52	0.06	0.43	0.02	0.45	0.01	3.53	0.04
	Bottom	0.68	0.00	0.52	0.00	0.60	0.02	0.78	0.01	0.34	0.02	0.65	0.18	0.97	0.02
Middlings	Top	1.65	0.25	0.90	0.30	0.80	0.17	0.35	0.15	0.18	0.08	0.12	0.10	0.58	0.22
	Middle	0.20	0.08	0.50	0.02	0.50	0.05	0.56	0.05	0.65	0.02	1.40	0.02	0.56	0.08
	Bottom	0.30	0.00	0.37	0.02	0.80	0.00	0.76	0.00	0.67	0.00	0.56	0.00	0.68	0.02

[a] The top, middle, and bottom (T, M, and B) locations are 1 ft below the surface, 3 ft from the surface (middle areas), and 1 ft above the bottom, respectively, of a 6 ft × 16 ft diameter paper laminate grain bin. Wheat samples were taken at five different areas at each level (center, N, S, E, and W) during each sampling round. The samples were subsequently composited for milling and baking purposes. Thus, each location (T, M, and B) is a composite of five subsamples.

and middle areas of the bin 3 days after application. Bread residues from top samples of wheat were 0.04, 0.04, and 0.13 ppm of CT in the upper crust, lower crust, and crumb, respectively. These were later reduced to 0, 0.2, and 0.01 ppm in bread from samples taken after 7 weeks.

In the middle samples taken 3 days after application, the CT residues were 0.01 ppm in the upper crust, lower crust, and crumb. For samples taken 1–7 weeks after fumigation, the CT residues were zero. Bread baked from flour milled from the bottom wheat samples showed the highest CT residues at 2 weeks after application: 0.01, 0.03, and 0.04 ppm for the upper crust, lower crust, and crumb, respectively. All other bread samples had no detectable CT residues, except for three samples that had 0.005 ppm of CT in each case.

The most surprising aspect is that CT residues were present at all. The high baking temperatures (227° (440°F) oven temperature) might be expected to desorb all traces of physically sorbed fumigant residues remaining in the dough. However, dough crumb temperatures within the outer perimeter of the loaf do not exceed 100°. It is conceivable that traces of CT gas were encapsulated or enveloped within gluten-encased air bubbles (spaces) during the baking process, and were thus preserved for residue determination. It is also possible that the low amounts of CT residue found in the bread were mainly due to enzymatic biodegradation that could convert the fumigant residues (CT, EDC, and EDB, if present) to inorganic Cl^- and Br^- during dough fermentation. Confirmation of this hypothesis will be sought in future investigations.

Comparative Results Obtained by Others. In the determination of CT and EDB residues reported herein, cold extraction with acetone of 10-g samples was used. CT residues were found in the fumigated wheat ranging from 72.6 to 3.2 ppm and in bread from 0.04 to 0 ppm, accompanied by EDB residues in the wheat ranging from 3.3 to 0 ppm, with no EDB residues found in bread. Different residue values obtained by other investigators may be due to differences in method of application, experimental conditions, nature and dosage of fumigant, variety and amount of grain fumigated, fumigation period, intensity and duration of aeration, analytical method, and other factors. Thus, Wit *et al.* (1969) experimentally fumigated three 75-kg sacks of wheat of 14% moisture content with a liquid mixture consisting of CT, EDC, and EDB in 10.2:8:1 w/w proportions at a rate of 8.5 imperial gal/1000 bu (38.6 l./27.3 metric tons) (*cf.* our use of 9:4.3:1 w/w proportions in applying Dowfume EB-5 at 4 imperial gal (18.2 l.)/1000 bu of wheat). Wit *et al.* (1969) found the descending order of magnitude of the residues in the wheat to be CT, EDC, and EDB. The residue levels were highly variable, and decreased rapidly several weeks after opening of the bin, and remained more or less constant thereafter. CT residues at the top, middle, and bottom of the sacks ranged from 62 to 23, 59 to 20, and 53 to 21 ppm, depending on the time after opening of the fumigation chambers. Similarly, EDC residues ranged from 43 to 23, 43 to 25, and 38 to 28 ppm, and EDB residues ranged from 30 to 5, 17 to 6, and 15 to 6 at the top, middle, and bottom of the sacks. Up to 0.07 ppm of CT was found in bread baked from flour milled from the treated wheat. The steam distillation method of Kennet and Heulin (1957), sample sizes of 200 g, and EC detection were used.

Scudamore and Heuser (1973) applied CT as a gas at 80 mg/l. of air to 1-kg amounts of wheat and corn on trays in a 170-l. steel fumigation chamber for 3 and 6 days at 25°. CT residues were determined by EC detection after cold extraction with acetone–water (4:1, v/v). Initial residues of 200–400 ppm of CT in whole grain were reduced to 1–10 ppm when aired for 6 months in thin layers at 25° and to 5–50 ppm at 10°. Residues in wheat disappeared more rapidly than those in corn. Grinding of the grain effected a sharp reduction in CT content, but the per cent loss due to aeration of ground grain was the same as that of whole grain. It was concluded that complete elimination of trace amounts of CT from fumigated grain was unlikely, even after milling. Scudamore and Heuser (1973) found that, even after 12 months of aeration, whole kernel wheat contained up to 4.7 ppm of CT residues. While this very useful research shows that CT residues can persist for long periods despite prolonged aeration, the experimental conditions, including use of CT as a gas to small amounts of grain, favored high initial levels of CT residues, and do not correspond to field conditions such as employed herein.

Quality Tests. Per cent germination of each of 110 wheat samples was determined as a sensitive index of change in wheat quality. No significant change or deterioration of seed quality could be discerned by this test. The recorded mixograph patterns showed no significant differences between the flour milled from each of the 21 composite samples of fumigated wheat and 3 control samples in the series.

Three of the 21 samples of flour from the fumigated grain showed a significant reduction in loaf volume. The reduction had no relationship to the CT and EDB residue values, which were quite small for the samples in question. There is no satisfactory explanation for this finding. Additional research along such lines should be undertaken under controlled conditions to establish a possible cause and effect relationship between nature of fumigant, dosage, contact time (storage period), storage temperature, moisture content of wheat, kind of fumigant residue, etc., and the loaf volume of bread baked from the flour.

Bioassays. In unreplicated tests with 30 cages each containing 25 adults and eggs of the red flour beetle, *Tribolium castaneum* (Herbst), and of the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), placed at the 15 bin locations, most of the adults and eggs were killed after 48 hr of exposure in the fumigated grain. The exceptions were some that revived after 5 days recovery in fresh flour. It was not possible at the time to obtain a second grain bin with identical properties to those of the test bin in order to replicate the insect bioassay tests. Because of the interaction of uncontrolled or uncontrollable factors, fumigants applied under field conditions generally yield insect mortality effects quite different from those obtained under laboratory conditions (Berck, 1964, 1971). Although the main objective of this investigation was to determine residue levels that result from application of commercially recommended dosages of Dowfume EB-5 under field conditions, it was reassuring to confirm that the CT–EDC–EDB gas concentrations manifested during the first 2 days had insecticidal effects.

A reduction of spc (standard or total plate count) and yeast and mold counts was obtained, particularly in samples taken from the middle and bottom areas of the bin. More detailed investigation of these aspects will be undertaken in future investigations (Berck and Pereira, 1974). There is presumptive evidence that EDC vapor may act to reduce microbiological population density (Hurtig, 1973).

Additional Observations. The use of cold solvent extraction at −18° with extension of extraction time to 10 days is simple and effective, and avoids losses that might occur at higher temperatures used to extract traces of volatile fumigant residues from the substrate. Interfering substances that were also extracted were at too low a level to interfere with the determination of CT or EDB residues from the wheat and wheat products in question.

Higher peaks were obtained with acetone–water (95:5, v/v), under the same conditions of extraction. A water content greater than 5% v/v caused erratic and drifting base lines with considerable background noise. Although higher peaks were obtained with 95% acetone, the lineari-

ty of response thus obtained in the range 0.05–10 ng for CT and EDB and in the range 1.5–40 ng for EDC was similar to that obtained with 100% acetone. Reproducibility of response was better with 100% acetone as the extraction solvent, which became the solvent of choice for extraction of cereal samples.

Recoveries by the method of standard addition by which amounts of 1–1000 μg of fumigant in standard solutions were added to acetone extracts of untreated wheat to a total volume of 10 ml showed that amounts ranging from 0.1 to 40 ng of CT and EDB and from 1.5 to 100 ng of EDC could be measured with 95–100% recovery by EC detection.

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Endosulfan Persistence in Soil and Uptake by Potato Tubers

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Studies on technical endosulfan incorporated into soil at a rate of 6.7 kg/ha showed that α-endosulfan decomposed fairly rapidly (50% in ~60 days) with the simultaneous formation of equivalent amounts of endosulfan sulfate which appeared to be relatively stable in soil. β-Endosulfan disappeared slowly (~50% in 800 days). Residues in potato tubers due to direct absorption from the soil, in the same season that endosulfan was applied at 6.7 kg/ha, were 0.3 ppm of endosulfan sulfate, 0.06 ppm of β-endosulfan, and 0.01 ppm of α-endosulfan in peel and 0.03 ppm of endosulfan sulfate in pulp. Eight foliar sprays, each applied at the rate of 0.6 kg/ha, resulted in residues of 0.01 ppm of endosulfan sulfate in peel and pulp.

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide) is recommended as an insecticide on a number of crops in Nova Scotia and repeated sprays may be applied each season depending on the crop and insect involved. Since endosulfan has been in use for more than 15 years a considerable amount of information is available on residues in plants and metabolism in animals (Maier-Bode, 1968). However, there does not appear to be any information in the literature dealing specifically with the persistence of endosulfan in soil.

The present study reports the persistence of technical endosulfan in sandy loam soil, the direct absorption from soil by potato tubers, and the tuber residues resulting from repeated foliar sprays of endosulfan.

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MATERIALS AND METHODS

Soil Treatments. Four plots (7 × 5 m) were established July 28, 1970 on a Somerset sandy loam (Cann *et al.*,