fortified natural broth and carrying it through the recovery process up to the point of the final precipitation. To test the first method six 25-ml. aliquots were taken and varying amounts of calcium hydroxide were added as shown in Table VII. The reaction proceeded slowly.

Table VIII illustrates the results of the second method, wherein calcium chloride and sodium hydroxide are added as the precipitating agent and shows that either method may be used, and neutralization need not be carried beyond a pH of 5 to 5.5. This will avoid the precipitation of calcium carbonate. To recover a higher percentage of α-ketoglutaric acid it is advisable to concentrate the filtrate and recycle it through the recovery process. The quality and purity of the salt can be improved by washing with several small portions of water before it is dried at 100° C. At this temperature the white monohydrate is obtained which is stable up to 170° C.

Table VIII. Results of Precipitation with Calcium Chloride and Sodium Hydroxide

NaOH added, Ml.	pH	Weight of Dry Precipitate, Grams	α-KG Concn. in Filtrate, μmoles/Ml.
1.97	3.60	0.73	...
2.34	4.08	1.20	97
2.66	4.80	1.26	87
2.84	5.57	1.20	90
2.86	5.86	1.28	88
2.90	6.38	1.17	96

Results

A number of small and several larger scale laboratory recovery runs have been made using the recovery process just described. The pertinent data are presented for the small scale (100- to 200-ml. initial volume of broth) runs in Table IX. In each case the starting material was a natural broth, in some cases fortified with extra α-ketoglutaric acid. All samples were acidified to a pH of 1.0 with the acid as indicated, and all precipitations are carried out by addition of calcium hydroxide to the final pH. Recovery is reported as per cent of theoretical for easy comparison.

These results represent no attempt to recover the α-ketoglutaric acid remaining in the filtrate after precipitation, and yields were presumed to be low because of the inevitable losses occurring in making filtration and separation on a small scale.

Several larger scale runs were also made; one involving a 4000-ml. sample, the other a 1300-ml. sample. Recovery in the first was 60%, and the product analyzed as 91% Ca α-KG.H_2O.

Better results were obtained in the second smaller run (1300-ml. sample) and the yield was 73%. The product was more thoroughly washed and analysis gave 100.8% of the monohydrated calcium salt. Again, no recovery from the filtrate was attempted.

Table IX. Recovery of α-Ketoglutaric Acid in Small Scale Runs

Initial α-KG Concn., μmoles/Ml.	Acid Used	Final pH	Recovery, %
209	H_2SO_4	5.0	69
222	HCl	4.5	41
226	HCl	4.9	63
239	HCl	4.5	61
239	HCl	4.5	60
240	HCl	4.7	60
305	HCl	5.0	62
322	H_2SO_4	5.2	69

Acknowledgment

The authors are indebted to Robert Anderson and Robert Rhodes, Northern Regional Research Laboratory, for their valuable assistance.

Literature Cited

(1) Berger, C., Witt, E. E. (to Campbell Soup Co.), U. S. Patent **2,841,616** (July 1, 1958).
(2) Friedemann, T. E., Haugen, G. E., *J. Biol. Chem.* **147,** 415 (1943).
(3) Kirk, R. E., Othmer, D. F., "Encyclopedia of Chemical Technology," Vol. **4,** p. 771, Interscience Encyclopedia Co., New York, 1949.
(4) Koepsell, H. J., Sharpe, E. L., *Arch. Biochem. Biophys.* **38,** 443 (1942).
(5) Koepsell, H. J., Stodola, F. H., Sharpe, E. L., *J. Am. Chem. Soc.* **74,** 5142 (1952).
(6) Meister, "Methods in Enzymology," Vol. **III,** p. 407, Academic Press, New York, 1957.

Received for review July 1, 1964. Accepted March 1, 1965. Work done under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act. Contract supervised by the Northern Utilization Research and Development Division, Agricultural Research Service.

FUMIGANT RESIDUES

Residues in Milled Wheat Products Resulting from Spot Fumigation of Mill Machinery with Halogenated Liquid Fumigants

M. E. GETZENDANER

Bioproducts Department, The Dow Chemical Co., Midland, Mich.

SANITARY practice in flour mills involves a constant vigilance for infestation of any insects within the mill or mill machinery. When a problem with insects does occur, the most common practice is to control it by fumigation. The liquid halogenated fumigants are effective where the machinery is involved, being introduced directly into the machinery.

Studies have been made on residues resulting from fumigation with liquid grain fumigants as direct fumigants for grain (*1*, *5*). However, data are lacking on residues resulting from spot fumigation of mills and subsequent start-up of the milling operation. The purpose of this report is to show the safety of the use of fumigants containing ethylene dibromide, carbon tetrachloride, and ethylene dichloride in mill machinery from the standpoint of residues in products made from wheat going through the mill.

Experiments were carried out in which three mills were fumigated with different mixtures of halogenated liquid fumigants. Samples of various process streams were taken intermittently after start-up of the mills, with subsequent determination of the amount of residue. The practice of setting off the early run and blending it back when the mill is on stream reduces the residue content to negligible levels.

Experimental

Fumigation with Dowfume EB-59. Dowfume EB-59 fumigant (Dowfume and EB-59 are registered trade-marks of The Dow Chemical Co.) has 100% active ingredients consisting of 59% ethylene dibromide, 32% carbon tetrachloride, and 9% ethylene dichloride.

Both the cleaning house and the mill proper were fumigated with Dowfume EB-59. A total of 5.5 gallons of fumigant was utilized in the cleaning house and 25 gallons were used in the flour mill.

DOSAGES. The dosages recommended on the label were followed as much as possible. Some deviations were made because of the varying sizes of mill machinery, such as boots, conveyors, and elevator legs. Table I gives actual amounts used throughout the mill.

Table I. Dosage and Application Schedule of Dowfume EB-59 in Flour Mill

Location in Mill	*Notes*	*Dosage, Fluid Ounces*
Elevator boots	8 inches wide (majority)	8
	>8 inches wide	12
Conveyors	For each 15 feet	4
Sifter sections		12 each
Dust collector trunks approx. 18 inch diameter	For each 10 linear feet	4
Bran dusters		8
Purifiers	2 conveyors each	4 per conveyor
Spouts	Metal	Not fumigated
Roll stands	Above gate	4
Roll stands divided	To each side	4
Roll hoppers		Not fumigated
Elevators,		Not fumigated
Heads		8 or 12 ounces depending on size.
Up and down sides		Applied 8 ft. from top of head
Flour bins, empty	Applied to bottom	8
Stocking dust collectors	On bottom	4

The spot fumigation of the mill was completed at 4:30 P.M. February 18 and the mill resumed operation at 7:30 A.M. February 20.

Temperatures throughout the mill during the time of the spot fumigation were between 70° and 85° F.

A spot fumigant applicator was used for applying the fumigant and periodic checks showed that dosages varied ±0.5 ounce.

The procedure followed by the mill when starting up after a spot fumigation is as follows:

The first half-hour run of patent and first and second clear flours is directed to a setoff bin, at the end of which time these flours are blended back into the mill stream at a ratio of about 8 parts of mill stream to 1 part of setoff.

The pre- and postfumigation samples taken were those given in Table II. Samples of the first and second clear flours during blending were not taken since blending was not to be done until a later date, because of the particular run being made in the mill at this time.

All samples obtained were immediately placed in polyethylene containers and inserted in metal cans with friction-type lids.

No methyl bromide was used during this fumigation.

The capacity of the mill fumigated is 10,000 cwt. in a 24-hour period.

Table II. Bromide Residues in Flour from Mill Fumigated with Dowfume EB-59

	Description		*Residues, P.P.M.*		
Sample	*Prefumigation*	*Postfumigation*	*Total Br*	*Organic Br*	*Inorganic Br av.*
A-1	Wheat		7, 8	0, 0	
A-2	Wheat		10, 8	1, 1	7
A-3		Wheat	1, 6	0, 0	
A-4		Wheat	4, 4	1, 1	3
B-1	Patent flour		5, 5	0, 0	
B-2	Patent flour		9, 11	0, 1	7
C-1	First clear		11, 12	0, 0	11
C-2	First clear		10, 12	1, 1	
D-1	Second clear		15, 12	1, 0	
D-2	Second clear		9, 6	0, 0	10
E-1		Patent	827, 933	1112[a], 1083[a]	
E-2		Patent	1107, 1077	1072, 962	...
F-1		First clear	..., 636	563, 544	
F-2		First clear	727, 700	710, 630	76
G-1		Second clear	1410, 1500	1090, 1112	
G-2		Second clear	1090, 1200	1047, 1051	215
H-1		Patent, blended 1/2	24, 22	18, 18	
H-2		hr.	21, 23	18, 18	5
K-1		Patent, blended	59, 57	40, 50	
K-2		1 1/2 hr.	60, 56	59, 60	6
N-1		Patent, blended 3	8, 5	2, 2	
N-2		hr.	13, 14	2, 2	8
O-1		First clear, blended	22, 20	2, 2	
O-2		3 hr.	6, 5	4, 4	10
P-1		Second clear,	5, 8	4, 8	
P-2		blended 3 hr.	27, 27	4, 4	12

[a] Organic bromide higher than total in sample E-1 probably because of necessity of rerunning total bromide after considerable time lapse.

Fumigation with Dowfume EB-15. Dowfume EB-15 inhibited fumigant (registered trade mark of The Dow Chemical Co.) is a mixture of the following composition by weight: $C_2H_4Br_2$ 20.4%, CCl_4 57.0%, $C_2H_4Cl_2$ 19.6%, and inert ingredients 3.0%.

This fumigant was applied in the mill at the maximum rates specified by the label. Table III gives the rates used and the method of application.

The mill was run dry before fumigation. A spot fumigant applicator, which was calibrated for proper delivery before use, was used for injection of the liquid fumigant. No fumigant was put on the sifters, as they might become plugged.

Table III. Dosage and Application Schedule of Dowfume EB-15 Inhibited in Flour Mill

Location	Method of Application	Dosage, Fluid Ounces
Elevator boots	Through hand-hole opening of slide spout, through slide in side of elevator boot, or through hole drilled in boot	6
Rolls (in each side)	Into spouts above	6
Reel and purifier conveyors	Pour along entire length	6
Bran and shorts dusters	Hand hole at top or any opening	12
Spouts	Into convenient hand slides	1 to 2 per floor length (for Mediterranean flour moth only)
Flour bins	Splash on wall near top	6/100 cu. ft.
Dust trunks	Any suitable opening	12 per trunk
Conveyors		
6- to 8-inch	At convenient points over entire length	1 to 1.5 per linear ft. but not less than 8 per conveyor
10-, 12-, and 16-inch	At convenient points over entire length	2 to 3 per linear ft. but not less than 16 per conveyor
Elevator heads	Pour around inside	12

Table IV. Resiudes of Organic Chloride and Bromides in Wheat Fractions from Fumigation of a Mill with Dowfume EB-15

		P.P.M. Residues of			
Product	Sampled at	Organic Cl	Organic Br	Total Br	Inorganic Br[a]
Wheat	Prefumigation	1, 0	0, 0	12, 11	12
Wheat (dirty)	Prefumigation	3, 1	0, 0	14, 12	13
1st break flour	Prefumigation	0, 0	0, 0	10, 10	10
	Start-up	22, 22	18, 18	27, 27	9
	Start-up + 15 min.	1, 0	3, 3	14, 16	12
1st sizings	Prefumigation	0, 0	0, 0	8, 10	9
	Start-up	42, 48	156, 144	156, 154	6
	Start-up + 15 min.	2, 2	3, 3	35, 38	34
1st middlings	Prefumigation	0, 0	0, 0	9, 10	10
	Start-up	58, 71	211, 220	252, 250	36
	Start-up + 15 min.	2, 2	6, 6	8, 10	3
Shorts	Prefumigation	0, 0	0, 0	6, 7	7
	Start-up	91, 95	258, 250	306, 310	54
	Start-up + 15 min.	11, 9	24, 22	37, 35	13
Bran	Prefumigation	0, 0	0, 0	7, 6	7
	Start-up	69, 62	158, 162	259, 248	104
	Start-up + 15 min.	1, 0	2, 2	12, 15	12
Patent flour	Prefumigation	0, 0	0, 0	11, 14	13
	Start-up	35, 36	69, 68	91, 93	24
	Start-up + 15 min.	10, 9	18, 17	31, 30	13
1st clear	Prefumigation	0, 0	0, 0	2, 5	4
	Start-up	19, 15	69, 68	80, 81	13
	Start-up + 15 min.	9, 9	28, 30	28, 30	0
2nd clear	Prefumigation	0, 0	0, 0	6, 5	6
	Start-up	43, 46	110, 106	208, 210	101
	Start-up + 15 min.	25, 25	69, 70	105, 103	34
Germ	Prefumigation	0, 0	0, 0	7, 8	8
	Start-up	8, 8	17, 17	73, 70	55
	Start-up + 15 min.	3, 3	5, 4	20, 21	16
Setoff bin	Start-up	34, 37	83, 85	97, 98	14
Blend	Beginning	8, 7	18, 20	37, 35	17
Blend	10 A.M.[b]	1, 1	1, 1	20, 21	20
Patent flour	1/2 hour postblend	3, 2	2, 3	9, 10	7
Patent flour	Before bleach	1, 1	2, 2	10, 12	9

[a] Inorganic = average of total − average of organic bromide.
[b] Mill on-grade, blend of 25 cwt. setoff to 400 cwt. on grade flour.

Following the fumigation, the mill was allowed to stand for about 40 hours before it was started up. Temperature of the mill was 65° to 75° F., during the fumigation. It was started at 7:30 A.M.

Samples of the various products, such as dirty and cleaned wheat, were removed from the mill prior to fumigation. The dirty wheat sample represented the wheat going into the mill on start-up after fumigation, while the clean wheat represented the wheat which had been going through the mill when it was run dry. The setoff flour as the mill went down before fumigation came from the clean wheat. Following fumigation, when the mill was started up, samples were removed from the mill streams as soon as the material started through the conveyors. Sampling doors were opened and a portion of the stream was removed over a period of several minutes, to fill a 1-gallon can. About 15 minutes later, the second samples were taken from the same streams in the same manner. A sample was removed from the setoff bin after start-up following fumigation. This represents the flour which was setoff as the mill was run dry, plus that which was in the mill during fumigation and that which went into the mill between start-up and on-grade operation. A sample was taken at 10 A.M., of the blend of the setoff flour with on-grade flour. Two samples were taken about 30 minutes after the blending was completed. Blending was done at the rate of 1 part of setoff to 16 parts of on-grade flour.

The postfumigation samples were put into 1-gallon friction top cans, which were tightly sealed immediately after sampling. They were shipped to Midland, Mich., a week after collection and then stored in a freezer until they were analyzed, within a few weeks.

EDB-60 Fumigation. Fumigant EDB-60 is a special mixture containing 59% ethylene dibromide, 33% carbon tetrachloride, and 8% ethylene dichloride.

The mill in preparation for fumigation was run almost dry after the incoming grain was shut off at the first break rolls, to reduce the quantity of flour and intermediate stocks lying in the equipment. The equipment at the tail end of the mill was still heavily loaded. The stagnant stocks lying below screws, in conveyors, on ledges, under the cups, in boots, etc., are periodically removed and diverted to feed. On this occasion the following equipment was cleaned: all sifter boxes, all sifter stubs, all roll hoppers, conveyors above packer bins, packer bins (inside), and flour conveyors on the deck.

The fumigant was introduced into the equipment and mill stream through small ports by means of a semiautomatic dispenser in which the liquid fumigant remained under 40 to 60 pounds' pressure at the nozzle. The application ports are relatively close together, so that good distribution of the concentrated vapors is assured. Normally, an 8-ounce shot (by measure) is applied at each port. A total of 30 gallons of EDB-60 was used in the mill and cleaning house area. This is at the rate of 0.5 gallon per 100 cwt. daily production. The inside temperature of the building and interior of equipment was held at about 74° to 78° F. The windows and doors of the building were not sealed, as is required for space fumigation. The exposure period from end of application to the start-up time was 38 hours. A normal low velocity wind prevailed.

Samples were drawn on the XN (straight grade) grade stream at the

Table V. Residues Found from Fumigation with EDB-60

Sample Number	Sampling Time, A.M.	Bromide, P.P.M.		
		Total	Organic	Inorganic
1	7	231, 228	41, 39	190
2	7:05	547, 556	147, 138	409
3	7:10	248, 247	103, 102	145
4	7:15	75, 77	27, 25	50
5	7:20	59, 61	46, 47	13
6	7:25	33, 31	57, 59	26
7	7:30	56, 55	28, 27	28
8	7:35	41, 44	7, 7	36
9	7:40	29, 26	8, 9	18
10	7:45	22, 25	8, 8	16
11	7:50	24, 22	3, 3	20
12	7:55	25, 26	17, 16	9
13	8	14, 19	8, 7	9
14	8:10	36, 39	6, 6	32
15	8:20	31, 37	5, 4	25
16	8:30	23, 27	17,15	9
17	8:40	17, 14	5, 6	11
18	8:50	17, 14	3, 3	12
19	9	27, 30	2, 2	26
20	9:30	24, 25	3, 4	21
21	10	25, 24	3, 2	22
22	10:30	14, 8	4, 4	7
23	2:30 P.M.	16, 17	3, 2	14

point where it is pneumatically conveyed to the storage bins. The sampling began at the time of mill start-up, 7 A.M. February 26, 1962. During the first hour, samples were taken at 5-minute intervals; the second hour, at 10-minute intervals; the next three, at 30-minute intervals. The last flour sample in the series was taken $4^1/_2$ hours later.

Analysis

The samples were analyzed for total bromide by the method of Shrader and coworkers (*6*). Organic bromide was determined by the Mapes and Shrader procedure (*3*). Organic chloride was determined by the distillation-combustion method of Mapes and Shrader (*2*). Recovery of ethylene dibromide and ethylene dichloride added to wheat and flour was in the range of 75 to 110% with from 5 to 93 p.p.m. added. Inorganic bromide was determined as the difference between total and organic bromide.

Results and Discussion

The results of the residue analyses from the fumigation with Dowfume EB-59 are given in Table II. In samples E, F, and G both total and organic bromide residues were high when the mill was started up immediately following the fumigation. This would be expected, as the first flour coming through would pick up the fumigant residing in the machinery. Starting with sample H, however, where the first flour was blended back into the run of the mill, the residues have dropped to just about the same level as the prefumigation samples. The inorganic bromide residue which is calculated as difference between the total and organic had dropped to a level of from 5 to 12 p.p.m. on the blended samples.

A fumigation to obtain data on residues resulting from the organic chloride components of the fumigant was carried out with Dowfume EB-15. This fumigant was chosen because of its large content of carbon tetrachloride and ethylene dichloride. Table IV gives the results of the analysis of the samples from the fumigation with Dowfume EB-15. In all cases, residues found after 15 minutes of mill operation were well below those found at mill start-up. This indicates an extremely rapid rate of dissipation of the fumigant. Only low levels of the fumigant were detected in the patent flour as a result of the fumigation—i.e., 7 to 8 p.p.m. of organic chloride and 18 to 20 p.p.m. of organic bromide when the blending began. These values rapidly dropped as the blending was completed.

Results from the EDB-60 fumigation are given in Table V. The organic bromide content of the first flour started at a high level, but dropped rapidly. After the first hour and a half, the levels of bromide residues dropped to a low, steady state value.

It is traditional in milling to divert the first flour after start-up to a setoff bin until the mill is "balanced." The flour is fed back into the stream slowly when the latter is considered to be of acceptable grade. Some mills follow a similar practice with feeds, setting off the first 20 minutes of run and feeding them back into the stream 48 hours later at a slow rate (*8*). This study indicates that this practice will reduce residue levels from mill fumigation with these fumigants to insignificant levels.

Stenger and Mapes (*7*) baked rolls which contained ethylene dibromide in flour as a result of milling wheat fumigated with liquid grain fumigants. They found that all of the organic bromide disappeared. Munsey and coworkers (*4*) baked rolls using flour to which carbon disulfide, carbon tetrachloride, ethylene dichloride, and ethylene dibromide were added. They found that "...even high levels of fumigants in flour will not persist as such in the bread."

On the basis of the above findings and the results reported here, it was concluded that the use of these fumigants to control insects in flour milling machinery would not result in harmful residues in the milled products.

Acknowledgment

The author is grateful to Bruce D. Heimark, The Dow Chemical Co., Minneapolis, for assistance in arranging for the fumigations.

Thanks are also due H. M. Baker and L. E. Swim, The Dow Chemical Co., Analytical Laboratories, who carried out the analytical determinations.

Without the wholehearted assistance of George Wagner, Pillsbury Co., and A. J. Mattis, International Milling Co., and their fine staff members, this work would not have been accomplished.

Literature Cited

(1) Lynn, G. E., Vorhees, F. A., Jr., *J. Assoc. Offic. Agr. Chemists* **40,** 163–209 (1957).
(2) Mapes, D. A., Shrader, S. A., *Ibid.*, **40,** 180–5 (1957).
(3) *Ibid.*, pp. 189–91.
(4) Munsey, V. E., Mills, P. A., Klein, A. K., *Ibid.*, **40,** 201–2 (1957).
(5) Olomucki, E., Bondi, A., *J. Sci. Food Agr.* **6,** 592–600 (1955).
(6) Shrader, S. A., Beshgetoor, A., Stenger, V. A., *Ind. Eng. Chem., Anal. Ed.* **14,** 1–4 (1942).
(7) Stenger, V. A., Mapes, D. A., *J. Assoc. Offic. Agr. Chemists* **40,** 196–201 (1957).
(8) Wagner, G. B., Pillsbury Co., Minneapolis, Minn., private communication, 1964.

Received for review November 27, 1964. Accepted June 28, 1965.