Project Name: On-Farm Fumigation of Almonds

TITLE : Project 75-I. Fumigation.

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I. OBJECTIVES & GOALS:

Methyl Bromide

To obtain approval and a label from the Environmental Protection Agency (EPA) for the use of methyl bromide as a fumigant for almond hulls and shells.

Hydrogen Phosphide

Since hydrogen phosphide is approved for use for post harvest almonds and animal feed, studies were made to find out if this fumigant could be used effectively to fumigate inhull almonds stored on the farm.

II. ABSTRACT:

Methyl Bromide

Information obtained during the 1974 studies was assembled and sent to the coordinator of the IR-4 program for review and transmittal to EPA. After reviewing the results, the coordinator requested additional information which is being assembled and will be forwarded to the coordinator as soon as possible. Methyl bromide can be used as a fumigant for almond hulls and shells as soon as EPA approves and provides a label.

Hydrogen Phosphide

In cooperation with the Superior Farming Co., Bakersfield, CA., 33 stacks of inhull almonds placed on firm dirt and covered with 6 mil. polyethylene sheeting were fumigated with hydrogen phosphide (Phostoxin) pellets. Dosages used were 25, 50, 100 and 165 pellets per 1000 cu ft and the exposure periods were

24 and 48 hrs. Egg and larval stages of the navel orangeworm were used to measure the effectiveness of the fumigations. This study would indicate that inhull almonds can be fumigated effectively on the farm if not less than 50 pellets are used with an exposure period of 48 hrs. Hydrogen phosphide is the only fumigant approved for use on almond shells and hulls at this time.

Methyl Bromide (1974 Studies)

During the 1974 harvest season, 11 stacks of inhull almonds were fumigated with methyl bromide. These stacks contained almonds that had just been removed from the orchards and the objective of the fumigation was to halt navel orangeworm development and thus reduce the damage to the almonds caused by this insect. The almonds under study were stacked on either cement slabs, black top or on firm dirt. Each stack was covered with 6 mil polyethylene sheeting. Four stacks were fumigated with methyl bromide applied at the rate of 1 lb per 1000 cu ft and 7 stacks at the rate of 2 lbs per 1000 cu ft. The early egg and larval stages of the navel orangeworm were used as the test insect and the exposure period was 24 hours. On the basis of this study it appeared that inhull almonds could be fumigated successfully on the farm with methyl bromide applied at the rate of 2 lbs/M cu ft for 24 hours. It was also observed that inorganic bromide residues were increased as a result of the fumigation. The greatest amount of residue was found in the shell, next greatest amount in the hull and least amount in the nutmeats.

Methyl bromide is approved as a post harvest treatment for almonds and a tolerance of 200 ppm of inorganic bromide established. This means that 200 ppm of inorganic bromide applies to the nutmeats after the shells have been removed and discarded. This, then, means that methyl bromide is not approved for use on the shells or hulls, as no registration and tolerance has been established. Since virtually all of the almond hulls are used for livestock feed, the hulls should not be fumigated with methyl bromide until approval

Protection Agency). The same is true of shells should they be used as food or incorporated into a feed formulation.

Data obtained from the 1974 crop studies were summarized and sent to the IR-4 project coordinator for submittal to EPA as a petition for a use tolerance for the use of methyl bromide as a fumigant for almond hulls through the government registration group, health and environmental, of the Dow Chemical Co., Midland, Michigan.

The following information was forwarded:

1. Statement of purpose
2. Procedure
3. Findings
4. Procedure used in determining inorganic bromide residues.

on the Farm with Methyl Bromide

I. The purpose of this study was to develop procedures to fumigate inhull almonds as soon as they are removed from the orchard in an effort to reduce the damage to the almond meats caused by the navel orangeworm, Paramyelois transitella (Walker) a severe pest of almonds.

II. Procedure -

Eleven stacks of inhull almonds were fumigated with methyl bromide on the farm during the 1974 harvest season. These included 5 stacks composed of Nonpareils, 3 stacks composed of Thompsons and 3 stacks of the Merced variety. The stacks of almonds occupied from 1,200 to 15,000 cu ft of space and were formed on smooth dirt or on cement slabs. Prior to fumigation, the tops of each stack were leveled to form a flat area from 6-10' wide extending the length of each stack, also two stages of the navel orangeworm were placed in three locations, fumigant sampling tubes, and temperature sensing equipment were placed in each of four locations within each stack. The end of one plastic tube was located above the flat area on top of each stack and the other end extended at least six ft beyond the bottom edge of each stack. Three thirty pound samples of almonds were taken at random from each stack.

Each stack was covered with 6 mil polyethylene sheeting, the edges of which were pressed against the substrate around the bottom of the stack by the use of 2"x6' canvas tubes filled with sand. This served as a seal to prevent the fumigant from

Methyl bromide was applied to each stack through the plastic tube, one end of which was attached to a measuring device on a 50 lb cylinder containing methyl bromide and the other end was mounted in the air space on top of the stack. The dosages applied were 1 and 2 lbs per M cu ft, and the exposure period was 24 hours.

Fumigant concentration and temperature readings were taken from the locations in each stack at intervals during the 24 hour period. Methyl bromide concentrations were determined by a fumiscope and the temperature measurements by a Tele-Thermometer.

At the end of the 24 hour exposure period the plastic sheeting was pulled from the stack, fumigant sampling leads, temperature probes and test insects were removed. Samples of inhull almonds were also taken in the same manner as before fumigation. These samples were used to determine the amount of inorganic bromide residue present in the almonds before and after fumigation as well as to measure the moisture content.

The method used to determine the inorganic bromide residue is described in Exhibit A and to determine the moisture content Exhibit B - sections 7.004 and 7.005, both of which are attached.

It would appear that under the conditions of this study inhull almonds can be fumigated effectively on the farm. A two pound dosage of methyl bromide per 1000 cu ft for 24 hours killed the two more resistant stages of the navel orangeworm placed within the stacks. Each dosage used increased the amount of inorganic bromide present in the almonds. The two pound dosage added more residue than the one pound dosage. The greatest amount of residue was found in the shells, next greatest amount in the hulls and least in the nutmeats.

The data obtained from this study is summarized in tables 1 through 5 which are enclosed. Table 1 contains methyl bromide concentrations, table 2 - inorganic bromide residues, table 3 - mortality of two stages of test insect, table 4 - temperature readings and table 5 - moisture content of almonds.

1:--Average concentration of methyl bromide in oz/M cu ft found at four locations during the 24 hour exposure period in piles of inhull almonds fumigated with methyl bromide at the rates of 1 and 2 lbs/M cu ft. Fresno, California

Fumigant sampling intervals	Average amount of methyl bromide found at each location within enclosure							
	Dosage of methyl bromide 1 lb/M cu ft				Dosage of methyl bromide 2 lbs/M cu ft			
	1[1]	2[2]	3[3]	4[4]	1[1]	2[2]	3[3]	4[4]
hours	oz/M cu ft	oz/M cu ft	oz/M cu ft	oz/M cu ft	oz/M cu ft	oz/M cu ft	oz/M cu ft	oz/M c
iately after ation	13	8	4	6	14.9	15.6	5.6	3.
1	8	5.7	7.3	3.7	22.7	16.8	17.2	10.
2	17.7	12.7	13	14.7	20.5	16	18.6	12.
4	21	18	22	30	18.5	13.2	15.5	11.
8	14.5	13	14.5	12	17.4	12	11.8	10.
12	9	11.5	11	10.8	7.6	8	7.4	6.
18	1.1*	--	0.9*	1.1*	10.3	9.3	8.7	6.
24	8.1	9	7.3	7.4	6.9	7.4	6.6	6
Average	11.47	10.57	10.04	9.96	15.18	12.6	11.59	8.

r space above almonds
2 ft. in almonds; upper right hand corner
nter of mass
ar floor-opposite corner from top
alysis by gas chromatography

e 2.--Average amount of inorganic bromide found in unfumigated inhull almonds and the amount added as a result of one fumigation when the fumigant was applied at the rates of 1 and 2 lb/M cu ft for 24 hours.

sage of thyl omide	Inorganic bromide residue present in the hulls			Inorganic bromide residue present in the shells			Inorganic bromide residue present in the nutmeats		
	before fumigation	amount added as a result of fumigation	total residue present	before fumigation	amount added as a result of fumigation	total residue present	before fumigation	amount added as a result of fumigation	total residu present
M cu ft	P.P.M.	P.P.M.	P.P.M.	P.P.M.	P.P.M.	P.P.M.	P.P.M.	P.P.M.	P.P.M
1	8.33	11.95	20.28	8.60	46.33	54.93	11.16	9.24	20.4
2	6.97	33.67	40.64	8.56	177.05	185.61	6.08	20.99	27.0

: applied at the rates of 1 & 2 lbs/M cu ft in piles of inhull almonds.
: Fresno, California 1974

Test Number	Mortality of two stages of navel orangeworm			
	Unfumigated		Fumigated	
	eggs	pupae	eggs	pupae
	percent	percent	percent	percent
			1 lb/M cu ft	
8	-	-	79.5	100
9	11.3	2.2	100.0	100
10	2.8	1.1	87.6	100
11	2.8	1.1	92.4	100
			2 lbs/M cu ft	
2	11.5	5.7	100.0	100
3	7.6	10.0	100.0	100
4	-	-	100.0	100
5	-	10.0	-	100
7	8.0	2.2	100.0	100

4:--Average temperature readings taken at four locations in 11 piles of inhull almonds during the 24 hr. exposure period. The almonds were fumigated with methyl bromide applied at the rate of 1 and 2 lbs. per M cu. ft. Fresno, California 1974

mperature reading ntervals	Average temperature readings taken in each location within the enclosure							
	Dosage of methyl bromide 1 lb/M cu. ft.				Dosage of methyl bromide 2 lbs/M cu. ft.			
	1[1]/	2[2]/	3[3]/	4[4]/	1[1]/	2[2]/	3[3]/	4[4]/
hours	°F	°F	°F	°F	°F	°F	°F	°F
iately after ation	91.8	97.3	75	74.3	124.2	97	79.1	81.4
1	96	100.3	80	79.3	134.5	92.3	85.1	82.25
2	124.7	104.3	80.5	74	140.7	103.8	90.3	90.9
3	111	120	--	73	111.25	123.5	108	95.8
4	86.3	98.3	77	75.7	119.3	102.7	79	79
8	71.5	87.8	67.8	68.5	90.8	93.7	77	80.9
12	125.5	83	76.5	69	90.9	99.6	78.5	78.7
18	114	92	--	72	87.3	91.8	72.8	68.6
24	--	--	--	--	109	101.8	90.5	88.9

rspace above almonds
2 ft in almonds; upper right hand corner
nter of mass
ar floor - opposite corner from top

5:--Average moisture content of inhull almonds before and after fumigation with methyl bromide. Fresno, California 1974

Test number /M cu ft	Moisture before fumigation: Hulls percent	Shells percent	Nutmeats percent	Composite percent	Moisture after fumigation: Hulls percent	Shells percent	Nutmeats percent	Com pe
8	8.6	7.1	4.2	7.0	6.2	7.2	3.0	
9	8.5	7.7	4.3	7.2	7.8	7.6	4.2	
10	9.7	7.8	4.2	8.3	11.4	8.1	4.4	
11	17.6	10.5	6.0	12.4	16.8	11.0	6.3	1
Total	44.4	33.1	18.7	34.9	42.2	33.9	17.9	3
Average	11.1	8.3	4.7	8.7	10.6	8.5	4.5	
s/M cu ft								
1	8.8	8.3	3.8	7.4	9.5	8.2	4.2	
2	9.0	7.2	4.4	6.4	9.6	7.6	4.5	
3	8.8	7.6	4.4	6.8	5.3	5.6	3.6	
4	7.9	7.8	3.9	7.1	8.0	7.0	4.0	
5	8.0	6.9	4.3	6.8	7.2	6.6	3.8	
6	8.0	7.6	4.2	7.6	6.8	6.9	3.7	
7	7.1	7.8	3.7	6.9	5.3	6.7	3.4	
Total	57.6	53.2	28.7	49.0	51.7	48.6	27.2	4
Average	8.2	7.6	4.1	7.0	7.4	6.9	3.9	
d Total	102.0	86.3	47.4	83.9	93.9	82.5	45.1	8
Average	9.3	7.8	4.3	7.6	8.5	7.5	4.1	

U.S. Dept. of Agriculture
Agricultural Research Service
Stored-Product Insects Research
Laboratory
5578 Air Terminal Drive
Fresno, CA 93727

Method 11-56-64
Nov. 9, 1964
Rev. Nov. 12, 1973
by Rita Chriss
Rev. Mar. 23, 1975
by Teresa Bedgood

TOTAL BROMIDE RESIDUE ON COMMODITIES FUMIGATED WITH METHYL BROMIDE

PRINCIPLE:

The total bromide analysis will detect the organic methyl bromide residues as well as its inorganic bromide breakdown products, with the results calculated as p.p.m. bromide ion. The analysis consists of converting the organic bromide to inorganic bromide, using alcoholic potassium hydroxide. The sample is then ashed in a muffle furnace to eliminate organic material. The bromide ion is oxidized, using sodium hypochlorite, to the bromate ion which is then titrated with a standard solution of sodium thiosulfate.

SENSITIVITY OF METHOD:

Two parts per million

1) Potassium hydroxide pellets (KOH), analytical reagent grade 2. [illegible]

2) Sodium hydroxide pellets (NaOH), A.R.G.

3) Potassium iodide (KI), A.R.G.

4) Sodium acid phosphate ($NaH_2PO_4 \cdot H_2O$), A.R.G.

5) Hydrochloric acid (HCl) - 12 N - A.R.G.

6) Sulfuric Acid (H_2SO_4) - 36 N - A.R.G.

7) Sodium Hypochlorite solution 4-6%, N.F. grade.

8) Sodium Formate (HCOONa), A.R.G.

9) Sodium Molybdate ($Na_2MoO_4 \cdot 2\ H_2O$), A.R.G.

10) Methyl Red - A.R.G.

11) Starch soluble - A.R.G.

12) Salicylic Acid - A.R.G.

13) Ethyl Alcohol - or Reagent Alcohol Formula 3A-95.

14) Sodium thiosulfate ($Na_2S_2O_3 \cdot H_2O$) - A.R.G.

15) Carbon disulfide (CS_2)

16) Potassium dichromate ($K_2Cr_2O_7$), - A.R.G.

17) Boiling chips.

A.R.G. - Analytical reagent grade

1) Nickel crucibles, 100 ml. with covers.*

2) Muffle furnace.

3) Hot plate.

4) Distilling apparatus:

   Friedricks condenser
   Round bottomed 3 neck flask - 3 liter with glass stoppers
   200°C thermometer with collar to fit one of flask fitting
   heating mantle to fit 3 liter flask - Briskeat

   or comparable set up.

5) Erlenmeyer flasks - 500 ml. (for daily use).

   Stoppered: 1,000 ml. (for storage of HCl)
   250 ml. (for Sodium Formate Reagent)
   500 ml. (for starch indicator)

6) Buret (Class A) - 50 ml.

7) Waring blendor.

8) Hobart food chopper, Model #4612, with 1/8" plate.

9) Standard hand meat chopper converted to motor (¼ h.p.) driven

10) Funnels, glass - 96 mm. short stem
    55 mm. short stem

11) Filter paper - Whatman #1, Qualitative, 11 cm.

12) Beakers - 400 ml.

13) Pipettes - TD volumetric - 25 ml., 10 ml.

14) Volumetric flasks - 100 ml., 200 ml., 1 liter and 2 liter.

15) Graduated cylinders - 5 ml., 10 ml., 25 ml., 50 ml., 100 ml., 200 ml., 1,000 ml. (1 liter).

16) Eye droppers with bottles - standard size (ca. 25 ml. capacity). Used for dispensing 1% Sodium Molybdate, Starch indicator, Methyl Red indicator, and 5% NaOH.

*Porcelain crucibles are not suitable as they are not alkali resistant.

17) Glass stirring rods - ca. length, 25 cm.

18) Wash bottles - for distilled water and ethanol.

19) Pestle and mortar.

1) Hydrochloric Acid - 6N - bromide free:

In a 3 liter distilling flask with boiling chips, add 1 liter of distilled water. To this add 1 liter of 12N HCl. Distill, discarding the first 10% (200 ml.) and last 10%. (to eliminate free bromine or hydrobromic acid)

Caution: Add the acid to the water - slowly, handling acid only with proper exhaust system and protective clothing. This distillation step may be eliminated if chemical control is run w/test samples.

2) Sodium Hydroxide solution - 5%:

Place 100 gm of NaOH pellets in a 2 liter volumetric flask. Dissolve in ca. 500 ml. distilled water. Allow to dissolve and cool, then bring to volume with distilled water.

3) Sodium Formate solution 50 gm./100 ml. water:

To a 250 ml. stoppered erlenmeyer flask, add 50 gm. sodium formate and 100 ml. distilled water. It will take some time to dissolve all the sodium formate.

4) Sodium Molybdate solution - 1%:

Place 1 gm. sodium molybdate in a 100 ml. volumetric flask containing 99 ml. of distilled water.

5) Sulfuric Acid - 6N:

In a 2 liter volumetric flask with ca. 1,000 ml. distilled water, carefully add 333.3 ml. of 36N H_2SO_4. Allow the solution to cool, then bring up to volume - 2 liters.

Caution: - Handle as you would HCl - 12N. When the acid is added to the water, it will generate enough heat to boil the water, therefore add slowly.

6) Alcoholic Potassium Hydroxide - 2.5%:

In a 2 liter volumetric flask dissolve 50 gm. KOH in ca. 500 ml. ethyl alcohol. Allow to cool then bring up to volume - 2 liters with ethyl alcohol.

Note: Potassium hydroxide pellets are slow to dissolve in ethanol. If reagent alcohol (denatured ethyl alcohol) is used, it will discolor to a yellow-orange.

7) Methyl Red Indicator:

First prepare a 0.1 N NaOH solution-Dissolve 0.4 gm. of NaOH in ca.

Grind 0.1 gm. methyl red with 4 ml. 0.1 N NaOH in a mortar. Pour into a 100 ml. volumetric flask and bring up to volume with distilled water.

Note: The methyl red will be difficult to get into solution.

8) Starch Indicator solution:

Mix 2 gm. soluble starch with 10 ml. of cold distilled water in a large beaker. Add 400 ml. boiling distilled water and stir. Boil again for a few minutes. Remove from heat and add 0.25 gm. of salicylic acid as a preservative. Transfer to a 500 ml. stoppered erlenmeyer flask.

9) Sodium Thiosulfate standard solution - 0.1 N:

Weigh 24.8000 gm. of sodium thiosulfate on the Mettler balance. Dissolve in ca. 300 ml. distilled water in a 1 liter volumetric flask. Bring volume up to 1 liter with distilled water.

Standardization: Prepare 0.1 N $K_2Cr_2O_7$ by dissolving 4.9037 gm. (weighed on the Mettler balance) in a few mls. of distilled water in a 1 liter volumetric flask. Bring volume up to 1 liter with distilled water. Pipette 25 ml. aliquots of $K_2Cr_2O_7$ and titrate as in the regular procedure (adding H_2SO_4, sodium molybdate, KI and starch-treating 0.1 N $K_2Cr_2O_7$ as the sample and 0.1 N $Na_2S_2O_4$ as the titrant).

Note: At start solution will be rusty brown. At the endpoint the solution will be turquoise blue instead of clear.

At least three aliquots of $K_2Cr_2O_7$ should be titrated. Then Calculate the normality of K_2Cr_2O as follows:

$$N\ Na_2S_2O_4 \cong \frac{.1\ N\ K_2Cr_2O_7}{.025\ l} \times \text{liters of } Na_2S_2O_4 \text{ used for titration.}$$

Where liters of $Na_2S_2O_4$ used for titration = ml. $Na_2S_2O_4$ used x .001 liters/ml.

The average of the three normalities is then to be calculated for use in sample calculation.

Add one ml. of CS_2 to the 0.1 N $Na_2S_2O_4$ solution as a preservative. Also keep tightly sealed to prevent evaporation.

10) Sodium thiosulfate - .005 N standard:

Prepare the day of use. Pipette 10 ml. of 0.1 N sodium thiosulfate standard solution into a 200 ml. volumetric flask. Bring up to volume. This is the working standard used for the sample titrations.

1. Grind about 100 gm. of sample to a fine state in the appropriate apparatus:

   a. Waring Blendor for commodities as almond (meats, soft shells or hulls), walnut meats etc.

   b. Hobart Grinder for raisins, figs and other non-pit dried fruits.

   c. Food Chopper for pit dried fruits as dates and prunes. These commodities should be ground 3-4 times to grind the pits to a fine state.

   Note: When using the Hobart Grinder or the Food Chopper, grind the control sample first, followed by the sample with the lowest expected residue. Continue with increasing residue samples. Between each sample, grind a small portion of the sample to be ground. This clears the chamber of the previous sample.

   If the approximate residues are unknown and could have a broad range, it may be necessary to clean the grinder between samples.

2. Weigh 7.0 gm. of sample into a 100-ml. nickel crucible (each sample should be run in triplicate).

3. Add 40 ml. of alcoholic potassium hydroxide to the crucible and allow to set for 1 hour.. (alcohol precipitates chlorides and alkali breaks CH_3-Br bond)

4. Add 9 gm. of NaOH pellets, stir into the sample. Rinse the stirring rod with ethyl alcohol allowing the alcohol to run into the crucible.

5. Place the crucibles on the hot plate. Set the temperature to 200°C and turn on the fume hood. It is important not to pre-heat the hot plate to drive off most of the volatiles.

6. When all the alcohol and water has evaporated, turn the temperature to high. Heat the sample at the high temperature for 2 hours or until the sample is charred.

   Note: For some products such as dates a slightly lower temperature is necessary to avoid excessive spattering. The fume hood should be on at all times.

   Charring prevents excessive flaming in the muffle furnace which could destroy bromide.

7. Transfer the crucibles into a muffle furnace at 600°C for 75 minutes. (to ash and oxidize organic matter)

8. Remove from the furnace, cool for a few minutes, andd 60 ml of distilled water. Break up the carbonized material as much as possible with a stirring rod, using a clean stirring rod for each sample. (to dissolve solids)

9. Set on a hot plate (set on 150-200°C, 30-60 min.) if necessary to dissolve the sodium hydroxide.

10. Under the fume hood, transfer the contents into a 400 ml. beaker, being careful not to loose any down the side of crucible. Add exactly 35 ml. of distilled 6N HCl to the crucible using it to wash down the walls of the crucible. Transfer the HCl from the crucible slowly into the beaker by pouring down a stirring rod. Stir continuously while transferring and until foaming stops. Rinse the crucible inside and out very carefully three times. Be certain all rinsings go into the beaker. (to neutralize NaOH)

11. Add a couple of boiling chips and reduce the volume to 100 ml. or less on a hot plate. (for ease of filtering and to boil off any peroxides)

12. Filter solution through Whatman #1 filter paper into a 500 ml. Erlenmeyer flask. Rinse the beaker three times with distilled water, adding each rinse to the filter. Then rinse the filter paper and filter with distilled water. Filtering removes NiOH and other insolubles.

13. Add two drops methyl red indicator, and 5.0 ml. of 6N HCl. The solution should now be acid. If it is not, add more 6N HCl dropwise until it is acid (red color).

14. Neutralize the solution with 5% NaOH, adding dropwise w/10 ml. pipette until neutral. At neutral pH the solution will be a yellow color. It is advisable to run a sample on a pH meter the first time to be certain you are close to pH 7.0.

    Note: Steps 13 & 14 are to make sure pH is neutral. If pH is not in neutral range, addition of sodium acid phosphate + sodium hypochlorite will result in a cloudy or black precipitate. The sample must then be refiltered (after step 16 A) cooling) before continuing. Use Whatman #1 filter paper.

15. Add 2 gm. of sodium acid phosphate and 10 ml. of sodium hypochlorite solution to the flask. Boil on the hot plate for about one minute.

(Procedure, cont'd)

16. Add 5 ml. of sodium formate solution and boil for an additional two minutes. The solution should be clear at this point.

Note: Proceed with/Step 17 to finish one flask at a time.

17. When the sample has cooled to room temperature add 4 drops of sodium molybdate solution, 0.5 gm. of potassium iodide (or 5 ml. of 10% sol. - 10g. in 100 ml. distilled water made daily), and 25 ml. of 6 N H_2SO_4. The solution will be yellow at this point.

18. Titrate immediately using the 0.005 N sodium thiosulfate, adding ca. 20 drops of starch indicator (add rapidly) just before reaching the endpoint -- slightly yellow. The starch will turn the solution dark blue. Titrate to the end point when the solution turns clear. The solution should remain clear at least 30 seconds for a true end-point. An easy check to determine if you have over shot the end-point is the length of time it takes the solution to oxidize to blue. This time span should not be more than 2 minutes.

Note: It is important to approach the end point slowly, adding less than a drop at a time when the end point is within 0.1 ml. (pale blue solution). Also, the solution will be oxidized by air, turning the solution blue again after the end point is reached. Therefore, it is important to titrate at a relatively constant rate from the addition of the reagents in Step #17 to the end point. Also it is important to have the solution at room temperature before proceeding to Step #17.

CALCULATION:

$$\text{Ppm } Br^- = \frac{13320 \times .005 \text{ (conc. of thiosulfate standard)}}{7\text{g sample}} \times \text{mls used of thiosulfate standard}$$

$$\text{Ppm } Br^- = 9.514 \times \text{mls used.}$$

1) $S_2O_3^{2-} + I_2 + H_2O \rightleftharpoons SO_4^{2-} + 2I^- + 2H^+$

2 moles I_2 react per mole $S_2O_3^{2-}$

2) $BrO_3^- + 6I^- + 6H^+ \rightleftharpoons Br^- + 3I_2 + 3H_2O$

3 moles of I_2 react per mole Br^-

$$\frac{1 \text{ mole } I_2}{1 \text{ mole } S_2O_3^{2-}} \times \frac{1 \text{ mole } Br^-}{3 \text{ moles } I_2} \times$$

Therefore 1 mole Br^- reacts equivalently as 3 moles $S_2O_3^{2-}$

$$1 \text{ ml } S_2O_3^{2-} \times \frac{.005 \text{ m moles } S_2O_3^{2-}}{\text{ml } S_2O_3^{2-}} \times \frac{1 \text{ m mole } Br^-}{3 \text{ m moles } S_2O_3^{2-}} \times \frac{79.9 \text{ mg } Br^-}{\text{m mole } Br^-}$$

Therefore 1 ml of .005 molar $S_2O_3^{2-}$ = 133.2 mg Br^-

Calculation:

$$\frac{13320 \text{ mg } Br^-}{\text{ml } S_2O_3^{2-}} \times \frac{.005 \text{ m moles } S_2O_3^{2-}}{\text{mls } S_2O_3^{2-}} \times \frac{\text{mls used } S_2O_3^{2-}}{7 \text{ g sample}} \longrightarrow \text{ppm } Br^-$$

Precautions:

Rx: $S_2O_3^{2-} + 2H^+ \longrightarrow H_2SO_3 + S$

Stirring should be efficient to prevent local excesses of thiosulfate because it is decomposed in acid solution.

Rx: $I^- \xrightarrow{air} I_2$

Titation should be performed rapidly to minimize air oxidation of the iodide.

The two above reactions also make it necessary that samples be titrated at a constant rate and that they be done one at a time after assition of the KI, H_2SO_4, and $Na_2MoO_4 \cdot 2H_2O$ in step 17.

OFFICIAL

METHODS OF ANALYSIS

OF THE

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7. Animal Feed

7.001 Sampling (1)—Procedure

Use slotted single or double tube, or slotted tube and rod, all with pointed ends.

Take ≥500 g sample, 1 kg preferred, as follows: Lay bag horizontally and remove core diagonally from end to end. Det. number of cores as follows: From lots of 1–10 bags, sample all bags; from lot of ≥11, sample 10 bags. Take 1 core from each bag sampled, except that for lots of 1–4 bags take enough diagonal cores from each bag to total ≥5 cores. For bulk feeds draw ≥10 cores from different regions; in sampling small containers (≤10 lb) 1 package is enough. Reduce composite sample to amt required, preferably by riffling, or by mixing thoroly on clean oil-cloth or paper and quartering. Place sample in air-tight container.

A sample from less than these numbers of bags may be declared an official sample if guarantor agrees. For samples that cannot be representatively taken with probe described, use other sampling means.

7.002 Preparation of Sample
Official Final Action

Grind sample to pass sieve with circular openings $\frac{1}{25}$" (1 mm) diam. and mix thoroly. If sample cannot be ground, reduce to as fine condition as possible. Do not grind molasses feeds.

Moisture—Official Final Action

I. Drying in Vacuo at 95–100° (2)

7.003 *Determination*

Dry amt sample contg ca 2 g dry material to constant wt at 95–100° under pressure ≤100 mm Hg (ca 5 hr). For feeds with high molasses content use pressure ≤70 mm Hg. Use covered Al dish ≥50 mm diam. and ≤40 mm deep. Report loss in wt as moisture.

II. By Distillation with Toluene (3)

7.004 *Apparatus*

Connect 250 ml flask of Pyrex or other resistant glass by means of Bidwell-Sterling moisture receiver to 500 mm Liebig condenser. Calibrate receiver, 5 ml capacity, by distg known amts H_2O into graduated column, and estg column of H_2O to 0.01 ml. Clean tube and condenser with chromic acid cleaning mixt., rinse thoroly with H_2O, then alcohol, and dry in oven to prevent undue amt H_2O from adhering to inner surfaces during detn.

7.005 *Determination*

If sample is likely to bump, add dry sand to cover bottom of flask. Add enough toluene to cover sample completely (ca 75 ml). Weigh and introduce enough sample into toluene to give 2–5 ml H_2O and connect app. Fill receiving tube with toluene, pouring it thru top of condenser. Bring to boil and distil slowly, ca 2 drops/sec, until most of the H_2O passes over; then increase rate of distn to ca 4 drops/sec.

When all H_2O is apparently over, wash down condenser by pouring toluene in at top, continuing distn short time to see whether any more H_2O distils over; if it does, repeat washing-down process. If any H_2O remains in condenser, remove by brushing down with tube brush attached to Cu wire and satd with toluene, washing down condenser at same time. (Entire process is usually completed within 1 hr.) Let receiving tube come to room temp. If any drops adhere to sides of tube, force them down, using Cu wire with end wrapped with rubber band. Read vol. H_2O and calc. to %.

★ *III. Drying without Heat over Sulfuric Acid (4)* ★

7.006 *Reagent*

Sulfuric acid.—Boil H_2SO_4 in large Kjeldahl flask 4 hr, close flask with stopper carrying $CaCl_2$ tube, and cool.

7.007 *Determination*

(*Caution: See* 51.015.)

Weigh 2–5 g sample into 5–10 cm diam. metal dish with tight-fit cover. (If subsequent fat detns are to be made, fat extn cones may be used.) Mix substances that dry down to horn-like material with fat-free cotton or other suitable material. Place 200 ml of the fresh H_2SO_4 in strong, tight vac. desiccator. Place uncovered dsh in desiccator and exhaust with vac. pump to pressure ≤10 mm Hg.

If pump is not available, place 10 ml ether in small beaker in desiccator and exhaust with H_2O filter pump. Between pump and desiccator interpose empty bottle next to desiccator and bottle of H_2O next to pump. Draw air from desiccator thru the H_2O and turn desiccator stopcock the instant H_2O begins to rise in tube leading from empty bottle.

Gently rotate desiccator 4 or 5 times during first 12 hr. After 24 hr, open desiccator, bubbling incoming air thru H_2SO_4. Place cover on dish and make first weighing. After weighing, place sample in desiccator contg fresh H_2SO_4 and exhaust as before. Rotate desiccator several times during interval and weigh again after suitable drying period. Repeat process to constant wt.

★ Surplus method—*see* inside front cover.

information is needed:

1. Raw data from which table 1 was compiled.

2. Raw data from which table 2 was compiled.

3. Define inhull almonds.

4. Identify stage of development of the almonds at harvest and fumigation, handling of almonds after fumigation, composition of the almond hull animal feed, (does it include the shells)?

5. If the recommended dosage of methyl bromide is 2 lbs per 1000 cu ft for 24 hours, the EPA will require residue determinations on the almond hulls fumigated at double the dosage or 4 lbs/M cu ft.

6. Need to know the details of the fumigation procedure, use of tarpaulin, application of methyl bromide, etc.

7. Need to know more about the feeding of the hulls, proportion of the diet, seasonal or year round, etc.

8. After the above information has been supplied and reviewed, determinations will be made as to what additional data may be required by EPA for the establishment of a tolerance and the registration of a label.

Methyl Bromide - 1975 Studies

One stack of inhull almonds, of the Thompson variety, was fumigated with methyl bromide at the rate of 4 lbs/M cu ft for 24 hours to obtain the residue data necessary for inclusion in the petition to be submitted to EPA. This fumigation was conducted in the same manner as those studies made in 1974. Briefly, the stack was located on firm soil, fumigant sampling tubes and temperature probes were placed within the mass of almonds and in the airspace above the stack, a black 4 mil plastic sheet was used to cover the stack; the edges of which were held firmly to the ground around the edge of the stack

with 2" diameter canvas tubes six feet long filled with sand The methyl bromide was introduced into the air space on top of the stack through ¼" O.D. plastic tubing. One end of the tubing was attached to a methyl bromide measuring device screwed onto the pressure cylinder in which the fumigant was stored. The other end was anchored within the air space and directed into an aluminum pan to prevent the methyl bromide from splashing on the almonds while the fumigant was introduced. Samples of almonds were taken at random throughout the stack before fumigation and after fumigation for use in determining the amount of inorganic residues present in the shells, hulls and nutmeats as well as the moisture content. The stack occupied 2,662 cu ft and the total amount of methyl bromide applied was 11 lbs. Fumigant concentrations were determined at the 4 locations within the stack with a fumiscope at intervals of 1, 2, 4, 8, 12, and 24 hours after the fumigant was introduced. Temperature readings in ^{o}F were taken with a Tele-thermometer at the same locations and at the same intervals.

Hydrogen Phosphide (1975)

Procedure:

During the 1975 almond harvest season 33 stacks of inhull almonds were fumigated with hydrogen phosphide. The study was conducted in cooperation with the Superior Farming Company, Bakersfield, Calif., whose representatives Bill Duncan, Art Foster and Bill Absher made the almonds and space available for the study and supplied some of the labor. The Phostoxin Sales Inc., Alhambra, Calif., furnished the fumigant, fumigant sampling lines and fumigant concentration detection equipment.

The inhull almonds were placed in stacks on firm soil.

The study consisted of three tests. The first involved twelve stacks of Nonpareil almonds, three of which were fumigated at the rate of 165 Phostoxin pellets per 1000 cu ft, with 100 pellets and 3 stacks with 50 per 1000 cu ft. Three stacks were used as controls. The exposure period was 48 hours. The same stacks were used in the second test and the same procedure was followed. The only difference was that the exposure period was 24 hours and there were 2 sets of controls. One was placed in 3 stacks that were covered with plastic and the other set was held at ambient temperature. In the third test 9 stacks of almonds were used which consisted of Merced and Mission varieties. Three were fumigated at the rate of 50 pellets and three with 25 pellets per 1000 cu ft for 48 hours. There were also 2 sets of controls. One set was placed in stacks covered with plastic and one held at ambient temperature.

In preparing the stacks for fumigation the top of each stack was leveled and two 100 lb burlap bags filled with inhull almonds were placed in the leveled area, one at each end of the stack. These sacks created an air space on top of the stacks when the plastic sheeting was put in place.

To measure the effectiveness of these tests, the egg and pupae stages of the navel orangeworm were placed in 3 locations within each stack, fumigant concentrations and temperature readings were taken from 4 locations at intervals of 1, 2, 4, 8, 12 and 24 hours after the fumigation began during the 24 hr exposures and at 1, 2, 4, 8, 12, 24,

36 and 48 hours during the 48 hour exposures. Composite samples of inhull almonds were taken at the time of fumigation to determine the moisture content of the almonds. Approximately 600 lbs. of navel orangeworm infested inhull almonds were thoroughly mixed and divided into two equal parts. One 300 lb lot was placed in the stacks and fumigated at the highest dosage and longest exposure and the remaining lot was not fumigated. Samples of almonds were taken from each of the two lots immediately after fumigation and at three week intervals to determine if there was an increase in navel orangeworm damage to those almonds that were not fumigated.

The navel orangeworm eggs used in all three tests were obtained from insects reared at a constant temperature of 80 ± 2°F and relative humidity of 60 ± 5% RH. In tests No. 1 and 2 the eggs were older than those in test No. 3. In excess of 100 eggs were placed in each sample.

The navel orangeworm pupae were reared under the same environment as were the eggs. Those used in the tests 1 and 2 were in the late pupal stage and in test No. 3 about mid pupal stage. There were 20 pupae in each sample in tests 1 and 2 and 30 per sample in test No. 3. Fumigant concentrations were determined by Auer and Drager tubes as well as by a portable gas chromatograph and the temperature readings were obtained with a Tele-thermometer. An Ohaus moisture tester was used to determine the moisture content of the almonds. The instrument was calibrated against the standard toluene extraction procedure. Fumigant and temperature readings were obtained from 4 locations with each stack e.g., 1, 2, 3 and 4. Number 1 was in air space above stack, 2 in the top corner one foot below surface of almonds and one foot in from sides, 3 as near the center of stack as possible and 4 opposite bottom corner from 2 - one foot from bottom and one foot in from sides. Test insects were placed in locations 2, 3, and 4 in each stack.

Methyl Bromide (1974 studies)

See III above.

Methyl Bromide (1975 studies)

A copy of the original data relative to the fumigant concentrations obtained and the temperature readings are presented in the following table.

(See attached table)

During the 8, 12 and 24 hour sampling intervals duplicate fumigant samples were taken. One was analyzed using a fumiscope and the other by gas chromatography.

Hydrogen Phosphide

The available data is summarized in the tables 1, 2 and 3. In table 1 may be found the average concentration of hydrogen phosphide in ppm and temperature in °F identified by test no., length of exposure, location in stack and dosage. Table No. 2 is a summary of the mortality of egg and pupal stage of the navel orangeworm on the basis of test no., dosage of fumigant and exposure period. In table no. 3 may be found a summary of the mortality of the test insect, stages resulting from exposure to conditions existing within stacks covered with plastic sheeting and at ambient temperature and humidity.

EXPOSURE: 24 hr

Methyl bromide concentrations determined by use of a portable fumiscope. A tele-thermometer used to measure temperat

STACK No. 1

SAMPLE INTERVAL	FUMIGANT CONCENTRATION LOCATION IN STACK								TEMPERATURE LOCATION IN STACK			
	1 [1]		2 [2]		3 [3]		4 [4]		1 [1]	2 [2]	3 [3]	4
hrs.	oz./m cu.ft.		oz./m cu.ft.		oz./m cu.ft.		oz./m cu.ft.		°F	°F	°F	°F
1	38		32		45		34		116	84.5	87.5	7(
2	40		42		53		35		114	86.5	86	7(
4	48		50		76		42		100	88	85	7I
8	36.1*	43	35.6*	46	35.2*	43	37.5*	38	70	85.5	84	7I
12	38.8*	36	37.7*	42	37.1*	40	38.9*	34	62	82.5	83.5	68.
24	26.2*	28	27.4*	38	26.8*	30	27.1*	26	122	82	83	6(
TOTAL=	101.1*	233	100.7*	250	99.1*	287	103.5*	209	584	509	509	410
AVG.=	34	39	34	42	33	48	34	35	97	85	85	6(
TOTAL(GAS + Fumiscope)=	334.1		350.7		386.1		312.5					
AVG.=	37		39		43		35					

* Methyl bromide concentrations measured by gas chromatography

1] Air space above almonds.

2] Corner 1ft. below surface of almonds and 1 ft. in from side.

3] As close to center of stack as possible

DOSAGE Pellets/M cu ft	AVG. FUMIGANT CONC. Location in Stack				AVG. TEMPERATURE Location in Stack			
	1/	2/	3/	4/	1/	2/	3/	4/
	Ppm	Ppm	Ppm	Ppm	°F	°F	°F	°F
				Test No. 1				
				48 hr Exposure				
165	194	184	165	170	110	99	94	82
100	97	119	90	89	-	97	92	83
50	34	50	40	33	-	95	99	93
				Test No. 2				
				24 hr Exposure				
165	105	182	110	108	114	99	92	80
100	69	137	71	68	-	95	91	89
50	31	46	24	29	-	101	97	86
				Test No. 3				
				48 hr Exposure				
50	79	72	73	87	120	102	81	90
25	26	27	28	29	-	97	77	80
Untreated					107	100	79	80

1/ Air space above almonds
2/ Top corner 1 ft below surface of almonds and 1 ft in from side
3/ As close to center of stack as possible
4/ Opposite bottom corner from (1/) above - 1 ft in from bottom and 1 ft in from side

fumigation tests.

Test No.	Dosage Pellets/M cu ft No.	Exposure period Hrs.	Mortality of Navel Orangeworm: Eggs: Hatched No.	Eggs: Unhatched No.	Fumigated in pupal stage: Pupae: Alive No.	Pupae: Dead No.	Adults: Alive No.	Adults: Dead No.
1	165	48	0	1939	0	175	0	7[1/]
	100	48	0	1441	0	170	0	10[1/]
	50	48	0	1405	0	173	0	7[1/]
	Untreated[2/]	48	641	785	0	42	102	47
	Total			1426				191
	%			55.0%				47.0%
2	165	24	0	1274	0	180	0	0
	100	24	0	1395	0	180	0	0
	50	24	0	1250	0	180	0	2[1/]
	Untreated[2/]	24	417	719	0	67	100	13
	Total			1136				180
	%			63.0%				44.0%
	Untreated[3/]	24	769	409	0	15	181	4
	Total			1178				200
	%			35.0%				10.0%

1/ Adults emerged just before fumigation
2/ Placed in stacks covered with plastic sheeting
3/ Held at ambient temperature and relative humidity

Test No.	Dosage Pellets/M cu ft No.	Exposure period Hrs.	Mortality of Navel Orangeworm: Eggs Hatched No.	Eggs Unhatched No.	Pupae Alive No.	Pupae Dead No.	Adults Alive No.	Adults Dead No.
1	Untreated[1/]	48	641	785	0	92	102	47
	Total			1426				191
	%			55%				47.0
2	Untreated[1/]	24	417	719	0	67	100	13
	Total			1136				180
	%			63%				44.0
2	Untreated[2/]	24	769	409	0	15	181	4
	Total			1178				200
	%			35%				10.0
3	Untreated[1/]	48	576	725	0	64	190	13
	Total			1301				267
	%			56%				29.0
3	Untreated	48	822	459	0	27	239	4
	Total			1281				270
	%			36%				12.0

1/ Placed in stacks covered with plastic sheeting
2/ Held at ambient temperature and relative humidity

Test No.	Dosage Pellets/M cu ft No.	Exposure Period Hrs.	Fumigated in Pupal Stage: Eggs Hatched No.	Eggs Unhatched No.	Pupae Alive No.	Pupae Dead No.	Adults Alive No.	Adults Dead No.
3	50	48	30	1547	0	271	0	0
	Total			1577				
	%		2.0%	98				
	25	48	340	1048	0	273	0	0
	Total			1388				
			24.0	76.0				
	Untreated 2/	48	576	725	0	64	190	13
	Total			1301				267
	%			56%				29.0%
	Untreated 3/	48	822	459	0	27	239	4
	Total			1281				270
	%			36%				12.0%

2/ Placed in stacks with plastic sheeting
3/ Held at ambient temperature and relative humdity

Methyl Bromide (1974 studies)

See III above

Methyl Bromide (1975 studies)

The amount of fumigant present was about what might be expected. It was interesting to note that at the end of one hour the fumigant was well distributed throughout the stack and at the end of 24 hours at least ½ of the fumigant remained. The inorganic bromide residue analyses and the moisture determinations are not completed.

The additional information requested by the IR-4 project coordinator is being assembled and as soon as the inorganic bromide residues, resulting from the above fumigation are available, the results will be put together and submitted for inclusion in the petition.

Hydrogen Phosphide (1975 studies)

On the basis of these tests it would appear that the fumigant concentrations were quite uniform within the stacks for each dosage exposure relationship. The temperature levels were high throughout the stacks of almonds and were particularly high in the space above the almonds.

None of the eggs hatched nor did the pupae survive in tests 1 and 2. There were some dead adults, but these had emerged from the pupal stage between the time the pupae were placed in the test cages and when they were fumigated.

In test No. 3, 2% of the eggs hatched that were exposed to a dosage of 50 pellets/M cu ft for 48 hours and 24% hatched when exposed to

The study also showed that the temperatures that existed within the plastic covered stacks produced mortality in both the egg and pupa stages.

Recommendations for future work:

1. Be prepared to obtain any additional information that may be required by the Coordinator of the IR-4 program or by EPA relative to obtaining approval and a label for the use of methyl bromide as a fumigant for almond shells and hulls.

2. To conduct additional studies with hydrogen phosphide to refine the dosage requirements for the use of pellets and to evaluate a new formulation of hydrogen phosphide that does not contain ammonia.

3. Prepare a fumigation manual for use by the Almond Industry.