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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Follow-up to Methyl Bromide Registration Standard.
Post Harvest Protocol, Interim Plant Metabolism
Report, Analytical Methods, and Storage Stability.
(I.D. No. 53201-1; Record No. 222,880; RCB No.
3890) MRID No.'s 405795-01, 406078-01, 406185-01

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TO: Jeffrey Kempter, Product Manager No. 32
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and

Toxicology Branch
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The Methyl Bromide Industry Panel (MBIP) has submitted a post harvest fumigation chamber pre-study aimed at determining the type of chamber to be used in generating the residue data required to support the reregistration of methyl bromide for post harvest fumigation. An interim report on the metabolism of methyl bromide by plants (post harvest), analytical methodology for the determination of residues of methyl bromide per se and inorganic bromide, and a storage stability study of fumigated commodities were also submitted.

Summary of Deficiencies Relating to the Present Submission

- RCB needs to receive the protocol used in the metabolism studies (for further details, see RCB's Comments/Conclusions, re: Metabolism studies).
- More information is needed with regard to methyl bromide and inorganic bromide analytical methodologies (for

further details, see RCB's Comments/Conclusions, re: methyl bromide and inorganic bromide analyses).

- ° The storage stability studies for inorganic Br⁻ are not complete; validation data are needed to support the reported residue levels.
- ° The raw data, calibration curves, or sample chromatograms have not been submitted. Also, fortification/recovery data and the limit of determination for the MeBr and inorganic bromide methodologies have not been provided.

MBIP will need to study carefully RCB's Comments/Conclusions, re: post harvest residue data.

Recommendations

RCB recommends that MBIP receive an unabridged copy of this review and that MBIP resolve all issues discussed in this review.

Detailed Considerations

Deficiency as cited in the Registration Standard

Nature of the Residue in Plants

- ° Representative raw agricultural commodities, harvested according to common commercial procedures, are to be fumigated with ¹⁴C-methyl bromide at or above the maximum registered rate and under conditions comparable to those specified in the label directions. Considering the relatively brief duration of these studies, the use of [¹⁴C, ⁷⁴⁻⁹⁰Br] double-labeled methyl bromide may be feasible...In addition to methyl bromide per se and bromide ion, methylated and brominated derivatives of natural plant constituents produced upon fumigation must be characterized, particularly such products as purine and pyrimidine bases which may be mutagenic. It is advisable to combine the metabolism study with storage stability and method recovery studies as an optimal means of validating the residue data. Representative members of at least the following crop groups [as defined in 40 CFR 180.34(f)] must be tested: root and tuber vegetables, tree nuts, cereal grains (corn and a small grain), citrus fruits, pome or stone fruits, and nongrass animal feeds. It is preferred that the major crops in the above or other groups be included.

In a telecon on 2/11/88, RCB (W. Hazel) told Verne White of MBIP that MBIP did need to send in a plant metabolism protocol--even if the metabolism study was already underway. [RCB has not yet received a copy of the protocol]

MBIP's Response

MBIP had informally presented preliminary findings of a corn

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metabolism study at the 10/15/87 meeting with RCB. After fumigation with ^{14}C -methyl bromide, approximately 0.5-1% of the bound activity had been found in the DNA of the corn. The methylated bases which were identified were: 7-methyl guanine, 1-methyl adenine, 3-methyl adenine, and 3-methyl cytosine.

The Registration Standard had cited the need for studies using radioactive bromine. However, the petitioner said that the use of radioactive Br would be impractical because several half lives would elapse before the samples could be analyzed.

MBIP has submitted an interim report on the plant metabolism studies. ^{14}C -methyl bromide was used to fumigate batches of corn, wheat, oatmeal, almonds, peanuts, alfalfa, almonds, corn, oranges, apples (Granny Smith) and potatoes (Kennebec). The fumigation of beef serum albumin was also investigated; the petitioner reports that earlier work had shown that the amount of methylation appears to be directly related to protein content.

In the plant metabolism studies, the final MeBr concentration was about 48 mg/L (3 lbs ai/1000 ft³). The samples were exposed to methyl bromide for 3 days. The proposed application rate for these commodities ranges from 3-5 lbs ai/ 1000 ft³, depending on the commodity; the proposed exposure time ranges from 2-24 hours. At the end of the treatment, the samples were aerated overnight or longer.

Peels from the oranges and potatoes were removed and dried in an evacuated desiccator. The inside portion of the oranges and potatoes were homogenized in a Waring blender with enough water to produce a slurry. The homogenates were frozen and lyophilized. The corn, wheat, oatmeal, almonds, peanuts, alfalfa, dried orange peels, and potato skins were ground in a coffee bean grinder and (except for the potato skins) were extracted with ethyl ether using a Soxhlet extractor.

The treated sample (after lyophilization, drying, and/or soxhlet extraction) was heated with 1N sodium hydroxide for 5 hours in the presence of methyl methionine sulfonium bromide. Five traps were connected in series to the system; the traps contained:

1. Water (ice-cooled)
- 2 and 3. Saturated mercuric cyanide
- 4 and 5. Saturated mercuric chloride.

A slow stream of nitrogen was passed through the system. Dimethyl disulfide was treated with sodium borohydride to produce methyl mercaptan, which was carried to Trap 2 with a stream of nitrogen.

Any methanol which formed as a result of basic hydrolysis would have been trapped in Trap 1 (water). The petitioner points out that any methanol present before the alkaline hydrolysis would have been removed during the Soxhlet extraction or during the drying steps. Carrier methanol was added to Trap 1, and 3,5-dinitrobenzoyl chloride was added to form methyl 3,5-dinitro-

benzoate.

Methylmethionylsulfonium derivatives are expected to decompose to dimethyl sulfide during alkaline hydrolysis and would have been trapped in Traps 4 and 5 (saturated mercuric chloride). Traps 2 and 3 (saturated mercuric chloride) were included in the system to trap decomposition products which are less volatile than dimethyl sulfide, such as methyl mercaptan.

The alkaline solutions were then neutralized, frozen, lyophilized, and the residues were hydrolyzed with 6N HCl to hydrolyze the protein. The acid hydrolysate was subjected to ion exchange chromatography on Dowex 50-HB (H⁺). The major portion of activity was associated with basic amino acids. Re-chromatography of these fractions on the same column (NH₄⁺ form) yielded a major peak of activity corresponding to "the elution pattern of histidine" and a minor peak "eluting in an area characteristic of arginine and lysine."

The petitioner states that methylmethionine sulfonium salts and other methylated amino acids decompose during 6N HCl hydrolysis; therefore the major study of the sites of N-methylation of amino acids was performed on material which had been treated with 1N NaOH. Although some amino acids are unstable to base, histidine, which had been shown in 1955 to be the preferred site of N-methylation, is stable to alkali; the petitioner assumed that the N-methylated histidine would also be stable.

The report states, rather confusingly, "For estimation of the radioactivity in the treated commodities, the insoluble portion after acid hydrolysis and the mercury complexes of dimethyl sulfide and methyl mercaptan, samples were combusted in an R.J. Harvey Instrument Corp... oxidizer... and the ¹⁴CO₂ collected...." The distribution of activity is summarized below.

Commodity	% Initial Activity			His	Lys + Arg	% Identified
	Volatiles after MeOH	(OH ⁻) MeSH	Hydrolysis (Me) ₂ S			
Wheat	22.2	1.9	31.5			57.7
	23.3	2.7	25.1			53.6
Oatmeal	18.9	3.9	17.9			41.8
	20.6	3.5	19.6			45.9
Peanuts	23.0	7.8	22.4	16.9	3.4	73.5
	24.0	7.0	24.2	18.1	3.9	77.2
Almonds	16.5	2.3	10.7	45.4	4.4	79.3
	17.4	4.8	11.4	42.5	4.1	80.2
	15.4	3.8	11.4	48.8	3.7	83.3
Apples	29.1	13.8	29.6			76.5
	25.5	14.4	28.6			71.0

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Commodity	% Initial Activity			His	Lys + Arg	% Identified
	Volatiles after MeOH	(OH ⁻) Hydrolysis MeSH	(Me) ₂ S			
Orange pulp	26.5	17.8	23.9			76.4*
	26.3	22.4	21.2			71.4
Orange peels	17.3	15.3	23.1			56.4
	19.2	28.1	23.7			58.1**
Corn	30.8	2.8	21.6	8.5	1.6	65.3
	30.9	2.0	22.8	---	---	57.2
Alfalfa	52.0	0.3	5.6			58.7
	55.9	0.5	6.1			63.9
Potato skins	13.3	6.7	27.6			50.4
	12.5	6.5	26.8			48.5
Potato pulp	7.4	20.4	23.8			54.1
Bovine serum albumin	42.1	0.5	3.2			46.6
	41.2	0.2	4.7			46.1

* Volatiles totaled 68.2%; the entry in the table was reported by the petitioner.

** Volatiles totaled 71%; the entry in the table was reported by the petitioner.

RCB's Comments/Conclusions, re: Metabolism Studies

RCB has not yet received a copy of the protocol used in the metabolism studies. From the interim report, it appears that the metabolism studies were aimed at identifying bound radioactive residues. According to the report, the insoluble portions remaining after acid hydrolysis and precipitation of the various mercury complexes were radioassayed by combustion analysis. The metabolic profiles of the matrices were delineated in terms of "Radioactivity, % of Initial," but the report does not describe the determination of the initial activity. It appears that no samples were counted until after drying (in an evacuated desiccator), lyophilization, and/or Soxhlet extraction. Volatile residues and non-polar residues would probably be lost during these treatments.

Metabolism studies are intended to delineate the composition of the total radioactive residue (TRR); this should include the contribution of the parent as well as volatile metabolites.

The petitioner will need to modify the protocol so that the distribution and composition of the total radioactive residue can be examined. RCB suggests that the petitioner also investigate the distribution and composition of the TRR as a function of

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aeration time.

The petitioner states that determining the sites of N-methylation of amino acids was performed on material which had been treated with 1N NaOH because of decomposition problems with 6N HCl. But the alkaline hydrolysis step was followed by hydrolysis in 6N HCl for 24 hours. Since some of the methylated amino acids may not be stable to acid and/or base, RCB suggests that the petitioner demonstrate whether such acids (e.g. arginine, lysine, tryptophane, cysteine, serine, threonine, etc.) are stable to the reaction conditions used by the petitioner. Also, the petitioner describes the peaks of activity following ion exchange chromatography as eluting in regions characteristic of arginine, lysine, and histidine. Did the petitioner mean methylated arginine, lysine, and histidine? In the final report, the petitioner will need to confirm the identification of the metabolites.

MBIP has reported that the use of radioactive Br would not be practical because several half lives would elapse before analysis. A fresh batch of MeBr would be needed for each fumigation. At the meeting of 10/15/87, MBIP suggested conducting a metabolism study on corn only, using radioactive Br and determining what per cent of the Br associates with DNA vs the per cent which ends up as inorganic Br.

Considering the large number of commodities used in the metabolism studies (10), RCB understands that preparing fresh batches of Me^xBr for every fumigation would not be practical. Therefore, RCB agrees that MBIP need investigate the fate of ^xBr in one commodity only. This commodity need not be corn, if the DNA from some other pertinent crop is more easily isolable. In addition to the amount of ^xBr bound to DNA and present as inorganic Br, RCB suggests that MBIP also determine the amount of ^xBr present as MeBr and as other organic bromine residues. RCB suggests that MBIP submit a protocol for this investigation.

Upon completion of the post harvest metabolism studies, RCB will defer to TOX on the need for regulating residues other than parent and iBR (such as the methylated bases). If TOX should conclude that additional residues need to be included in the tolerance expression, further appropriate residue data may be needed.

Deficiency as cited in the Registration Standard

Residue Analytical Methods

The available methods are not adequate for data collection or the enforcement of tolerances for inorganic bromides because appropriate fortification/recovery data were not submitted...The King et al. method will be considered acceptable for enforcement of tolerances for methyl bromide per se, should they be established, once a successful method trial has been conducted...A question exists as to the validity of all residue data for bromide ion generated using any of the available methods...To validate the residue data, the following must be submitted:

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- ° Bromide analysis- Fortification/recovery data validating the methods of Shrader et al.,...and Abdalla and Lear as well as Method SSL 57.3, Method ML-AM-69-57, and the WIL 84:7 procedure. Individual values must be reported for each food/feed analyzed.
- ° Methyl bromide analysis-[The deficiencies regarding methyl bromide analyses referred to methods other than the King method and to the possible need for additional methods to determine any residues of concern identified in the plant metabolism studies.]

MBIP's Response-Methyl Bromide Analysis

MBIP has submitted two procedures, Procedure I, "MeBr by Headspace GC. Modified King Headspace Method (1)" and Procedure II, "Inorganic Bromide Analysis with Ion Selective Electrode."

A modified King headspace procedure has been successfully validated at the Beltsville lab (PP #5F3300, memo of W.J. Hazel, 5/28/87). Additional modifications were reported in the present submission. These modifications are briefly described below.

1. The petitioner specifies the amount of water which should be added to 50 g samples of various commodities before blending.
2. The lid of the blender is lined with neoprene; washers are placed on the outside of the neoprene around the bulkhead union to provide a good seal. Diagrams of the blender lid are provided.
3. The sample is blended for 3 minutes at high speed (not one minute at low speed).
4. In order to withdraw a sample of headspace, the syringe is first purged with ultrapure air, 20 ml of ultrapure air are collected in the syringe, the air is injected into the blender container, the syringe is pumped 4-5 times to mix the vapors, and the sample is extracted by allowing the internal jar pressure to push up the plunger on the syringe.
5. In order to spike samples, 20 ml of headspace is removed and retained before blending the sample. The appropriate amount of MeBr vapor is added to the jar with a second syringe, and the vacuum in the jar is satisfied with the removed headspace in the first syringe.
6. Directions are provided so that the % water in a commodity can be determined.
7. A capillary column was used (Poropak GSQ 30m x 540 um), and the instrument parameters were different from the originally reported parameters.

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RCB's Comments/Conclusions, re: Methyl Bromide Analysis

In this latest revision, the oven temperature to be used and the amount of water to be added to the sample depend upon the commodity. Although MBIP has provided instructions on determining the water content of the sample, the analyst is not told what should be done with this information until the end of Procedure II, which describes inorganic bromide analysis. MBIP will need to provide a summary at the beginning to explain that methyl bromide and inorganic bromide (iBr) may be determined successively but that the water content of the sample must be known before the bromide content can be determined. MBIP will also need to submit fortification/recovery data and sample chromatograms to validate the residue data and describe any precautions taken during maceration of the sample to prevent loss of the volatile MeBr. RCB suggests that the loss of MeBr during maceration could be minimized by maintaining a temperature of <3.6°C (MeBr bp) during this process; spiked samples should be macerated and analyzed to determine whether significant amounts of MeBr are lost during the maceration procedure which is used.

If the requested validation data are adequate, RCB will recommend that the modifications be published in the Pesticide Analytical Manual, Vol II, as a letter method.

Inorganic Bromide Analysis

After a headspace sample has been successfully injected for the determination of MeBr, the blended sample is transferred to centrifuge bottles and permitted to stand for 25 minutes. The samples are centrifuged (2000 rpm) and 50 ml of the supernatant is poured into plastic beakers. One milliliter of 5M NaNO₃ is added to each beaker along with a stir bar. Before the analysis of each sample, the Double junction electrode (Scientific Products H3725-2) is calibrated using two working standard solutions of KBr. The instrument reading is then taken for the sample. An aliquot of working standard is added to the sample, and the instrument reading is again taken. Two more aliquots of standard are added, and the reading is taken after each addition. The concentration of added Br⁻ is plotted on the x-axis vs the meter reading (ppm based on external standard). The per cent recovery is calculated from the slope of the least squares line through the 4 data points. The actual solution concentration is the negative of the concentration intercept on the x-axis.

The inorganic Br⁻ concentration is calculated from the following equation:

$$\text{Br}^- \text{ (ppm)} = \text{sol'n conc.} \times \left[\frac{\text{ml H}_2\text{O added} + \% \text{ H}_2\text{O}}{\text{wt of sample}} \right] \times \frac{100}{\% \text{ recovery}}$$

RCB's Comments/Conclusions, re: Inorganic Bromide Analysis

The description of the methodology should specify if the ion

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selective electrode can be attached to an ordinary pH meter.

The description of the analysis of bromide should be rewritten so that the operations and calculations are more comprehensible.

The current instructions refer to both the standards and the test material as "samples." When standards are being measured, they should be referred to as standards. The current directions say to "Add 1 ml of working standard #1 to sample beaker"; confusion may arise because both standards and test materials are called samples.

The directions should clarify that the determination of bromide is carried out by first measuring the test sample, adding three consecutive aliquots of standard to the test sample, and plotting the 4 resulting points vs the concentration of added Br⁻. RCB suggests that it would be helpful to provide an explanatory summary preceding the step-by-step instructions. A sample calculation of Br⁻ should be included in the revised version to illustrate the use of the graph and the equation. The MBIP should verify that the submitted equation is correct.

The limit of determination was not specified. MBIP should provide the limit of determination and should support the claimed limit of determination with appropriate fortification and recovery data. Without this information, RCB cannot judge the adequacy of the method for the collection of data.

If the validation data are adequate, RCB will recommend that the ion selective electrode method be published in PAM II as a letter method, after MBIP has rewritten the method so that the instructions can be more easily understood.

Deficiency as cited in the Registration Standard

[The Registration Standard concluded that the storage stability data for methyl bromide per se is adequate. The data indicated that methyl bromide residues are lost when stored in ziplock plastic bags. The Registration Standard concluded therefore that analyses must be conducted as soon as possible (perhaps within 12 hours) after sampling and/or that samples must be stored in impermeable containers. If stored in leakproof containers, analysis of headspace samples as well as the sample itself may be required if preliminary studies indicate that a significant amount of methyl bromide in the treated sample volatilizes into the gaseous phase during a typical storage period. The Standard stated that a problem may arise in estimating the volume of the gaseous phase once the sample has been introduced. Spiked samples of each crop should be handled exactly like the treated samples to determine the loss between treatment and analysis.]

No data are available concerning the storage stability of the

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inorganic Br⁻ residues. The following data are required:

- Representative fumigated and/or spiked commodities must be analyzed for iBr at various intervals following the end of the aeration time prescribed on registered labels. The end of aeration will represent time zero. Untreated controls should be included as a means of determining background levels of iBr. A validated method of analysis must be used.
- All residue data (iBr and methyl bromide per se) in this Standard must be accompanied by data regarding storage length and conditions of storage of samples analyzed. These data must be accompanied by data depicting stability of residues under the conditions and specified intervals.

A letter to V. White of MBIP from J. Kempter (5/8/87) stated that an acceptable storage to analysis interval would be one in which at least 80-90% of the MeBr present at collection remains.

MBIP Response

MBIP has submitted a storage stability study involving walnuts, rice, and strawberries. The walnuts and rice were fumigated at a rate of 3 lbs ai/1000 ft³ for 24 hours (proposed use from PP #5F3300, tree nuts-3.5 lbs ai/1000 ft³, 24 hrs exposure; cereal grain-3 lbs ai/1000 ft³, 24 hrs exposure). Strawberries were fumigated at a rate of 3 lbs ai/1000 ft³ for 4 hrs (proposed rate from PP #5F3300, 1-3 lbs ai/1000 ft³ for 3-4 hrs).

The walnuts and rice were aerated for 24 hours, including 2 hours of forced aeration in the chamber with the fan on. The strawberries were aerated for one hour (forced aeration). The samples were then placed in glass canning jars equipped with rubber ring metal lids, and the jars were placed in ice chests containing dry ice. The commodities were sampled at time 0, 8, 16, 24, and 48 hours.

Separate sets of jars were used for each sampling time; 4 replicates were analyzed for each interval. The jars were removed from the ice chests, and the samples were weighed out and analyzed for MeBr (modified King procedure, see page 7) and inorganic bromide (selective ion procedure, see page 8). Because of the high residue levels of MeBr in walnuts and strawberries (> 220 ppm for walnuts; >50 ppm for strawberries), it was necessary to use a flame ionization detector instead of an electron capture detector. Instead of using the Poropak GSQ column (30 m x 540 um), the registrant used a column described as "an 8' x 118" stainless steel column packed with 5% silicone Dow 200 on poropak 100/120."

The methyl bromide residue data are tabulated below. All control samples exhibited <0.01 ppm.

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Methyl Bromide (ppm)

Storage time	0 Hr	8 Hr	16 Hr	24 Hr	48 Hr
Walnuts	150-183	131-204	186-221	162-172	175-196
Avg	168	176	207	167	187
Rice	0.58-0.70	0.44-0.54	0.46-0.51	0.40-0.51	0.40-0.52
Avg	0.63	0.49	0.49	0.44	0.46
Strawberries	34.3-39.5	44.1-55.1	30.6-44.8	33.0-42.4	29.4-46.0 (44 Hr)
Avg	36.4	49.4	40.1	37.5	35.4 (44 Hr)

The inorganic bromide residue data are tabulated below.

Inorganic Bromide (ppm)

Storage time	0 Hr	8 Hr	16 Hr	24 Hr	48 Hr
Walnuts	63.0-90.4	58.0-69.3	66.4-78.6	56.6-73.1	60.8-86.1
Avg	75.2	64.9	71.2	68.0	76.4
Check	7.7-25.0				
Rice	16.2-29.2	24.9-32.8	24.2-57.51	38.8-49.2	20.2-25.1
Avg	24.4	23.7	39.2	45.4	22.8
Check	2.3-13.3				
Strawberries	27.1-37.8	48.4-53.5	53.9-70.8	45.5-62.1	29.4-46.0 (44 Hr)
Avg	33.1	52.4	60.5	53.2	35.4 (44 Hr)
Check	13.4-21.7				

RCB's Comments/Conclusions

No recovery data or standard curves were submitted. Before RCB can reach any conclusion on the adequacy of the methods, MBIP will need to provide fortification and recovery data for the determination of both methyl bromide and inorganic bromide.

However, the submitted data appear to indicate that MeBr residues do not dissipate significantly from strawberries and walnuts during 48 hours of storage at dry ice temperatures. MeBr residues on rice do appear to dissipate; MeBr levels 8, 16, 24, and 48 hours after treatment averaged 78, 78, 70, and 73% respectively of the initial residue level. Since rice is much more finely divided than the other commodities, dissipation of the residues is not surprising. The data also indicate that 80-90% recoveries after storage will not be obtainable with rice. The headspace over the stored rice samples was not analyzed so that it was not possible to determine whether MeBr residues had desorbed from the rice into the headspace. Since the loss amounted to 30%, at

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most, RCB does not consider analysis of the headspace in the storage jars to be necessary for rice.

Since residue levels appeared to degrade with rice, RCB emphasizes that for the residue trials it will be necessary to generate a decline curve to cover the storage period for each commodity. Storage stability data covering the interval from sampling to analysis are generally required. In this particular case, involving a gaseous analyte, RCB is extremely reluctant to translate data from one commodity to another. The rate of dispersion of the gas from the samples could be governed by the amount of wax (natural or applied) on the surface, the surface to volume ratios, maturity of the commodities, storage time before fumigation, or other unforeseen parameters.

RCB notes that the chosen commodities, rice, strawberries, and walnuts, may not have required maceration before analysis. If the samples were macerated, RCB needs to know what precautions were taken to minimize the loss of MeBr during maceration. Although the samples selected for these storage stability studies may not have required maceration, many other commodities, such as grapefruit, cantaloupes, etc., will obviously need maceration.

The column used to carry out the methyl bromide analyses was described as "an 8' x 1/8" stainless steel column..." RCB assumes that MBIP meant 1/8 inch, but this should be verified.

MBIP also needs to explain why it was necessary to use a packed column with an FID for the storage stability study, when even higher levels of MeBr were determined using the usual capillary column with EC detection in the study entitled, "Post Harvest Chamber Fumigation Pre-study Using Methyl Bromide as a Fumigant." Both the storage stability study and the fumigation chamber study were conducted by Bolsa Research Associates.

Deficiency as cited in the Registration Standard

The Registration Standard cited the need for residue data reflecting pre-plant and/or post-harvest uses for the following crop groups:

- Root and Tuber Vegetables
- Leafy Vegetables (except Brassica)
- Brassica
- Legume Vegetables (succulent and dried)
- Fruiting Vegetables (except Cucurbits)
- Cucurbits
- Citrus Fruit
- Pome Fruit
- Small Fruit and Berries
- Tree Nuts
- Cereal Grains*

Grass Forage, Fodder, and Hay (no currently
registered use)
Nongrass Animal Feeds*
Herbs and Spices

Residue data on the following miscellaneous crops are also needed:

Asparagus
Avocados*
Cocoa Beans*
Coffee Beans*
Copra*
Cottonseed*
Mangoes (no currently registered use)
Okra*
Papayas (no currently registered use)
Peanuts*
Pineapples
Pistachio nuts*
Pomegranates (no currently registered use)

* Residue data reflecting post-harvest treatments only; otherwise residue data reflecting both post-harvest and pre-plant fumigation are required.

Residue data on the representative crops from the various crop groups are required. If processed commodities are associated with raw agricultural commodities, usually a processing study is needed. However, if no detectable residues of MeBr result after exaggerated treatments, processing studies may not be required. The exaggerated treatment rates should reflect the theoretical concentration factor; e.g., if there are no detectable residues on corn following a 25 X treatment rate (the theoretical concentration factor for corn oil), then a corn processing study probably will not be required. The Registration Standard also emphasized that if multiple treatments are allowed under the proposed uses, then residue data reflecting multiple treatments will be required.

RCB did not consider the label statement requiring that the commodity be analyzed for inorganic bromide before retreatment, or if the treatment history were unknown, to be practical. Therefore, MBIP needs to submit either a more practical label restriction or residue data reflecting the greatest number of treatments expected.

Also, the Registration Standard stipulated that the post-harvest fumigation studies should reflect the various commercial practices (tarpaulin fumigation, chamber fumigation, vacuum chamber fumigation, etc.).

[The label submitted with PP #5F3300 had categorized all the uses according to crop groupings. Several registered uses of methyl bromide were thereby omitted. If it is MBIP's intent to support

applications to asparagus, peanuts, copra, cottonseed, cocoa beans (and processed commodities), okra, pineapples, and pistachios, appropriate residue data will need to be submitted for these commodities. See PP #5F3300, memo of W. Hazel, 2/19/86]

[RCB pointed out that the dosage rates and/or exposure times in the use proposed in PP #5F3300 differed from the uses previously registered for a number of crops. These discrepancies appeared to arise because of the use of crop group application rates on the label submitted with PP #5F3300. The affected individual crops were: carrots, Jerusalem artichoke, rutabaga, potato, yam, salsify, sweet potato, corn, popcorn, sweet corn, sorghum, barley, grapes, cucumbers, squash (winter and summer), succini, peas (particularly succulent peas), pimentos, peppers, eggplant, and tomatoes (see memo of W. Hazel, PP #5F3300, 2/19/86). MBIP should verify that the use proposed in PP #5F3300 represents the intended use rate. Dosage ranges were given for the small fruit and berries group and for the legume vegetables group. MBIP should explain whether these ranges apply to all members of each group.]

MBIP's Response

MBIP has submitted a pre-study, aimed at determining what type of fumigation equipment should be used. Four types of enclosures were investigated: a plastic tarpaulin, a wood walled room, a modified intermodal container, and an intermodal container modified as a vacuum chamber.

The tarp chamber consisted of PVC pipe covered with 4 mil black plastic. The black plastic was taped to the cement floor with silver duct tape. The temperature during fumigation ranged from 65-80°F. The chamber measured 8'x 7'x 6'.

The walls and ceiling of the room were constructed with 2"x6" studs, which were covered with Visqueen. The Visqueen was covered with 1/2" plywood. The floor of the room was concrete. The junctions of the wall were sealed with caulking. The door consisted of a plastic tarpaulin 1/32" thick. The tarp edges were sealed with 2"x6" studs wedged into place with wooden wedges. The bottom edge of the tarp was sealed to the floor with sand snakes and loose sand. The dimensions of the chamber were 22'x 22'x 14'. The temperature outside the room was 70°F during fumigation.

The fumigation vault was constructed of 1/4" thick steel. The inside walls of the container were lined with 3/8" exterior plywood. The floor consisted of 1 3/4" marine plywood. The interior of the vault had been coated with epoxy paint. The dimensions of this intermodal container were 8' x 8' x 20'.

The vacuum chamber consisted of an intermodal container which had been modified; an innerwall of 3/8" steel was attached to the wall of the container with 10" I-beams. The space between inner

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and outer walls was filled with R-9 insulation. The outside of the chamber was covered with aluminum siding. The dimensions of the inner chamber were 19' x 6'7" x 6'7". A vacuum equaling 26 inches of mercury (660 mm Hg) was pulled on the chamber prior to introduction of the MeBr. The chamber was filled and evacuated with air 4 times before opening the chamber for removal of the fumigated commodities for aeration.

Walnuts, wheat grain, potatoes, and carrots were placed in each of the fumigation enclosures and fumigated at 3.5 lb. per 100 ft³ for 24 hours. The proposed application rates are:

Walnuts	3.5 lbs ai/1000 ft ³	24 hr exposure
Wheat	3.0 lbs ai/1000 ft ³	24 hr exposure
Potatoes	3.0 lbs ai/1000 ft ³	4 hr exposure
Carrots	3.0 lbs ai/1000 ft ³	4 hr exposure

As soon as it was safe to remove the commodities, the samples were taken to the Bolsa Research Associates laboratory, where they were left to aerate in an area enclosed by a wooden fence. Aeration was unassisted. The following aeration periods were observed:

Walnuts	24.5 hours
Wheat	24 hours
Potato	23.5 hours
Carrot	23 hours

The samples were analyzed at the end of the aeration period for methyl bromide and inorganic bromide. The decrease in levels of methyl bromide inside the fumigation enclosures within a 24 hour period were determined as a measure of chamber tightness. The tightness of the vacuum chamber was measured by how well it maintained a vacuum.

The loss of methyl bromide or a vacuum from the various fumigation enclosures is tabulated below.

Chamber	Initial MeBr Level (ppm)	Final MeBr Level (ppm) t = 24 hr	% Lost
Tarp	5847	1376	76.5
Room	13816	9951	28.0
Vault	12336	9766	20.8
	Initial Vacuum	Final Vacuum	
Vacuum	26 in. Hg	20.75 in. Hg	20.2

The methyl bromide levels found in commodities fumigated in the various chambers are tabulated below. All check samples exhibited MeBr levels of <0.01 ppm.

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Commodity	Mean MeBr Residue Level (ppm)			
	Tarp	Room	Vault	Vacuum
Carrots	10.14	146.9	160.2	173.0
Rel. Std. Dev.	52%	24%	17%	22%
Potatoes	0.011	130.8	296.4	403.6
Rel. Std. Dev.	31%	19%	10%	11%
Wheat	0.498	5.79	5.35	14.42
Rel. Std. Dev.	55%	32%	30%	43%
Walnuts	243.4	551.3	559.5	819.5
Rel. Std. Dev.	18%	17%	14%	8%

The inorganic bromide (iBr) levels as a function of fumigation enclosure are given below.

Commodity	Mean iBr Residue Level (ppm)				
	Tarp	Room	Vault	Vacuum	Check
Carrots	39.36	57.27	41.21	56.16	9.50
Rel. Std. Dev.	8%	11%	13%	13%	12%
Potatoes	21.00	48.42	67.92	59.34	17.26
Rel. Std. Dev.	5%	27%	15%	12%	18%
Wheat	29.57	60.18	35.80	53.03	20.54
Rel. Std. Dev.	5%	10%	11%	6%	13%
Walnuts	88.70	133.7	98.00	166.8	32.61
Rel. Std. Dev.	7%	27%	11%	4%	10%

MBIP concluded that the MeBr residue levels tended to increase as the tightness of the chamber increased; the highest residue levels for all four commodities were found with vacuum chamber fumigation.

Inorganic bromide residues were highest in 2 out of the 4 samples fumigated in the vacuum chamber.

Although the vacuum chamber represented the worst case regarding MeBr residue levels in the commodities tested, MBIP contends that MeBr residues are statistically the same for the vault and vacuum chamber fumigations for most of the crops analyzed. Because of the limited use of commercial vacuum fumigation chambers, MBIP intends to use the vault type of chamber for generating the requisite residue data.

RCB's Comments/Conclusions

MBIP has not submitted the raw data, the calibration curves, or

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sample chromatograms to validate the residue studies. Also, fortification/recovery data and the limit of determination for the MeBr and bromide methodologies have not been provided. However, the submitted data (average values, based on an unspecified number of replicates) clearly indicate that the vacuum chamber represents the worst case, with respect to MeBr residue levels. Therefore, MBIP will need to submit residue data on those crops which may be subjected to vacuum fumigation.

The Registration Standard had required residue data reflecting vacuum chamber fumigation for those crops for which this use was registered. However, the label submitted with PP #5F3300 does not specify which commodities may be fumigated in vacuum chambers, although a footnote to Table 1 does refer the applicator to the APHIS treatment manual for additional rates and commodities. The manual does not recommend vacuum fumigation for all the commodities listed in the table.

If it is the petitioner's intent to permit vacuum chamber fumigation for all commodities in Table 1, then residue data reflecting vacuum chamber fumigation are required for these commodities. Since MBIP contends that vacuum fumigation is of limited use, MBIP has the options of revising the label to restrict vacuum fumigation to only those crops, such as tree nuts, where vacuum fumigation is of use, and generating appropriate residue data on those crops, or of eliminating vacuum chamber treatment from the label altogether.

In addition to using the vault and, where appropriate, the vacuum chamber to generate the residue data, some data reflecting the fumigation of trucks, trailers, or vans should be submitted. Data in RCB's files indicate that residues may be higher after the fumigation of trucks.

MBIP should also consider the following factors when generating residue data.

1. RCB's guidelines as put forth in its review of the almond protocol (memo of W. Hazel, 11/3/87), apply to all residue tests. The tests should be conducted at maximum label rates and represent actual commercial fumigation events in all respects, such as MeBr introduction, temperature, humidity, air circulation, packaging, load factor, and aeration and storage conditions. For example, grapes may be packaged in lugs containing wood shavings, which, according to the APHIS plant protection manual, are highly sorbent. Also, many commodities are stored cold after fumigation. Moreover, the residue data should reflect the range of temperatures expected during fumigation, or MBIP should demonstrate that the fumigation temperatures chosen represent the worst case. RCB notes that the APHIS manual uses lower rates with higher fumigation temperatures, but there is no tie-in of the rate and the fumigation temperature on the label submitted

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with PP #F3300.

2. After fumigation, MeBr levels should be monitored in various parts of the loaded vault before sampling. Load factors typical of commercial operations should be used.
3. According to United Fresh Fruit and Vegetables, many commodities are sometimes waxed, such as apples, avocados, bell peppers, cantaloupes, cucumbers, eggplants, citrus, melons, parsnips, passion fruit, peaches, pineapples, pumpkins, rutabagas, squash, sweet, potatoes, tomatoes, and turnips. Dan Botts (Florida Fruit and Vegetables) contacted the Indian River Citrus League and learned that citrus may be washed and waxed before fumigation. Therefore residue data of waxed and unwaxed commodities should be generated where appropriate.
4. The residue data should encompass a range of sizes of a commodity when appropriate. For example, data on both tomatoes and cherry tomatoes should be generated. If MeBr residues adhere to the surface, higher levels could result on the cherry tomatoes.
5. Residue data reflecting multiple applications are required when appropriate. MBIP will need to explain how it determined the number of applications for each commodity.
6. If certain commodities are generally stored before fumigation, some of the residue data should reflect representative storage periods and temperatures before fumigation.

It has been reported in the literature that the storage temperature prior to fumigation may effect the amount of fumigant absorbed by the commodity [W. B. Sinclair and D.L. Lindgren, "Factors Affecting the Fumigation of Food Commodities for Insect Control," J. Econ. Entomology, 51 (6): 891-900 (1958).]
7. If certain commodities are generally picked green, the residue data should reflect residues in both green and mature fruit. Sinclair and Lindgren (see above) also reported that the amount of fumigant sorbed by the commodity could depend upon its stage of maturity.
8. The use of MeBr in grain elevators could lead to higher residue levels in grain dust than in the grain itself. Grain dust is a cattle feed item. Therefore residue data on grain dust is also required. [At the time the Registration Standard was written, RCB was not aware of the potential for concentration of residues in the grain dust.]
9. The residue data should reflect the analyses of a representa-

tive proportion of bruised or stemless commodities. Data in RCB's files indicate that certain fumigant levels are higher in such fruit.

10. RCB reiterates that if tolerances are proposed on the basis of residue levels following a period of aeration, MBIP will need to demonstrate that the aeration period is appropriate (i.e., that the commodity will not be available for sampling by the FDA before the aeration period has elapsed).
11. After fumigating samples contained in packing cartons, samples to be analyzed should be selected from various sections of the cartons. Data contained in RCB's files indicate that samples from different sections of a package may contain different residue levels following fumigation.
12. The aeration temperatures should be specified. RCB suggests that the coolest feasible temperatures for each commodity be investigated. MBIP has the option of revising the label to specify a minimum aeration temperature if it can demonstrate that such a label restriction is practical.
13. MBIP should heed RCB's comments contained in previous memos and in the Registration Standard regarding the generation of residue data for post harvest use.

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