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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Follow-up to Methyl Bromide Registration Standard.
Amendment of 2/9/89. Revised Plant Metabolism
Protocols (DEB #5001)

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Background

The registrant, the Methyl Bromide Industry Panel (MBIP), has submitted interim metabolism reports, which have been reviewed in DEB's memos of 7/14/88, 11/3/88, and 2/9/89. At the 11/10/88 meeting with the registrant, DEB also discussed the need for characterizing the total radioactive residue (TRR) and for determining whether residues such as 5-bromouracil are present.

Summaries of DEB's Comments, re: Revised Protocol

A more detailed account of DEB's Comments may be found in the main body of this review.

1. If the data indicate that 5-BrU may be present, DEB recommends that confirmation be sought by a method which is basically

different from the method which originally detected 5-BrU.

2. If there is little variation in residue levels from replicate fumigations, the ppm MeBr from the cold treatments could be added to the ppm of chemically bound MeBr equivalents from the previously submitted metabolism studies, to yield an estimate of the TRR and the MeBr contribution to the TRR. The ppm in both cases should be based on the weight of the commodities before lyophilization and/or extraction.
3. If there is significant variation between replicate fumigations, the metabolism studies may need to be repeated in order to determine the contribution of the parent to the TRR.
4. DEB emphasizes that the commodities in the cold and hot experiments must be treated in exactly the same manner, if the MeBr levels from the cold studies are to be added to the bound residues estimated from the hot studies.
5. The residue data may be generated by the Heuser-Scudamore method, as long as the method is validated by recovery data, and chromatograms.
6. When the ¹⁴C MeBr studies are repeated, the commodities should be dosed at the same level as in the other hot and cold experiments. Samples from these studies should also be analyzed for MeBr by GC before being ground, defatted, and combusted. In this way, the contribution of MeBr to the TRR can be directly measured.
7. MBIP should heed DEB's Comments contained in previous memos and in the Registration Standard regarding metabolism studies. MBIP is especially referred to DEB's memo of 2/9/89, which reviewed interim reports and articles. This review cited 15 issues which should be considered in delineating the nature of the residue.

Recommendations

DEB recommends that the protocols be modified to take into account the issues contained in DEB's Comments/Conclusions in this review. Where appropriate, the registrant should also consider DEB's Comments/Conclusions from previous memos and the Registration Standard. MBIP is especially referred to DEB's memo of 2/9/89, which cited 15 issues which should be considered in delineating the nature of the residue.

Background--5-Bromouracil

The Methyl Bromide Registration Standard had stipulated that the registrant should determine whether brominated purines and

pyrimidines such as 5-bromouracil (5-BrU) arise from the post-harvest use. The registrant argued that the short half-life of radioactive bromine precluded labeling studies (amendment of 9/22/88), and DEB agreed. DEB suggested that the registrant use some other means to determine whether 5-BrU is a terminal residue.

At the meeting of 11/10/88, Dr. A. Starratt (Agriculture Canada), who is conducting the metabolism studies, said that it might be possible to analyze for 5-BrU with HPLC and electrochemical detection.

Revised Protocol for 5-Bromouracil

It won't be possible to use electrochemical detection for the analysis of 5-BrU; the required potential is too high. Other HPLC methods have been described in the literature; one article reports a sensitivity of 20 ng.

Wheat grain, potatoes, and yeast RNA will be fumigated with MeBr; the yeast RNA will serve to provide experience and as a basis for comparison. Uracil from nucleic acids and from free base and nucleoside pools will be isolated and examined for the presence of 5-BrU. The nucleic acids will be isolated by methods which the registrant has used previously. Check samples will be spiked with 5-BrU to ascertain that 5-BrU in the presence of plant constituents can be detected with an appropriate degree of sensitivity. The registrant will examine the stability of 5-BrU towards the hydrolytic conditions necessary to liberate this residue. If evidence for the formation of 5-BrU is found, its presence will be confirmed by using other HPLC systems, GC, or GC/MS.

Precise details of the protocol cannot be given, as the exact approach followed will depend upon the circumstances encountered.

DEB's Comments/Conclusions

The protocol described by the registrant represents the best approach for determining whether 5-BrU is formed, given the impracticality of using radiolabeled Br.

If the data indicate that 5-BrU may be present, DEB recommends that confirmation be sought by a method which is basically different from the method which originally detected 5-BrU. That is, GC or MS may be used to confirm the presence of 5-BrU, if that residue were originally found by HPLC. However, NP-HPLC may be used to confirm the presence of a residue detected by RP-HPLC.

Background--Accounting for Total ¹⁴C Radioactive Residue

In its 7/14/88 review (memo of C. Deyrup), DEB emphasized that metabolism studies should attempt to account for the total radioactive residue (TRR), not just the chemically bound residues. Since the commodities treated with ¹⁴C MeBr had been lyophilized

or soxhlet extracted before counting, only chemically bound ^{14}C had been investigated.

At the meeting of 11/10/88, DEB discussed the plant metabolism studies further with Dr. Starratt, who agreed to determine the contribution that MeBr per se and other volatile residues make to the TRR and has submitted a revised protocol.

Revised Protocol--Accounting for Total ^{14}C Radioactive Residue

The same kinds of commodities treated in the previously submitted metabolism studies will be treated with unlabeled methyl bromide in the same manner as before. After fumigation, the commodities will be aerated for one hour in a hood at room temperature. Samples taken at that time, and 1, 2, 4, and 10 days after treatment, will be analyzed for MeBr, methanol, and methyl chloride. The analytical results reflecting a one hour aeration period, together with the previously generated data on chemically bound residues, would approximate the total residue. Earlier work has shown that the level of chemically bound residues remains stable over a 6-month period.

MeBr residues will be determined by the Scudamore and Heuser method, rather than by the King headspace procedure, because the laboratory has had a great deal of experience with the former method, and because that method is better suited for the analysis of small sample amounts.

Corn, almonds, and apples will be treated with ^{14}C MeBr; samples taken one hour, 4 days, and 10 days following treatment will be ground and defatted as in the previously submitted metabolism studies. The total radioactive residue, corresponding to the level of chemically bound residues, will then be determined by combustion.

DEB's Comments

The registrant had submitted an interim metabolism report with the amendment of 11/17/88. The review of this amendment (2/9/89, memo of C. Deyrup) contains observations which are relevant to the present submission.

DEB stated, "... it may not be necessary to repeat all the radiolabeled metabolism studies. DEB suggests that the registrant fumigate the various commodities with MeBr (unlabeled); the fumigations should exactly mimic the conditions used in the metabolism studies. As recommended at the 11/10/88 meeting, the commodities should be aerated for 1-2 hours, since similar aeration periods are encountered in commercial practice. The levels of MeBr could then be determined by the King headspace method. It would be necessary to determine MeBr levels from several fumigations so that a coefficient of variation could be determined. If there is little variation

in residue levels from replicate fumigations, the ppm MeBr could be added to the ppm of chemically bound MeBr equivalents to yield an estimate of the TRR and the MeBr contribution to the TRR. The ppm in both cases should be based on the weight of the commodities before lyophilization and/or extraction."

"If there is significant variation between replicate fumigations, the metabolism studies will need to be repeated in order to determine the contribution of the parent to the TRR."

DEB emphasizes that the commodities in the cold and hot experiments must be treated in exactly the same manner, if the MeBr levels from the cold studies are to be added to the bound residues estimated from the hot studies; this includes aeration times. It would not be appropriate to add MeBr levels from commodities which have been aired for 1 hour to the TRR from commodities aired for 10 hours, or stored for 6 months. In any case, according to data presented in the amendment of 11/17/88, the level of bound residues appeared to increase with storage time at room temperature.

The residue data may be generated by the Heuser-Scudamore method, as long as the method is validated by recovery data, and chromatograms.

In the new studies using ¹⁴C MeBr, the commodities should be dosed at the same level as in the other hot and cold experiments. Samples also should be analyzed for MeBr by GC before being ground, defatted, and combusted. In this way, the contribution of MeBr to the TRR can be directly measured. [The registrant may prefer to use the King headspace method for these analyses in order to minimize ¹⁴C contamination of the equipment.]

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