



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OCT 16 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#4F3148/FAP#4H5443 - Bayleton on Tomatoes; and
PP#5F3224/FAP#5H5458 - Baytan on Wheat and Grapes
[No RCB Number]. Evaluation of Method Trial Results
(K. Zee memos of April 4, 1985, and September 3,
1985).

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[Signature]

RCB had requested that EPA's Analytical Chemistry Laboratory (ACS, COB, BUD) run method trials for Mobay Chemical Corporation methods designed to quantitate residues of Bayleton and its metabolites Baytan (KWG-0519), KWG-1323, and KWG-1342 (also a metabolite of Baytan) in tomatoes (Method No. 80488) and milk (Method No. 69531) (see M. Firestone memo of November 7, 1984, re: PP#4F3148/FAP#4H5443).

I. Method No. 80488 - Plant Commodities Analysis:

A. Principles of Analysis:

Bayleton and metabolites are extracted from tomatoes by a methanol-water mixture which is filtered to remove solids. The filtrate is evaporated to the aqueous phase which is incubated in a 37 °C water bath for 16 to 18

hours with a cellulase enzyme solution. After incubation, Bayleton and the metabolites are then extracted with dichloromethane (DCM). The DCM extract is evaporated to dryness and the residue is dissolved in chloroform and cleaned up by gel permeation chromatography and florisil chromatography. After the florisil column cleanup, KWG 1342 and KWG 1323 fractions are derivatized to an ester by trifluoroacetic anhydride. The ester is analyzed by GLC with a N/P detector. Bayleton and KWG 0519 can be measured directly by GC without derivatization.

B. Modifications to Method No. 80488:

According to the Analytical Chemistry Section (ACS) memo of April 4, 1985:

In November of 1984, ACS received a July 27, 1983 revised edition of the method which was tried without success. The company submitted a January 24, 1985 revision in order "to clarify description of gel permeation column (p. 3) and to clarify the stated purpose for addition of Appendix IV." The revision was received by ACS on or about February 12, 1985, and other than taking three days to analyze a set of four samples, gave satisfactory recoveries.

According to the ACS memo of September 3, 1985:

We tried to use Du Pont's HPLC 8000 instrument instead of ABC's gel permeation instrument as specified in the method. HPLC instrument could not do the work. We had to borrow Mobay's gel permeation instrument to complete the trial.

C. Results:

All control values are reported as < 0.01 part per million (ppm). Recoveries from tomato samples fortified at 0.05 and 0.20 ppm each with Bayleton, Baytan, KWG-1323, and KWG-1342 are tabulated below:

Chemical	% Recovery - Range	(Average)
Bayleton	80-100	85
Baytan	75- 80	79
KWG-1323	80- 80	80
KWG-1342	80- 95	87

D. RCB's Comments/Conclusions re: Method No. 80488:

RCB concludes that Mobay Method No. 80488 is adequate for enforcement of the proposed tolerances for residues

of Bayleton and its metabolites in tomatoes (see Deficiency 2b re: PP#4F3148/FAP#4H5443) and Baytan and its metabolite in grapes and wheat (see Deficiency 4a re: PP#5F3224/FAP#5H5448).

However, in lieu of the modifications cited above, the petitioner will need to submit four copies of the final (January 24, 1985) revision of Mobay Method No. 80488 as tried out by EPA's method trial laboratory. This revised method will be submitted to FDA for inclusion in PAM-II.

Upon receipt of the January 24, 1985 revised Method No. 80488, RCB could conclude that Deficiencies 2b and 4a of PP#4F3148/FAP#4H5443 and PP#5F3224/FAP#5H5448, respectively, are considered resolved.

II. Method No. 69531 - Animal Commodities Analysis:

A. Principles of Analysis:

Bayleton and its metabolites are extracted from milk using methanol in a Polytron blender. After vacuum filtration and rotary evaporation, the extract is cleaned up by passing it through XAD-4 ion exchange resin. Elute the column with methanol and concentrate by rotary evaporation. The residue is hydrolyzed by refluxing with concentrated hydrochloric acid, which converts the compounds to p-chlorophenol. The resulting phenol is steam distilled and further cleaned up by acid/base partitioning. An internal standard of 3,4-dichlorophenol is added before it is derivatized by 2,4-dinitrofluorobenzene. The samples are assayed using gas chromatography/mass spectrometry, monitoring the ions 127 and 161 AMU.

B. Modifications to Method No. 69531:

There are no reported modifications to Mobay Method No. 69531 dated June 6, 1981.

C. Results:

All controls are reported as < 0.01 ppm. Recoveries from milk samples fortified at 0.04 and 0.10 ppm each with Bayleton, Baytan, KWG-1323, and KWG-1342 are tabulated below:

Chemical	% Recovery - Range	(Average)
Bayleton	88-107	96
Baytan	66-112	89
KWG-1342	79-88	84
KWG-1323	70-97	84

D. Other Considerations re: Method No. 69531:

ACS has previously run a method trial using Method No. 69531 in which beef liver and egg samples were fortified (see E. Greer, Jr. memo of November 29, 1982, re: PP#1F2474/FAP#1H5292). The following comments were reported:

1. Method appears to be satisfactory and the recoveries at the requested fortification levels were generally very good.
2. It is highly suggested that an antifoam reagent (Dow-C was used in this laboratory) be added in steps IB-3 for eggs and IIB-4 for liver and eggs to facilitate rotary vacuum evaporation. Excessive foaming was encountered in the absence of antifoam, and evaporation step IIB-4 took in excess of 8 hours. This change was made with the concurrence of a representative of Mobay Chemical Corporation.
3. Interferences with the internal standard peak were observed on the standard and sample chromatograms during the egg analyses. These could have been introduced by one or more of the many reagents used in the method.
4. The optional alumina column cleanup was determined to be unnecessary.

RCB previously concluded that this method was acceptable for enforcement purposes (see A. Smith memo of December 16, 1982, re: PP#2F2665).

RCB's Comments/Conclusions re: Method No. 69531:

RCB reiterates its earlier conclusion that adequate methods are available for enforcement of Bayleton/Baytan tolerances for animal commodities, thus, Deficiencies 2c of PP#4F3148/FAP#4H5443 and 4b of PP#5F3224/FAP#5H5458 are now considered adequately resolved.

Copies of Mobay Method No. 69531 dated June 8, 1981, will be submitted to the editors of PAM if this was not done previously in conjunction with PP#2F2665.

III. Recommendation:

RCB continues to recommend against the Bayleton tolerances for tomatoes and its processed fractions proposed in PP#4F3148/FAP#4H5443 because of the numerous deficiencies pending (see Deficiencies 2a, 4a, 4b, 4c, 4d, and 5 as cited in M. Firestone memo of March 20, 1985).

RCB continues to recommend against the Baytan tolerances for wheat, grapes, their processed fractions, and animal commodities proposed in conjunction with PP#5F3224/FAP#5H5448 for the reasons cited under Conclusions 1a, 1c, 2a, 2b, 5b, 5c (i-vi), 5d (i-iv), and 6 of M. Firestone memo dated June 6, 1985.

The petitioner will need to submit four copies of the January 24, 1985 revision of Method No. 80488 to RCB for inclusion in PAM-II.

cc: R.F., Circu, MPFIRESTONE, EAB, EEB, PP#4F3148, FDA
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