File: PP #2F1218

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PP #2F1218 (FAP #2H5004). Benomyl on grapes.
Amendment of 1/31/73.

Coordination Branch
and Toxicology Branch, RD

In our original evaluation (see memo of Dr. R. J. Hummel, 5/12/72), we stated that for a favorable recommendation, we would require the following:

1. Additional data indicating whether STB and BUB are formed in tank mixes, in crops (grapes and raisins) and in alkaline soils.

2. Residue data for the feed by-products, dried grape pomace and raisin waste.

3. A poultry feeding study.

In response, the petitioner has submitted both residue data for grapes, grape pomace, and raisin waste and a poultry feeding study and has amended Section B by including a label restriction against tank mixing benomyl with alkaline pesticides such as basic copper sulfate, Bordeaux mixture, or lime sulfur. In addition, a tolerance of 0.1 ppm for residues of benomyl and its metabolites containing the benzimidazole moiety in eggs, meat, fat, and meat by-products of poultry and a food additive tolerance of 70 ppm for residues in dried grape pomace have been proposed.

Analytical Method

In the proposed method of analysis for grape pomace and raisin waste, residues of benomyl and its two primary metabolites, methyl 2-benzimidazole carbamate (MBC) and 2-aminobenzimidazole (2-AB), are determined as 2-AB and expressed as ppm benomyl. The method used is the fluorometric procedure of Pease and Holt, JAOAC, 54, 1399 (1971) which was discussed in detail in our original review. The following validation data for benomyl are submitted :

Substrate	Fortification Level (ppm)	Recovery (%)	Blanks (ppm)
Wet Grape Pomace	0.1-2.0	60-90(av. 74)	0.1-0.3
Raisin Waste	0.13, 5.0	140, 89(av. 115)	0.2

No validation data are submitted for dried grape pomace; however, based on the wet pomace data, we expect that recoveries of benomyl from dried pomace will be adequate.

No recovery data are presented for the possible benomyl metabolites from raisin waste and grape pomace. However, recoveries of MBC averaged 84% from beans and sugar beets (PP #1F1010) and 56% from celery at fortification levels of 0.2-10 ppm. Recoveries of 2-AB from peaches, celery, muskmelons, and bananas ranged from 50-72% at fortification levels of 0.2-2 ppm. We would expect similar recoveries of these metabolites from raisin waste and grape pomace.

We conclude that the fluorometric procedure, as modified by AMS, is adequate to enforce tolerances on dried grape pomace and raisin waste.

Residue data for the benomyl conversion product STB are obtained using a liquid chromatographic procedure with a sensitivity of 0.1 ppm. Recoveries from grapes at fortification levels of 0.1-1 ppm range from 75-120% (av. 90%) and from wet grape pomace at fortification levels of 0.2-1 ppm, from 50-120% (av. 77%). As discussed under Residue Data, STB is not considered to be a component of terminal plant residues.

The proposed method of analysis for poultry tissues and eggs is a modified version of the liquid chromatographic procedure used to determine residues of benomyl and its animal metabolites in beef tissues and milk. This method has been discussed in detail in connection with PP #1F1010 (see memo of W. J. Boodee, 3/31/71).

The method consists of an initial hydrolysis of the sample in aqueous acid to convert residues of benomyl to its metabolite MBC followed by extraction into ethyl acetate and cleanup by solvent-solvent partition. Residues of 4-hydroxy MBC, 5-hydroxy MBC, and combined benomyl/MBC are determined by cation exchange liquid chromatography as three separate peaks.

This method, with modifications, has been successfully tested by AMS on milk and beef liver. Adequate recoveries were obtained with benomyl at the 0.1 ppm level (blanks <0.02 ppm) and with 4-hydroxy MBC and 5-hydroxy MBC at the 0.05 ppm level (blanks <0.01 ppm). The method, as modified by AMS, determines residues of 4- and 5-hydroxy MBC at an inlet pressure of 500 psi and in a separate injection, determines combined residues of benomyl/MBC at 1800 psi.

The following validation data are submitted:

Commodity	Fortification Level (ppm)	Recoveries (%) benomyl/MBC	4-OH MBC	5-OH MBC
Eggs	0.02-0.2	80-100	40-80	60-70
Breast	0.02-0.2	54-85	45-100	42-100
Fat	0.04, 0.2	105, 85	35, 50	70, 74
Liver	0.04, 0.2	70, 88	85, 88	__, 70*

	Blanks (ppm) Benomyl/MBC	4-OH MBC	5-OH MBC
Eggs	<0.02	<0.02	<0.02
Breast	<0.02	<0.02	<0.02
Fat	<0.02	<0.02	<0.02
Liver	<0.02	<0.02	0.06

*Corrected for blanks.

Recoveries of 4- and 5-hydroxy MBC are somewhat low and variable. The probable cause of the low recoveries is the occurrence of these peaks on the tail (or base of the tail) of the previousby eluting solvent peak. This same problem was encountered in the petitioner's meat and milk studies and similar variable recoveries were reported. However, in the method tryout by AMS, recoveries in milk and beef liver were consistent and all above 70%.

Therefore, we conclude that recoveries from poultry tissues and eggs will be adequate and that the method is suitable to enforce the proposed tolerances for eggs and poultry tissues other than liver. Due to the high control value for 5-OH MBC in liver, we do not consider the method adequate to enforce a tolerance of 0.1 ppm in liver. The interfering material in liver appears as a well defined peak with the same retention time as 5-OH MBC. Apparent residues of 5-OH MBC in liver from the two feeding levels are reported as 0.06 and 0.09 ppm and may be due entirely to interference. Assuming that the interfering peak is due to a natural constituent of liver rather than to sample contamination, additional methodology with improved cleanup will be necessary for a favorable recommendation.

Residue Data

In the course of gathering grape samples for preparation of grape pomace and raisin waste, additional data reflecting residues of benomyl on grapes were obtained. These data support our previous conclusion that residues of benomyl in or on grapes resulting from the proposed use will not exceed the proposed tolerance of 10 ppm.

Grapes containing benomyl residues of 0.12-3.7 ppm were analyzed for residues of STB. No detectable residues (<0.1 ppm) were found. No data for STB residues in raisins are submitted. However, since ca. 99% of the grapes dried for raisins are sun-dried in the field under the same climatic conditions that they are grown, we would not expect residues of STB to form on drying grapes to raisins. There are no data for residues of BUB on grapes or raisins; however, on the basis of recently submitted data, we have concluded (PP #1F1033, Hummel, Reed, and Gee, 10/25/72) that STB and BUB will not be formed in tank mixes, plants, postharvest dips and sprays, and soils.

Grapes bearing aged benomyl residues of 0.12-3 ppm were pressed for juice. The pressing processes were not described but it was stated that three samples of wet pomace were prepared commercially and five samples were prepared in the laboratory. Benomyl residues in the wet pomace ranged from 0.26-6 ppm and were 1.4-2.8X those on the grapes. No detectable residues of STB (<0.1 ppm) were found in wet pomace. The juice fractions were not analyzed. However, since benomyl residues concentrate in the pomace, we can conclude that benomyl residues in the juice fractions (grape juice or wine) will not exceed those on grapes.

No residue data are submitted for dried grape pomace. However, since the percentage moisture in each sample of wet pomace is given, it is possible to calculate the residue level on dried pomace. Assuming a dried pomace of 5% moisture and no loss of benomyl residues on drying, the concentration factors for benomyl residues in going from grapes to dried pomace range from 3.7-12.5. Therefore, we conclude that the proposed food additive tolerance of 70 ppm (7X the proposed grape tolerance) is not adequate to cover benomyl residues in dried grape pomace. A tolerance level of 125 ppm would be more appropriate.

Data submitted in connection with PP #1F1033 indicate that benomyl can be converted to STB on heating. When an aqueous slurry of a 50% benomyl wettable powder was stirred at a constant temperature of 50°C (122°F), conversions of benomyl to STB of 1, 4, 15, and 47% were reported at 0, 1, 6, and 25 hours, respectively. No detectable amount of BUB (<0.1%) was found after 25 hours and only a trace amount (0.7%) after 49 hours. Since wet pomace is dried at elevated temperatures, typically 212-280°F, there is the possibility that residues of benomyl are converted to STB during the drying process. (On the basis of the above thermal data, we expect that conversion of benomyl residues to BUB on drying would be negligible.) We relayed this concern to the petitioner (see memo of conference, P. Chichilo, 8/3/72) and he indicated that residue data for STB on dried grape pomace would be obtained. However, no such data are submitted. In order to determine if STB will be a component of the residue in dried grape pomace, we will require data showing whether residues of benomyl are converted to STB during the commercial drying of wet pomace.

Residue data are submitted for three samples of raisin waste. Following two applications of benomyl at 0.66X the maximum proposed rate, benomyl residues on raisin waste are 4.6, 2.8, and 1.4 ppm at PHI's of 20, 29, and 32 days, respectively. Only in one of these studies were residue data obtained for the corresponding grapes. In that, grapes contained benomyl residues of 0.12 ppm and the resulting raisin waste, 4.6 ppm. Thus, this single study would indicate that benomyl residues increase by a factor of ca. 40 in going from grapes to raisin waste. This factor seems unusually large and we are reluctant to base a food additive tolerance on this single value.

No food additive tolerance for raisin waste has been proposed. We will require additional residue data for raisin waste and an appropriate food additive tolerance if necessary.

Meat and Milk

In our original evaluation, we were unable to draw any conclusions about secondary residues in meat and milk due to the absence of residue data for dried grape pomace and raisin waste. From the feed items with established tolerances for benomyl, it was recently concluded (PP #1F1033, F. D. R. Gee, 1/6/72) that the maximum daily intake of benomyl residues by cattle would result from a diet consisting of 1/3 dried apple pomace containing 70 ppm, 1/3 sugar beet tops containing 15 ppm, and concentrates containing <1 ppm. A tolerance of 0.1 ppm was established to cover residues of benomyl and its metabolites containing the benzimidazole moiety (calculated as benomyl) in meat and milk resulting from the feeding of this diet, which contains total benomyl residues of ca. 30 ppm.

Dried grape pomace may comprise up to 20% of the diet of beef and dairy cattle and may contain maximum benomyl residues of 125 ppm. Thus, the feeding of dried grape pomace would contribute maximum benomyl residues of 25 ppm to the diet. Although the residue data for raisin waste are still inadequate, raisin waste normally constitutes only 5% of the cattle diet and, relative to dried grape pomace, would not be expected to be a major contributor of benomyl residues to the diet. Grape by-products and dried apple pomace are not likely to be fed simultaneously to cattle. The substitution of dried grape pomace (at 20%) for dried apple pomace (at 33%) in the composite diet above results in no change in the dietary intake of benomyl residues.

Therefore, we conclude that the feeding of dried grape pomace and raisin waste will not increase benomyl residues in meat and milk to levels higher than the established tolerance of 0.1 ppm.

Poultry and Eggs

Three groups of 8 laying hens each were fed diets containing benomyl at levels of 0,5 and 25 ppm for 28 days. Samples of eggs and feces were taken at weekly intervals and composited for each group. Four hens from each group were sacrificed either at the end of the feeding period or after a withdrawal period of one week and composite samples of liver, fat, muscle, and skin collected. All samples were analyzed for residues of 4-hydroxy MBC, 5-hydroxy MBC, and benomyl/MBC by a liquid chromatographic procedure.

No detectable residues (<0.02 ppm) of 4-hydroxy MBC or benomyl/MBC were found in poultry tissues and eggs. Detectable residues of 5-hydroxy MBC were found in eggs at the 25 ppm level (0.03-0.06 ppm) but were not found (<0.02 ppm) at the 5 ppm level or at the end of the withdrawal period. Apparent residues of 5-hydroxy MBC are reported as 0.06 ppm in liver samples from the control study, the 5 ppm feeding study and the withdrawal study and as 0.09 ppm in the liver sample from the 25 ppm feeding study. As noted in the Analytical Method, we are unable to determine what part, if any, of these apparent residues are attributable to real residues of 5-hydroxy MBC.

Dried grape pomace is the only poultry feed item with established or proposed tolerances for residues of benomyl. Since it may constitute up to 5% of the poultry diet, the maximum daily intake of benomyl residues will be 0.05 x 125 ppm or <u>ca</u>. 6 ppm. We consider the feeding at this level to be Category 2 of Section 180.6(a) with respect to both poultry tissues and eggs.

We conclude that the proposed tolerance of 0.1 ppm will be adequate to cover residues of benomyl and its metabolites containing the benzimidazole moiety (calculated as benomyl) in eggs and poultry tissue other than liver. We are unable to determine the adequacy of the proposed tolerance for residues in liver due to deficiencies in the proposed method of analysis.

Other Considerations

The benomyl conversion product STB contains the benzimidazole moiety but is not considered to be an animal metabolite of benomyl. Our conclusions regarding the adequacy of the tolerances for meat, milk, poultry, and eggs are based on the presently known residues of benomyl. The finding of significant residues of STB in dried grape pomace may alter these conclusions.

Conclusions

1a. The proposed analytical methods are adequate to enforce tolerances on dried grape pomace, raisin waste, eggs, and poultry tissues other than liver.

b. The proposed analytical method is not adequate to enforce a tolerance on poultry liver. Additional methodology with improved cleanup is necessary.

2a. The proposed food additive tolerance of 70 ppm is not adequate to cover benomyl residues in dried grape pomace. A tolerance of 125 ppm would be more appropriate.

b. The residue data for raisin waste are inadequate for us to determine the appropriate tolerance level. We will require additional residue data for raisin waste and an appropriate food additive tolerance, if necessary.

c. The level of benomyl residues in grape juice and wine will not exceed that in grapes.

3a. STB and BUB will not be components of the terminal residue in grapes, raisins, and wet grape pomace. BUB will not be a component of the terminal residues in dried grape pomace.

b. Benomyl can be converted to STB on heating. In order to determine if STB will be a component of the residue in dried grape pomace, we will require data indicating whether residues of benomyl are converted to STB during the commercial drying of wet pomace.

4a. Residues in meat and milk will not exceed the established tolerance of 0.1 ppm.

b. The proposed tolerance of 0.1 ppm will be adequate to cover residues of benomyl and its metabolites containing the benzimidazole moiety (calculated as benomyl) in eggs and poultry tissue other than liver. We are unable to make a conclusion concerning the adequacy of the proposed 0.1 ppm tolerance for residues in liver due to deficiencies in the proposed method of analysis.

5. Our conclusions regarding the adequacy of the tolerances for meat, milk, poultry, and eggs are based on the presently known residues of benomyl. The finding of significant residues of STB in dried grape pomace may alter these conclusions.

Recommendations

For the reasons stated in Conclusions 1b, 2a, 2b, 3b, and 4b, we recommend that theproposed tolerances not be established.

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