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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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SUBJECT: FAP#1H5282. Bayleton on apple and grape byproducts. Evaluation of the analytical method and residue data

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Mobay Chemical Corporation, Agricultural Chemicals Division proposes the establishment of temporary tolerances for residues of the fungicide Bayleton [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone] and its metabolite, -(4-chlorophenoxy)- -(1,1-dimethylethyl)-1H-1,2,4-triazol-1-ethanol (KWG 0519) in wet apple pomace at 4 ppm, dry apple pomace at 2 ppm, raisin trash at 7 ppm, wet grape pomace at 2.5 ppm, dried grape pomace at 3 ppm and grape juice and wine at 2 ppm.

Temporary tolerances of 1 ppm for residues of Bayleton in or on apples and grapes were recently established pursuant to PP#0G2300. These tolerances will expire 12/31/82. The current EUP is limited to fruit intended for the fresh market only. PP#1G2432 proposing 0.1 ppm tolerances for residues in or on wheat and barley is currently pending.

Conclusions

1a. The fate of Bayleton in apples and grapes has been adequately delineated for the purpose of the proposed temporary tolerances. The residues of concern are Bayleton and its metabolite KWG 0519. For a future permanent tolerance for residues on grapes an additional grape metabolism study in which about 90% of the activity remaining on the grape is identified or characterized will be required.

1b. We can consider Bayleton, per se, and its metabolite KWG 0519, the residues of concern for the purpose of the proposed temporary tolerances. However, for any future permanent tolerances the animal metabolites KWG 0519 acid, KWG 1342 and KWG 1323 will have to be included in the tolerance expression for meat, milk, poultry and eggs (see attachment for structures).

2. Adequate methodology and validation data have been submitted to demonstrate that residues of Bayleton, per se, and its metabolite KWG 0519 can be determined in wet and dried apple and grape pomaces, raisins, and raisin waste.

3a. The additional data reflecting the residue levels in or on fresh apples and grapes presented in this submission support our previous conclusion that the established tolerances are appropriate. These additional studies now provide adequate geographical representation for the grape use.

3b. The available apple processing data demonstrate that residues of Bayleton, per se, and its metabolite KWG 0519 in wet and dried apple pomace will not exceed the respective proposed tolerances for these commodities. However, we recommend that the higher level of 4 ppm be established for both wet and dried pomace to prevent unnecessary proliferation of tolerance levels.

3c. Two grape processing studies are available and their results are conflicting. The first study indicates no concentration of residues in grape juice or wine while the second study indicates a 2X concentration in grape juice. The second study also indicates a 2X concentration in wet grape pomace, which is not consistent with finding increased levels in grape juice. For the purpose of this temporary tolerance proposal only, we are willing to conclude the processing data indicate that the proposed tolerances for residues in grape juice, wet grape pomace, dried grape pomace and raisin waste are needed. However, to avoid unnecessary proliferation of tolerance levels, we recommend that the higher of the two grape pomace tolerances be established for both commodities. No food additive tolerance is recommended for wine because the Agency does not have the legal authority to set pesticide tolerances for alcoholic beverages.

4. The available animal feeding data demonstrate that the proposed use falls under Category 2 of Section 180.6 (a). Therefore, temporary tolerance proposals (in an amendment to PP#0G2300) for residues of Bayleton in meat, milk, poultry and eggs will be required. The available feeding data indicate that a level of 0.01 ppm would be appropriate.

Recommendations

1. We recommend against the establishment of the proposed tolerances for the reason cited in Conclusions #3b, #3c and #4.

2. For a favorable recommendation the following will be required:

   1) Deletion of the proposed food additive tolerance for residues in wine.

   2) Proposal of a 4 ppm food additive tolerance for residues in apple pomace instead of the separate levels now proposed for wet and dry apple pomaces.

   3) Proposal of a 3 ppm food additive tolerance for residues in grape pomace instead of the separate levels now proposed for wet and dry grape pomaces.

   4) Proposal of temporary tolerances of 0.01 ppm in milk, eggs and meat byproducts of cattle, goats, hogs, horses, poultry and sheep.

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3. For a future permanent tolerance the following will also be required:

1) A more detailed description of the manufacturing process indicating the solvent medium, the base used and any other significant reaction parameters.

2) A grape radiotracer metabolism study in which about 90% of the activity remaining in the grape is identified or characterized.

3) Inclusion of the animal metabolites designated KWG 0519 acid, KWG 1342 and KWG 1323 in the tolerance for meat, milk, poultry and eggs.

4) Analytical methodology and validation data for the parent compound in poultry and for both the free and conjugated forms of the animal metabolites in meat, milk, poultry and eggs.

5) Additional bovine feeding data reflecting the levels of the free and conjugated animal metabolites that result in tissues and milk from the ingestion of Bayleton and KWG 0519.

6) Poultry feeding reflecting the levels of Bayleton, per se, and the free and conjugated animal metabolites in poultry tissues and eggs.

7) Possibly the proposal of higher tolerance levels to cover the levels of metabolites in meat, milk, poultry and eggs.

Formulation

Bayleton is to be formulated as a 50% wettable powder containing:

[redacted]

The inert ingredients of the proposed formulation are all cleared under Section 180.1001(c) or (d).

The ingredients of Technical Bayleton are listed as follows:

1. Bayleton 87% minimum

[redacted]

INERT INGREDIENT INFORMATION IS NOT INCLUDED

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

For the purpose of the proposed temporary tolerances we anticipate no residue problems resulting from the impurities present in the technical product at the above levels.

A summary of the manufacturing process stating that [redacted] was submitted in support of PP#0G2300. We reiterate our previous conclusion that for the purpose of a temporary tolerance, this description of the manufacturing process is adequate. However, for a future permanent tolerance, a more detailed description indicating the solvent medium, the base used and any other significant reaction parameters will be required:

Proposed Use

Experimental Use Permits (3125-EUP-165 and 3125-EUP-169) have been granted for the use of Bayleton on apples and grapes. Bayleton may be applied to apples at rates ranging from 0.25 to 1 lbs per 100 gallons of spray solution. A maximum of 0.5 lb. a.i. per acre may be applied per application, and not more than a total of 2 lbs. a.i. per acre are to be applied in a single crop season. There is no PHI. A restriction against the feeding or grazing of treated cover crops has been imposed.

Bayleton may also be applied to grapes at rates of 1 to 3 oz. a.i. per acre. At least 20 gallons of spray solution for ground applications and 10 gallons of spray solution for aerial applications are required. Applications may be repeated as needed up to a total of 9 oz. a.i. per acre per crop season.

The uses cited here are the same as those previously accepted pursant PP#0G2300 except that the fresh market only limitations have been deleted.

Nature of the Residue

Plants:

The fate of Bayleton on apples was investigated in a radiotracer study using both a benzene ring and triazole ring labeled compounds. A total of 82 apples on a single apple tree was treated individually at the rate of 15 mg/100 ml (which is equivalent to 2 oz./100 gal, or 2 times the maximum proposed rate). The tree was covered by a polyethylene tent throughout the study. Samples of the apples treated with triazole labeled material were taken 1 hour and 3, 7, 14, 21, 28, 35, 42 and 49 days after treatment, while additional benzene ring labeled samples were collected at 1 hour and 7, 14 and 35 days post-treatment.

The apples were initially subjected to several benzene rinses. The rinses were collected and analyzed. The apples were then separated into peel and pulp portions and ground with dry ice. Subsamples of each portion were analyzed for total activity.

The peel and pulp samples were analyzed by the following procedure: Two 50-gram subsamples of pulp (12 grams for peel) were separately blended with acetone-water (2:1, 100 ml and 50 ml, respectively) and filtered. The remaining solids were reblended with 150 ml chloroform and filtered. The filtrates were combined in a separatory funnel and partitioned. The water phase, designated as aqueous I, was radioassayed. The lower organic phase was passed through sodium sulfate and evaporated to dryness. The residue was redissolved in hexane and partitioned with acetonitrile (ACN). The lower phase (ACN) was drained into a second separatory funnel containing hexane and repartitioned. The process was repeated with another pass of ACN. The ACN fractions were combined, evaporated and redissolved in 2-5 ml of chloroform (organic I) and subjected to TLC. Negligible amounts of ^{14}C were found in the hexane fractions.

The solids from above (solid I) were reblended in 70% methanol and filtered. The filtrate was evaporated to water and labeled aqueous II.

Subsamples of the solids (solid II) wer combusted to determine residue levels and subjected to further analysis where ^{14}C levels were high enough.

For the identification of additional activity, aqueous II fractions were partitioned with chloroform:acetone (2:1), both fractions radioassayed and the organic phase subjected to TLC. Peel solids were further analyzed by a 2-hour reflux in 70% methanol, then filtered. The filtrate was evaporated to remove the methanol. The resulting solution (aqueous IV) was partitioned with chloroform:acetone as before. The remaining water phase (aqueous V) was radioassayed, the organic phase (organic III) was evaporated to dryness, the residue redissolved in 2-5 ml chloroform and analyzed by TLC.

The aqueous and solid fractions were subjected to both acid and enzymatic hydrolysis and analyzed by TLC or combusted to determine total activity.

The thin-layer chromatography was performed on silica gel plates using the following solvent systems:

(2:1)	Benzene: Ethyl acetate
(10:5:4:1)	Ethyl acetate: methylene chloride: toluene: ethanol
(1:1)	Benzene: Ethyl acetate

Total activity in the apples decreased from approximately 0.83 ppm on day 0 to 0.15 on day 21. No further decline in total activity occurred after day 21. Growth dilution only accounted for a small portion of the decline because the average increase in weight of the course of the experiment was 2%. Therefore, the data indicate that volatilization of the Bayleton residues is a principal source of residue decline. The data also show very similar levels for the two labeled Bayletons, thus indicating that the metabolites contain both the benzene and triazole rings.

Initially about 83% of the total residue of 0.83 ppm was solublized with the benzene washes, but after 28 days only 5 to 10% of the activity was removed by the benzene. On day zero about 90% of the activity present was parent compound. After 14 days Bayleton per se accounted for about

40 to 50% of the total activity. The organo-soluble fractions accounted for virtually all the activity on day 0; however this level declined steadily to 74% during the first four weeks and then remained virtually constant for the duration of the experiment. The aqueous soluble fraction accounted for no more than 11% of the activity present throughout the experiment. The portion of activity that remained unextracted ranged as high as 7% on day 49. On day 49 Bayleton accounted for about 13% of the 0.15 ppm remaining. The principal metabolite, 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazole-1-yl)-2-butanone (trade name KWG0519) comprised an additional 49% of the activity. A third metabolite that represented 4-5% of the residue was not identified. The hydrolysis studies yield small amounts of parent compound and KWG 0519. No attempt was made to characterize the aqueous soluble compounds.

In conclusion, a level of activity corresponding to approximately 0.15 ppm Bayleton remained 49 days after the application of 2 times the maximum proposed rate. Approximately 13% of this activity was Bayleton per se and an additional 51% was determined to be the principle metabolite KWG 0519. The remaining activity was only characterized as unidentified organo-soluble metabolites (18%), aqueous soluble metabolites (11%) and unextractable activity (7%). We consider the fate of Bayleton on plants adequately delineated to support the proposed temporary tolerances. However, for the future permanent tolerances a radiotracer study on grapes, which identifies or characterizes about 90% of the residue will be required.

Animals:

A single oral dose of 25 mg ring labeled ^{14}C Bayleton/KG body weight was administered to two male and two female rats. Approximately 80% of the activity was excreted after 7 days. A variety of metabolites were observed in both urine and feces. Residues in fat tissues ranged as high as 45 ppm and peaked 4-8 hours after treatment. Residue levels in other tissues peaked earlier and with lower concentrations. At seven days the highest reported values were found in liver tissue at levels up to 0.14 ppm. G enerally Bayleton, per se, and its metabolite KWG0519 accounted for 60-75% of the activity in tissues.

A radiolabeled cow feeding study using a benzene ring labeled and a triazole ring labeled ^{14}C Bayleton was conducted to determine the fate of Bayleton in ruminants. The benzene ring labeled material was administered in a single dose at the rate of 0.14 mg/kg while the triazole ring labeled material was fed for five days at the rate of 10 mg/kg.

In the chronic study samples of blood, urine, feces and milk were taken daily before each dose and eight hours later and the animal sacrificed thirty minutes after the last dose. In the single dose study, the levels of labeled Bayleton in the blood were monitored and the animals sacrificed within the ten minutes after the observed peak. Samples of liver, kidney, fat, and muscle tissues were taken in both experiments. The samples were subjected to solvent extraction, enzyme hydrolysis and thin layer and gas-liquid chromatography. The levels of activity were determined by either a liquid scintillation counter or a radiochromatogram scanner.

The cow given the single dose excreted 50% of the dose in seven hours and 87% of the dose in 3 days. Only a small portion (6.6%) of the activity was detected in the feces. Bayleton showed little or no tendency to concentrate in tissues. It was rapidly absorbed into the blood stream, conjugated and removed from circulation by the kidneys. Glucuronic acid conjugates of both KWG 1323 and KWG 1342 (see the chart of metabolites attached) comprised most of the activity found in urine. The residue levels in the tissues from this treatment were so low they could not be identified. An additional animal was dosed at 10 mg/kg but sacrificed just after residue levels in the blood reached a maximum level. This experiment produced levels as high as 15 ppm in kidney, 4 ppm. In fat, 3.7 ppm in liver, and 0.36 ppm in muscle tissue. Three principal metabolites were detected: KWG 1323, KWG 1342 and KWG 0519 acid. However no information is reported on their relative quantities or the portion of the total residue they represent.

Similar studies were conducted with male and female pigs. Essentially the same metabolic pathways were found in the pig as in the cow. The pigs were dosed at 5 mg/kg in both the single and multiple dose studies. The animals were sacrificed just after residue levels in the blood reached maximum values (3 hours after the last dose). Tissue residue levels ranged as high as 4 ppm in kidney, 3.1 ppm in liver, 1 ppm in fat and 0.4 ppm in muscle. Bayleton, per se, accounted for only 0.6%, 3.4% and 24% of the activity in kidney, liver and fat respectively. The same metabolites found in bovine tissues were also found in the porcine tissues. None of the metabolites accounted for any more than 36% of the residue in any tissue. KWG 0519 was the principal metabolite in liver and fat, while KWG 1323 accounted forthe largest portion of the residue in kidney. Approximately 80% of the activity activity in kidney and fat and 70% of the activity in liver was identified. The levels found in muscle tissue were too low to characterized.

In conclusion the three animal metabolism studies demonstrate that Bayleton is rapidly metabolized and excreted with little or no tendency to concentrate in tissues. We consider the fate of Bayleton in animals adequately delineated for the purpose of the proposed temporary tolerances. KWG 0519, KWG 0519 acid, KWG 1323 and KWG 1342 are the principal metabolites found in animal tissues. Considering the relatively low burden of residues expected in the diet of livestock ingesting feed items processed from treated apples and grapes. We are willing for the purpose of a temporary tolerance only to conclude that Bayleton, per se, and KWG0519 are the residues of concern. For a future permanent tolerance, the above animal metabolites will have to be included in the tolerance expression.

Analytical Methods

A gas chromatographic method for the determination of Bayleton on apples has been developed and is presented in Report No. 54166. The method involves grinding the apples with dry ice; extraction with acetone and methylene chloride; filtration of the extract; partitioning of the extract against a sodium chloride solution; evaporation of the organic layer to dryness; florisil column chromatography and determination of Bayleton, per se and metabolite, KWG 0519, with a gas chromatograph equipped with a thermionic detector.

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The validation data for apples includes samples of various varieties of apples and apple products including wet and dried pomace. Recoveries from samles fortified with both compounds at levels ranging from 0.05 to 0.10 ppm Bayleton ranged from 72 to 110% and averaged 91.6% with a standard deviation of 9.3%. Control values were generally less than 0.01 ppm.

Validation data have also been submitted for the use of the proposed method on grapes and grape products have also been submitted. Recoveries of residues of Bayleton, per se, and KWG0519 from samples of grapes, grape juice and wine raisins, raisin waste, wet gr ape pomace and dried grape pomace fortified at levels of 0.05 ppm and 0.1 ppm ranged from 71 to 112 and averaged 88.6%. Control values were reported as less than 0.01 ppm.

An analytical method for the determination of Bayleton and its metabolite, KWG 0519, in animal tissues has been developed and is presented in Report 68705. The tissues a e extracted with methanol using a Polytron blender, and after vacuum filtration and evaporation of the solvent, the residue is partitioned between hexane and acetonirile. Further clean-up is achieved using methylene chloride-water partition and Florisil column chromatography. After concentration, the extract is analyzed by GLC employing a alkali flame ionization detector in the nitrogen sensitive mode.

With this method, recoveries of Bayleton and KWG 0519 from liver, kidney, muscle and fat samples fortified at 0.1 and 0.05 ppm ranged from 76-116% and 64-90%, respectively. All control values were reported as <0.01 ppm. The control peaks actually corresponded to levels approaching 0.002 ppm. We consider this analytical procedure suitable for enforcement of a 0.01 ppm tolerance for residues of Bayleton and KWG 0519 in meat, fat and meat byproducts.

Recoveries of Bayleton and KWG 519 from milk samples fortified at 0.1 and 0.05 ppm ranged from 79-89% and 80-88%, respectively. All of the control values were reported as <0.001 ppm. The chromatograms of milk control samples did not show any peak with the same retention time as Bayleton and KWG 0519. We consider this procedure suitable for the enforcement of a 0.001 ppm tolerance for residues of Bayleton and KWG 0519 in milk.

GLC confirmatory methods employing column prackings of a different polarity and a Hall electrolytic detector have also been submitted. An interference study was conducted with all the nitrogen containing compounds with tolerances in milk or meat (Report 68887). The study showed that these compounds did not interfere with the determination of Bayleton or KWG 0519 by the proposed methods.

We consider the proposed methods adequate to determine residues of Bayleton, per se, and KWG 0519 in meat and milk. As discussed in the Nature of the Residue above, the animal metabolites will have to be included in any permanent tolerance proposal. Therefore, appropriate analytical methodology and validation data. For both the free and conjugated forms of these metabolites.

Residue Data

The petition includes residue data for fresh apples and grapes and a variety of processing products. Temporary tolerances were established to cover residues in apples and grapes at 1 ppm resulting from the same application patterns pursuant to PP#0G2300. The additional residue data presented here also indicates that the established 1 ppm tolerances are appropriate.

A processing study was conducted with apples treated 10 times at a rate of 0.125 lbs a.i. per acre in Geneva, N.Y. The fresh unwashed apples bore residues of 0.23 ppm. Combined residue levels of Bayleton, per se, and KWG 0519 were 0.07 ppm in juice, 0.92 ppm in wet pomace and 0.45 ppm in dried pomace. The calculated concentration factors are therefore 4X for wet pomace and 2X for dried pomace. Thus, considering the established 1 ppm tolerance for residues in apples, the proposed 4 ppm food additive tolerance for residues in wet pomace and the 2 ppm level for dried pomace are adequate to cover the expected levels; however, to avoid unnecessary proliferation of tolerance levels we recommend that the 4 ppm level be established for both commodities.

Two processing studies were conducted with grapes. The first study involving grape juice and wine (discussed in our review of PP#0G2300) indicated there was no concentration of residues in either of these two commodities. An additional study has been submitted in the subject petition. In the new study grapes treated 3 times at rates up to 0.25 lbs a.i. per acre were processed into grape juice, raisins, raisin waste wet pomace and dried pomace. The fresh fruit was harvested on the day of the last application and shown to bear residues ranging from 0.16 to 0.50 ppm. Upon processing the highest concentration factors calculated were 1.8X for juice, 0.25X for raisins, 2.1X for wet pomace and 2.5X for dried pomace and 7.0X for raisin trash. Based on these values and considering the established 1 ppm tolerance for fresh grapes, the petitioner proposes a 2 ppm tolerance for residues in grape juice and wine, a 2.5 ppm and 3.0 ppm tolerances for residues in wet and dried pomace, respectively, and a 7 ppm tolerance for residues in raisin trash.

The new study indicates that residues concentrate in both grape juice and wet grape pomace. This result is paradoxical and conflicts with the previous processing study. However, for the purpose of the proposed temporary tolerance we will recommend that the proposed tolerance for grape juice be established. However, for a future permanent tolerance an additional grape processing study must be conducted to determine if residues actually concentrate in juice or wet pomace. We cannot recommend that the wine tolerance be established because the Agency has no authority to establish pesticide tolerances for alcoholic beverages. The levels proposed for wet and dried pomaces are probably adequate, but to avoid unnecessary proliferation of tolerance levels, we recommend that a 3 ppm level for for wet or dried pomace be proposed.

Meat, Milk, Poultry and Eggs

The proposed uses involve the livestock feed items apple pomace, grape pomace and raisin waste. As discussed above residues are not expected to exceed 4, 3 and 7 ppm respectively. The feeding of apple pomace at a level of 50% of the diet or raisin waste at a level of 30% will result in a maximum practical burden in the livestock diet of approximately 2 ppm. It is unlikely that the livestock diet would contain more than one of these commodities.

A bovine feeding study was conducted to determine the level of residues that will result in tissues from the ingestion of Bayleton and KWG 0519. The two compounds were fed to nine dairy for 28 days in equal proportions at levels totaling 25, 75 and 250 ppm (three animals at each level). No gross toxicological effects were observed from any of the feeding levels.

None of the muscle, liver or kidney samples from the 75 and 250 ppm treatment levels contained detectable levels (<0.01 ppm) of Bayleton or KWG 0519. Residues were found in the fat samples at levels up to 0.024 ppm and 0.016 ppm from the 250 ppm and 75 ppm dose levels, respectively. No detectable residues were found in any tissue sampless from the 25 ppm treatment level. One milk sample from the highest treatment level contained trace residues of 0.0013 ppm. All other milk samples contained no detectable residues (<0.001 ppm).

The petitioner proposes no tolerances for residues in meat, milk, poultry or eggs; obviously concluding that the proposed use falls under Category 3 of Section 180.6(a). We do not concur. The study demonstrates that at exaggerated feeding levels residues of Bayleton are found in milk and tissues, further no poultry feeding data are available. Therefore, it is our judgement that there is a reasonable likelihood of less than detectable levels resulting from the expected ingestion levels. Thus, an amendment to PP#0G2300 proposing method sensitivity tolerances of 0.01 ppm for residues in meat, milk, poultry and eggs will be required for a favorable recommendation.

No poultry feeding data are available; however, the poultry radiotracer study indicates that excretion patterns and resulting residue levels in poultry tissues are similar to those in cows. Therefore, since the proposed uses only involve minor poultry feed items, and the expected ingestion level of Bayleton residues is much less than the dose levels in the metabolism studies and the bovine feeding experiments, we can also conclude for the purpose of this temporary tolerance only that 0.01 ppm tolerances for poultry and eggs would be adequate to cover any anticipated residues of Bayleton, per se, and KWG 0519.

As discussed above for a future permanent tolerance the principal animal metabolites (designated KWG 0519 acid, KWG 1323 and KWG 1342) will have to be included in the tolerance expression. Therefore, analytical methodology and validation data for both the free and conjugated metabolites, bovine and poultry feeding data reflecting the levels will be required. Poultry feeding data reflecting the levels of Bayleton, per se, and KWG 0519 will also be required for a permanent tolerance.

General Metobolic Pathway of Bayleton in Animals