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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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SUBJECT: PP#1G2432 Bayleton on wheat and barley. Evaluation of the analytical methods and residue data.

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Mobay Chemical Corporation, Agricultural 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, β-(4-chlorophenoxy)-α-(1,1-dimethylethyl)-1H-1,2,4-triazol-1-ethanol (KWG 0519) in wheat and barley grain at 0.1 ppm.

Temporary tolerances of 1 ppm for residues of Bayleton in or on apples and grapes were recently established pursuant to PP#0G2300. FAP#1H5282 proposing temporary tolerances for residues of Bayleton in apple and grape processing products at levels as high as 7 ppm is currently pending.

The proposed experimental programs involve the treatment of approximately 1250 acres this year, and 2100 acres next year with a total of 1675 active ingredient.

Conclusions

1a. The fate of Bayleton in wheat and barley has been adequately delineated for the purpose of the proposed temporary tolerances. The residues of concern are Bayleton, per se, and its metabolite KWG 0519. For a future permanent tolerance an appropriate metabolism study on grains 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 tolerance. However, for a future permanent tolerance 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 wheat and barley grains at the level of the proposed tolerance.

3a. The residue data adequately demonstrate that residues of Bayleton, per se, and its metabolite KWG 0519 resulting from the proposed use in wheat grain will not exceed the proposed tolerance.

3b. No residue data are presented for barley. Therefore, for a favorable

recommendation either the submission of appropriate residue data for barley, or deletion of the of the proposed tolerance for barley from Section F and the use on barley from Section B and the EUP will be required.
3c. No tolerances have been proposed for residues in or on the forages and the straws of wheat and barley; however, a label restriction against the grazing or feeding of fodder and forage has been imposed. The treated straws must be included in this restriction for a favorable recommendation. Appropriate tolerance proposals for these comodities will be required for a future permanent tolerance.

3d. We are not requiring milling fraction data for the proposed temporary tolerance. However, for a future permanent tolerance appropriate validation and residue data for wheat and barley milling fractions will be required.

4. The available feeding data demonstrate that the proposed use in, conjuction with the uses on apples and grapes we have previously recommended for, falls under Category 2 of Section 180.6(a). These data also indicate that secondary residues in meat, milk, poultry and eggs from the feeding of treated grains will not exceed the tolerances we recommended for in connection with the apple and grape uses. (See the review of FAP#1H5258 dated 2/27/81 by John Worthington.)

Recommendations

1. We recommend against the establishment of the proposed temporary tolerances for the reasons cited in Conclusion 3b and 3c above.

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

   a) Inclusion of straw in the label restriction against the feeding or grazing of treated forage and fodder.

   b) Either the submission of appropriate residue data for barley, or deletion of the proposed tolerance for barley from Section F and the use on barley from Section B and the EUP.

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

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

   b) Deletion of the label restriction against the feeding or grazing of treated forage, fodder, and straw; the proposal of appropriate tolerances for these comodities; and the submission of supporting residue data.

   c) Submission of additional recovery data from samples of both wheat and barley fortified at 0.1 ppm that show significantly less variation.

   d) A wheat or barley metabolism study.

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e) Inclusion of the animal metabolites, KWG 0519 acid, KWG 1342, and KWG 1323 in the tolerance expression for meat, milk, poultry and eggs. Therefore, appropriate analytical methodology and validation data for both the free and conjugated forms of the metabolites; bovine and poultry feeding data reflecting the levels of the above metabolites in meat, milk, poultry and eggs; and possibly the proposal of higher tolerance levels.

f) Poultry feeding data reflecting the levels of Bayleton, per se, and KWG 0519 in eggs and poultry tissues.

g) Additional wheat residue data reflecting the residue levels that will result from the low volume aerial applications.

h) Submission of appropriate methodology, validation data and residue data to determine residue levels in milling fractions.

Detailed Considerations

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]

For the purpose of the proposed temporary tolerances, we do not anticipate any 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 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

Bayleton is proposed for use on wheat and barley to control powdery mildew and various rusts at rates ranging from 1 to 4 oz. a.i. per acre. Additional applications are to be permitted, but the total amount of Bayleton applied per season is not to exceed 8 oz. a.i. per acre.

Minimium spray volumes of 20 gallons per acre for ground applications and 5 gallons for aerial applications are required. A 60 day preharvest interval is also required. A label restriction against the feeding or grazing of fodder and forage has been imposed. There is no mention of treated straw. The treated straws must be included in this restriction for a favorable recommendation.

Such a restriction is not considered practical for a permanent tolerance. Therefore, appropriate tolerance proposals for these comodities will be required for a future permanent tolerance.

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 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 soilds (solid II) were 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 radioassyed 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 assayed, 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
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 occured 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 0.5 ppm remaining. The principal metabolite, 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-

triazole-1-yl)-2-butanone (trade name KWG 0519) 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, 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 metabolite, KWG 0519. The remaining activity was only characterized as unidentified organo-soluble metabolites (18%), aqueous soluble metabolites (11%), and unextractable activity (7%).

For the purpose of the proposed temporary tolerances, we can conclude the fate of Bayleton on plants has been adequately delineated and that Bayleton, per se, and KWG 0519 are the principal residues of concern. However, for any future permanent tolerances on wheat or barley, a metabolism study on one of these crops 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 60% 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. Generally Bayleton, per se, and its metabolite KWG 0519 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 procine 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 for the largest portion of the residue in kidney. Approximately 80% of the activity in kidney and fat and 70% of the activity in liver was identified. The levels found in muscle tissue were too low to characterize.

Radiolabeled Bayleton metabolism studies were also conducted in poultry. Laying hens were dosed at 2.4 mg/kg in a single oral dose. Virtually all of the activity was elimiated within 24 hours. Six hours after treatment 1.18 ppm, 0.26 ppm and 0.30 ppm were found in kidney, liver, muscle and fat, respectively. Ninety hours later no activity was detectable (<0.01 ppm) in any tissues. KWG 0519 acid was the principal residue found in liver and kidney. KWG 0519 and KWG 1342 were the major components of the residue in muscle fat and eggs. Approximately 75% of the activity in liver and kidney, 90% of the activity in gizzard muscle and fat and 80% of the activity in eggs was characterized.

In conclusion the three animal studies demonstrate that Bayleton is rapidly metabolized and excreted with little 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. (See the attached table of structural formulas). Considering the relatively low burden of residues expected in the diet of livestock from ingesting treated feed items, we are willing to conclude, for the purpose the proposed temporary tolerances only, that Bayleton, per se, and KWG 0519 are the residues of concern. For any future permanent tolerance the animal metabolites listed above will also have to be included in the tolerance expression.

Analytical Methods

The analytical method developed to determine Bayleton in apples is also proposed as the enforcement method for wheat and barley. The procedure is

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a gas chromatographic method which involves the grinding of the sample 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 its metabolite, KWG 0519, with a gas chromatograph equiped with a thermonic detector.

Validation data for the determination of residues in wheat samples fortified at levels of 0.05 ppm are presented. Recovery values for Bayleton, per se, ranged from 58% to 114% and averaged 80.3% with a standard deviation of 22.3%. Recovery values for KWG 0519 ranged from 58% to 136% and averaged 88.0% with a standard deviation of 24.7%. Reported control values ranged from <0.02 ppm to <0.04 ppm. We consider the reported recovery values marginally acceptable, but only because the fortification levels were at half the proposed tolerance. The 0.05 ppm fortification level is quite low compared a control value of 0.04 ppm. For a future permanent tolerance additional recovery data from samples of both wheat and barley fortified at 0.1 ppm that show significantly less variation will be required. Also for a future permanent tolerance methodology and validation data for wheat and barley milling fractions will be required.

The analytical method developed for the determination of Bayleton and its metabolite, KWG 0519, in animal tissues, presented in Report 68705 and discussed in detail in the review of FAP#1H5282 (See the memo of 2/27/81 by John Worthington). In that review we concluded that the proposed gas chromatographic method was suitable to determine residues of Bayleton and its metabolite, KWG 0519, in animal tissues at a level of 0.01 ppm.

The analytical method was also developed for the determination of Bayleton and its metabolite, KWG 0519, in milk, presented in Report 68798 and discussed in detail in the review of FAP#1H5282 (See the memo of 2/27/81 by John Worthington). In that review we concluded that the proposed method was suitable to determine residues of Bayleton and its metabolite, KWG 0519, in milk at a level of 0.001 ppm.

Interference studies and confirmatory procedures for the meat and milk methods were also discussed in the above mentioned review. We concluded that the proposed methods adequate to determine residue levels of Bayleton and its metabolite, KWG 0519, in animal tissues and milk. However, as discussed in the Nature of the Residue Section above, for any future permanent tolerance the animal metabolites will have to be included in the tolerance expression. Therefore, appropriate analytical methodology and validation data for both the free and conjugated forms of the metabolites in meat, milk, poultry and eggs will be required for any future permanent tolerances.

Residue Data

Residue data from seven states and Canada are presented for wheat. No data have been submitted for barley. Wheat was treated twice with ground applications at either 4 or 6 oz a.i. per acre 13 to 76 days before harvest.

The highest level found was 0.09 ppm on a sample harvested 13 days after the last treatment. All of the reported results including those from exaggerated application rates were less than the proposed tolerance. It is our judgement that for the purpose of the proposed temporary tolerance, the available residue data demonstrate residue levels in wheat resulting from the proposed use will not exceed the proposed temporary tolerance. For a future permanent tolerance additional residue data reflecting low volume aerial applications will be required.

No residue data are presented for barley. The petitioner has requested that we extend the wheat data to barley. We are unwilling to do this; and therefore, for a favorable recommendation either the submission of appropriate residue data for barley, or deletion of the of the proposed tolerance for barley from Section F and the use on barley from Section B and the EUP will be required.

Meat, Milk, Poultry and Eggs

A bovine feeding study was conducted in support of FAP#1H5282 to determine the level of residues that will result in tissues and milk from the ingestion of Bayleton and KWG 0519. This study was discussed in detailed in the review of FAP#1H5282 and at that time we concluded that the apple and grape uses fall under Category 2 of Section 180.6(a). Thus, in conjuction with those uses which would result in a dietary burden of approximately 2 ppm, we recommended for the establishment of temporary 0.01 ppm tolerance for residues in milk, eggs and the meat, fat, and meat byproducts of cattle, goats, hogs, horses and sheep. The use proposed here will not contribute significantly to the existing dietary burden. Therefore, it is our judgement that the above meat, milk, poultry, and egg tolerances will be adequate to cover any secondary resulting from the uses proposed here.

As discussed in the Nature of the Residue Section above, for any future permanent tolerance the animal metabolites will have to be included in the tolerance expression. Therefore, bovine and poultry feeding data reflecting the levels of animal metabolites in meat, milk, poultry and eggs and possibly the proposal of higher tolerances levels will required any future permanent tolerances. Poultry feeding data reflecting the levels of Bayleton, per se, and its metabolite, KWG 0519, will also be required for a permanent tolerance.

cc: Reading file
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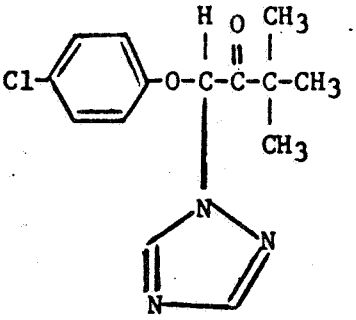
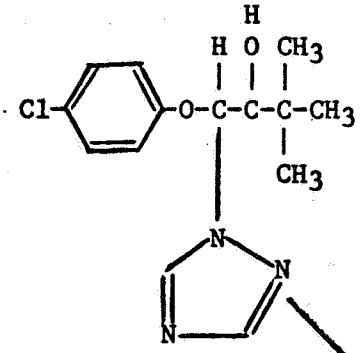
Reviewer
FDA
PP# No.
TOX
EEB
EFB
Randy Watts

TS-769:Reviewer:JMWorthington:JMW:Date: 2/24/81 Rm:810:CM#2
RDI:Section Head:RJH:Date:2/25/81:RDS:Date:2/25/81

General Metobolic Pathway of Bayleton in Animals

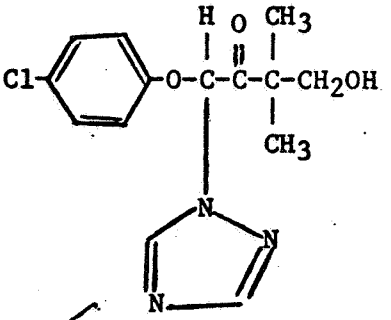


BAYLETON



KWG 0519

Baytan (S#127201)



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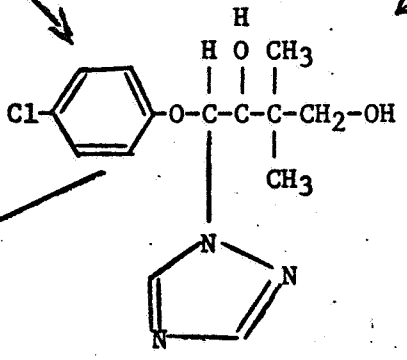
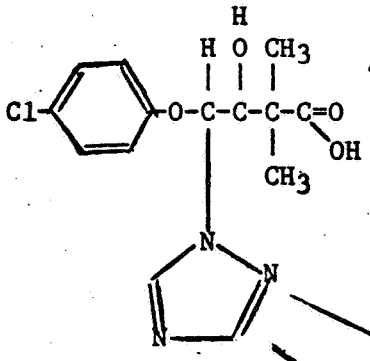


KWG 1342



KWG 0519 ACID

CONJUGATION

ELIMINATION