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PP#0G2300 Bayleton on Apples, Grapes and Pears. Evaluation of the analytical methods 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-dimethyl-1(1H-1,2,4-triazole-1-yl)-2-buthanone and its metabolite, β-(4-chlorophenoxy)-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-ethanol, in or on apples and pears at 0.75 ppm and in or on grapes at 1 ppm.

No other tolerances for Bayleton have been proposed or established. The proposed experimental programs involve the treatment of appoximately 290 acres of grapes (at rates up to 3 oz. a.i. per acre) and approximately 185 acres of apples and pears at rates up to 1 oz. a.i. per acre).

Conclusions

1a. The fate of Bayleton in plants is adequately delineated for the purpose of the proposed tolerances. Bayleton per se and its metabolite β-(4-chlorphenoxy)-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-ethanol, are the principal plant metabolites.

1b. The fate of Bayleton in animals is also adequately delineated for the purpose of the proposed temporary tolerances.

2a. Adequately validated enforcement methodology is available to determine residues in apples and grapes.

2b. No validation data has been submitted for the analysis of pears.

3a. The available residue data demonstrate that residues in or on apples from the proposed use will not exceed the proposed 0.75 ppm tolerance level; however, it is our judgment that 1 ppm would be a more appropriate level.

3b. The available grape residue data indicate that the proposed 1 ppm tolerance for residues in or on grapes is appropriate.

3c. The available residue data for grape juice and wine indicate that the 1 ppm tolerance level will also cover residues in these commodities.

3d. No residue data have been submitted to determine residue levels in wet or dried apple and grape pomaces or raisin waste.

3e. No residue data have been presented for the use of Bayleton on pears. Such data and an a corresponding appropriate tolerance proposal for pears will be required for a favorable recommendation.

4. For the reasons cited in Conclusion 3d we cannot categorize the proposed use as written under Section 180.6(a). However, for the purpose of the proposed temporary tolerances in conjunction with the limited experimental program to be conducted, we consider a fresh market only label restriction appropriate.

However, such a restriction has not been included in the use for grapes. If such a restriction were imposed we could categorize the proposed use under Section 180.6(a)(3).

5. A detailed description of the manufacturing process, a statement of purity and a complete list of impurities is required.

6. The use for apples and pears indicates that Bayleton may be tank mixed with other fungicides, insecticides and miticides. Such general recommendations for tank mixes are not acceptable.

7. The label restriction prohibiting the grazing of treated cover crops should also prohibit the feeding of these crops.

Recommendations

1. We recommend against the establishment of the proposed temporary tolerances for the reasons cited in conclusions 2b, 3a, 3d, 3e, 4, 5, 6 and 7.

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

a). Appropriate validation data for the analysis of pears by the proposed method.

b). Proposal of a 1 ppm tolerance level for apples.

c). Residue data for pears reflecting the proposed use and an appropriate tolerance proposal to cover the resulting residues.

d). If a fresh market only label restriction is not imposed, residue data for wet and dried grape pomace, and raisin waste. And if these data indicate that food additive tolerances for the commodities are needed, proposal of appropriate food additive tolerances will also be required.

e). Also, if the fresh market only label restriction is not imposed, livestock feeding studies that determine the levels of the significant animal metabolites that will result from the ingestion of treated feed items.

f). A detailed description of the manufacturing process.

g). A statement of purity for technical Bayleton and a complete list of impurities.

2

h). Elimination of the general statement that Bayleton may be tank mixed with other fungicides, insecticides and miticides. If tank mixes are to be recommended on the label, specific directions for each tank mix must appear on the label. Further, residue data reflecting the application of any proposed tank mixes will also be needed.

i). Inclusion of a restriction against the feeding of treated cover crops on the proposed label.

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

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

b). Additional residue studies on grapes which will provide adequate geographical representation.

c). Residue data for wet and dried apple and grape pomaces and raisin waste, developed using appropriately validated methodology.

d). Development of analytical methodology to determine the principal animal metabolites in animal tissues.

e). Appropriate animal feeding studies to determine the levels of secondary residues that will result in meat, milk, poultry and eggs.

4. We also suggest that an alternative confirmatory procedure be developed for a future permanent tolerance. The techniques used in the interference study (Report No. 68006) should provide the basis for an alternative procedure.

Detailed Considerations

Formulation

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

[redacted]

The inert ingredients of the proposed formulation are all cleared under Section 180.1001 (c) or (d). However, no information on the technical product has been provided. For a favorable recommendation a detailed description of the manufacturing process, a statement of purity and a complete list of impurities will be required.

Proposed Use

Bayleton is proposed for use on apples and pears to control powdery mildew and cedar-apple rust at rates ranging from 0.25 to 1 oz a.i. per 100 gallon

of spray solution. A maximum of 1 lb a.i. per acre may be applied per application and not more than a total of 2 lbs a.i. are to be applied per crop season. No preharvest interval is required. A restriction against the grazing of cover crops has also been included. This restriction should also prohibit the feeding of cover crops. A fresh market only label restriction for the apple and pear use has also been imposed.

The use for apples and pears indicates that Bayleton may be tank mixed with other fungicides, insecticides and miticides. Such general recommendations for tank mixes are not acceptable. The proposed label must specifically indicate the pesticides and the application rates that are recommended for tank mixes. Further for any future permanent tolerance, residue data reflecting the recommended tank mixes will be required.

Bayleton is also proposed for use on grapes to control powdery mildew and black rot 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. A 14 day preharvest interval is required and a maximum total application of 9 oz a.i. per acre per crop season is permitted.

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 labelad 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) 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 radioassayed and the organic phase subjected to TLC. Peal 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
	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 solubilized 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 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, 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. 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 (6.5%) 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 for the largest portion of the residue in kidney. Appropximately 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 eliminated 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 metabolism studies demonstrate that Bayleton is rapidly metabolized and excreted with little or no tendency to concentrate in tissues. KWG 1323, KWG 1342, KWG 0519 acid and KWG 0519 were the principal metabolites found in tissues. We consider the fate of Bayleton in animals adequately delineated for the purpose of the proposed temporary tolerances.

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 dectector.

The validation data for apples includes samples of various varieties of apples and apple products including wet and dried pomace. Recoveries from samples fortified with both compounds at levels ranging from 0.05 to 0.10 ppm Bayleton ranged from 72 to 110% and averaged 91.8% 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 KWG 0519 from samples of grapes, grape juice and wine fortified at levels of 0.05 ppm ranged from 80 to 107 and averaged 89.5% with a standard deviation of 9.4%. Control values were reported as less than 0.01 ppm.

No validation data have been submitted for pears appropriate recovery and control data for the analysis of pears will be required for a favorable recommendation.

A confirmatory procedure for Bayleton and the KWG 0519 metabolite has been proposed. The procedure is based on determining the partitioning coefficients for the two compounds between the liquid phases in two liquid-liquid systems. It is our judgment that these techniques would present practical difficulties in typical use for the following reasons: 1) the substrates involved may significantly effect the partitioning coefficients 2) it seems unlikely that similar compounds will have significantly different partitioning coefficients; 3) the system depends on the precision of GLC quantitative measurements to determine the identity of the compounds. The development of the alternative confirmatory procedure is suggested for a future permanent tolerance. We suggest the techniques used in the interference study (Report No. 68006) to determine Bayleton and its principal plant metabolite in the presence of pesticide residues that interfere with the enforcement method would be appropriate. (See below).

Report No. 68006 describes an interference study which was conducted to determine if any of 61 nitrogen containing compounds that are registered on apples, grapes and grasses would interfere with the enforcement procedure. Nine of the compounds produced peaks that interfered with the analysis. Three of the nine peaks were resolved with the use of an alternate column. The interference from the remaining six compounds was eliminated by the use of a Hall Electrolytic Conductivity Detector operated in the halogen mode. The study demonstrates that adequate methodology is available to determine Bayleton residues in the presence of the pesticides currently registered for use on apples and grapes.

Residue Data

Apples: Twelve residue studies were conducted in five states to determine the levels of Bayleton residues in on apples from the proposed use. Six to eleven applications were made to apples at a 2 oz. a.i. per 100 gals (2 times the maximum proposed) rate. The apples were generally harvested 0,1,3, 7 and 14 days after treatment. On day zero the whole apple residue values ranged from 0.25 to 0.50 ppm. The half life of Bayleton residues appeared to be approximately 8 days. An experiment was also conducted in which apples were treated thirteen times at four times the maximum proposed rate. On day 1, residue levels of 1.34 ppm were found. The above studies demonstrate that the proposed 0.75 ppm tolerance would be adequate to cover the resulting residue levels but it is our judgment that a tolerance proposal of 1 ppm would be more appropriate. No residue data for apple processing fractions have been submitted.

Samples of apple peel and pulp from the radiotracer studies were placed in frozen storage for 132-420 days. The pulp samples initially had almost no residues but the peel samples contained 0.21 ppm Bayleton per se and 0.18 KWG 0519. Levels after 420 days were within 95% of the initial values. The experiment demonstrates that Bayleton residues in apples held in frozen storage showed little decomposition after more than one year.

Grapes: Two residue studies conducted in California and Oregon to determine the levels of Bayleton residues that will result in grapes from the proposed use. Grapes were treated 2 or 3 times at the rate of 3 oz

a.i./A. The highest residue level reported after the required 14 day PHI was 1 ppm. For the proposed of the proposed temporary tolerance, we consider the available data adequate to demonstrate that the proposed tolerance is appropriate. For a future permanent tolerance additional residues which provide adequate geographical representation will be required.

Grape wine and grape juice made from grapes treated three times with 3 oz. a.i. per acre and harvested 0 and 7 days after the last treatment were analyzed for Bayleton residues. The highest reported values were 0.25 ppm. No analyses of wet or dry grape pomace were presented.

No residue data for pears have been submitted. The petitioner has requested that we extend the apple data to pears. We are unwilling to do this; therefore for a favorable recommendation the appropriate residue data that determine the residue levels that will result in or on pears from the proposed use are required.

Meat, Milk, Poultry and Eggs

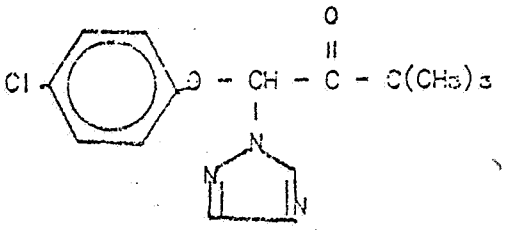
The proposed uses involve several livestock feed items: apple pomace, grape pomace and raisin waste. No experiments have been done to determine the level of residues in these commodities. No animal feeding studies have submitted to determine the principal animal metabolites. For the purpose of the proposed temporary tolerances in conjunction with the limited experimental program we would consider a fresh market only label restriction an alternative to the submission of the above information. For a future permanent tolerance residue data determining the residue levels in wet and dried apple and grape pomace and raisin waste; submission of adequately validated analytical methodology to determine Bayleton and its principal metabolites in animal tissues; appropriate animal feeding studies; and proposal of appropriate tolerances to cover residues in meat, milk poultry and eggs.

Attachment

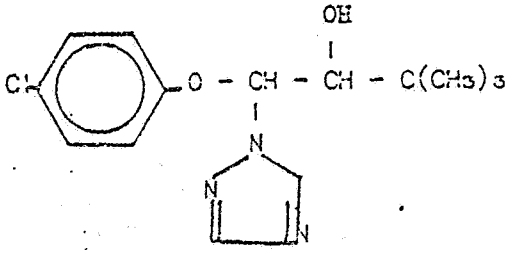
cc: Reading file
Circu
FDA
Reviewer
TOX
EEE
EFB
PP# NO.
Watts

TS-769:RCB:Reviewer:J.M. Worthington:LDT:X77324:CM#2RM:810 Date:3/28/80:
RDI:Section Head:Acting ARB Date:3/27/80:RDS:Date:3/27/80

Structural Formulas for Bayleton and its Metabolites



BAYLETON



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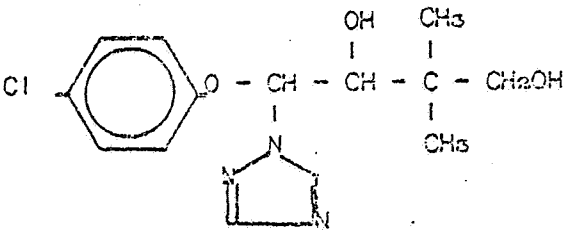

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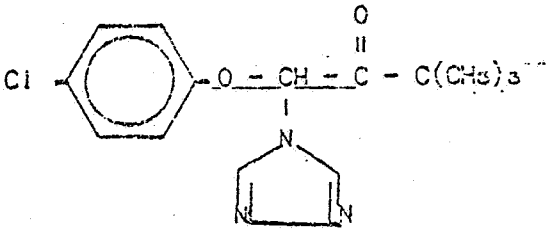
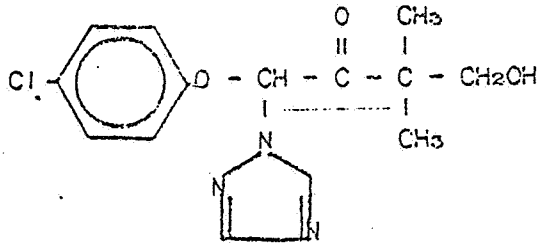


BAYLETON SYMMETRICAL ISOMER



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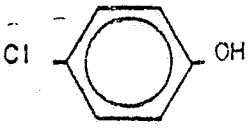
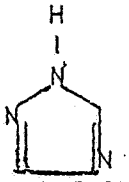


p-CHLOROPHENOL



1,2,4-TRIAZOLE