1-19-79

DATE: January 19, 1979

SUBJECT: PP# 8G2068. BAS 352F on strawberries. Evaluation of analytical method and residue data.

FROM: G. P. Makhijani, Chemist, Residue Chemistry Branch
Hazard Evaluation Division TS-769

1/19/79

TO: PM Team 21 and TOX

THRU: Chief, Residue Chemistry Branch

The BASF Wyandotte Corp. proposes a temporary tolerance for residues of the fungicide BAS 352F, "3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2, 4-oxazolidinedione", (Tradename Ronilan), in or on strawberries at 5 ppm.

BAS 352F is a new pesticide and this is the first submission for this product.

The proposed experimental program will use 3,000 lb actual ingredient to treat 400 acres in 21 states.

Conclusion

1(a) The metabolism and or degradation route of the parent compound and the nature of terminal residues in strawberries have been adequately delineated.

(b) For the purpose of this petition, the metabolism in animals is adequately delineated.

2. The proposed analytical methods are adequate for enforcement of the proposed tolerance.

3. The proposed temporary tolerance of 5 ppm for total residues of BAS 352F and its metabolites containing the 3,5-dichloroaniline moiety in or on strawberries is adequate.

4. Since no feed items are involved, the proposed tolerance falls into Category 3 of Section 180.6(a) with respect to secondary residues in meat, milk, eggs and poultry.

Recommendations

Toxicological considerations permitting, we recommend that the proposed temporary tolerance for residues of BAS 352F in or on strawberries at 5.0 ppm be established. Since the method determines BAS 352F and all metabolites containing the 3,5-dichloroaniline moiety, the tolerance should be expressed in terms of 3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione and its metabolites containing the 3,5-dichloroaniline moiety. The petitioner

has no objections to this Section F revision (telecon, Dr. R. Hummel/Dr. D. Yoder, 1/15/79).

For a permanent tolerance, we will require additional residue data including data for tank mixes with captan and benomyl.

Detailed Considerations

Formulation

Vinclozolin (BAS 352F) is to be imported from West Germany and the technical product is 93% pure. The principal impurities consist of:

[redacted]

At the levels indicated, we do not expect a residue problem from these impurities.

The synthesis of Vinclozolin is carried out [redacted]

BAS 352F is marketed as a 50% wettable powder under the tradename "Ronilan".

The inert ingredients consist of [redacted]

All the inerts are exempt under Section 180.1001.

Proposed Use

Vinclozolin is a contact fungicide and is to be used for the control of botrytis fruit rot on strawberries. It is to be applied at the rate of 0.5 to 1 lb of ai/A in not less than 100 gallons of water/A to obtain a thorough coverage of fruit and foliage, using ground type (spray boom), equipment. Multiple applications permitted, there is no PHI.

At locations where fungus disease such as anthracnose or leaf scorch occur together with botrytis, tank mixtures of Vinclozolin, at 0.5 lb ai/A with either captan at 2 lb act/A or benomyl at 0.25 lb act/A are recommended. These rates for captan and benomyl are 0.5X the currently registered maximum rates.

Nature of Residues

Metabolism studies using ^{14}C uniform ring-labelled BAS 352F were carried out on strawberries, grapes, rats and soils. The "Metabolic Pathway" summary

INERT INGREDIENT INFORMATION IS NOT INCLUDED

is shown in Fig 1 (attached) and includes all intermediary metabolites to date in strawberries, grapes, rats and soil.

Plant metabolism

(a) Strawberries

Two studies have been carried out in the open air section of the isotope greenhouse in W. Germany. In the first study, potted strawberry plants were sprayed @ 1 kg ai/ha in the form of 0.1% spray solution. The plants were treated on 3 different dates and the fruits were sampled starting on the last day of treatment and continuing for 31 days. Total activity, expressed as ppm BAS-352F, declined from an initial level of 2.6 ppm to 0.2 ppm at day 31.

The proportion of total residues made up of parent compound decreased with the passage of time. Seven days after the last treatment, the proportion of intact compound was still over 85%, by 31 days it had fallen to apx 31%. There was a reciprocal increase in the proportion of polar degradation products during the course of the trial.

The metabolism of BAS 352F is shown graphically in figure 1 along with the structures of degradation products A, B, D and E. These were characterized by thin layer chromatography. Small amounts of metabolites B, D and E also occur in the free form but metabolite A is present only as a conjugate.

The petitioner's analytical method is designed to determine BAS 352F and its metabolites containing the 3,5-dichloroaniline moiety. To validate the method, strawberries containing total activity equivalent to 0.2 ppm (24 day PHI) were analyzed by the proposed method. Total residues as determined by the method were essentially the same as those determined radiometrically.

The second study was an extension of the first study and was carried out to find out if more frequent fungicide treatment would result in accumulation of residues in fruit and if the metabolism differs qualitatively under these conditions. Potted strawberry plants were treated at 1 lb of ai/A in the form of 0.1% spray solution. The plants were treated 5 times at weekly intervals and ripe fruits were sampled, starting from 6 days and continuing up to 39 days after the last treatment. It was noted that the residues in strawberries were rapidly decreasing. Six days after the last treatment, a radio active residue level of 3.3 ppm was found; this decreased to 0.6 ppm after 20 days. The percentage of the residue accounted for as parent compound declined from 80% at day 6 to 25% at day 39. Besides the active ingredients, small amounts (about 1% of total residue) of metabolite E and S occur in free form. Metabolites B and T represent the main portion (38%) of metabolized material but occur only in conjugated form. After the cleavage of the conjugates with 2 N methanolic HCl, metabolite D is detectable, though this degradation product may result as an artifact upon hydrolysis. The portion of conjugated metabolites increase from 12% after 6 days to 38% after 39 days.

As additional validation of the analytical method, 10 strawberries samples (6 to 39 days PHI) were analyzed by the proposed method. Total residues as determined by the method were in close agreement with those determined radiometrically.

Grapes

The metabolism study in grapes is not as extensive as that in strawberries, but the first report shows that the initial degradation is similar in both the crops. However, metabolism of the parent compound seems to proceed more slowly in grapes. Following 3 applications at a rate of 2 kg/ha and a 28 day PHI, the total radioactive residue found in grapes was 90% extractable with methanol. Sixty percent of the radio activity in the methanol extract was present in the form of intact BAS 352F. The remaining radioactive residues were polar metabolites that were extracted with ethyl acetate from aqueous solution. These polar metabolites did not yield any non-polar cleavage products under the influence of hydrolysing enzymes. Methanolic HCl hydrolysis produced 3,5-dichloroaniline and small amounts of intact BAS 352F as determined by TLC. Further studies are being carried out to characterize the polar metabolites in the remaining 30% of the initial methanol extraction.

Animal metabolism

Results of two metabolism studies have been reported in rats.

In the first study ^{14}C-labelled BAS 352F was fed @ 40 mg ai/kg body weight per day to rats (body weight ca 200 g) for seven days. After daily oral feeding of BAS 352F, excretion of radioactivity was fairly rapid and was similar in both male and female rats. Apx 43% and 50% of the daily administered dose were excreted in urine and feces, respectively, during each day of the dosing period. Six days after the final dose (12 days after the first dose) means of 47% and 54% of the total had been excreted in urine and feces, respectively, and means of 0.1% and 0.04% of the total dose were retained in the gastrointestinal tract and liver resp. No radioactivity was detected in the remaining carcass at six days after the final dose nor was any detectable radioactivity excreted in the expired air during 24 hours after the final dose. The major metabolite in the fecal extracts was tentatively identified by mass spectrometry as being metabolite F, N-(3,5-dichlorophenyl)-2-methyl-2,3,4-trihydroxybutyramide. It was also shown that the glucuronide conjugate of this metabolite was the major radioactive component excreted in urine and bile. Unchanged parent was found in fecal extracts but not in urine.

The second study was carried out with a view to confirm these findings and increase the knowledge of the metabolism of BAS 352F. Rats were dosed with 40 mg of active ingredient per kg body weight orally over a period of 5 days. Urine and feces were collected during the experiment. Four hours after the last application, the animals were killed and organs samples were taken (liver, kidney, fat, blood, muscle) and frozen. Metabolite F

was identified as the main metabolite of BAS 352F. It was excreted in the feces in the free form and in urine of a conjugate. After cleavage of the conjugates in urine with an enzyme mixture, there were numerous other degradation products in addition to metabolite F. The metabolism apparently proceeds through the metabolic pathways F→H→K→N→D (See Fig. 1). Although small amounts of metabolites A, B, D and E were detected, metabolite F represented 80% of the residue in blood, 50% in the liver and 50% in the kidney. As in plants and soil, the detectable and metabolic product was metabolite D.

We conclude that for the purpose of this temporary tolerance, the above studies adequately delineate the metabolism of BAS 352F in animals.

Analytical Methods

The metabolism studies submitted by the petitioner have shown that the principal metabolites found in strawberries contain the 3,5-dichloroaniline moiety (ca. 92%). Therefore, the petitioner's residue method is based on the determination of 3,5-dichloroaniline. Upon alkaline hydrolysis, the parent and metabolites degrade to form free 3-5-dichloroaniline which is quantitatively isolated from the hydrolysis mixture by steam distillation and is collected in 1 N sulfuric acid solution. The 3-5-dichloroaniline is extracted from the aqueous distillate by partitioning with chloroform at 2 controlled pHs. The chloroform extracts are dried over sodium sulfate and the 3-5-dichloroaniline is derivatized by using a 4% solution of chloroacetyl chloride in anhydrous chloroform. After derivatization, the mixture is concentrated to dryness and all traces of chloroform and excess chloroacetyl chloride are removed by repetitive additions of hexane and subsequent concentration. The derivative is dissolved in ethyl acetate and measured by GLC equipped with ^{63}Ni electron capture detector. The total residue is expressed in terms of BAS 352F equivalents. This method after slight modification in the extraction step, can also be used for residue analysis in soils. The method is sensitive to 0.05 ppm.

The petitioner has submitted data to show that (a) the method is very specific and no interference will result from any other compound registered for use on strawberries and soils and

(b) the conversion of BAS 352F and its metabolites to 3-5 dichloroaniline and the conversion of 3-5-dichloroaniline to its acylated derivative are efficient and consistent over a wide concentration range.

A total of 119 recovery values for BAS 352F ranging from 0.05 ppm to 20.0 ppm were run both in strawberries and soil. The overall recovery efficiency of the method was 90% with a standard deviation of ± 18%. The recoveries of metabolites B, D, and E from strawberries averaged 93 ± 7% and from soil, 94 ± 10%.

The above material has not been evaluated in the EPA labs. It should be validated when the petitioner applies for permanent tolerances. We conclude that the method submitted by the petitioner is adequate to enforce a temporary tolerance for residues of BAS 352F and its metabolites containing the 3,5-dichloroaniline moiety in or on strawberries.

Residue Data

Storage stability

Individual strawberry control samples were fortified with BAS 352F at 0.2 and 5.0 ppm stored in the freezer at -15°C and analysed at monthly intervals (for 6 months) to determine stability of BAS 352F residues. Recoveries ranged from 81-99% (av. 87 ± 10%) for 0.2 ppm level and 79-91% (av. 83 ± 5%) for 5.0 ppm level. Results are not yet available for metabolites in strawberries. All storage stability studies are designed to last for 2 years.

Field Studies

Residue data are submitted from 6 studies carried out in 5 states, scattered throughout the entire USA. The residue program involved 175 treated samples, 5 different varieties and application rates of 0.25, 0.5, 1.0 and 2.0 lb ai/A (0.25 X to 2X, the max proposed rate). The number of applications of BAS 352F varied from 1 to 22 at intervals of 3-23 days depending on various climatic conditions. The berries were picked from 0-28 days after the last application.

In one of the studies carried out in California, only one application was made with BAS 352F at the rate of 0.25, 0.5, 1.0 and 2.0 lb ai/A and the samples were analyzed at 0, 1, 3 and 5 days from the date of application. At 0.25 lb ai/A, the initial residue at 0 days was 0.43 ppm, which dropped to 0.17 ppm on the 5th day; at 0.5 lb ai/A, the initial residue at 0 days of 0.59 ppm dropped to 0.30 ppm. At 1.0 lb ai/A after the 5th day, it dropped from 1.70 ppm to 0.66 ppm and at 2.0 lb ai/A on the 5th day it dropped from 4.40 ppm to 2.43 ppm. Based on these values, the half-life of BAS 352F is between 3-5 days.

The max. residue value found in strawberries sampled immediately after multiple treatments at 0.25 lb, 0.5 lb, 1.0 lb ai/A were 1.60 ppm, 2.90 ppm and 4.20 ppm, respectively, while at twice the proposed rate of 1 lb ai/A, it was 7.80 ppm.

The petitioner has not given any residue data on tank mixtures of BAS 352F with captan or benomyl, applied to strawberries. For the permanent tolerance, we will need this data.

Overall, we conclude that the available data are adequate to show that total residues of BAS 352F resulting from the maximum proposed use will not exceed the proposed 5.0 ppm temporary tolerance.

Meat, Milk, Poultry and Eggs

No animal feed items are involved in this petition. Hence, there is no likelihood of secondary residues of BAS 352F in meat, milk, poultry and eggs.

The use should be classified as Section 180.6(a) 3.

G. P. Makhijani

cc: EEE, TOX, FDA, CHM (3)
TS-769:GPMAKHIJANI:mer:Rm 108:WSMK:X62610:1/19/79
RDI:RJHUMMEL:1/18/79:JGCUMMINGS:1/18/79

FIGURE 1: SUMMARY OF METABOLISM OF BAS 352 F IN STRAWBERRIES, GRAPES, SOIL, AND RATS