6-12-96

MEMORANDUM

SUBJECT: Imazalil. Case 2325. Metabolism on Bananas From Post-Harvest Treatment. MRID 43965701. CBRS 17087. DP Barcode:D224876.

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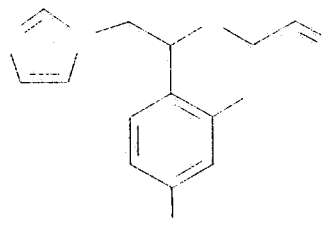
Janssen Pharmaceutica has submitted a metabolism study of imazalil on bananas for reregistration. Imazalil is 1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole.



Chemical Number: 111901
Chemical Name: Imazalil

IMAZALIL.033

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The structures of actual and potential metabolites are shown on pages 7-9.

CONCLUSIONS AND RECOMMENDATION

The metabolism of imazalil on bananas following the post-harvest treatment of the fungicide is understood. The major residue is imazalil, with minor amounts of metabolites containing the 2,4-dichlorophenyl group.

The metabolism of imazalil on citrus and wheat is also understood (see CBRS 14111, L. Cheng, 9/22/94 & 11295, L. Cheng, 1/10/94).

The data requirement for 171-4(a) is satisfied and the current tolerance expression is appropriate. The registrant should be informed that either banana or wheat matrices from the metabolism studies should be used to radiovalidate the PAM enforcement method.

Detailed Considerations

Banana plants were grown in a greenhouse. Radiolabeled imazalil sulfate, labeled with carbon-14 uniformly in the 2,4-dichlorophenyl ring, was synthesized in the laboratories of Cambridge Research Biochemicals, Billingham, Cleveland, England. The test compound had a specific activity of 186 μCi/mg base equivalent and was purified to a radiochemical purity of 99.5% at Janssen. CBRS notes that the identity and purity of the material is supported by spectroscopic and chromatographic analyses.

The radioactive material was diluted with unlabeled imazalil sulfate to a specific activity of 12.1 μCi/mg base-eq. The mixed material was initially dissolved in ethanol which was evaporated and replaced with de-mineralized water. The resultant solution was mixed with 6% w/w alum ($AlKO_8S_2 \cdot 12H_2O$) to yield a final volume of 6 liter and a concentration of 599 mg carbon-14 imazalil base equivalent per liter by counting and 1% (w/v) alum. The formulation was prepared on the morning of dipping.

The radioactivity was measured before and after the dipping of each cluster of bananas by counting 50-μL samples of the solution in quadruplicate. The concentrations before and after the last dipping (585 mg/L) process did not change very much.

Two bunches of bananas (Grand Nain Carvendish) were harvested around April 1995 and reached the research facilities of Janssen, Beerse, Belgium in 35 minutes. The bunches were dehanded into 23 clusters in a water tank. The clusters were rinsed for 20 minutes and then drained for 3 minutes.

Individual clusters were weighed before dipping in the treatment solution in a beaker for 1 minute. The bananas were then drained for 2 minutes over the beaker before drying in a ventilation hood. The treated clusters were vacuum packed in plastic bags and properly labeled. A total of 21 clusters from the 2 bunches were dip-treated, and the remaining 2 clusters served as control and samples for method validation. These clusters were packaged in two cartons (12 and 11 including one control in each). The weight of the dip solution was measured before each dipping and after the last dipping.

One carton was used to simulate short (10 day) shipping and ripening. The

carton of bananas arrived at the testing facility with a temperature of 21 C but cooled to 15 C within 24 hours. The clusters remained at this temperature (14-15 C) for 5 extra days to simulate a 5-day transport. Vacuum was then broken and ethylene gas was released in the chamber to yield a concentration of 2800 ppm (1000 parts of ethylene per million parts of air or 1.144 g C_2H_4/m^3 air). The air in the chamber was gently agitated for 5 minutes to create a homogeneous concentration of ethylene. After 31 hours, the carton of bananas was transferred to a different room for one day at ambient temperature to simulate marketing conditions.

The second carton of bananas was similarly treated to mimic the long shipping and ripening scheme (36 days). The bananas were kept at 14-16.5 C for 20 days to simulate longer transport duration. The vacuum packs were opened and ethylene was introduced at 2800 ppm. After 25 hours, the bananas were kept in a climate room for (intended) 7 days. However, after 6 days, the bananas were still not sufficiently ripe for easy peeling. Hence a second ethylene treatment (3100 ppm) was applied for 24 hours. The bananas were left at ambient temperature for one more day.

Clusters of bananas (both cartons) were assigned numbers according to dipping order. The ten clusters from each carton were divided into two groups of 5. One group was homogenized as whole bananas while the remaining group from the same carton (shipping and ripening scheme) was carefully peeled and homogenized to yield banana pulp.

For homogenization of whole bananas, the crowns and tips were cut off and the bananas were cut into 3 to 4 pieces. The crowns and tips were ground up into a powder with dry ice. The cut-up banana pieces were homogenized in a laboratory mixer with dry ice and mixed again after the addition of the powder from crowns and tips. Two portions of ≈100 grams and one portion of 20- to 25- gram of the homogenized material were placed in plastic sample bottles and stored at -20 C until analysis.

Peeled bananas were each cut into 3 to 4 pieces and homogenized with dry ice. Two portions of 100-g and one portion of 20- to 25-g of the homogenate were placed in plastic sample bottles and stored at -20 C until analysis.

Half of the bananas in each carton were mixed to yield control samples of whole banana and banana pulp. Aliquots of the blank whole banana and banana pulp were fortified with carbon-14 imazalil at 0.003 ppm and 0.75 ppm. Recoveries were 0.003-0.004 ppm at the lower level and 102% at the higher level.

One whole banana and one pulp sample from each scenario were extracted with methanol. After centrifugation, the precipitates were extracted with methanol and centrifuged again. The precipitates were washed with methanol for the last time and filtered. The extracts were pooled and 0.25 mL aliquots were diluted with water and radiocounted. Samples were analyzed in 3 months.

The total radioactive concentrations in whole bananas and banana pulp are summarized in Table 1. The report attributed the apparent higher TRR's in bananas in the 36-day study to smaller bananas used.

Table 1. Total Radioactive Residue (TRR) of Imazalil and Metabolites in Whole Bananas and Banana Pulp

10-Day Simulation	PPM	36-Day Simulation	PPM
Whole bananas			
cluster 3	3.109	cluster 12	3.688
8	3.317	14	6.113
13	3.717	18	5.091
17	3.961	20	6.596
23	3.153	22	2.966
mean	3.451		4.891
± SD	0.372		1.549
Banana pulp			
cluster 1	0.152	cluster 2	0.759
6	0.085	4	1.349
11	0.046	7	1.127
15	0.088	9	0.791
21	0.024	16	0.622
mean	0.079		0.930
± SD	0.049		0.299

The extracts were analyzed by reverse phase HPLC with radioactivity detector. Unchanged radioactive imazalil matched will in retention time with cold imazalil. For confirmation, the metabolism samples were also co-chromatographed with a mixture of imazalil and a number of potential degradation products (cold compounds, UV detection) in 2 solvent systems.

By HPLC analysis of the methanol extracts, imazalil accounted for 77-98% (calculation by CBRS after corrected for HPLC column recoveries of 83-89%) of the residue in whole bananas and banana pulp. Several other minor components were also detected. These are summarized in Table 2 below.

Table 2. Distribution and Characterization of Residues in Whole Bananas and Banana Pulp

Compound	10-Day Simulation	36-Day Simulation

	whole cluster 3 (3.017 ppm) %TRR, ppm	pulp cluster 6 (0.080 ppm) %TRR, ppm	whole cluster 12 (3.348 ppm) %TRR, ppm	pulp cluster 7 (1.070 ppm) %TRR, ppm
imazalil	81.9, 2.47	68, 0.054	69.1, 2.313	81.8,0.875
1	0.9, 0.028	<4, <0.003	1.6, 0.053	3.7, 0.040
2	0.3, 0.009	<4, <0.003	<0.1, <0.003	<0.3,<0.003
3	1.1, 0.035	<4, <0.003	1.7, 0.058	0.9, 0.010
4	0.4, 0.012	<4, <0.003	0.8, 0.026	<0.3,<0.003
5	1.4, 0.042	<4, <0.003	3.9, 0.130	<0.3,<0.003
Total	86, 2.596	68, 0.054	77.1, 2.580	86.4, 0.925

For identification of metabolites, an aliquot of the whole banana homogenate (cluster 12) was extracted 3 times with methanol. The extract was evaporated to dryness under reduced pressure at 45 C. The residue was treated sequentially with 0.01N HCl, 13.5N ammonia, and 12N HCl. "This mixture was transferred to another recipient, and the remaining residue was further dissolved in 50 ml 0.1N NH_3 and again made acidic with 0.842 ml 12N HCl. The latter solution was also transferred to the same recipient as the first one." (CBRS: we assume the sentences to mean that the supernatant was decanted and the residue was treated again with ammonia and hydrochloric acid. The supernatant from the second treatment was combined with the first extract.) The recovery was 89.6% for this dissolution process. The major part of this aqueous phase was concentrated on a column of polystyrene resin (Amberlite® XAD-2, Serva). The resin was initially washed with distilled water and then eluted with methanol. The second and third methanol eluate were combined and contained 89.7 of the applied radioactivity. The eluates were evaporated to dryness and the residue dissolved in DMSO. The metabolites were identified by LC/MS using HPLC.

By HPLC, compound 1 showed a mixture of at least two products, one eluted with R42639 or the oxalate salt of R42243. Compound 3 co-eluted with R14821, and compound 5 co-eluted with R44177, R44179, and R55609 in one solvent system but only with R44177 in a second system. The report stated that imazalil, compound 1, compound 3 and compound 5 had been confirmed by LC/MS (electrospray); compounds 2 and 4 accounted for <0.05 ppm or <10% TRR and had not been identified. Mass spectra of the reference compounds were not submitted.

The report included a metabolic scheme proposing three possible degradation routes: epoxidation of the double bond leading to the diol (R42243 or compound 1); dealkylation of the propenyl group (R14821 or compound 3); and ring opening of imidazole group (base of R44177 or compound 5).

CBRS comment: The predominant residue found in the whole bananas and pulp is imazalil. Minor amounts of despropenyl imazalil (R14821), bishydroxylated imazalil

(R42243), and imidazole ring ruptured imazalil (base of R44177) also have been detected (by HPLC retention time). Since mass spectra of the reference compounds have not been included in the report for comparison, we consider these compounds to be not rigorously identified. However, the absolute identity of these three compounds is not critical since they account for less than 5% of the TRR present in whole bananas and banana pulp.

cc:Circ, RF, List B, Cheng, D. Monos (SRRD)
RDI:ARRathman:6/11/96:RBPerfetti:6/11/96
7509C:CBRS:LCheng:CM#2:RM804E:4/23/96:[redacted]05:IMAZALIL\PLNTMETA