1-3-95

**MEMORANDUM**

SUBJECT: Imazalil. Case No. 2325. Metabolism on Banana - Postharvest Treatment. Review of Protocol. No MRID No. CBRS No. 14816. DP Barcode:D209996.

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THROUGH: Andrew R. Rathman, Section Head
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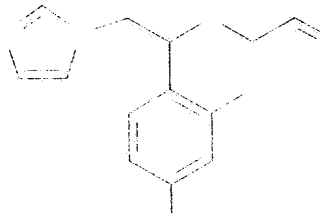
Janssen Pharmaceutica has submitted a banana metabolism study protocol for reregistration of imazalil. The registrant has asked the Agency to review the protocol because of the complexities involved in the postharvest treatment of bananas. A previous banana metabolism study submitted by Janssen was determined to be inadequate (L. Cheng, 5/5/94, CBRS No. 13072). Imazalil is 1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole.



IMAZALIL-022

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4

Chemical Number: 111901
Chemical Name: Imazalil

The undated study protocol was assigned R023979/ENV343, with a subject title of "Imazalil: nature of residue on bananas after post-harvest treatment." The metabolism study will be carried out in compliance with Good Laboratory Practice (GLP) regulations. Auditing will be performed by a Quality Assurance Unit of Janssen Research Foundation. The following is a summary of the submitted protocol.

Bananas (Grand Nain Carvendish) will be grown in a greenhouse of a commercial grower in Koningshooikt, Belgium. One week after flower shooting, the bud (the male part of the inflorescence) will be removed and full bunches (CBRS notes that each bunch consists of several hands of bananas) will be harvested 13 to 18 weeks after shooting. Two bunches will be shipped to the company research facilities at Beerse, Belgium (transport time of <30 min), dehanded into clusters in a watertank, and then washed with water. Individual clusters (6 to 8 bananas) will be immersed in the treatment solution contained in a 10-liter glass beaker for one minute.

Imazalil, uniformly labeled in the phenyl ring with carbon-14, having a specific activity of >10 $\mu$Ci per mg and a chemical purity of >97%, will be used. The treatment solution (600 mg imazalil per liter) will be prepared by dissolving radiolabeled imazalil sulfate in 6 liters of distilled water containing 1% alum. The actual concentration will be calculated from activity counting (liquid scintillation or LSC) and the specific activity of the sulfate salt. The radiochemical purity will be checked by radio-HPLC analysis.

Excess treatment solution will be drained from the bananas back to the beaker for 2 minutes. The treated clusters will be vacuum packed in plastic bags and labeled. Enough bananas from the two bunches will be dip treated to fill 2 packaging cartons (18 Kg each). The weight of the treatment solution will be determined before each immersion and after the last immersion. Imazalil concentration in the residual treatment solution will be checked by radiocounting.

One carton will be placed in a climate control room (14 C) for 5 days to simulate transport. The vacuum packs will be opened and the bananas exposed to ethylene emanating from a ripe apple for 4 days to simulate commercial ripening conditions. Bananas will then be kept at ambient temperatures for one day to simulate market conditions. The second carton will be kept in a climate control room (14 C) for 20 days and ripening conditions for 8 days. Bananas will be kept at ambient temperatures for 2 days to simulate market conditions.

For sample grouping, 10 clusters from each carton will be divided into two groups of 5 depending on the treatment order. Alternately treated clusters will be grouped together. One group of clusters will be homogenized as whole bananas, while

the second group from the same carton (ripening scheme) will be carefully peeled and homogenized to yield banana pulp. Therefore, two sets of 5 samples of whole bananas and 5 samples of banana pulp will be generated.

For sample preparation of whole bananas, the crowns and tips of individual bananas are removed, cut into 3 to 4 pieces, placed in a laboratory mixer, and ground with dry ice. The crowns and tips are chopped into a powder, mixed with the peel and pulp, and homogenized to a paste. Two 100-gram samples are placed in plastic sample bottles and stored frozen until analysis.

For peeled bananas, these are cut into 3 to 4 pieces and homogenized with dry ice to a paste. Again, two 100-gram samples are removed and stored frozen until analysis.

Concentrations of radioactivity in whole and peeled bananas will be determined by LSC. Replicates (four) will be freeze dried first before combustion and LSC. All samples will be counted for at least 10 minutes or 100,000 counts, whichever occurs first. Samples with low activity may be counted for more than 10 minutes. Samples will be reanalyzed if individual replicates differ by more than 10% of the mean value, except when the mean is <100 dpm. Background activity (BKG) will be determined in quadruplicate for each batch of samples analyzed. The limit of detection (LOD) is defined as $BKG+3(BKG/0.8t)^{½}$ and the limit of quantitation (LOQ) as $BKG+10(BKG/0.8t)^{½}$, and "t" is the time for activity counting in minutes. As an example, a counting time of 10 minutes and a background of 20 dpm would translate to a LOD of 5 dpm and an associated LOQ of 16 dpm. For a sample of 100 mg, this would yield a LOD of 0.002 ppm and a LOQ of 0.007 ppm.

With respect to metabolite characterization, no additional work (extraction, characterization or identification) will be performed if any sample from the 4 groups of treated bananas contains <0.01 ppm total radioactive residue (TRR). When the TRR in any samples from the four groups exceeds 0.01 ppm, one sample from that group will be subjected to extraction at room temperature, extraction at elevated temperature, and chemical and/or enzyme hydrolysis. The trigger level for this sequence of tiered approach is 0.05 ppm or 10% of TRR.

Any extract or hydrolysate containing <0.01 ppm radioactivity will not be further analyzed. Organosoluble fractions containing between 0.01 and 0.05 ppm activity will be chromatographed, and those containing >0.05 ppm will be profiled by chromatography. For identification of metabolites in fractions containing >0.05 ppm residue, a set of authentic reference standards of potential metabolites (see Appendix to the protocol, 2 pages) will be compared through normal phase and reverse phase HPLC. Metabolites of interest will be isolated and purified by preparative HPLC and examined by mass spectroscopy with or without prior enzyme or chemical hydrolysis. Sample analysis will be initiated within 30 days of homogenization. The types of

various instruments, equipments, detectors, and solvents to be used for the metabolite characterization and identification are also described.

The final report will identify or provide the following: scientific staff, location of the experimental facilities, protocol changes, dates of initiation and termination of various study phases, information or data for the climate control room (transport simulation), and ripening and market scenario, material balance expressed in terms of dpm/g, ppm equivalents of imazalil and TRR, rationale for metabolite assignment, chromatograms, spectra, sample calculations, archive location, and Quality Assurance and GLP statements.

The registrant plans to complete the metabolism study by December, 1995.

CBRS comments

The protocol is adequately described and contains all pertinent information, including structures of the major potential metabolites. One suggestion is that conditions for transport simulation and ripening scenario should encompass those practiced in Central and South Americas. It should be emphasized that any metabolite that is present at >10% TRR or >0.05 ppm, whichever is higher, must be identified. CBRS also recommends treatment at exaggerated rates to facilitate identification/characterization of residues. We have no objection to the protocol submitted for the banana metabolism study.

Attachment: 2 pages of chemical structures

cc(Attachment):Circ, SF, RF, List B File, Cheng
RDI:ARRathman:12/27/94:MSMetzger:12/27/94
7509C:CBRS:LCheng:CM#2:RM810D:12/20/94:04:IMAZALIL\METPROTOCOL