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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OCT 18 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT : Review of Protocols for Benomyl Bound Liver Residues in Response to 3(c)(2)(B) Data Call-In. No Accession Number. RCB No. 1407.

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The petitioner, E. I. duPont de Nemours & Co., Inc., is requesting our comments on two alternative protocols for benomyl residues designed to answer the question of "plateau" levels for bound residues in the liver. The question of bound residues was raised in connection with PP#6F1748 and was part of the Agency's 3(c)(2)(B) Data Call-In letter of May 14, 1985 and a meeting with company representatives on August 26, 1985 (see S. Malak's memorandum of meeting on benomyl, August 29, 1985).

During our meeting, referred to above, the petitioner wanted clarification on what kind of information we wanted for our risk assessment for benomyl. They were told we wanted to know: (1) whether benomyl and benomyl metabolites in liver tissue reach a plateau in beef liver, (2) what is the maximum level of bound and total residues in the liver if a plateau is reached, and (3) the length of time it takes to reach a plateau when livestock are fed at the maximum expected exposure levels. For the present uses of benomyl, we have calculated an appropriate 1X feeding level of approximately 45 ppm (PP #6F1748, memo of E. Haeberer, 8/15/84).

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Permanent tolerances are currently established for the combined residues of the fungicide benomyl (methyl 1-(butyl carbamoyl)-2-benzimidazolecarbamate) and its metabolites containing the benzimidazole moiety(calculated as benomyl) in/on several raw agricultural commodities at 0.1 to 50 ppm, including 0.1 ppm in the fat, meat and meat byproducts of cattle, goats, hogs, horses and poultry, 0.2 ppm in the liver of poultry, and 0.1 ppm in milk (40CFR 180.294). A proposal to raise the milk tolerance to 1 ppm and to establish a tolerance of 4 ppm in the liver of cattle, goats, hogs, horses and poultry is currently pending.

The following is a summary of each of the two protocols submitted by duPont:

Protocol A, entitled "Determination of the Plateau Level of Bound Benomyl Residues in Goat Liver". The overall objectives of the protocol are to feed ^{14}C-benomyl to three goats, sacrifice them at different time intervals, and determine the radioactivity in the livers as a function of the number of days they were dosed. The experimental design of protocol A calls for dosing of three nonlactating goats twice daily with capsule containing approximtely 25 mg of [2-^{14}C] benomyl embedded in a larger gelatin capsule. This dose rate is equivalent to 50 ppm benomyl in the average daily feed.

Protocol B, entitled "Determination of the Persistance of Bound Benomyl Residues in Goat Liver". This is actually a depuration experiment wherein three goats are preconditioned on technical benomyl and given a single ^{14}C-benomyl dose. The goats are then sacrificed at different time intervals in order to determine the persistence of liver residues once benomyl has been recovered from the diet. The experimental design of protocol B calls for dosing three nonlactating goats twice daily for five days using capsules containing 25 mg of technical benomyl (a mixture of radiolabled and nonradiolabled benomyl) contained in a small gelatin capsule.

Both Protocols: The radioactivity levels in both protocols will be determined in the animal's blood using Liquid Scintillation Counter (LSC), until a plateau is reached, at which time the animal is sacrificed. The second and third goats will be sacrificed 5 and 10 days, respectively, after the first goat. The entire liver in each animal will be collected within 24 hours of the last respective dose.

Liver samples will be lyophilized and homogenized. Triplicate aliquots will be analyzed by combustion and the released $^{14}CO_2$ will be determined by LSC. Liver samples will be subjected to treatment with Raney nickel, acid hydrolysis, and extraction

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with ethyl acetate. Level of residual bound radioactivity (in aqueous and solids remaining) will be determined by combustion and LSC. Known metabolites will be determined by a suitable chromatographic technique in the organic extract.

The final report in both protocols will include: Concentration of the radioactivity in the liver expressed in ppm, recovery of dosed radioactivity in the liver, data on the extraction of known metabolites from the remaining bound residues in the liver, and determination of the plateau level (in protocol A) or the Persistence (in protocol B) of bound residues in the liver.

Our Comments and Recommendations

Neither protocol A or B may address the stated objectives as written which are: (1) whether benomyl and benomyl metabolites reach a plateau level in beef liver, (2) what is the maximum level of bound and total benomyl residues in the liver if a plateau is reached, and (3) the length of time it takes to reach a plateau when livestock are fed at the maximum expected exposure levels. Protocol A is designed to address the plateau level in goat liver. The test, however, may not be adequate. Therefore, the experimental design of protocol A may be modified along the following suggestions to insure addressing the stated objectives. If our objectives are not met, a follow-up test may be necessary taking into considerations the results of the first test.

Suggested Modifications in the Experimental Design of Protocol A

1. The level of feeding of 50 ppm is approximately equivalent to an appropriate 1X feeding level of 45 ppm. In addition to the 1X dose, a second set of goats may be fed an exaggerated dose of 10X (450 ppm).

2. One point we are not sure of, however, is the relationship beween benomyl residues in the blood and that in the liver. The protocol assumes that maximum benomyl residues in the blood is indicative of the maximum residues in the liver. This may or may not be true. The protocol calls for sacrificing the second and third animals at 5 and 10 days after sacrificing the first animal. Since pesticide residues in milk, and perhaps the blood, are expected to plateau at 4-7 days from the onset of the feeding study, this means that the proposed study could be terminated at about 14-17 days. In feeding studies used to determine the maximum expected residues, however, the duration of the experiment usually continues for 30 days. Therefore,

we recommend that the second and third animals may be sacrificed at 10 and 20 days after sacrificing the first animal.

3. The experimental design should include two animals per time point for a total of six goats/dose level and controls.

4. Our objectives are best addressed using cattle rather than goats, since cattle liver is the commodity of concern to the Toxicologists. If the petitioner conducts this study with goats, they should be aware that a study using cattle may be necessary.

5. A material balance should be provided showing the percentage of the bound residues relative to total benomyl residues.

Additional Comments

This protocol was discussed with Dr. Marion Copley of the Toxicology Branch.

TS-769:RCB:RM810:CM#2:S.Malak:X577-7377:10/8/85.
RDI:E.Errico:10/16/85:RDSchmitt:10/16/85.
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