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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Reregistration of Propanil: Interim Rice Metabolism Study; Chemical No. 28201; Branch No. 8703; DP Barcode No. D157863

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On behalf of the Propanil Task Force, NPC, Inc. has submitted interim results from a rice metabolism study being conducted to support the reregistration of propanil. Rice metabolism studies were required in the Registration Standard dated 12/87.

Propanil, 3',4'-dichloropropionanilide, is a herbicide which may be applied pre- and post-emergence. Tolerances are established for residues of propanil and its metabolites (calculated as propanil) in 40 CFR 180.274 for numerous commodities including rice at 2 ppm and rice straw at 75 ppm. Feed additive tolerances are established in 40 CFR 186.1875 for residues of propanil in rice bran, rice hulls, rice polishings, and rice mill fractions all at 10 ppm.

CONCLUSIONS

1. Propanil and 3,4-dichloroaniline (DCA) were found in rice shoots (4 weeks post-treatment), and rice bran. Metabolites were not determined quantitatively.

2. Most of the radioactivity in rice straw, rice hulls, and rice bran is associated with hemicellulose.

3. Approximately 5-30% of the total radioactive residues (TRR) can be extracted by the residue analytical method. Since metabolites were not quantified, it cannot be determined whether the analytical method is capable of recovering all of the residues of concern.

RECOMMENDATIONS

Additional work is required before this study can be considered acceptable. Sample storage intervals and conditions must be described in detail, especially time between harvest and processing, and associated conditions. Metabolite identifications must be confirmed using a second, dissimilar technique, preferably mass spectrometry. All fractions, including acidic/basic and enzyme hydrolysates, which contain significant radioactivity should be characterized. A justification should be provided if the registrant does not analyze these fractions. A more specific HPLC detection system such as a radioactivity monitor should be used. Metabolites should be quantitatively determined.

The flow charts for each commodity describing extraction techniques, chromatography systems used, sample weights, and dpm values were very helpful when reviewing this submission and should be included in the final report. They should be checked carefully as a few typographical errors were found.

The registrants should clarify their intentions regarding the enforcement analytical method. The radiovalidation was conducted on a method which is different from either of the enforcement methods. Quantification of metabolites determined to be of concern based on the metabolism study should be compared to the enforcement and residue collection methods. If a revised method is proposed as an enforcement method then an independent method validation must be submitted before the Agency can conduct a validation.

DETAILED CONSIDERATIONS

In-Life Phase

An early harvest variety of rice was sown in plastic pots kept in a greenhouse. Twenty-three days after planting each pot was treated with [^{14}C]-propanil (ring-labeled) at a rate of 3 lb ai/A soil application and 3 lb ai/A foliar application (1X rate). Plant samples were taken pre- and post-treatment, 4 weeks and 8 weeks post-treatment, and at mature harvest (110 days PHI). At harvest rough grain was removed from the shoots and air dried. The rough grain was shelled to produce hulls and brown rice. The rice was milled to provide the bran and milled rice. All samples were frozen and shipped to Xenobiotic Laboratories for metabolite characterization.

Extraction and Metabolite Characterization

The total radioactive residues (TRR) were determined by combustion/liquid scintillation spectrometry (LSS) and are presented in Table 1.

Table 1. Total Radioactive Residues from Rice treated with ^{14}C-Propanil

Sample	Total Radioactive Residues (ppm ^{14}C-propanil equivalents)
Rice shoots (4 wks)	7.02
Rice shoots (8 wks)	1.14
Rice shoots (mature, dry)	1.51
Rice straw	1.22
Rice grain (rough)	0.44
Rice grain (milled)	0.25
Rice hulls	0.72
Rice Bran	1.55

Extraction of soluble residues was done using chloroform, methanol, and water. Post-extraction solids were subjected to hydrolysis using acid, base, protease, amylase, or amylglucosidase. Further extractions were done on the hydrolysates. HPLC and/or TLC was used to analyze fractions containing significant radioactivity. Various fractionation schemes were used to characterize radioactivity which may have been incorporated into natural products such as protein, starch, pectin, hemicellulose, cellulose, and lignin. Results of these extractions are presented in Table 2.

Table 2. Characterization of ^{14}C-residues in Rice Commodities

	Rice Shoots[1]	Rice Straw	Rice Hulls	Rice Bran	Milled Rice
TRR, ppm[2]	7.02	1.22	0.72	1.55	0.245
Hexane Soluble	4.94% (0.35)[3]	0.90% (0.01)	1.58% (0.01)	8.28% (0.13)	1.10% (0.003)
ACN Soluble	10.35% (0.73)	12.67% (0.15)	7.47% (0.05)	3.91% (0.06)	1.53% (0.004)
MeOH/H_2O Soluble	24.35% (1.71)	12.43% (0.15)	5.86% (0.04)	23.08% (0.36)	5.32% (0.013)
Protein	11.82% (0.83)	7.04% (0.09)	6.71% (0.05)	14.23% (0.22)	25.3% (0.062)
Starch	1.75% (0.12)	1.53% (0.02)	0.96% (0.007)	2.35% (0.04)	62.3% (0.15)
Pectin	46.79% (3.29)	7.89% (0.10)	5.40% (0.04)	8.06% (0.13)	4.48% (0.011)
Hemicellulose		44.7% (0.54)	44.62% (0.32)	30.76% (0.48)	
Lignin		8.76% (0.11)	22.55% (0.16)	9.14% (0.14)	
Cellulose		4.08% (0.05)	4.85% (0.04)	0.18% (0.003)	
Sample Recovery	97.63%	98.13%	94.3%	75.2%	126.2%

[1]Rice shoot samples collected at 4 weeks post-treatment.
[2]Total radioactive residues reported as ^{14}C-propanil equivalents.
[3]Percent TRR. ^{14}C-propanil ppm equivalents reported in parentheses.

Many of the HPLC chromatograms could not be interpreted by the registrant due to interfering peaks in the regions of interest. Identification could probably be improved if radioactivity monitoring detection is used instead of the UV system at 254 nm. Ideally both detectors should be used in series.

Most of the TRR from the milled rice grain is associated with the starch fraction. Results of HPLC and TLC analyses could not be interpreted, primarily due to significant interferences. The registrant should attempt to characterize some of the starch related hydrolysates such as fraction AQ-5 or EtOAc-1.

Propanil and DCA were found in the organosoluble and MeOH/H_2O soluble rice shoot (4 weeks) fractions. Identifications were not confirmed, nor were they reported quantitatively. Identifications of metabolites should be confirmed by a dissimilar technique such as mass spectrometry (MS). Most of the radioactivity was associated with the combined pectin, hemicellulose, lignin, and cellulose fractions.

Most of the radioactivity in the rice straw, rice hull, and rice bran samples was associated with the hemicellulose fraction. It does not appear any of the rice straw hydrolysates were characterized by any chromatographic techniques. A DCA glucose conjugate was found in the rice straw MeOH/H_2O fraction. Propanil and DCA were found in MeOH/H_2O and organosoluble fractions of rice bran. Identifications were not confirmed, nor were they reported quantitatively.

Residue Analytical Method

Rice commodity samples were analyzed by an analytical method which is similar to Method II in PAM II. The samples were subjected to hydrolysis with 6 N NaOH in a reaction flask connected to a continuous extractor (with isooctane). Method II specifies spectrophotometric detection, but in this study the isooctane extract was analyzed by HPLC-RAM (radioactivity monitor) or subjected to further clean-up on a silica gel column and analyzed by GC with NPD detection for DCA residues. Results are presented in Table 3.

Table 3. Radiovalidation of Method for Determination of DCA

Matrix	%TRR Recovered in Isooctane Phase[1]	ppm ^{14}C-Propanil Equivalents HPLC-RAM	ppm ^{14}C-Propanil Equivalents GC-NPD
Rice Straw	29.30	0.278	0.384
Rice Hulls	26.92	0.184	--[2]
Rice Bran	27.46	0.345	0.739
Rough Rice Grain	25.06	0.097	0.147
Milled Rice Grain	4.84	N/A[3]	N/A

[1]As determined by HPLC-RAM.
[2]Could not be quantitated due to significant interference.
[3]Not analyzed due to insufficient radioactivity.

CBRS cannot determine if this method is adequate since the registrant has not quantitatively determined the total DCA residues in the metabolism study. Approximately 5-30% of the TRR can be extracted into isooctane with this method. It appears that the GC analysis quantifies more DCA than the HPLC-RAM method because of matrix interference. A more extensive clean-up step is required. When samples are fortified with [^{14}C]-propanil greater than 97% recovery is obtained, as determined by HPLC-RAM. Fortified samples were not analyzed by the GC technique. Fortifications should be done with DCA as well.

Radiovalidation of the actual enforcement method is required. If the registrant intends to revise the enforcement method an independent method validation (as per PR Notice 88-5, 7/15/88) of the enforcement method is required before an Agency validation can be conducted.

cc: CLOlinger (CBRS), Circulate, Reg. Std. File, RF, SF, C. Furlow (PIB/FOD)
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