List A File 8-8-94

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

SEP 8 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Propanil. List A Reregistration Case No. 0226/Chemical ID No. 028201. Propanil Task Force Submission to Upgrade a Rice Metabolism Study [Guideline Ref. No. 171-4(a)] and Rice Field Trials [Guideline Ref. No. 171-4(k)]. MRID Nos. 43285401 and 43282801. CBRS No. 14030. DP Barcode No. D205676.

FROM: Christina B. Swartz, Chemist
Reregistration Section II
Chemistry Branch II: Reregistration Support
Health Effects Division (7509C)

THRU: William J. Hazel, Ph.D., Section Head
Reregistration Section II
Chemistry Branch II: Reregistration Support
Health Effects Division (7509C)

TO: Esther Saito, Chief
Reregistration Branch
Special Review and Reregistration Division (7508W)

On behalf of the Propanil Task Force (PTF), NPC Inc. has submitted a rice metabolism study entitled "Metabolism of ^{14}C-Propanil in Rice: Metabolite Analysis and Quantitation in Various Parts of Rice Plant," 6/23/94 [MRID No. 43285401] and a magnitude of the residue study in rice entitled "Magnitude of the Residues of Propanil in or on Rough Rice Grain Treated with Propanil 4EC at 4 lb Plus 4 lb or 6 lb ai/A," 6/14/94 [MRID No. 43282801].

Tolerances are established for the combined residues of propanil (3',4'-dichloropropionanilide) and its metabolites (calculated as propanil) in or on barley grain (0.2 ppm); barley straw (0.75 ppm); cattle, goats, hogs, horses, poultry and sheep, (fat, meat, MBYP), 0.1 ppm; eggs (0.05 ppm); milk (0.05 ppm); oat grain (0.2 ppm); oat straw (0.75 ppm); rice (2 ppm); rice straw (75 ppm); wheat grain (0.2 ppm); and wheat straw (0.75 ppm) [40CFR §180.274]. There are no food/feed additive tolerances established under 40 CFR §185 and §186.

Background

The nature of the residue in plants is not adequately understood. Wheat and rice metabolism studies were required in the 8/26/87 Residue Chemistry Chapter of the propanil Reg. Std. A

wheat metabolism study was submitted to the Agency and deemed unacceptable, but may be upgraded with the submission of additional data [CBRS No. 9528, 4/2/92, J. Abbotts; and CBRS No. 11742, 11/30/93, F. Fort]. A rice metabolism study submitted in response to the Reg. Std. was also deemed unacceptable, but potentially upgradable, contingent upon the results of the following [CBRS No. 10228, 12/14/92, S. Funk]:

(1) The aqueous fractions from the strong base hydrolysis/distillation of bran, hulls, straw, raw grain, and milled grain must be characterized, and major components must be identified by 2 independent methods, MS strongly recommended;

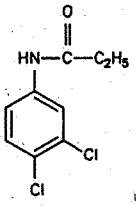
(2) The aqueous fraction from the bran extract must be characterized using another technique (in addition to TLC);

(3) Additional work to characterize/identify the acetonitrile fraction from rice straw (13 %TRR) must be conducted (TLC, LC/MS); HPLC indicated that there were 3 to 5 major components;

(4) Additional work must be done with the methanol/water ($MeOH/H_2O$) fraction of rice straw (12 %TRR), which had been characterized as polar compounds using only TLC; and

(5) The %TRR in the grain starch fraction attributable to ^{14}C-glucose should be determined or estimated.

The current submission (MRID No. 43285401) addresses these deficiencies. The structure of propanil is shown below:



propanil

Conclusions

Rice Metabolism Upgrade

1. The performing laboratory conducted all of the additional work required in the 12/14/92 S. Funk memo, and the submission was accompanied by adequate supporting raw data and documentation.

2. The performing laboratory was able to demonstrate that radioactive propanil residues were stable in frozen storage during the interval between the inital analysis and the later analyses conducted to upgrade the initial study.

3. Radioactivity in the aqueous distillate of rice grain was found to be either incorporated into or strongly conjugated with amino acids. It was estimated that 4.75% of the rice grain TRR (0.011 ppm) was incorporated into ^{14}C-glucose in the starch fraction of rice grain. Although only 1.53 % of the rice grain TRR was conclusively identified, CBRS concludes that the registrant has demonstrated that propanil is eventually broken down by plants, enters the carbon pool, and is incorporated into biomolecules.

4. Aqueous distillates of rice bran and straw contained some DCA residues either bound or conjugated with macromolecules. Although these metabolites would not be determined by the analytical method, CBRS can make a worst-case risk assessment based on the fact that 22% of the total propanil residue in rice straw is released as DCA. Rice bran is not considered to be an animal feed item, and therefore there is no concern about the nature of the residues not determined by the analytical method.

5. Radioactivity in the MeOH/H_2O fractions of rice bran and straw was multicomponent, and metabolites were characterized as sugars and conjugates with DCA. Radioactivity in the ACN extract of rice straw was multicomponent, and metabolites were partially characterized as DCA-related.

6. When the results of the submission to upgrade the wheat metabolism study (due to be submitted to the Agency by 8/31/94) are reviewed, the HED Metabolism Committee will be consulted to determine which propanil residues require regulation.

Rice Field Trials

7. Six field residue trials were conducted in AR (2), LA (2) and TX (2), principal rice growing areas in the U.S. representing 68-70% of national rice production (Agricultural Statistics, 1990). In each field trial, one test plot was treated with two ground (postemergence) applications of propanil at 4 lb ai/A 11 to 15 days apart, while the other test plot received one ground (postemergence) application of propanil at 6 lb ai/A, close to the end of tillering. The application rates and timing used are representative of registered labels and typical agricultural practice.

8. Rice and straw were harvested 67 to 80 days after the second treatment (4lb + 4lb) or 56 to 58 days after the single treatment (6lb). Adequate supporting documentation and information relating to the application/field portion of the study were provided in the final report.

9. The method used to analyze rice grain and straw samples obtained from the field trials is EN-CAS Method No. ENC-9/90, dated June 7, 1991; the method quantitates base-

releasable propanil residues as DCA. Residues are quantitated using a gas chromatograph (GC) equipped with an alkali flame nitrogen/phosphorous (N/P) detector. The limit of quantitation (LOQ) for rice grain and straw is 0.01 ppm.

10. Method validation data in rice were reviewed by the Agency and deemed acceptable. In the subject submission, concurrent method recoveries were obtained using fortified control rice grain and straw samples; procedural recoveries ranged from 71 to 120%, with an average of 91 $\pm$ 11% (n=11).

11. Rice grain and straw samples were held in frozen (-7 to -27 °C) storage for 29 to 60 days. Previously submitted rice storage stability data demonstrated that propanil residues are stable in frozen rice grain for up to 525 days, and in rice straw held at ambient temperature for up to 235 days.

12. Residues in rice grain ranged from 0.04 to 2.20 ppm; the sample containing the highest residue level of 2.2 ppm, which is higher than the established tolerance of 2 ppm, was obtained from a TX plot treated with 1 application of 6 lb ai/A, and harvested 56 days later. **CBRS recommends the registrant propose an increased tolerance of 3 ppm in or on rice grain.**

13. The data submitted and discussed in the Reg. Std. indicated that residues concentrate 5X in the rice bran, and therefore **CBRS recommends that the registrant propose a feed additive tolerance of 15 ppm in or on rice hulls (grain tolerance, 3 ppm X 5).**

14. Residues in rice straw ranged from 0.23 to 30.0 ppm; the highest residue was found in an AR sample harvested 56 days after treatment with 6 lb ai/A. Although the maximum residue of 30 ppm found in rice straw was much less than the established tolerance of 75 ppm, CBRS is reluctant to recommend for a decreased tolerance in rice straw without additional data from MS and CA, the states from which over-tolerance samples were harvested in previous studies.

15. Since aspirated rice grain fractions (grain dust) is no longer considered to be a significant animal feed item (refer to the "Updated Livestock Feed Tables," 6/94), the requirement for submission of data depicting propanil residues in rice grain dust no longer applies.

Recommendation

CBRS concludes that the rice metabolism study has been upgraded to acceptable, with the submission of additional data. Much of the previously unidentified radioactivity was partially characterized, characterized or identified. In rice grain, residues not determined by the analytical method are more than likely not of regulatory concern. However, the HED Metabolism Committee will be called upon to determine which metabolites are of regulatory concern once the wheat metabolism study has been upgraded.

The rice field trials are adequate, and indicate that the tolerance in rice grain should be increased to 3 ppm (see Conclusion 12). In addition, CBRS recommends that the registrant propose a feed additive tolerance of 15 ppm in or on rice hulls, based on a grain tolerance of 3 ppm, and a concentration factor of 5X in rice hulls (see Conclusion 13).

DETAILED CONSIDERATIONS

Nature of the Residue in Rice (Guideline Ref. No. 171-4(a)

The original rice metabolism study (MRID Nos. 42382901 and 42382902) was reviewed in detail in the 12/14/92 S. Funk memo (CBRS No. 10228). Briefly, rice was treated foliarly at a 1X rate; harvested samples included mature straw and rough grain which was processed into hulls, bran, and milled rice. Samples were subjected to a modified Bligh-Dyer extraction, yielding ACN, hexane, aqueous and post-extraction solid (PES) fractions. The PES were then subjected to sequential treatment with protease, amylase, EGTA, KOH, thioglycolic acid/sodium hydroxide (NaOH), and acid. Solvent extractions released a small percentage of the TRR, indicating a high level of bound and/or conjugated residues. Rice straw and milled fractions were also subjected to analysis using a modification of the existing analytical enforcement procedure, but only the residues in the resultant hexane fractions were identified. Based on the TLC and HPLC analyses conducted by the registrant, the deficiencies cited above were noted by the CBRS reviewer.

The completion date of the original study was 10/28/91 for the in-life phase, and 6/29/92 for the analytical phase. In order to verify the TRRs in rice matrices, the study to upgrade the original metabolism study included combustion of retained samples. The results are found in Table 1.

Table 1. Determination of the TRR in Rice Matrices.[1]

Matrix	Straw	Bran	Milled Grain	Rough Grain	Hull
1994 Value	2.078	2.826	0.539	0.545	0.684
1992 Value	1.510	1.551	0.245	0.483	0.708

[1] Results are reported in ppm, ^{14}C-propanil equivalents.

The performing laboratory did not provide any explanation for the higher TRR values obtained during the later analysis. The distribution of the TRR between the acetonitrile, hexane, and MeOH/H_2O extracts and the post-extraction solids (PES) determined in the original study and in the subsequent attempt to upgrade the study are shown in Table 2. It was concluded that radioactive residues had remained stable in frozen storage.

Table 2. Distribution of the TRR in Rice Straw and Bran Extracts.[1]

Fraction	Acetonitrile	Hexane	MeOH/H_2O	PES (Bound)
Straw (1994)	9.55	2.17	19.84	68.44
Straw (1992)	8.59	1.30	16.37	73.74
Bran (1994)	8.79	8.28	22.16	65.53
Bran (1992)	3.91	3.52	23.08	64.72

[1] The numbers are presented as %TRR found in a particular fraction.

Extraction

The performing laboratory conducted a Nielsen-Kryger (strong base) distillation of mature rice straw, rice bran, rough rice grain, milled rice grain and hulls. Since a sufficient quantity of rice bran was not available, the post-extraction solids (PES) remaining after the modified Bligh-Dyer extraction of the rice bran were used in the distillation. Samples were placed in distillation flasks with antifoam, NaOH and hexane; once the flasks boiled, the distillation was continued for 20 to 25 hours. Samples were cooled for 30 minutes, and the hexane and aqueous fractions were collected and frozen to facilitate separation of the layers. The hexane layer was removed, and the aqueous fraction rinsed with hexane. The combined hexane fractions were analyzed using HPLC.

The aqueous fraction was centrifuged and the supernatant and/or the entire mixture filtered to yield a second aqueous fraction and post-extraction solids (PES). The aqueous filtrate/supernatant was neutralized prior to HPLC analysis. Neutralized hydrolysates were cleaned up on sulfonic acid cartridges, eluted with water, and subjected to preparative HPLC in order to isolate major components. Neutralized aqueous fractions from the rice bran PES and milled rice grain were cleaned up on amino cartridges, rather than sulfonic acid cartridges. Aqueous eluates from the cartridges were used for preparative HPLC.

Rice straw and bran samples were once again subjected to the modified Bligh-Dyer extraction, as was done in the original study. Fractions obtained from the extraction included an ACN-soluble fraction, a hexane-soluble fraction, an MeOH/H_2O fraction, and a PES fraction. The results of the extraction are shown in Table 2 (above), and were used to verify stability of propanil residues in rice matrices during frozen storage.

Analytical Methods Used to Characterize/Identify Metabolites

Percent distributions of components in hexane fractions from the Nielsen-Kryger distillations were determined using a solid-cell radioactive monitor. Radioactivity in extracts was determined using liquid scintillation counting (LSC) or combustion followed by LSC of the trapped $^{14}CO_2$. Reversed-phase high performance liquid chromatography (RP-HPLC) was used to directly analyze extractable and hydrolyzed residues, but also to separate and isolate components that were then further analyzed using RP-HPLC, normal-phase HPLC (NP-HPLC) and TLC. Radioactivity in HPLC eluates fractionally collected every 30 seconds was determined using LSC, and reconstructed HPLC chromatograms were generated. UV chromatograms indicated significant matrix effects, which resulted in tailing during HPLC analysis, and consequently resulted in incomplete resolution of metabolites in extracts analyzed using HPLC.

Three different HPLC conditions (I, II and III) were used in the analysis of propanil metabolites. Retention times (R_t) of metabolite reference chemicals under the three different conditions were provided. Preparative (RP-HPLC) chromatography eluates were collected at one-minute intervals, and subsequently analyzed using NP-HPLC under different chromatographic conditions. Specific conditions were adequately described in the report.

Metabolite reference standards for which HPLC retention times were provided included propanil, N-3,4-dichlorophenyl-glucosylamine, N-3,4-dichlorophenyl-Na-glucuronate amine, 3,4-dichloroaniline-N-sulfamic acid potassium salt, 2-amino-4,5-dichlorophenol, 3′,4′-dichlorolactanilide, 3′,4′-dichloroacetanilide, 3,4-dichloroaniline, and numerous sugars.

Normal phase thin layer chromatography (TLC) in either one or two dimensions was used for qualitative confirmation and characterization of isolated metabolites. Six different solvent systems were used, and were adequately described by the registrant. The R_f values for metabolites reference chemicals under the six different conditions were provided. Reference chemicals and metabolites were visualized with UV light. Sugar standards were visualized by spraying the plates with H_2SO_4/anisaldehyde/HOAc, 1:5:50, followed by heating. TLC radiochromatograms were obtained using a FUJIX BAS 100 Bio-imaging Analyzer System; plates were scanned from 70 minutes to 240 hours depending on the level of radioactivity.

Results

Nielsen-Kryger Distillation (Response to Deficiency 1 cited above)

The results of the Nielsen-Kryger distillation are presented in Table 3 (see below). The percent of the TRR in rice straw, rice bran PES, rice hull, rough rice grain and milled rice grain recovered in the hexane fraction was similar to that found in the isooctane fraction in the original study. In addition, the %TRR identified as DCA (in the hexane fractions) was similar to that determined in the original experiment (isooctane fractions). CBRS was concerned that the radioactivity in the aqueous base hydrolysate was not identified in the original study, though it contained from 32% of the TRR for rice hulls to 73% of the TRR for milled grain. As in the

original study, the results of the Nielsen-Kryger distillation indicated that a significant percentage of the TRR (for rice straw, bran PES, hull, rough grain and milled grain) was found in the aqueous hydrolysate.

The rice straw aqueous hydrolysate comprised 63.01% of the rice straw TRR (1.31 ppm). HPLC analysis of the neutralized aqueous hydrolysate revealed 2 major polar components; subsequent 2D-TLC analysis of one of the peaks was inconclusive, since no discernible spot was seen due to low radioactivity. The other peak was subjected to normal phase HPLC, and found to contain more than one component; TLC analysis could not be done due to matrix interference. The performing laboratory stated that the components could have been DCA strongly attached to humic acid, or polyphenolic molecules or polymerization products of the metabolites formed during the hydrolysis.

The aqueous basic hydrolysate obtained from the distillation of the rice bran PES contained 56.83 %TRR (1.05 ppm); HPLC analysis of the hydrolysate revealed one polar peak; the neutralized fraction, cleaned up on an amino cartridge and analyzed by normal phase HPLC, contained 3 peaks isolated by preparative HPLC. Subsequent TLC analysis of 2 of the 3 peaks was not fruitful, since co-existing matrices caused interference, and limited the amount of radioactivity that could be applied to the plates. One of the peaks was subjected to 2D-TLC with glucose, galactose and mannose standards, but the peak did not co-chromatograph with any of the standards. As with the rice straw, the performing laboratory concluded that the components of the aqueous hydrolysate could be either DCA strongly attached to humic acid or polyphenolic molecules of plant origin or polymerization products of the metabolites formed during strong base hydrolysis.

The basic aqueous hydrolysate obtained from the distillation of the milled rice grain contained 91.12% of the grain TRR (0.491 ppm), which showed up as one polar peak on the reconstructed HPLC chromatogram. Once the hydrolysate was neutralized and cleaned up on an amino cartridge, no discernible radioactive peak was seen; the sample was then subjected to preparative HPLC, and the fractions collected every minute. One fraction containing the highest radioactive residue was derivatized (acetylated), but the reconstructed chromatogram of the acetylated mixture also did not show a discernible peak. The TMS-derivative of the fraction was prepared, and the ion-chromatogram obtained for the mixture. Mass spectra were obtained for each peak on the total ion chromatogram, and compared with reference mass spectra. This procedure demonstrated that some of the coeluting matrices were amino acids, such as leucine, glycine, isoleucine and short chain organic acids. However, no known propanil metabolites (containing the 3,4-DCA moiety) were identified in the fraction.

HPLC analysis of the rough rice grain neutralized basic fraction (54.48 %TRR, 0.297 ppm) did not yield identification of known propanil metabolites. As with the rice straw and the rice bran PES, the performing laboratory concluded that the components of the aqueous hydrolysate could be either DCA strongly attached to humic acid or polyphenolic molecules of plant origin or polymerization products of the metabolites formed during strong base hydrolysis.

The neutralized aqueous basic fraction from rice hulls (47.19 %TRR, 0.323 ppm) was analyzed using HPLC and found to contain one major polar peak. The neutralized fraction was subjected to preparative HPLC, and some of the pooled fractions were analyzed using NP-HPLC; however, no discernible radioactive peak was identified.

Table 3. Results of the Nielsen-Kryger Extraction.

Sample ID	Nielsen-Kryger Extraction					% DCA in Hexane by HPLC	% TRR as DCA	% TRR as DCA[6] (previous)
	^{14}C-Distribution (%TRR)[1]							
	Solids[2]	AQ-2[3]	AQ-1[4]	Hexane[5]	% Recovery			
Straw	12.63	63.01	N/A	24.36	94.47	78.85	19.21	22.80
Bran PES[7]	13.33	56.83	0.79	29.05	103.18	88.93	25.83	22.23
Hull	24.05	47.19	0.29	28.47	106.50	86.98	24.76	19.97
Rough Grain	23.49	54.48	0.94	21.10	98.61	92.86	19.59	22.03
Milled Grain	3.90[8]	91.12	0.45	4.53	99.47	91.38	4.14	4.84[9]

[1] Normalized distribution, with the exception of the rice bran sample.

[2] Solids remaining after base hydrolysis.

[3] Basic hydrolysate.

[4] Aqueous fraction partitioned from the hexane fraction.

[5] Hexane fraction of the aqueous distillate.

[6] Determined in the original study, and reported in the 12/14/92 S. Funk memo.

[7] Since there was no bran left over for the hydrolysis, the PES from the solvent extraction was used.

[8] The filter paper was combusted with the solids, and is therefore included in the TRR.

[9] In the original study, DCA was assumed to comprise 100% of the hexane fraction TRR, but was not actually determined due to low radioactivity.

Analysis of the MeOH/H_2O Fraction from Rice Bran (Response to Deficiency 2, cited above)

Four radioactive peaks were found following HPLC analysis of the filtered MeOH/H_2O fraction of rice bran. This fraction contained 22.16 % of the bran TRR (0.626 ppm); residues in the fraction were characterized as polar using TLC in the original study. The four peaks elucidated using HPLC were labeled Metabolites E, H, J and L. The peak containing metabolite L (0.087

ppm) was eluted and then analyzed using 2D-TLC, and was subsequently characterized as either sucrose or mannose; based on HPLC data, it was concluded that Metabolite L was sucrose.

The peak containing Metabolite E (0.034 ppm) was eluted and analyzed using 2D-TLC, but the results were inconclusive due to trailing. The fraction was subjected to mild base hydrolysis (0.25 N NaOH), and DCA was identified (using TLC) as the major aglycone in the hydrolysate. Similar results were obtained for Metabolite H (0.467 ppm), indicating that metabolites E and H were conjugates of DCA.

The peak containing Metabolite J (0.039 ppm) was also eluted and analyzed using 2D-TLC; DCA was identified in the eluate via co-chromatography. Using different solvent systems, the peak was shown to contain both polar and nonpolar radioactive spots. The nonpolar spot was thought to be a result of hydrolysis of the original polar metabolite. Following mild base hydrolysis (0.25 N NaOH) of the fraction, DCA was identified as the major aglycone. Hence, Metabolite J was also characterized as a conjugate of DCA.

Analysis of the MeOH/H_2O Fraction from Rice Straw (Response to Deficiency 4, cited above)

The MeOH/H_2O fraction from rice straw was filtered and subjected to preparative HPLC; 6 different peaks were observed, and designated peaks 1 and 2, and Metabolites F, G, I and K. The peak 1 and 2 eluates were analyzed using HPLC, and found to contain 4 peaks, designated Metabolites A through D. Upon analysis of the eluates using 2D-TLC, Metabolites A through D were found to be slightly more or less polar than co-chromatographed mono-, di-, tri- or tetrasaccharides.

Subsequent 2D-TLC analysis of eluates containing Metabolites G, I and K did not reveal the identities of the metabolites. Following mild base hydrolysis (0.25 N NaOH) of the eluates, all of the fractions were found to contain DCA, indicating that some of the metabolites were conjugates of DCA. A subsample of the rice straw MeOH/H_2O fraction was subjected to the Nielsen-Kryger distillation. The hexane fraction was found to contain 24.73% of the starting radioactivity, while the remaining radioactivity was found in the aqueous fractions. HPLC analysis of the hexane fraction identified DCA as 82% of the hexane fraction radioactivity. Analysis of the neutralized aqueous hydrolysate showed only one peak, in the region of Peaks 1 and 2 (see above), indicating that Metabolites F, G, I and K are conjugates of DCA.

Analysis of the ACN Fraction from Rice Straw (Response to Deficiency 3, cited above)

The ACN fraction (9.55 %TRR, 0.198 ppm) was cleaned up on a C-18 column and the MeOH eluate subjected to preparative HPLC, revealing 4 different peaks. Two of the peaks were identified (via co-chromatography with reference standards) as dichloroacetanilide (2.47 %TRR, 0.052 ppm) and DCA (2.81 %TRR, 0.059 ppm). Metabolite M was isolated using HPLC and subjected to mild base hydrolysis; 2D-TLC analysis of the hydrolysate indicated that the metabolite could be hydrolyzed to DCA. Metabolite N was analyzed in a similar fashion; the only radioactive component of the mild base hydrolysate (determined using 2D-TLC) was DCA,

indicating that the metabolite was a DCA-related compound.

%TRR in Grain Starch Attributed to ^{14}C-Glucose (Response to Deficiency 5, cited above)

In the original study, the rice grain PES remaining after the Bligh-Dyer extraction procedure was subjected to enzyme hydrolysis with amylase, yielding an enzyme hydrolysate and PES-2. The hydrolysate was then subjected to enzyme hydrolysis with glucosidase, to yield a starch hydrolysate, which was subsequently cleaned up using ion-exchange chromatography, concentrated, and finally acetylated. Chromatograms resulting from HPLC analysis of each of the fractions were provided in the original report. In the current submission, the performing laboratory calculated the percentage of the TRR in the grain starch attributable to ^{14}C-glucose using the following equation:

$$\%A \times \%B \times \%C \times \%D \times 10^{-6} = \% \text{ glucose}$$

$$(31.10 \times 79.13 \times 56.80 \times 34.00) \times 10^{-6} = 4.75\%$$

where,

A = %TRR released from PES by amylase
B = % recovery in the starch hydrolysate after glucosidase hydrolysis
C = % recovery in the neutral fraction after cleanup of the starch hydrolysate
D = % acetylation yield of the starch hydrolysate (based on HPLC/LSC data)

CBRS Comments

The performing laboratory conducted all of the additional work required in the 12/14/92 S. Funk memo. Sufficient raw data and sample chromatograms accompanied the submission to upgrade the metabolism study. The laboratory first attempted to demonstrate the stability of the radioactive residues under frozen conditions. Although the TRR determination yielded much higher results during the later analysis, the % distribution of the radioactivity between the various extracts was similar to that found in the original study. Similarly, the distribution of radioactivity between the distillation fractions following Nielsen-Kryger distillation was the same as was found during the original study. When various extracts from the later extraction/analysis were analyzed using HPLC, the profiles were the same as those obtained with the first analysis. The storage stability of the radioactive residues in rice matrices was adequately demonstrated by the registrant.

The Nielsen-Kryger distillation procedure is used in the existing and modified enforcement methods, and is similar to the modified Bleidner extraction that was used in the initial study. Since residues in the resultant aqueous basic hydrolysate are not quantitated in these methods, CBRS wanted to ascertain that the aqueous residues were not of regulatory concern. The results of attempts to characterize these residues were inconclusive, since none of the radioactivity was identified. The aqueous fraction of the milled grain hydrolysate was demonstrated to contain

^{14}C-amino acids which co-eluted with other unidentified radioactivity. Other aqueous distillates (i.e. straw, bran, and rough rice grain) were believed to contain DCA strongly attached to humic acid or polyphenolic molecules or polymerization products of the metabolites formed during the hydrolysis.

As required, the registrant estimated that 4.75% of the TRR was incorporated into ^{14}C-glucose in the starch fraction of rice grain. The analysis of the aqueous distillate from the Nielsen-Kryger distillation demonstrated radioactivity was incorporated into or conjugated with amino acids. Based on the results of the two experiments, CBRS concludes that the registrant has demonstrated that propanil is eventually broken down by plants, enters the carbon pool and is incorporated into biomolecules. It is also likely that propanil metabolites are either strongly conjugated with or entrapped by macromolecules.

Radioactivity in the MeOH/H_2O fractions of rice bran and straw was found to be multicomponent, and metabolites were characterized as sugars and conjugates with DCA. In rice straw, it is likely that some of these metabolites would be determined by the analytical method (i.e. are base-releasable), while others would not. The radioactivity in the ACN extract of rice straw was multicomponent in nature, and 2 of the metabolites were partially characterized as DCA-related.

Even though the residues in the aqueous distillates of rice bran and straw more than likely contained some DCA either bound or conjugated with macromolecules, CBRS is not concerned that these residues are not quantitated using the proposed analytical method (i.e. the Nielsen-Kryger distillation, in which residues in the hexane fraction are quantitated), since rice bran is not considered to be an animal feed item, and since rice straw constitutes a maximum of 10% of the beef/dairy cattle diet, and is not considered to be a poultry or swine feed item. Furthermore, a worst-case risk assessment can be made based on tolerance level residues in straw and the results of the Nielsen-Kryger distillation showing that 22% of the total propanil residues in straw are released as DCA.

The overall summary of the results of the rice metabolism study (including additional information obtained from the submission to upgrade the study) are presented in Table 4. Since the TRR determination for the second study yielded different results, the % distribution of metabolites in the MeOH/H_2O and ACN fractions of rice bran and straw were normalized to the TRR values obtained in those fractions from the original study.

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Table 4. Percent Distribution of Propanil Metabolites in Rice Matrices.[1]

Metabolite	Rice Matrix (TRR, determined in original study)[2]			
	Grain (0.234 ppm)	Bran (1.551 ppm)	Hulls (0.703 ppm)	Straw (1.218 ppm)
Extractable Residues (Identified)				
Propanil/Nonpolar	1.53	0.48	0.47	ND
3,4-dichloroaniline	ND[3]	0.24	0.37	2.81
3,4-dichloro-glucosylamine	ND	ND	1.96	1.89
3′,4′-dichloro-acetanilide	ND	ND	ND	2.47
Total ID'd	**6.28**[4]	**0.72**	**2.80**	**7.17**
Extractable Residues (Characterized)				
Polar Unknown	5.32	3.19[5]	3.07	5.56
Conjugates of DCA	ND	19.89	ND	6.87
Bound Residues (Characterized)				
Protein Related	25.30	14.23	6.71	11.82
Starch Related	62.27	2.35	0.96	1.75
% as ^{14}C-glucose	4.75	NA[6]	NA	NA
Pectin Related	4.48	8.06	5.40	46.79
Hemicellulose Related		30.76	44.62	
Lignin Related		9.14	22.55	
Cellulose Related		0.18	4.85	
Total Bound	92.05	64.72	85.09	60.36
Total Partially Characterized	**92.62**[7]	**87.80**	**88.16**	**72.79**
Total ID'd + Characterized	**98.90**	**88.52**	**90.96**	**79.96**

[1] Information was taken from both the original study and the submission to upgrade the study.

[2] The TRR is expressed as ppm, ^{14}C-propanil equivalents.

[3] ND = Not detected.

[4] Includes 4.75 %TRR as glucose, which was identified (bound, rather than extractable).

[5] Includes Metabolite L, which was characterized as sucrose.

[6] NA = Not analyzed.

[7] Does not include the 4.75% of the TRR which was identified as glucose in the fraction containing starch-related radioactivity.

Magnitude of the Residue in Rice Grain and Straw (Guideline Ref. No. 171-4(k)

The Propanil Task Force submitted a report entitled "Magnitude of the Residues of Propanil in or on Rough Rice Grain Treated with Propanil 4EC at 4lb Plus 4lb or 6lb ai/A," dated 6/14/94 (MRID No. 43282801). The analytical portion of the study was conducted by EN-CAS Laboratories of Winston-Salem, NC.

The test substance used in the field trials was a 4EC formulation of propanil, containing 4 lbs ai/gal. Currently registered labels allow postemergence applications of 3 to 6 lbs ai/A, with a maximum single application rate of 6 lbs ai/A, and a maximum seasonal rate of 8 lb ai/A. The pesticide is applied when weeds are actively growing, up until the end of tillering (approximately 30 to 45 days after rice is planted). No PHI is specified on the label, nor is there a specified interval between treatments when more than one application is made.

Field Trials

Six field residue trials were conducted in AR (2), LA (2) and TX (2), principal rice growing areas in the U.S. representing 68-70% of national rice production (Agricultural Statistics, 1990). These locations are found in field trial regions 4 and 10, discussed in the recent Agency guidance entitled "Number and Location of Domestic Crop Field Trials," dated 6/94. Each field trial location consisted of one control plot and 2 test plots; one test plot was treated with two ground (postemergence) applications of propanil at 4 lb ai/A, while the other test plot received only one ground (postemergence) application of propanil at 6 lb ai/A. The application rates and timing used are representative of registered labels and typical agricultural practice.

The two 4lb applications were made 11 to 15 days apart, with the first application made 30 to 34 days after planting, and the second application 45 to 50 days after planting. The 6lb application was made 56 to 69 days after planting, close to the end of tillering. Untreated and treated plots were separated by adequate buffer zones, and ranged in size from 1620 to 4897 square feet. Soil and water samples obtained from the sites were analyzed and adequately characterized. The ground applications to growing rice plants were made using CO_2 backpack equipment, in spray volumes ranging from 15 to 19 gallons per acre (gpa). Paddies were drained prior to application, and then reflooded 24 hours following application.

Rice and straw were harvested 67 to 80 days after the second treatment (4lb + 4lb) or 56 to 58 days after the single treatment (6lb). Untreated control samples were harvested first, to prevent contamination of control samples. Each plot was divided into zones (A, B and C), and samples consisting of 1.0 lb of rice straw and 2.5 lbs of rough rice grain were harvested from random locations within the zones. Rough rice and mature straw samples obtained at harvest were then frozen and shipped frozen to the performing laboratory for analysis.

Analytical Methodology

The method used to analyze rice grain and straw samples obtained from the field trials is entitled "Analytical Method for the Determination of Propanil as Base Releasable 3,4-Dichloroaniline (DCA) in Soil, Water, Crayfish (edible portion), Rough Rice Grain, Polished Rice Grain, Rice Hulls, Rice Bran, and Rice Straw and the Determination of Base Releasable 3,4-Dichloroaniline from N-(3,4-Dichlorophenyl)-D-glucosylamine (DCA-glucose) in Crayfish," EN-CAS Method No. ENC-9/90, dated June 7, 1991.

Rice grain and straw samples are hydrolyzed with sodium hydroxide (NaOH), which results in conversion of propanil and metabolites containing the 3,4-DCA moiety to 3,4-DCA. The hydrolysate is steam distilled for 16 hours using a Nielsen-Kryger apparatus, and the hexane and water fractions separated. The hexane fraction is then cleaned up on a silica gel column which has been pre-conditioned with hexane. The aqueous phase is washed with hexane, and the hexane wash added to the column. Residues are eluted from the column using hexane/ethyl acetate (EtOAc) [75/25, v/v]. Residues are quantitated using a gas chromatograph (GC) equipped with a DB-17 or DB-1701 column and an alkali flame nitrogen/phosphorous (N/P) detector. Residues are determined as 3,4-DCA, and calculated as the parent, propanil. The limit of quantitation (LOQ) for rice grain and straw is 0.01 ppm.

The analytical method has been validated in rice commodities by EN-CAS laboratories. Method validation data in rice were reviewed by the Agency and deemed acceptable (memo, R.B. Perfetti, 6/22/92; CBRS No. 9589, DP Barcode No. D175886). In the present residue study, concurrent method recoveries were obtained using fortified control rice grain and straw samples. These fortifications were made at 0.01, 0.05, 1 and 2 ppm. The procedural recoveries ranged from 71 to 120%, with an average of 91 ± 11% (n=11).

Results

Results of the analysis of field trial samples are presented in Table 5.

Table 5. Base-Releasable Propanil Residues in Rice Grain and Straw[1].

Site	Apple. Rate[2]	PHI[3] (Days)	Residue, Rough Grain (ppm)		Residue, Straw (ppm)	
			Replicates	Average	Replicates	Average
AR	4 + 4	67	0.08; 0.11; 0.12	0.10 ± 0.02	2.10; 2.80; 4.50	3.13 ± 1.23
		67	0.04; 0.07; 0.10	0.07 ± 0.03	0.79; 0.90; 3.30	1.66 ± 1.42
	6	56	1.30; 0.71; 1.20	1.07 ± 0.32	23.0; 13.0; 21.0	19.0 ± 5.3
		56	0.33; 0.47; 0.90	0.57 ± 0.30	15.0; 19.0; 30.0	21.3 ± 7.8
LA	4 + 4	80	0.07; 0.06; 0.09	0.07 ± 0.02	0.38; 0.37; 0.37	0.37 ± 0.01
		73	0.12; 0.12; 0.09	0.11 ± 0.02	0.47; 0.36; 0.31; 0.26	0.35 ± 0.09
	6	65	1.30; 1.30; 1.30	1.30 ± 0.0	4.30; 4.30; 3.70	4.10 ± 0.35
		58	1.20; 1.10; 1.0	1.10 ± 0.10	4.0; 6.60; 5.20	5.27 ± 1.30
TX	4 + 4	74	0.10; 0.14; 0.09	0.11 ± 0.03	1.60; 2.90; 1.10	1.87 ± 0.93
		74	0.05; 0.04; 0.05	0.05 ± 0.01	0.82; 0.51; 0.47	0.60 ± 0.19
	6	56	1.60; 1.60; 2.20	1.80 ± 0.35	23.0; 27.0; 24.0	24.7 ± 2.08
		56	1.00; 0.66; 1.00	0.89 ± 0.20	5.20; 4.80; 7.00	5.67 ± 1.17

[1] The residue analytical method determines all base-releasable propanil residues as DCA, which are then calculated as the parent, propanil.

[2] Either 2 applications were made 11 to 15 days apart at 4 lb ai/A (4 + 4), or a single application was made prior to the end of tillering (6).

[3] PHI = Pre-harvest interval.

Storage Stability

Rice grain and straw samples were held in frozen (-7 to -27 °C) storage either at the test site, during transit, or at the analytical laboratory for 29 to 60 days. Adequate chain of custody information was included in the study report. According to the residue chemistry chapter of the propanil Reg. Std. (8/26/87), rice storage stability data demonstrate that propanil residues are stable in frozen rice grain for up to 525 days, and in rice straw held at ambient temperature for up to 235 days. These data were generated using a Rohm and Haas colorimetric method which was deemed adequate for data collection purposes, but not for enforcement purposes due to its inability to distinguish between substituted anilines.

Both the enforcement method and the method used in the subject rice field trial study include an initial strong base hydrolysis step. Therefore CBRS concludes that the previously submitted storage stability data are adequate to support the subject rice field trial residue data.

CBRS Comments

The Agency's intent in requiring additional rice residue data (see the 8/26/97 Reg. Std.) was to determine if the data support the established tolerances on rice and straw. Some of the samples obtained from MS and CA in the earlier field trials had tolerance-exceeding residues, but this was presumably due to spray drift from other applications. In order to confirm the required tolerances, the Agency required the submission of additional field trial data (including aerial and ground applications) from all the major rice growing areas. While CBRS would have preferred to see data from the two areas from which samples containing over-tolerance residues were taken, the study is adequate regarding geographic representation.

The field trials were adequate with respect to the plot sizes, application rates and procedures, sample harvest, reporting of weather, soil and water conditions, and sample maintenance. The analytical laboratory report was complete. Adequate supporting sample chromatograms were included, along with sample calculations and raw data.

One TX rough rice grain sample contained propanil residues of 2.2 ppm, which is higher than the established tolerance of 2 ppm; this sample was obtained from a plot treated with 1 application of 6 lb ai/A, and harvested 56 days later. The highest residue in rice straw was found in an AR sample harvested 56 days after treatment with 6 lb ai/A. The data indicate that the rice grain tolerance should be increased to 3 ppm. Although the maximum residue found in rice straw was 30 ppm, much less than the established tolerance of 75 ppm, CBRS is reluctant to recommend for a decreased tolerance in rice straw without additional data from MS and CA, the states from which over-tolerance samples were harvested in previous studies.

The data submitted and discussed in the Reg. Std. indicate that residues concentrate 5X in the rice bran, and therefore CBRS recommends that the registrant propose a feed additive tolerance of 15 ppm in or on rice hulls (grain tolerance, 3 ppm X 5). Since aspirated rice grain fractions (grain dust) is no longer considered to be a significant animal feed item (refer to the "Updated Livestock Feed Tables," 6/94), the requirement for submission of data depicting propanil residues in rice grain dust no longer applies.

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