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

DEC 14 1992

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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Reregistration of Propanil. 171-4(a): Nature of the Residue in Rice. DP Barcode D180736. MRID Nos. 42382900 - 42382902. CBRS No. 10228.

From: Stephen Funk, Ph.D., Chemist S.A. Funk
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On behalf of the Propanil Task Force, whose members are Cedar Chemical Corp., Retzloff Delta Co., and Rohm & Haas Co., NPC, Inc. of Sterling, VA has submitted a final report on the metabolism of propanil in/on rice. Interim results were previously reviewed (C. Olinger, 02/14/92, CBRS No. 8703), and the following recommendations were made for upgrading the study:

1. Sample storage intervals and conditions must be detailed, especially the period from harvest to processing.

2. Metabolite identifications must be confirmed by a second, dissimilar technique, preferably mass spectrometry.

3. All fractions which contain significant radioactivity should be characterized, including acid/base and enzyme

hydrolysates.

4. The hplc should be equipped with a more selective detector than the uv. A radiochemical detector is appropriate.

5. Flow charts (for each commodity) showing extraction paths, sample weights, and dpm values should be submitted.

6. Intentions for the enforcement analytical method should be clarified. Radiovalidation was conducted with a method dissimilar from the enforcement methods.

Propanil, or N-(3,4-dichlorophenyl) propionamide, is a contact type selective postemergence herbicide. Tolerances are established (40 CFR 180.274) for residues of propanil and its metabolites, calculated as propanil, in/on barley (grain, straw), oats (grain, straw), wheat (grain, straw), rice, rice straw, and animal commodities. Feed additive tolerances have been established (40 CFR 186.1875) for residues of propanil and its metabolites in/on rice bran, rice hulls, rice polishings, and rice mill fractions, each at 10 ppm.

In addition to the present rice metabolism study, a wheat metabolism study has been submitted and reviewed (J. Abbotts, CBRS No. 9528, 04/02/92). Additional work, possibly including a new study, was required. The registrant responded with a plan for conducting additional work on existing wheat metabolism fractions (S. Funk, CBRS No. 10448, 12/14/92).

The structure of propanil (and the proposed metabolic pathway) is shown in Figure 1, a duplication of the registrant's Figure 36, page 87.

Conclusions

1. The submitted rice metabolism study is not acceptable for the reasons stated in Conclusion Nos. 3 - 5, but may be upgraded by the submission of the following additional data:

a. 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 two independent analytical methods. The use of ms is strongly recommended.

b. The aqueous fraction (24% of trr, 0.36 ppm) from the extraction of bran must be characterized by techniques in addition to tlc. Characterization as polar materials that did not migrate in tlc is inadequate. Major components must be identified by two independent analytical methods.

c. The acetonitrile fraction from rice straw (13% of trr, 0.16 ppm) was characterized by hplc as a complex mixture of compounds. No additional work was performed. The chromatogram indicates that there may be 3 to 5 major components. Additional characterization (tlc, lc/ms) is required, and any major components should be identified.

d. The methanol/water fraction of the rice straw (12% of trr, 0.15 ppm) was characterized by tlc only as polar compounds. Additional characterization is required. Any major components must be identified.

e. The percentage of trr in the grain starch fraction attributable to ^{14}C-glucose should be determined or estimated.

2. The analytical method may be adequate for the determination of free and conjugated propanil, but the method differs significantly from the existing enforcement methods. The existing method used gc and required derivatization of the distillate. The new method does not require derivatization and uses hplc or gc/npd, with the hplc procedure being free of interferences found with gc. An independent laboratory method validation will be required if the registrant is proposing the method as regulatory (NPC, Incorporated letter of 04/22/92 to SRRD). CBRS previously recommended for a delay in imposing this requirement for a closely related method for animal commodities (L. Cheng, CBRS No. 9807, 05/28/92). One of the reasons for the delay was the need to establish the metabolites to be regulated. Acceptable plant metabolism studies have not been submitted, and both the rice and wheat metabolism study results must be considered in determining the residue to be regulated. Also, an acceptable enforcement method does exist in PAM, Vol 2, Method I. Therefore, no independent laboratory validation of the new method will be required at this time.

3. The majority of the radiolabel was released from rice grain (109%), straw (92%), hulls (81%), and bran (83%) by a combination of solvent extraction and enzyme and chemical hydrolyses. Solvent extraction (organic and aqueous) released only a minority of the total radioactive residue: 8.4% in rice, 34% in bran, 15% in hulls, and 32% in straw. Only propanil (0% - 1.5% of trr) and DCA (0% - 0.2% of trr) were tentatively identified. Confirmatory analyses were not provided. Fractions containing $\geq$ 10% of the trr and $\geq$ 0.05 ppm were not adequately characterized. These include the acetonitrile and water/methanol fractions of rice straw and the methanol/water fraction of rice bran (see 1b, 1c, 1d).

4. The majority of the radiolabel remained with the post-solvent extraction solid for straw (74%), hulls (85%), bran (65%), and grain (92%). The registrant interprets this as inclusion of the radiolabel in natural compounds, either bound or catabolized and reincorporated. These non-extractable residues were characterized

as protein, starch, pectin, hemicellulose, lignin, and cellulose through a series of enzyme and chemical hydrolyses. For grain, about 72% of the trr was characterized as starch, and this result from protease/amylase digestion was confirmed by amyloglucosidase digestion. The presence of ^{14}C-glucose in the starch fraction of grain was shown by hplc and gc/ms. This proves some degree of propanil breakdown and reincorporation, but no quantitative results were provided. Efforts should be made to quantitate the percentage of starch radiolabel in grain attributable to ^{14}C-glucose (see 1e). Significant portions of the radiolabel in bran (28%), hulls (63%), and straw (51%) were characterized as hemicellulose, protein, and lignin. No compounds were identified in any of these hydrolysates.

5. Separate experiments in which treated grain commodities were refluxed with 6N NaOH for 6 hours and steam-distilled into isooctane revealed that significant portions of the radiolabel were released as polar, nondistillable compounds. The aqueous base contained from 32% of the trr for rice hulls to 73% of the trr for milled grain. The aqueous-soluble fractions of raw grain and straw must be characterized to determine if the compounds present are types anticipated from catabolism/incorporation and/or binding of propanil (see 1a).

The distillates contained 5% of the trr for milled grain and about 30% of the trr rice straw, hull, bran, and crude rice grain. This is consistent with a maximum 5% of the trr in refined grain and 30% of the trr in straw and the other milled fractions being attributed to free or conjugated propanil. The values correlate with the soluble percentages of trr: milled rice, 8%; bran; 34%; hulls, 15%; straw, 26%.

Recommendations

CBRS recommends that the registrant be requested to supply the additional data detailed in Conclusion 1, a - e. This will entail additional work with the metabolism study extracts and will serve to characterize/identify extracts containing major portions of the radiolabel and to characterize possible degradates of bound residues produced from strenuous base hydrolysis.

Detailed Considerations

In-Life Phase

The in-life phase laboratory was the Rice Research Station at the Louisiana State University Agricultural Center, Crowley, LA. The propanil test substance was uniformly radiolabeled with ^{14}C in the aromatic ring and had a specific activity of 20.56 μCi/mg and a radiochemical purity > 98%. The material was not diluted with natural abundance propanil. Plastic pots were lined with plastic and filled with local paddy soil and fertilized. Tebonnet rice was

sown on the soil surface and covered with 0.5 inch of soil. The pots were top-watered. Twenty-three days after planting, each pot was treated with ^{14}C-propanil at an application rate of 3 lbs./acre (0.5X) to the soil and 3 lbs./acre (1X) foliarly. The total application rate was 6 lbs./acre ^{14}C-propanil per pot. Higher application rates were phytotoxic (F. D. Griffith, Jr., DEB No. 5191, 10/25/89). The pots were placed in a greenhouse with controlled temperature and supplemental lighting. The plants were flooded 24 hours after treatment, per the approved protocol (F. D. Griffith, Jr., RCB No. 4053, 08/01/88).

Foliage samples were taken pretreatment, immediately posttreatment, 4 and 8 weeks posttreatment, and at maturity (110 days posttreatment). Rough grain was removed by hand and air-dried for one week to a stabilized moisture content of 10 - 12%. The rough grain was processed into hulls, bran, and milled rice. All samples were frozen (-20° C) and shipped frozen to the analytical laboratory.

Analysis Phase

Extractions/Hydrolyses and TRR Determinations

The determination of total radioactive residues and the characterization and identification of metabolites was conducted by XenoBiotic Laboratories, Inc. (XBL), Princenton, NJ. The results of the total radioactive residue determinations were previously presented (C. Olinger, CBRS No. 8703, 02/14/92). Levels ranged from 7.02 ppm in 4 week rice shoots to 0.25 ppm in milled rice grain. Rice straw contained 1.22 ppm ^{14}C-propanil equivalents.

Milled rice, straw, hulls, 4-week shoots, and bran samples were extracted with methanol/water/chloroform (11/5/5). The chloroform layer was concentrated to about 3 ml and partitioned with hexane/acetonitrile (1/1). The methanol water layer was evaporated to yield the aqueous soluble phase. The radioactivity levels in each fraction, including the PES-1, and the initial sample weights are provided in flow charts. The majority of the radiolabel for the mature samples remained with the PES-1, ranging from 65% in bran to 92% of the trr in grain.

The PES-1 from each crop fraction was incubated sequentially at 37° C with protease (AQ-1 protein and PES-2) and amylase (AQ-2 starch and PES-3). The residue (PES-3) was treated with ethylene bis(oxyethylenenitrilo)tetraceteic acid (EGTA) to release pectin (AG-3). The residue (PES-4) was treated with 24% KOH to yield AQ-4 (hemicellulose) and a residue (PES-5). PES-5 was treated with thioglycolic acid/sodium hydroxide to produce an aqueous fraction (AQ-5) and PES-6 (cellulose). The AQ-5 was acidified to yield a lignin aqueous phase (AQ-6) and a residue (PES-7). The grain was not processed beyond the PES-3 stage because of low radioactivity levels. The distribution of the radiolabel is summarized in Table

1.

Table 1: Distribution of the Radiolabel in Extracts and Hydrolysates					
Fraction	Milled Rice (% TRR)	Bran (% TRR)	Hulls (% TRR)	Straw (% TRR)	4-Week Shoots (% TRR)
Initial Matrix	100 (0.234 ppm)	100 (1.551 ppm)	100 (0.703 ppm)	100 (1.218 ppm)	100 (7.02 ppm)
Hexane	1.05	7.41	1.52	0.906	4.85
Acetonitrile	1.46	3.49	7.19	12.8	10.2
Water/Methanol	5.87	23.5	6.06	12.4	2.39
Protein	28.4	13.7	6.58	7.14	11.8
Starch	72.3	2.20	0.94	1.46	17.0
Pectin	5.20[1]	5.64	4.85	7.60	45.4[1]
Hemicellulose		23.1	40.0	43.9	
Lignin		4.84	23.0	7.76	
Cellulose		0.11	5.39	4.62	
Total Release/ Total Recovery	109/114	83/84	81/96	92/99	46/92
[1] PES from amylase treatment.					

The radiolabel released ranged from 46% in rice shoots to 92% in rice straw. Only a minority of the radiolabel was released by solvent extractions (8% grain, 15% hulls, 26% straw, and 34% bran). The registrant contends that this indicates a high level of conjugated and/or bound residues. Protease and amylase released most of the post-extraction solid radiolabel from grain; 62% of the trr was starch-related compounds and 25% was protein-related compounds. For hulls, bran, and straw the largest fractions of the trr were associated with hemicellulose and lignin.

Characterization/Identification of Soluble Metabolites

The various solvent extracts were analyzed by tlc and/or hplc. The tlc was conducted on silica gel plates using combinations of four solvent systems: (1) acetonitrile/toluene (10/90); (2) 2-propanol/ammonium hydroxide (80/20); (3) 1-propanol/ammonium hydroxide (80/20); (4) acetic acid/acetonitrile/ethylacetate (4/48/48). Solvent systems 2 and 1 were used for two-dimensional tlc of non-polar extracts. Solvent system 2 or 3 was used for polar extracts. Co-chromatography with reference standards was used for characterization/identification. Plates were scanned with an Ambis Image Scanner. Reference standards (no radiolabel) were visualized with iodine vapor or uv.

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The hplc analyses were conducted with a Zorbax Rx, 4.6 mm X 25 cm, column. Both uv (254 nm) and radioactivity detectors were used. A solvent program progressing from 90% A (2.5 mM tetrabutylammonium phosphate in water) and 10% B (acetonitrile) to 100% B over 50 minutes, at 1 ml/minute total flow, was utilized.

The hplc radiochromatogram for the acetonitrile extract of rice grain showed numerous peaks, the major peak of which may have been propanil. The methanol/water extract contained polar materials, which were shown not to be 3,4-dichloroaniline-N-sulfonic acid, N-(3,4-dichlorophenyl)-D-glucosylamine, 3,4-dichloroaniline glucuronate, or 3,4-dichloroaniline (DCA). The tlc chromatograms were presented. No quantitative data were produced.

For rice shoots, two-dimensional tlc of the methanol/water extract showed the presence of DCA-glucose conjugate and polar materials at the origin. The acetonitrile fraction was shown (tlc) to contain DCA (about 4% of trr, 0.3 ppm), propanil (not quantified), an unknown, and polar materials.

The hplc radiochromatogram of the rice straw acetonitrile fraction revealed numerous peaks, none of which were identified. The hexane fraction was not analyzed, and two-dimensional tlc of the methanol/water extract revealed a trace of N-3,4-dichlorophenyl-glucosylamine and most of the radioactivity at the origin.

No definitive characterizations or identifications were made on the rice bran extracts, but propanil (0.5% trr) and DCA (0.2% trr) may have been present in the acetonitrile fraction (hplc). The water/methanol fraction (24% of trr) showed only polar compounds (tlc).

Two-dimensional tlc of the methanol/water fraction from rice hulls showed DCA and DCA-glucosylamine, according to the registrant, but the tlc presented does not support this claim.

Little of the extracts was identified, no identifications were confirmed, and quantitation was usually absent.

Characterization of the Post Solvent Extraction Solid

An ethyl acetate extract (13% trr, 0.03 ppm) of the amylase-released hydrolysate from rice grain was analyzed by tlc. A nonpolar unknown was found. Low radioactivity levels precluded additional characterization. The remaining aqueous phase (48% trr,, 0.12 ppm) was freeze-dried, cleaned on gpc, and analyzed on tlc. Results were inconclusive. The aqueous phase was also refluxed with acid or base, but no radioactivity could be extracted with acetonitrile.

To confirm the protease/amylase sequential digestion, another sample of the post-extraction grain solid was digested with

amyloglucosidase, releasing 69% or 0.17 ppm of the trr. This compares with 62% of the trr released by amylase. Both are starch-specific enzymes.

In a separate experiment from the extraction scheme described above, milled rice after solvent extraction and containing 93% of the trr was treated with amylase. About 31% of the trr was released by amylase (starch-related). The amylase hydrolysate was treated with glucosidase, and 26% of the trr was recovered in the glucosidase hydrolysate. This hydrolysate was subjected to ion-exchange chromatography with 57% recovery. A hplc/lsc analysis revealed ^{14}C-glucose. Chromatograms of the hydrolysate, ^{14}C-glucose, and a mixture of hydrolysate and ^{14}C-glucose were presented. The hydrolysate peak coeluted with ^{14}C-glucose. Acetylated derivatives of the hydrolysate and hydrolysate plus ^{14}C-glucose were prepared and analyzed by hplc/lsc. Both showed the acetylated glucose derivative, but the hydrolysate showed additional peaks. GC/MS analyses of the acetylated ^{14}C-glucose and the acetylated starch hydrolysate revealed nearly identical spectra for the peak in question. Spectra were provided. The registrant concludes that some portion of the propanil was catabolized and reincorporated into glucose. No quantitative data were presented.

Residue Analytical Method

Rice grain, straw, hull, bran, and hemicellulose fractions from the metabolism study were analyzed by a modification of the existing analytical enforcement procedure. Control samples (5 g) of grain, straw, hull, and bran were fortified with ^{14}C-3,4-dichloroaniline (about 5 µg, 885,880 dpm). Samples were base hydrolyzed in 6N NaOH for 16 hours in a flask connected to a continuous extractor. Released organic compounds were steam-distilled into isooctane. After completion of the hydrolysis, radioactivity was determined in the base, in the isooctane, and in the residual solid. The isooctane fraction was concentrated and analyzed by hplc/ram for 3,4-dichloroaniline. The isooctane extract was also chromatographed on silica gel and analyzed for DCA by gc/npd. Representative chromatograms were not supplied.

About 38% of the radiolabel in the bran hemicellulose aqueous phase of bran was distilled into the isooctane. For hulls and straw, the amounts were 34% and 43%, respectively. This suggests that the majority of the radiolabel in the hemicellulose fractions was bound and/or highly polar.

Results for the grain, straw, hull, and bran are summarized in Table 2. The majority of the radiolabel remained with the residual solid and aqueous layer in all cases. For grain only 5% of the radiolabel was distilled into the isooctane, indicating high levels of catabolized/reincorporated ^{14}C and/or bound propanil released as

polar compounds. Most (≥ 77%) of the isooctane fraction radiolabel was associated with DCA. The ^{14}C-DCA recovery in isooctane ranged from 96.9% in control rice hulls to 111.6% in control milled grain.

Table 2: Base Hydrolysis/Solvent Extraction of Rice Fractions

Fraction	TRR Distribution (%)[1]			Recovery (%)	DCA (%)[2]	Propanil (ppm)	
	Residual Solid	Aqueous Base	Isooctane (distillate)			hplc	gc
Rice Straw	23.1	35.4	29.3	87.8	22.8	0.278	0.384
Rice Hull	24.5	31.9	26.9	83.3	20.0	0.184	-[4]
Rice Bran	8.00	32.2	27.5	67.7	22.2	0.345	0.623
Rice Grain	5.90	36.4	25.0	67.4	22.0	0.097	0.147
Milled Grain	15.4	73.0	4.84	93.2	-[3]	-	-

[1] Not corrected (normalized) for method recovery.
[2] Hplc analysis.
[3] Not analyzed because of low ^{14}C-activity.
[4] Interference.

Proposed Metabolic Path

The registrant proposes the metabolic path shown in Figure 1, a reproduction of the registrant's Figure 36. It is proposed that propanil is converted to 3,4-dichloroaniline (DCA). DCA is catabolized, conjugated with glucose, and complexed with carbohydrates and lignin (via phenyl ring oxidation). ^{14}C-Propanil, ^{14}C-DCA, and ^{14}C-glucose have been identified among the metabolites.

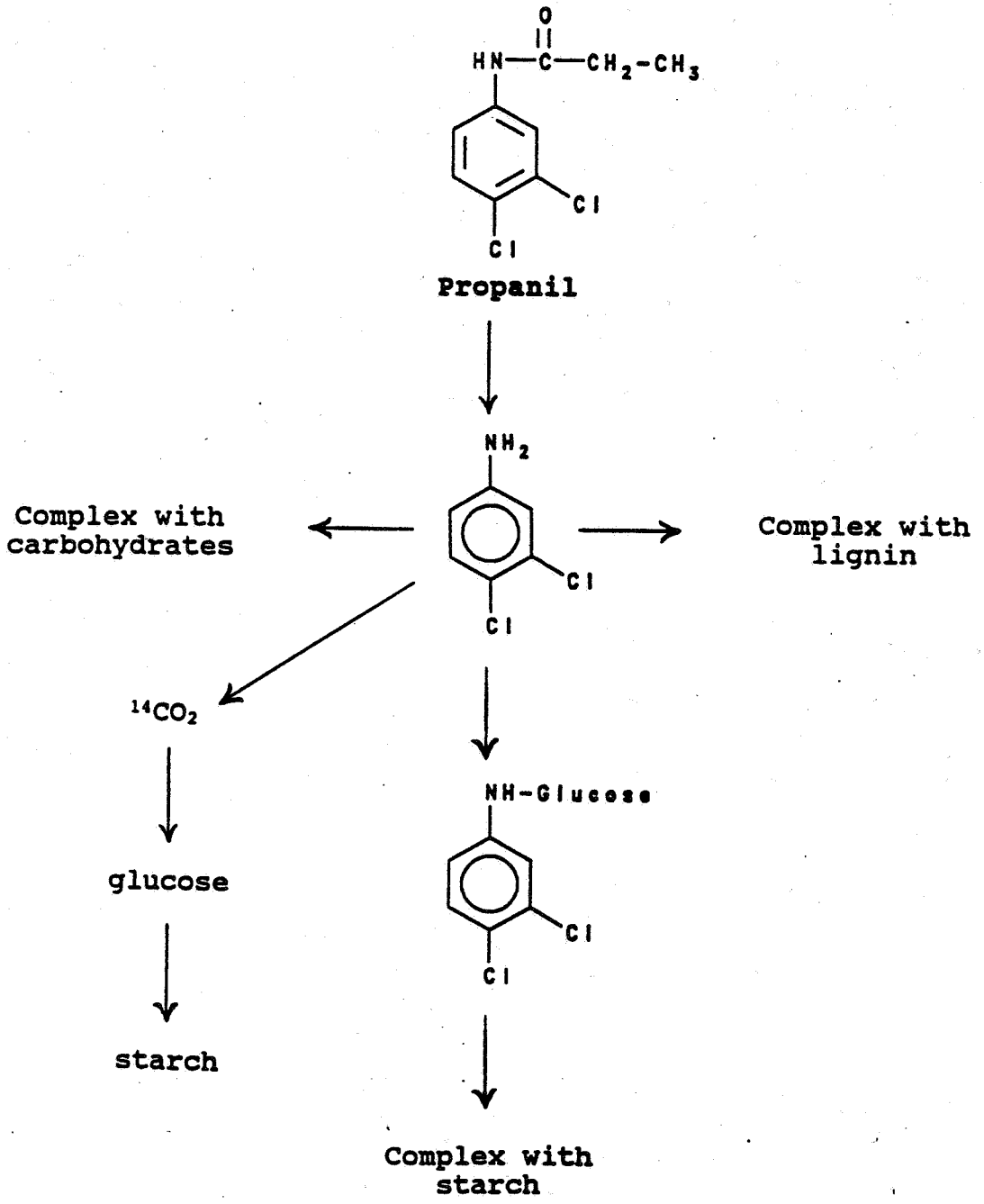
cc: Propanil Reregistration File, RF, circ., S. Funk.

RDI: A. Rathman:12/08/92:M. Metzger:12/10/92:E. Zager:12/10/92:

H7509C:CBRS:S.Funk:305-5430:CM#2:RM803:SF(1192.3):11/12/92.

Figure 1

Proposed Metabolic Pathways of Propanil in Rice



PROPANIL (CASE No. 226)
TENTATIVE RESIDUE CHEMISTRY DATA SUMMARY THROUGH 11/18/92[1]
REASSESSMENT OF U.S. TOLERANCES AND POTENTIAL FOR HARMONIZATION WITH CODEX[2]

Guideline Number and Topic[3]	Are data requirements satisfied?	MRID(s)[4]
171-3 Directions for use		
171-4(a) Plant Metabolism	N[5,6]	42382901, 42382902, 42209200,42209201
171-4(b) Animal Metabolism	N[7,8]	41755001,41755301 41848801,41983901
171-4(c) Residue Analytical Methods - Plants	N	
171-4(d) Residue Analytical Methods - Animals	N	
171-4(e) Storage Stability	N	
171-4(k) Crop Field Trials		
171-4(k) Cereal Grains Group		
Barley [see 171-4(l)]	N	
Oats [see 171-4(l)]	N	
Rice [see 171-4(l)]	Y[9]	42237101, 42237201
Wheat [see 171-4(l)]	N	
171-4(k) Forage, Fodder, and Straw of Cereal Grains		
Barley forage and straw	N	
Oats forage and straw	N	
Rice straw	Y[10]	42237301
Wheat forage and straw		
171-4(l) Processed Food/Feed		
Rice	N[11]	42417401
Wheat	N	
171-4(j) Meat/Milk/Poultry/Eggs	N	
171-4(f) Potable Water	Y [12]	42200401,42200501
171-4(g) Fish	N[1314]	42301001, 41448901, 41849101
171-4(h) Irrigated Crops	Y	
171-4(i) Food Handling Establishments	N/A	
171-5 Reduction of Residues	N/A	

[1]Registration Standard issued 12/87. No Reregistration Standard Update issued.

[2] There are no Codex MRL's proposed or established for propanil.

[3]N/A = Guideline requirement not applicable.

[4]MRIDs that were reviewed in the current submission are designated in shaded type.

[5] CBRS# 8703, 2/14/92 (C. Olinger): Interim rice metabolism report. Additional information is needed.

CBRS No. 10288, 11/18/92, S. Funk, MRID Nos. 42382901 and 42382902: Final rice metabolism report. The study is not acceptable, but may be upgraded by performing additional analyses of certain extracts and by analyzing the aqueous phases from severe base hydrolysis of grain and straw. The amount of ^{14}C-glucose found in the grain starch should be determined/estimated.

[6]CBRS 9528, 4/2/92 (J. Abbotts): Wheat metabolism study. Additional information is needed. Only parent is characterized, representing no more than 13% of the TRR.

[7] CBRS# 7622, 2/21/92 (C. Olinger): Metabolism in poultry is adequately understood. Additional information on the methodology, a lab validation and a method trial are needed.

[8] CBRS#'s 7960 and 8522, 3/18/92 (R. Perfetti): Additional information regarding radioactive residues milk and fat are needed in order to upgrade this study.

[9]CBRS # 9589, R. Perfetti, 6/22/92; The data for rice grain is acceptable. However a higher tolerance is needed. Ten ppm would be adequate. When the higher tolerance is established for rice grain, then the food/feed additive tolerances for processed fractions must also be revised.

[10]CBRS # 9589, R. Perfetti, 6/22/92; Residue data on rice straw indicate that the established tolerance is adequate. No additional data on straw is needed.

[11](CBRS No. 10362; COlinger; 11/6/92) A rice processing study was submitted and was considered adequate. Existing feed additive tolerances must be revised once the nature of the residue has been adequately delineated. Concentration factors should be based on the rice processing study described in the Reregistration Standard. The requirement for a rice grain dust processing study remains outstanding.

[12] CBRS #9541, RBP (8/28/92). Provided the Registrant amends the propanil label to prohibit the discharge of rice paddy water within 60 days of the last application there is no need for an MCL for propanil residues in water or for tolerances in irrigated crops. A 14 day interval between applications should be specified on the label. The current label directions for ground applications should be deleted.

[13] CBRS#'s 7960 and 8522, 3/18/92 (R. Perfetti): The metabolism of propanil in crayfish is adequately understood. Magnitude of the residue data in fish and shellfish are required.

[14] RBP, CBRS # 9876, 9/10/92. This study does not fulfil the requirements for residue chemistry data on crayfish. Only one application was performed, representing 0.5x the maximum label rate. Harvest was performed one year after treatment, rather than at 7 months. The registrant should conduct a new study reflecting two applications of propanil at 4 lb ai/A each and a 7-month harvest interval, which would be the minimum treatment-to-harvest interval, given April application and November crayfish harvest.

The data indicate that base releasable 3,4-DCA, expressed as propanil equivalents, reaches a level of 0.07 ppm in crayfish harvested approximately one year after a single application at 4 lb ai/A to rice paddy water. A tolerance for propanil residues in crayfish should be proposed when the crayfish residue data base is complete.

cc: S. Funk (1192.37); Propanil Reregistration Standard File; L. Rossi, SRRD.

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