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

OCT 24 1995

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Propanil. List A Reregistration Case No. 0226/Chemical ID No. 028201. *Propanil Task Force Submission of a Confined Rotational Crop Study*, and a Method Radiovalidation Study for Rotational Crop Matrices [Guideline Ref. No. 165-1].

MRID Nos. 42963001 and 43355201.

CBRS Nos. 12739 and 14594.

DP Barcode Nos. D196301 and D208552.

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On behalf of the Propanil Task Force (PTF), NPC Inc. has submitted a confined rotational crop study entitled "Confined Rotational Crop Study with ^{14}C-Propanil: Analysis of Soil and Plant Samples," 10/5/93 [MRID No. 42963001]. The in-life portion of the study was conducted by Pan-Agricultural Laboratories, Inc. of Madera, CA. Analysis of soil and plant samples obtained from the study was conducted by XenoBiotic Laboratories, Inc.; of Plainsboro, NJ.

An addendum to the study, consisting of a method radiovalidation study for rotational crop matrices, was also submitted for review. The study is entitled "Determination of 3,4-Dichloroaniline in Crop Matrices from the ^{14}C-Propanil Confined Rotational Crop Study by the Proposed Enforcement Analytical Method," and was assigned MRID No. 43355201 (DP Barcode No. D208552; CBRS No. 14594). The performing laboratory for the radiovalidation study was

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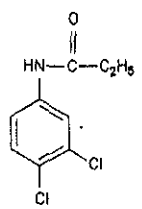
EN-CAS Analytical Laboratories, Winston-Salem, NC.

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) [40 CFR §180.274]. There are no food/feed additive tolerances established under 40 CFR §185 and §186.

The nature of the residue in rice is adequately understood (memo, C. Swartz, 9/8/94, CBRS No. 14030, DP Barcode No. D205676). Radioactivity not identified as base-releasable DCA was characterized as either sugars or conjugates very strongly bound to DCA. Radiolabeled glucose was identified as 4.75% of the TRR. Additional data required to upgrade the wheat metabolism study are currently under review in CBRS (CBRS No. 14597; MRID No. 43372201; DP Barcode No. D208555). Once the data have been reviewed, the residues to be regulated will be determined by the HED Metabolism Committee.

The nature of the residue in ruminants and poultry is adequately understood; propanil is hydrolyzed, oxidized, and then converted to conjugated metabolites of parent and 3,4-dichloroaniline (DCA). Metabolites 3,4-DCA-N-sulfamic acid; 4,5-dichloro-2-aminophenol-O-sulfonic acid; 4,5-dichloro-2-aminophenol-N-sulfamic acid; and 3′,4′-dichloro-6′-O-sulfonic acid-acetanilide were all greater than 10 %TRR in poultry and ruminant tissues, but cannot be converted to DCA, and therefore will not be detected using the current enforcement method. The HED metabolism committee will need to be consulted.



propanil

Recommendation

The confined rotational crop and radiovalidation studies submitted by the Propanil Task Force are acceptable. Previously submitted field rotational crop studies have shown that rotational crop tolerances are not necessary. Registered labels should indicate a plantback interval of 60 days for all rotational crops. No additional data are required under Guidelines 165-1 and 165-2.

Conclusions

1. Loam soil was placed in livestock feeding troughs in a greenhouse, and treated with uniformly ring-labeled ^{14}C-propanil at 1X the maximum registered rate of 6 lb ai/A. The rotational crops soybeans and sorghum were seeded in the soil at 30-, 157-, and 365-day plantback intervals. Bermudagrass was seeded at plantback intervals of 157 and 365 days.

2. The submission included all pertinent information regarding the soil type, greenhouse temperature, irrigation water applied, the test substance and application, and maintenance pesticides applied to control aphids and mites. Adequate measures were taken to separate control soil/crops from those treated with ^{14}C-propanil.

3. Immature (forage) and mature soybean (beans, pods and straw) and sorghum (grain and straw) samples were collected. Bermudagrass was sampled at various intervals throughout the study, but was never allowed to grow to a length of greater than 4 inches. Samples were frozen immediately after harvest.

4. Frozen plant samples which had been ground in a mill were combusted in triplicate, and the trapped $^{14}CO_2$ quantitated using liquid scintillation counting (LSC), in order to determine the total radioactive residues (TRRs).

5. In soybeans, the highest TRR obtained was 0.40 ppm in the straw from the 30-day plantback plot. The highest residue in grain sorghum was 0.31 ppm in grain from the 30-day plantback plot. The TRRs in bermudagrass ranged from 0.16 to 0.26 ppm (157-day and 365-day plantback intervals). Since all crops had TRRs greater than 0.01 ppm in samples harvested at all plantback intervals, additional work was required to determine the nature of the radioactive residues.

6. Analytical methods used to determine the nature of the radioactive residues included both one- and two-dimensional thin layer chromatography (TLC) using 4 different solvent systems, as well as high performance liquid chromatography (HPLC) in 2 different gradient solvent systems. Wherever radioactive residues were too low to be accurately quantitated using HPLC, eluates were collected and analyzed using LSC to generate reconstructed radiochromatograms.

7. Metabolites were identified via co-chromatography with nonradiolabeled standards; standards were visualized using UV light. HPLC retention times (R_t) and TLC R_f values were provided for propanil and reference standards in all solvent systems. Adequate sample chromatograms were submitted.

8. Radioactivity was extracted from rotational crop samples using a modified Bligh-Dyer extraction procedure. Radioactivity was partitioned into chloroform ($CHCl_3$) and methanol/water ($MeOH/H_2O$), with post-extraction solids (bound residues) remaining.

Radioactivity in the $CHCl_3$ fraction of mature soybeans was partitioned between hexane and acetonitrile (ACN). The majority of the radioactivity in all matrices remained bound, with 3 to 30% of the radioactivity extracted into the solvent fractions. Most of the extracted radioactivity was found in the $MeOH/H_2O$ fractions.

9. In order to release bound radioactivity from the post-extraction solids (PES) of soybean, sorghum and bermudagrass, the performing laboratory conducted sequential hydrolyses consisting of a protease hydrolysis, followed by a weak acid hydrolysis, and finally a strong base hydrolysis. Residues released into the aqueous hydrolysates were partitioned with ethyl acetate (EtOAc).

10. Protease and weak acid hydrolysis released roughly the same percentage of the TRR in all matrices; however, 10 to 20% of the TRR remained bound following strong base hydrolysis.

11. Due to low levels of radioactivity in the $CHCl_3$ fractions, only the extractable radioactivity in $MeOH/H_2O$ fractions was further analyzed using TLC and/or HPLC. None of the radioactivity in the $MeOH/H_2O$ fractions was identified; however, radioactivity was partially characterized as multi-component polar metabolites.

12. The registrant demonstrated that radioactivity in the combined hexane fractions of the $CHCl_3$ extracts of soybeans from all 3 rotations was not propanil.

13. The storage stability data submitted by the registrant do not strictly support the results of the confined rotational crop study, since the stability of the metabolites found in rotational crops was not demonstrated. However, based on the stability data for propanil, *per se*, CBRS concludes that no further data are necessary to support this study. Refer to the detailed considerations.

14. None of the radioactive residues in rotational crops were identified; however, residues were partially characterized as polar and multicomponent. In rotational crops, it is likely that, as in the case of rice, propanil residues were either strongly conjugated or incorporated into macromolecules.

15. A radiovalidation study was conducted using a previously-submitted method that quantitates residues as free and base-releasable 3,4-DCA (3,4-dichloroaniline); the method is the proposed enforcement method.

16. Method validation data submitted along with the radiovalidation data demonstrated that propanil is adequately recovered from rotational crop matrices using the analytical method. Average recoveries from soybeans (beans and straw), sorghum (grain and straw) and bermudagrass ranged from 73-82% following fortification with 0.01, 0.05 and 0.5 ppm propanil.

17. In sorghum grain samples from all 3 rotations, and bermudagrass from the 2 rotations harvested, radioactive residues quantitated as free and base-releasable DCA were <0.01 ppm. In sorghum straw, radioactive residues quantitated as DCA were 0.01 to 0.03 ppm. In soybeans, radioactive residues quantitated as DCA were 0.02-0.08 ppm, while DCA residues in straw were 0.04-0.08 ppm.

18. Field rotational crop studies were submitted to the Agency. Based on review of these studies, CBRS concludes that additional limited field trials are not required. Propanil residues were not detected (<0.05 ppm) in crops that were rotated at 2 weeks, with the exception of one soybean sample, which had a residue of 0.08 ppm; this sample was considered to be anomalous. However, to assure that no propanil residues of concern would be found in soybeans or any other rotational crop, registered labels should indicate a plantback interval of 60 days for all rotational crops.

DETAILED CONSIDERATIONS

Confined Rotational Crop Study: MRID No. 42963001

In-Life Portion of the Study

Untreated loam soil was obtained from a rice paddy located in Merced California, and shipped to the test facility to be used in the confined rotational crop study. The untreated soil was placed in animal watering/feeding troughs, each consisting of 28.07 ft^2, to be used for untreated control and treated rotational crop plots. The troughs were divided into 3 equal subplots, into which rotational crops would be planted. The soil in the troughs was conditioned in the greenhouse for 15 days prior to treatment with the test substance. Untreated control soil to be planted with control rotational crops was kept in a separate portion of the greenhouse.

The test substance was prepared by placing 1.81 g (36.63 mCi) of uniformly ring-labeled ^{14}C-propanil in 150 ml methanol (MeOH). The application rate was 6 lb ai/A, which is equivalent to the maximum single application rate allowed on currently registered labels; the test substance was broadcast over the soil using a CO_2 backpack sprayer. Prior to planting of rotational crops into the treated soil, the soil was tilled to a depth of approximately 2 inches.

The rotational crops soybeans and sorghum were planted into the subplots 30, 157 and 365 days after the soil was treated. Due to poor germination, soybeans had to be replanted after the 157- and 365-day plantings; the replant intervals were 174 and 391 days after pesticide application. Bermudagrass was also planted at 157- and 365-day plantback intervals. The use of soybeans, sorghum and bermudagrass as rotational crops was discussed in the protocol submitted to the Agency, and reviewed in the EFGWB memo of E. Conerly dated 1/30/90.

Both sorghum and soybeans were harvested at immature (forage) and mature stages. The forage samples consisted of the aerial portions of the plant. At maturity, the entire plant was pulled, the soil shaken off the roots, and the roots trimmed; for sorghum, grain was separated from the

straw, while soybeans were separated into beans, pods and straw. Bermudagrass was harvested at four scheduled sampling intervals, however the grass was trimmed whenever the growth exceeded 4 inches. Samples were harvested as 3 replicates from each subplot; samples were frozen immediately and then shipped frozen to the analytical laboratory.

Detailed information was provided regarding the maintenance pesticides used to control mites and aphids, the amount of irrigation water (obtained from an on-site well) used to water the crops, the ambient temperatures in the green house, cultivation of the plots, and shipping of the samples.

Determination of Total Radioactive Residues (TRRs) in Rotational Crops

Uniform replicate samples were obtained by grinding the frozen plant samples in a Tekmar® Analytical Mill. During the early portion of the study, only subsamples of the replicates were processed for TRR determination, while the remainder of the replicate was stored unprocessed; for samples harvested later in the study, the entire replicate was processed at the time of TRR determination, and remainder of the processed replicate was stored frozen. Total radioactive residues (TRRs) were determined via combustion of the samples in duplicate, followed by liquid scintillation counting (LSC) of the trapped $^{14}CO_2$. The results of the TRR determinations are presented in Table 1. Since the TRR in all crops harvested from the 365-day plantback plots contained residues greater than 0.01 ppm, additional work was required to determine the nature of the propanil residues found in these crops.

Table 1. Total Radioactive Residues (TRRs) in Rotational Crop Matrices.[1]

Crop Part	30-Day Plantback	157-Day Plantback[2]	365-Day Plantback[3]
Soybeans[4]:			
Forage	0.17	0.05	0.31
Beans	0.21	0.20	0.26
Pods	0.17	0.17	0.28
Straw	0.40	0.14	0.26
Sorghum[5]:			
Forage	0.12	0.04	0.04
Grain	0.31	0.10	0.06
Straw	0.20	0.07	0.09
Bermudagrass[6]:			
Forage	NA[7]	0.17	0.26
	NA	0.18	0.16

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

[2,3] For soybeans, the 157-day and 365-day samples had to be re-seeded due to poor germination; therefore the true plantback intervals for these samples were 174- and 391-days.

[4] For soybeans, the days between planting and harvest were as follows: for the rotational crop interval of 30 days, the harvest was 67 days after planting for forage, and 126 days for mature plant parts; corresponding harvest days for the other intervals were 85 and 151 days after planting, and 66 and 100 days after planting, respectively.

[5] For sorghum, the days between planting and harvest were as follows: for the rotational crop interval of 30 days, the harvest was 67 days after planting for forage, and 126 days for mature plant parts; corresponding harvest days for the other intervals were 109 and 186 days after planting, and 54 and 147 days after planting, respectively.

[6] Bermudagrass was harvested at intervals of 102, 165, 21, and 153 days after planting.

[7] NA = not applicable; the bermudagrass plot was fallow for the 30-day plantback interval.

Analytical Methods Used to Characterize/Identify Radioactive Residues in Rotational Crops

Soil and plant extracts obtained from the confined field study were analyzed using both thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Both one- and two-dimensional TLC analyses were conducted on plant extracts, using four different solvent systems. Radioactivity on TLC plates was measured quantitatively using the AMBIS imaging system, and the levels verified by scraping spots from the plates, and measuring radioactivity using LSC. Extracts were also analyzed using HPLC with two different gradient solvent systems. Where possible, radioactivity was monitored using the HPLC system; however, when radioactivity was low, eluates were collected at 30- or 60-second intervals, and reconstructed chromatograms were produced from the results of liquid scintillation counting of the radioactivity in the eluates.

Radioactive residues were identified via co-chromatography with nonradiolabeled standards. The submission included the chemical names, structures, TLC R_f values (in all solvent systems), and HPLC retention times (R_t) of propanil and metabolites. These included 3,4-dichloroaniline (3;4-DCA); *N*-(3,4-dichlorophenyl)-glucosylamine (DCPGA); 3,3′,4,4′-tetrachloroazobenzene (TCAB); and 3,3′,4,4′-tetrachloroazoxybenzene (TCOAB). Nonradiolabeled standards were detected using UV light (HPLC and TLC).

Prior to analysis of certain plant extracts, various cleanup procedures were used to remove some of the co-extractable plant materials. This generally entailed a C_{18} column followed by two sulfonic acid columns. In addition, preparative chromatography was used, in which certain aqueous fractions were subjected to normal phase TLC, and the major radioactive bands scraped and eluted with MeOH.

Extraction of Radioactivity from Rotational Crop Matrices

All plant samples except mature soybean beans were extracted using a modified Bligh-Dyer procedure. Equal portions of each of the sample replicates were composited and blended, and then extracted (blended) with methanol (MeOH)/H_2O. Chloroform ($CHCl_3$) was added, and the mixture blended again. After filtration, solids were re-blended with $CHCl_3$, and the filtrates combined and then separated into MeOH/H_2O and $CHCl_3$ fractions. Radioactivity in the fractions was quantitated using LSC, and the fractions were concentrated and stored frozen for further analysis. Solids were allowed to dry prior to combustion of subsamples, and then frozen.

Mature soybeans were extracted similarly, however the $CHCl_3$ fraction was then concentrated and then mixed with a solvent mixture consisting of ACN saturated with hexane and hexane (1:1, v:v). The mixture was shaken in a separatory funnel, and the layers separated to yield ACN and hexane fractions. The hexane layer was extracted again with ACN saturated with hexane, and the hexane fractions combined. The fractions were concentrated, analyzed using LSC, and the fractions frozen for further analysis.

In soybean forage, straw and pods, 3 to 8% of the radioactivity was extracted into the $CHCl_3$ fraction, while 3 to 30% of the TRR was extracted into the MeOH/H_2O fraction; approximately 55 to 80% of the radioactivity was bound in the post extraction solids. In beans, up to 20% of the radioactivity was extracted into the $CHCl_3$ fraction, but the majority of the radioactivity was bound. Similar extraction profiles were observed in sorghum matrices, with the exception of the grain. Approximately 95% of the radioactivity in the 30-day plantback grain was bound, but this decreased to approximately 75% bound in the 365-day plantback grain. Distribution of radioactivity in bermudagrass extracts was similar to the forage and straw of soybeans. In all three rotational crops, most of the radioactivity could not be solvent-extracted, and therefore attempts were made to release the bound radioactivity from the post extraction solids (see below). Results of the extraction of radioactivity from plant matrices are presented in Tables 2, 3 and 4.

Table 2. Distribution of Radioactivity in Extracts from the 30-Day Plantback Interval.[1]

Fraction ID	Soybeans: %TRR (ppm)[2]				Grain Sorghum: %TRR (ppm)[3]		
	Forage/97 (0.17)	Straw/156 (0.40)	Beans/156 (0.20)	Pods/156 (0.17)	Forage/97	Straw/156	Grain/156
$CHCl_3$[4]	5.29 (0.01)	5.39 (0.02)	19.04 (0.04)	7.31 (0.01)	8.93 (0.01)	9.32 (0.02)	1.04 (<0.01)
ACN			2.67 (<0.01)				
Hexane			17.0 (0.034)				
$MeOH/H_2O$	29.24 (0.05)	19.08 (0.08)	18.11 (0.04)	3.01 (<0.01)	26.19 (0.03)	26.08 (0.05)	3.53 (0.01)
PES	65.47 (0.11)	75.53 (0.30)	62.85 (0.13)	89.68 (0.105)	64.88 (0.08)	64.60 (0.13)	95.44 (0.30)
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.01
% Recovery of Radioactivity[5]	101.84	104.17	110.38	89.53	98.35	87.72	94.61

[1] Only data for soybeans and grain sorghum are presented, since bermudagrass was not rotated at the 30-day interval.

[2] Data are presented as %TRR (ppm, ^{14}C-propanil equivalents); for each soybean plant part, the number of days after treatment (DAT) that samples were harvested is presented, along with the TRR in the sample.

[3] Data are presented as %TRR (ppm, ^{14}C-propanil equivalents); for each sorghum plant part, the number of days after treatment (DAT) that samples were harvested is presented, along with the TRR in the sample.

[4] In soybeans, the $CHCl_3$ fraction was further partitioned into ACN and hexane fractions.

[5] Demonstrates loss or gain of radioactivity through partitioning/fractionation.

Table 3. Distribution of Radioactivity in Extracts from the 157-Day Plantback Interval.[1]

Fraction ID	Soybeans: %TRR (ppm)[2]				Sorghum: %TRR (ppm)[3]			Bermudagrass: %TRR (ppm)[4]	
	Forage/259 (0.05)	*Straw/325* (0.14)	*Beans/325* (0.20)	*Pods/325* (0.17)	*Forage/266* (0.04)	*Straw/343* (0.08)	*Grain/343* (0.10)	*259 DAT* (0.17)	*322 DAT* (0.18)
$CHCl_3$[5]	3.33 (<0.01)	4.07 (<0.01)	20.77 (0.04)	7.16 (0.01)	16.58 (<0.01)	5.01 (<0.01)	4.92 (<0.01)	21.18 (0.04)	12.23 (0.02)
ACN			3.45 (<0.01)						
Hexane			17.32 (0.035)						
MeOH/H_2O	22.46 (0.01)	25.19 (0.03)	16.92 (0.03)	13.21 (0.02)	13.59 (<0.01)	29.22 (0.02)	5.35 (<0.01)	14.93 (0.03)	14.42 (0.03)
PES (Bound)	74.22 (0.04)	70.74 (0.10)	62.31 (0.12)	79.63 (0.14)	69.83 (0.03)	65.77 (0.05)	89.73 (0.09)	63.89 (0.11)	73.35 (0.13)
Total	100.01	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
% Recovery of Radioactivity[6]	116.19	100.46	106.75	97.17	111.07	101.99	96.19	99.15	109.60

[1] Since some of the soybeans were re-planted, the actual rotational interval for these plants was 174 days.

[2] Data are presented as %TRR (ppm, ^{14}C-propanil equivalents); for each soybean plant part, the number of days after treatment (DAT) that samples were harvested is presented, along with the TRR in the sample.

[3] Data are presented as %TRR (ppm, ^{14}C-propanil equivalents); for each sorghum plant part, the number of days after treatment (DAT) that samples were harvested is presented, along with the TRR in the sample.

[4] Data are presented as %TRR (ppm, ^{14}C-propanil equivalents); bermudagrass was harvested at various intervals, for which the days after treatment (DAT) are shown in the table, along with the TRR in the sample.

[5] In soybeans, the $CHCl_3$ fraction was further partitioned into ACN and hexane fractions.

[6] Demonstrates loss or gain of radioactivity through partitioning/fractionation.

Table 4. Distribution of Radioactivity in Extracts from the 365-Day Plantback Interval.[1]

Fraction ID	Soybeans: %TRR (ppm)[2]				Sorghum: %TRR (ppm)[3]			Bermudagrass: %TRR (ppm)[4]	
	Forage/457 (0.31)	Straw/491 (0.26)	Beans/491 (0.26)	Pods/491 (0.26)	Forage/419 (0.04)	Straw/512 (0.09)	Grain/512 (0.06)	386 DAT (0.26)	518 DAT (0.16)
$CHCl_3$[5]	7.31 (0.02)	5.82 (0.02)	15.77 (0.04)	8.10 (0.02)	12.95 (0.01)	4.07 (<0.01)	13.57 (<0.01)	9.79 (0.03)	6.66 (0.01)
ACN			3.45 (<0.01)						
Hexane			12.32 (0.032)						
MeOH/H_2O	13.40 (0.04)	17.70 (0.05)	27.52 (0.07)	25.32 (0.07)	24.63 (0.01)	28.38 (0.03)	11.39 (<0.01)	11.49 (0.03)	21.25 (0.03)
PES (Bound)	79.28 (0.25)	76.48 (0.20)	56.71 (0.15)	66.58 (0.19)	62.41 (0.03)	67.55 (0.06)	75.05 (0.05)	78.73 (0.20)	72.09 (0.12)
Total	*99.99*	*100.00*	*100.00*	*100.00*	*99.99*	*100.00*	*100.01*	*100.01*	*100.00*
% Recovery of Radioactivity[6]	96.08	126.34	107.96	94.44	93.01	102.41	105.54	94.09	95.01

[1] Since some of the soybeans were re-planted, the actual rotational interval for these plants was 391 days.

[2] Data are presented as %TRR (ppm, ^{14}C-propanil equivalents); for each soybean plant part, the number of days after treatment (DAT) that samples were harvested is presented, along with the TRR in the sample.

[3] Data are presented as %TRR (ppm, ^{14}C-propanil equivalents); for each sorghum plant part, the number of days after treatment (DAT) that samples were harvested is presented, along with the TRR in the sample.

[4] Data are presented as %TRR (ppm, ^{14}C-propanil equivalents); bermudagrass was harvested at various intervals, for which the days after treatment (DAT) are shown in the table, along with the TRR in the sample.

[5] In soybeans, the $CHCl_3$ fraction was further partitioned into ACN and hexane fractions.

[6] Demonstrates loss or gain of radioactivity through partitioning/fractionation.

Extraction of Bound Radioactivity from the Post Extraction Solids (PES)

Selected samples of the post extraction solids of mature soybean and sorghum straw and bermudagrass, containing the highest levels of radioactivity, were subjected to enzymatic, acidic and basic hydrolyses. First, the solids were incubated with protease for 24 hours at 37 °C, and the hydrolysate filtered to yield PES-1 and AQ-1 fractions; both fractions were analyzed using LSC. Selected AQ-1 fractions were extracted with ethyl acetate (EtOAc), yielding AQ-3 and EtOAc-1 fractions.

Subsequently, PES-1 was subjected to a weak acid hydrolysis by refluxing with 0.1 N HCl for one hour. After cooling, the filtrate (AQ-2) and the solids (PES-2) were analyzed using LSC.. Once again, the aqueous filtrate was partitioned with EtOAc to yield aqueous (AQ-3) and EtOAc (EtOAc-1) fractions, which were then analyzed using LSC. Aqueous (AQ-3) fractions were neutralized to pH 7, concentrated, and then cleaned up on a C_{18} column. Radioactivity was eluted with MeOH and water, and the eluates combined, concentrated, and stored frozen for further analysis.

Finally, the PES-2 fraction was subjected to a strong base hydrolysis by refluxing in 1 N NaOH for one hour. After cooling, the hydrolysate was filtered, yielding PES-3 and AQ-4 fractions. The aqueous fraction was neutralized and extracted with EtOAc to yield EtOAc-2 and AQ-5 fractions, which were subsequently analyzed using LSC. The AQ-5 fraction was cleaned up on a C_{18} column as described above. Results of attempts to release bound residues from the post extraction solids are presented in Table 5.

Table 5. Release of Bound Residues from Rotational Crop Post Extraction Solids (PES).[1]

Matrix/DAT[2]	% TRR in PES (ppm)[3]	Protease[4]	Acid[5]	Base[6]	PES[7]
Soybean straw/156 (0.40)	75.53 (0.300)	15.45 (0.062)	11.05 (0.044)	19.83 (0.079)	29.21 (0.117)
Soybean straw/325 (0.14)	70.74 (0.099)	4.98 (0.007)	8.54 (0.012)	20.54 (0.029)	26.68 (0.037)
Soybean straw/491 (0.26)	76.48 (0.200)	14.27 (0.037)	16.38 (0.043)	23.49 (0.061)	22.34 (0.058)
Sorghum straw/156 (0.20)	64.60 (0.129)	15.48 (0.031)	16.34 (0.033)	18.92 (0.038)	13.86 (0.028)
Sorghum straw/343 (0.07)	66.37 (0.046)	8.45 (0.006)	17.56 (0.012)	21.80 (0.015)	18.56 (0.013)
Sorghum straw/512 (0.09)	67.55 (0.061)	18.03 (0.016)	15.34 (0.014)	23.93 (0.022)	10.25 (0.009)
Bermudagrass/322 (0.18)	73.35 (0.132)	19.25 (0.034)	22.43 (0.040)	18.83 (0.034)	12.84 (0.002)
Bermudagrass/518 (0.16)	72.09 (0.115)	14.81 (0.024)	18.02 (0.029)	23.86 (0.038)	15.39 (0.025)

[1] Radioactivity is reported as %TRR (ppm, ^{14}C-propanil equivalents).

[2] DAT = days after [soil] treatment; the TRR in the matrix is reported in parentheses.

[3] The %TRR in the post extraction solids (PES) is shown, along with the ppm radioactivity.

[4] The %TRR released following protease hydrolysis (ppm).

[5] The %TRR released following weak acid hydrolysis (ppm).

[6] The %TRR released following strong base hydrolysis (ppm).

[7] The %TRR remaining bound following sequential hydrolyses (ppm).

Results of Attempts to Characterize and Identify Radioactivity in Rotational Crop Matrices

Since most of the extractable radioactivity was found in the MeOH extracts, efforts to identify radioactivity began with these fractions. Prior to analysis, MeOH extracts from soybean, sorghum and bermudagrass were cleaned up using solid phase (C_{18}) and sulfonic acid columns. Radioactivity was eluted from the columns using water and/or MeOH.

Soybean straw extracts from the 3 plantback intervals showed similar multi-metabolite profiles upon analysis using TLC and HPLC. Although none of the radioactivity in these extracts was identified, both TLC and HPLC results demonstrated the presence of polar metabolites, in up to 7 different chromatographic regions. Although some of the regions may have contained more than one metabolite, the levels were generally close to or less than 0.01 ppm. MeOH extracts of sorghum forage from the first and third rotations were analyzed using RP-HPLC, and found to contain polar metabolites; no further work was done due to the low levels of radioactivity in these fractions (0.04 and 0.05 ppm).

MeOH extracts of the third rotation soybeans and pods were analyzed using TLC. The soybean pods MeOH extract had a profile similar to that of the MeOH extracts of straw, i.e. seven different regions of radioactivity were characterized. TLC analysis of the bean MeOH extract did not result in clean separation of the radioactivity.

The $CHCl_3$ fractions of bean extracts from the 3 rotations were concentrated and partitioned between hexane and ACN, with most of the radioactivity being found in the hexane fraction. The registrant stated that TLC analysis of the hexane fractions was not possible due to the high fat content in the concentrated fractions. However, since fortification experiments demonstrated that propanil in the hexane fraction was efficiently partitioned into the ACN fraction, the registrant concluded that the radioactivity partitioned into the hexane fraction could not be propanil.

Residues released from the bound fractions of soybean straw following protease, acid, and base hydrolyses were characterized as primarily polar water-soluble residues. Attempts to analyze neutralized aqueous fractions (following acid and base hydrolysis) were hampered by problems with the C_{18} cleanup.

HPLC and TLC analysis of cleaned up MeOH fractions from sorghum straw yielded results similar to those obtained with the soybean straw, i.e. seven different regions of radioactivity were found. However, due to matrix effects, further analysis was not possible. The MeOH fraction from sorghum forage was analyzed using HPLC, which showed polar metabolites with a retention time range of 3.3 to 4.9 minutes. No further analysis was done. Since the extractable radioactive residues in grain had decreased to less than 0.01 ppm in the third rotation, no further work was done with sorghum grain. As with the soybean fractions, radioactivity released from the bound fractions via protease, acid and base hydrolyses was not identified, but was partially characterized as primarily polar water-soluble residues.

The MeOH fraction of bermudagrass harvested 322 days after treatment (R2) was analyzed using TLC following cleanup as described above. The TLC profile was similar to that of the R1 soybean straw sample, i.e. seven different areas of radioactivity were found, with each area corresponding to less than 0.01 ppm radioactivity. Although the MeOH extract of R3 bermudagrass was also analyzed using TLC, good separation could not be obtained due to matrix effects. HPLC analysis of both R2 and R3 MeOH extracts showed that polar metabolites were present. Bound residues released following protease, acid and base hydrolyses were characterized as polar water-soluble residues.

In addition to the work described above, TLC analyses were conducted with all the MeOH soybean straw, sorghum straw, and bermudagrass extracts, in which the reference compounds were TCAB (tetrachloroazobenzene) and TCOAB (tetrachloroazoxybenzene). None of the extracts contained metabolites which corresponded to these reference chemicals.

Based on the results of attempts to characterize and/or identify radioactivity in rotational crops planted into propanil-treated soil, the registrant concluded that terminal crop residues did not include propanil, indicating that the parent chemical and any soil degradates are metabolized by plants into degradates more polar than the parent compound. TLC analysis of plant extracts indicated the presence of multiple metabolites, most of which were present at less than 0.01 ppm.

CBRS comments on the adequacy of the degree to which radioactive residues were characterized follows a discussion of the radiovalidation data submitted by the Task Force (see below).

Storage Stability

The performing laboratory conducted a one-year fortification experiment, in which samples of sorghum, soybeans and bermudagrass from the first and last rotations were fortified with ^{14}C-propanil and held in frozen storage for up to a year; samples were extracted and the ACN fractions analyzed after one month and after one year of frozen storage. Although the fortified samples showed only the presence of propanil in the ACN fractions after one year of storage, the experiment does not demonstrate stability of radioactive residues in the relevant plant matrices. In order to demonstrate storage stability, the laboratory should have provided the Agency with HPLC chromatograms of MeOH extracts analyzed at various intervals corresponding to storage intervals incurred during the study.

Radiovalidation Using Rotational Crop Matrices (MRID No. 43355201)

The radiovalidation study was conducted using a previously submitted method entitled "Analytical Method for the Determination of Propanil as Base-Releasable 3,4-Dichloroaniline (DCA) in Soil, Water, Crayfish, 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." An appendix of the subject study included a complete description of the analytical method, as well as method validation data for the matrices

cited in the title. These data have been previously reviewed by the Agency, and therefore are not reviewed herein.

The analytical method consists of a strong base hydrolysis, conducted using 5N NaOH and a small quantity of hexane in a Nielson-Kryger distillation apparatus. The hexane fraction is then cleaned up and analyzed, using gas chromatography with nitrogen-specific detection, for residues of dichloroaniline (DCA). The DCA residues determined by the method include free DCA, as well as base-releasable DCA, which are reported as propanil. Prior to initiation of the radiovalidation portion of the study, the method was validated in bermudagrass, soybean and sorghum matrices.

Method Validation

Control samples of soybean straw and grain, sorghum straw and grain, and bermudagrass generated in the rotational crop study were shipped to the performing laboratory. Each sample type was fortified with propanil in duplicate at 0.01, 0.05 and 0.50 ppm. Residues were detected as 3,4-DCA, and reported as propanil equivalents. Equipment used and instrument conditions were adequately described. Sample calculations, raw data and chromatograms were included in the submission. Method validation data are presented in Table 6.

Table 6. Method Validation Data for Rotational Crop Matrices

Fortification Level (ppm)	% Recovery, Soybeans		% Recovery, Sorghum		% Recovery, Bermudagrass
	Beans	Straw	Grain	Straw	
0.01	72, 82	83, --	86, 63	116, 71	79, 88
0.05	71, 74	96, 85	60, 65	74, 71	77, 85
0.50	81, 64	77, 68	79, 83	76, 78	77, 79
Average	74.0 ± 6.7	81.8 ± 10.3	72.7 ± 11.3	81.0 ± 17.4	80.8 ± 4.6

Although there were several low recoveries, i.e. less than 70%, overall recoveries were within the range considered acceptable by the Agency. Procedural recoveries were conducted during analysis of the radiolabeled rotational crop samples, in which two control samples were fortified with propanil at 0.01 to 0.50 ppm. These recoveries were generally comparable to those found in the method validation portion of the study, with an average of 87.0 ± 19.2% (n=17). Average recoveries from sorghum grain and straw were higher, but were more variable than in the other matrices. The procedural recoveries support the findings of the method validation study: the analytical method adequately recovers propanil as 3,4-DCA from rotational crop matrices.

Analysis of Rotational Crop Samples (Radiovalidation of the Rotational Crop Study)

The method described above is the proposed enforcement analytical method, and therefore it was used to determine what portion of the rotational crop TRRs would be quantitated as free and base-releasable DCA. Samples analyzed using the method included sorghum (straw and grain) and soybean (straw and beans) from all 3 rotations, and bermudagrass from the second and third intervals. In addition to GC analysis of the hexane distillates for residues of 3,4-DCA, total ^{14}C residues in the distillates were measured using liquid scintillation counting (LSC).

Results

No significant differences were observed between the levels of radioactivity in the hexane distillates and the residues determined as free and base-releasable DCA. Therefore, there were no radioactive residues extracted into hexane which were not determined by the analytical method. In all three matrices, the free and base-releasable DCA residues constituted a relatively minor portion of the TRR. Therefore, there were residues, presumably remaining in the aqueous fraction, which are not determined by the analytical method. A comparison of rotational crop TRRs and residues determined using the proposed analytical enforcement method is presented below.

Table 7. Comparison of Free and Base-Releasable DCA (Calculated as Propanil) Residues and TRRs in Rotational Crop Matrices.[1]

Crop Part	30-Day Plantback		157-Day Plantback		365-Day Plantback	
	TRR	Propanil, ppm	TRR	Propanil	TRR	Propanil
Soybeans:						
Beans	0.21	0.03 (14.3%)	0.20	0.02 (10.0%)	0.26	0.08 (30.8%)
Straw	0.40	0.05 (13.9%)	0.14	0.04 (28.6%)	0.26	0.08 (30.8%)
Sorghum:						
Grain	0.31	<0.01 (<3%)	0.10	<0.01 (<10%)	0.06	<0.01 (<16%)
Straw	0.20	0.03 (15.0%)	0.07	0.01 (14.3%)	0.09	0.02 (22.2%)
Bermudagrass:						
Forage	NA	--	0.18	<0.01 (<5.9%)	0.16	0.01 (6.3%)

[1] TRRs are expressed in ppm, ^{14}C-propanil equivalents. The proposed analytical enforcement method quantitates residues as 3,4-DCA (free and base-releasable), which are then calculated as the parent, propanil. Propanil residues are reported in ppm, with the %TRR recovered using the proposed enforcement method.

CBRS Comments

The in-life portion of the confined rotational crop study is adequate with respect to the crops used, application of the test substance, description of harvest techniques, and supporting documentation including crop maintenance and rainfall. The analytical portion of the study is also adequate; the performing laboratory followed Agency guidelines with respect to the degree of attempted identification/characterization of radioactive residues in rotational crop matrices. However, none of the radioactivity in rotational crops was identified.

The submitted storage stability data do not strictly support the results of the confined rotational crop study, since stability of propanil metabolites was not demonstrated in rotational crop extracts that were analyzed in the study. However, since residues of ^{14}C-propanil, *per se* were stable for up to one year, no additional data are required. CBRS concludes that the confined rotational crop study is acceptable. Although none of the radioactivity was identified, residues were partially characterized as polar and multicomponent. As with rice, bound residues constituted a major portion of the total radioactive residue; in rice, the registrant demonstrated that radioactivity was incorporated into ^{14}C-glucose. In rotational crops, it is likely that, as in the case of rice, propanil residues were either strongly conjugated or incorporated into macromolecules.

The radiovalidation study is also adequate. Method validation data submitted with the radiovalidation data demonstrate adequate recovery of propanil residues (quantitated as 3,4-DCA) from rotational crops fortified with propanil using the proposed enforcement method. Radiovalidation data have shown that, in soybeans (from all 3 plantback intervals), radioactive residues (i.e. free and base-releasable DCA) exceed 0.01 ppm. However, previously submitted data reviewed in the R. Cook memo dated 4/24/79 showed no accumulation of propanil residues in rotational crops [barley (grain); corn (grain); lettuce; sugarbeets (roots and tops); and sunflower seeds] at a plantback interval of 2 weeks (1X application rate). Although 1 soybean sample contained residues of 0.08 ppm, this is considered to be anomalous. However, to assure that no propanil residues of concern would be found in soybeans or any other rotational crop, registered labels should indicate a plantback interval of 60 days for all rotational crops. No additional data are required for Guidelines 165-1 and 165-2.

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