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

NOV 15 1995

MEMORANDUM

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Propanil. List A Reregistration Case No. 0226/Chemical ID No. 028201. Rohm and Haas Submission to Upgrade a Wheat Metabolism Study [Guideline Ref. No. 171-4(a)]. MRID No. 43372201. CBRS No. 14597. DP Barcode No. D208555.

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THRU: Ed Zager, Chief
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Rohm and Haas has submitted a study entitled "Additional Work on Metabolism of ^{14}C-Propanil in Spring Wheat," 8/19/94 [MRID No. 43372201]. The report is supplemental to a previously submitted spring wheat metabolism study [MRID No. 42209201]. The analytical portion of the subject submission was conducted by Xenobiotic Laboratories, Inc. of Plainsboro, NJ.

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.

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 rice metabolism study submitted in response to the Reg. Std. was deemed unacceptable, but was subsequently upgraded (memos, C. Swartz, 9/8/94, CBRS No. 14030, DP Barcode No. D205676; and S. Funk, 12/14/92, CBRS No. 10228). Approximately 60 to 92% of the rice

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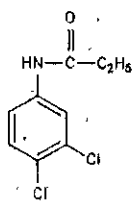
radioactivity was found in the bound fractions. Although attempts to identify much of the radioactivity were inconclusive, the registrant demonstrated that propanil is broken down by plants, enters the carbon pool, and is incorporated into biomolecules. It is also likely that propanil metabolites are either strongly conjugated with or entrapped by macromolecules.

A wheat metabolism study was submitted to the Agency and deemed unacceptable, but could be upgraded with the submission of additional data [CBRS No. 9528, 4/2/92, J. Abbotts; CBRS No. 10448, 12/14/92, S. Funk; and CBRS No. 11742, 11/30/93, F. Fort]. The original review (J. Abbotts) required the registrant to further characterize fractions designated as unknown(s), and to conduct base hydrolysis and steam distillation on wheat samples. The registrant submitted a proposal to upgrade the study which was reviewed by S. Funk (12/14/92), who concluded that (a) the lignin fraction must be further characterized, via characterization/identification of radioactivity following base hydrolysis/steam distillation; and (b) following base hydrolysis and steam distillation of straw and grain, radioactivity in the aqueous base, the distillate and the residual solids must be determined, and characterized/identified if the radioactivity exceeds 10% of the TRR in the sample.

The registrant responded with another proposal, which was reviewed by F. Fort (11/30/93). The registrant agreed to do the following:

- further characterize the aqueous extract (18 %TRR) from wheat straw;
- analyze samples using the current enforcement method; and
- following base hydrolysis/steam distillation, radioactivity in the residual solids, the aqueous base, and in the distillate will be determined, and the radioactivity further characterized/identified if the radioactivity in the fraction exceeds 10% of the sample TRR. Samples to be analyzed in this manner include final harvest straw, grain, and the lignin fraction from final harvest straw.

CBRS concluded that the proposal as outlined above would address the concerns cited in previous memoranda. The current submission (MRID No. 43372201) is the completion of the registrant's proposal. The structure of propanil is shown below:



propanil

Recommendation

The wheat metabolism study has been upgraded to acceptable. The required additional work showed that the unidentified radioactivity in the aqueous fraction consisted of multiple polar metabolites, some of which were conjugates with DCA. The proposed enforcement method was adequately validated in wheat straw, but highly variable recoveries were obtained with the grain. [No additional data are required, since the proposed enforcement method was validated in wheat grain in a cold study]. Based on rice, wheat and animal metabolism studies, the HED Metabolism Committee will be asked to make a determination will be made regarding the residues to be regulated.

Conclusions

1. The registrant conducted the additional work as stated in the proposal reviewed in the 11/30/93 F. Fort memo.

2. Re-determination of the TRR and re-extraction of radioactive residues demonstrated that there was no significant decomposition of propanil residues in the wheat samples during the additional storage time incurred between the original study and the subject study.

3. A sufficiently large sample of final harvest wheat straw was re-extracted, and separate aliquots of the aqueous fraction (AQ-1) were subjected to C-18 column clean-up, followed by cellulase, acid or base hydrolysis.

4. Samples of final harvest straw, wheat grain, and the lignin fraction from final harvest straw were analyzed using the proposed enforcement method (Nielsen-Kryger distillation).

5. The AQ-1 MeOH fraction, cellulase, acid and base hydrolysates, as well as the aqueous distillates and base hydrolysates remaining following the Nielsen-Kryger distillation were analyzed using RP-HPLC and TLC. Adequate representative HPLC and TLC chromatograms were included in the submission. The hexane distillate of the Nielsen-Kryger distillation was analyzed using both HPLC and GC/MS. Instrumental conditions were adequately described for HPLC, TLC and GC/MS.

6. The proposed enforcement method was adequately validated in the final harvest wheat straw. Highly variable recoveries were obtained with the wheat grain; however, the method was adequately validated in wheat grain in a cold study.

7. The additional work conducted by the registrant did not result in identification of a significant portion of the radioactive residues. The aqueous fraction from wheat straw was characterized as multicomponent polar residues, some of which consisted of DCA conjugates.

8. In the final harvest wheat straw, a maximum of 4.0 %TRR (0.08 ppm) was identified as propanil (in the organic extracts), and 4.1 %TRR (0.08 ppm) was identified as DCA; an additional 1.04 %TRR (0.02 ppm) was identified as DCPGA, or *N*-(3,4-dichlorophenyl)-glucosylamine.

9. Although the additional work conducted by the registrant did not confirm that incorporation of the radiolabel occurred in wheat, this metabolic pathway was demonstrated in rice, and therefore CBRS concludes it is likely to have occurred to some extent in wheat.

DETAILED CONSIDERATIONS

Re-Determination of the TRR

Since the original study was conducted in 1988, the performing laboratory reanalyzed the wheat straw and grain samples for radioactivity. Samples were combusted in triplicate, and the trapped $^{14}CO_2$ analyzed using liquid scintillation counting (LSC). The results of the original TRR analysis, and the analysis conducted in the subject study, are presented in Table 1.

Table 1. Total Radioactive Residues.[1]

	Straw (ppm)	Grain (ppm)
Original study	1.92	0.16
Reassay (subject study)	2.00	0.11

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

The submission also included a summary of the extraction profiles from the original study as well as from the subject study. The profiles were similar enough to demonstrate that no significant degradation of residues occurred between the original study and the subject study.

Analytical Methods Used to Characterize/Identify Radioactive Residues

Liquid fractions were analyzed for total radioactivity directly using liquid scintillation counting (LSC). Solids and some liquid fractions were analyzed via combustion followed by LSC of the trapped $^{14}CO_2$. Extracts were analyzed using reversed-phase high performance liquid chromatography (RP-HPLC) using 2 different solvent systems. Because of the low levels of radioactivity involved, eluates were collected every 30 seconds, the eluates analyzed using LSC, and reconstructed radiochromatograms prepared (background noise was subtracted to facilitate interpretation). Reference compounds were analyzed using the same conditions, and the retention times (R_t) included in the submission.

One-dimensional, normal phase thin layer chromatography (TLC) was used to confirm

identification of metabolites made using HPLC, and to characterize unidentified radioactivity. Two different solvent systems were used, and metabolite/reference compounds were spotted on the plates and the ratio-to-front (R_f) values reported. TLC plates were visualized using UV light; for radioactive samples, radioactivity on the plates was quantitated using an AMBIS imaging system or a FUJIX BAS Bio-Imaging System.

Certain fractions obtained after Nielsen-Kryger distillation were analyzed using gas chromatography (GC) with nitrogen/phosphorous (N/P) detection. Chromatographic conditions were adequately described. Other fractions were analyzed using GC-Mass spectrometry (GC-MS). Once again, chromatographic/spectrophotometric conditions were adequately described.

Adequate supporting chromatograms, calculations and documentation were submitted in support of metabolite identification/characterization.

Extraction of Radioactive Residues

Radioactive residues were extracted from the wheat straw in the same manner as in the original study. Briefly, final harvest straw samples were stirred with acetonitrile (ACN), filtered, and the solids extracted with MeOH/H_2O overnight and filtered. Solids were again extracted overnight with MeOH/H_2O and filtered; combined MeOH/H_2O fractions were concentrated and partitioned with dichloromethane (DCM), yielding organic and aqueous fractions. Fractions were concentrated and stored frozen; remaining solids were stored frozen. In order to obtain sufficient aqueous fractions from the wheat straw for the additional analysis required by the Agency, the performing laboratory extracted 4 separate straw samples.

Aqueous fractions were cleaned up using C_{18} column chromatography. Radioactive residues were eluted with water, MeOH, and 0.1N HCl. In all fractions analyzed, approximately 80% of the eluted radioactive residues were found in the MeOH fraction. MeOH fractions from the first, third and fourth extractions were combined and concentrated. The second extract was not concentrated. Subsamples of the MeOH eluate, or aqueous fraction (noted as AQ-1 in the original and subject studies) were then subjected to cellulase, acid (1N HCl), and base hydrolysis (weak hydrolysis at 2N NaOH followed by hydrolysis at 5N NaOH).

The cellulase hydrolysate was partitioned with DCM to yield aqueous and organic phases. Acid hydrolysis was conducted on 2 separate aliquots of the AQ-1 extract. First, one aliquot was hydrolyzed for 1 hour using 1N HCl, neutralized, and extracted twice with DCM. The resultant DCM fraction was concentrated for analysis. The aqueous fraction was made basic, and then extracted twice with DCM; the DCM fraction was again concentrated for analysis. The second hydrolysis was conducted in the same manner, however the initial DCM fraction was extracted with 1N HCl, divided into 3 parts, and concentrated using different methods in order to minimize the loss of radioactivity. The acidic aqueous fraction was then neutralized and partitioned twice with DCM. The acid hydrolysate was divided into 2 portions, the larger of which was partitioned twice with ethyl acetate (EtOAc) and once with *n*-butanol; all fractions were stored frozen prior to analysis.

Following base hydrolysis of the AQ-1 fraction with 1N NaOH, the hydrolysate was partitioned twice with DCM. The resultant aqueous fraction was made more basic, and partitioned twice with DCM; the combined DCM fractions were frozen for analysis. The basic aqueous fraction was then subjected to strong base hydrolysis in 5N NaOH overnight. The basic hydrolysate was extracted with DCM, and the DCM fraction concentrated for analysis. The remaining aqueous fraction was sequentially extracted with EtOAc and *n*-butanol. The aqueous fraction was then acidified, extracted with DCM and then EtOAc; fractions were concentrated and frozen for analysis.

To isolate the lignin fraction of final harvest wheat straw, the performing laboratory subjected the post-extraction solids to soxhlet extraction with methanol, α-amylase enzyme hydrolysis, cellulase hydrolysis, protease hydrolysis, and incubation with H_2SO_4. Solids remaining, containing the insoluble lignin fraction, were maintained frozen. Adequate measures were taken to ensure enzyme activity.

Results of Attempts to Characterize/Identify Residues in the Aqueous Fraction (AQ-1)

Following C_{18} column clean-up of the AQ-1 fraction of wheat straw, the MeOH eluate (containing 9.48 %TRR, or 0.19 ppm) was analyzed via HPLC and TLC. The MeOH eluate was found to contain at least 3 unidentified polar metabolites, but the radioactive peaks did not correspond to any of the HPLC retention times for known reference compounds. TLC analysis of the MeOH fraction did not yield further information. Based on the results of the rice metabolism study, in which mild base hydrolysis was conducted on the polar metabolites in the MeOH/H_2O fraction, the registrant stated that the unidentified polar metabolites in the wheat straw AQ-1 could be DCA tightly bound to humic acid or other plant macromolecules.

The organic fraction of the cellulase hydrolysate (comprising 2.2 %TRR, or 0.04 ppm) was concentrated and analyzed using HPLC; approximately 60% of the radioactivity was lost upon concentration. Remaining radioactivity was characterized as polar metabolites, all of which were $<$0.01 ppm, using TLC and HPLC. The aqueous fraction of the hydrolysate (15.26 %TRR, 0.31 ppm) was analyzed with similar results--radioactivity was partially characterized as multiple polar metabolites, each comprising a small fraction of the TRR.

Following acid hydrolysis, only 8.84 % of the wheat TRR (0.18 ppm) could be extracted into the organic phase. Attempts were made to minimize losses of radioactivity upon concentration for analysis (see discussion above); however, small amounts of radioactivity were lost regardless of the method used. HPLC and TLC analyses were conducted on the organic phase, resulting in the identification of, at most, 0.75 %TRR ($<$0.015 ppm) as DCA. The remainder of the radioactivity was partially characterized as multicomponent polar metabolites. The aqueous fraction of the acid hydrolysate (8.62 %TRR, or 0.17 ppm) formed a viscous fraction with a precipitate upon concentration, and therefore no chromatographic analyses could be conducted. The second aliquot aqueous phase was partitioned, and radioactivity was partially characterized as metabolites ranging from non polar to polar water-soluble compounds. Another aliquot of the second aliquot aqueous phase was cleaned up on a C_{18} column, eluted with MeOH, and

analyzed using HPLC and TLC; the TLC plate indicated the presence of DCPGA, or *N*-(3,4-dichlorophenyl)-glucosylamine, at 1.04 %TRR (0.020 ppm).

The combined DCM fractions (4.95 %TRR, 0.10 ppm) of the base hydrolysate were analyzed using TLC and HPLC, and were found to contain DCA at 1.81 %TRR (0.04 ppm). Strong base hydrolysis of the aqueous fraction of the base hydrolysate release 2.29 %TRR (0.05 ppm) as DCA, determined via TLC and HPLC analysis. The aqueous fraction of the strong base hydrolysis was partitioned, but the fractions could not be further analyzed due to the low levels of radioactivity. A total of 4.1 %TRR (0.09 ppm) was identified as base-releasable DCA. The extraction profiles of the aqueous hydrolysate indicate that the hydrolyzed radioactivity was multicomponent.

Nielsen-Kryger Distillation of Final Wheat Straw, Grain, and the Lignin Fraction of Straw

The enforcement method used to quantitate propanil residues as free and base-releasable DCA is the Nielsen-Kryger base hydrolysis/steam distillation. Briefly, 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 method was validated with control wheat straw and grain samples obtained from the metabolism study. Wheat straw was fortified with propanil at 0.2, 0.5, and 2.0 ppm; wheat grain was fortified with propanil at 0.01, 0.1, and 1.0 ppm. Lignin samples obtained from the untreated control straw were fortified with 0.01, 0.1 and 2.5 ppm propanil. Recoveries were generated concurrently with the analysis of the ^{14}C-propanil-treated samples. Recoveries from straw ranged from 93.5 to 110%, while recoveries from grain ranged from 61 to 200%. Recoveries from lignin were the most variable, ranging from 111.6 to 400%.

Although the method was not adequately validated in the wheat grain in the subject study, adequate validation data for wheat grain were submitted in conjunction with wheat field trials (refer to the C. Swartz memo dated 9/22/94, CBRS No. 13729). The submission included sample chromatograms indicating interfering peaks and coeluting matrices. The method was adequately validated in wheat straw. Although the method was not adequately validated in the lignin fraction from wheat straw, this is not a problem since lignin is not a rac.

Results of the Nielsen Kryger Distillation

The results of the Nielsen Kryger distillation are presented in Table 2.

Table 2. Results of Nielsen Kryger Distillation.

Sample ID	Nielsen Kryger Distillation					
	^{14}C-Distribution %TRR (ppm)					
	Solids[1]	Hydrolysate[2]	Aqueous[3]	Hexane[4]	% Recovery[5]	% as DCA[6]
Wheat straw (2.0 ppm)	14.08 (0.28)	58.33 (1.17)	1.37 (0.03)	26.22 (0.52)	117.38	34 (0.68 ppm)
Wheat grain (0.11 ppm)	42.75 (0.05)	53.95 (0.06)	0.73 (<0.01)	2.58 (<0.01)	96.59	(<0.01 ppm)
Lignin (0.52 ppm)[7]	22.48 (0.12)	48.68 (0.25)	0.54 (0.01)	26.77 (0.14)	71.34	N/A

[1] Solids remaining after base hydrolysis.

[2] Basic hydrolysate--constitutes remaining aqueous fraction in the hydrolysis pot.

[3] Aqueous fraction of the distillate.

[4] Hexane fraction of the distillate.

[5] Percent recovery of the radioactivity through the distillation procedure.

[6] % DCA as determined via GC/MS. It was not possible to determine the %DCA in the lignin hexane distillate, due to interfering peaks.

In accordance with the registrant's proposal to upgrade the study, the performing laboratory conducted additional experiments to characterize the radioactivity found in the basic hydrolysates. Basic hydrolysates of the wheat straw and the lignin fraction of wheat straw were subjected to C_{18} column clean-up, eluted with MeOH, and analyzed using RP-HPLC and TLC. Due to the low levels of radioactivity involved and/or coeluting matrices, however, TLC analysis did not result in identification of radioactive residues. Reconstructed HPLC chromatograms indicated the presence of multiple components, eluting from 20 to 35 minutes. The basic hydrolysate from grain was not further analyzed, since the level of radioactivity was so low (0.06 ppm).

The hexane/EtOAc fractions of wheat straw, grain and the lignin fraction of wheat straw were analyzed for DCA using GC and GC/MS. Analysis for DCA in the lignin fraction was complicated by interference, and therefore the results were not definitive. Analysis for grain was complicated by low levels of radioactivity, but demonstrated that DCA was <0.01 ppm. Results for wheat straw showed that all of the radioactivity found in the hexane distillate was attributable to DCA. Adequate supporting GC chromatograms and mass spectra were submitted.

CBRS Comment

The degree to which the registrant attempted to characterize and/or identify radioactive residues is adequate. The additional work required by the Agency was conducted by the registrant, and was supported by adequate chromatograms, calculations, and other documentation. No

additional work is required to upgrade the wheat metabolism study.

In the final harvest wheat straw, a maximum of 4.0 %TRR (0.08 ppm) was identified as propanil (in the organic extracts), and 4.1 %TRR (0.08 ppm) was identified as DCA; an additional 1.04 %TRR (0.02 ppm) was identified as DCPGA, or *N*-(3,4-dichlorophenyl)-glucosylamine. Aqueous residues in wheat straw were partially characterized as polar and multicomponent; such metabolites would likely be sugars and conjugates with DCA. Similar conclusions were made regarding the nature of the radioactivity found in the aqueous base hydrolysate fractions of wheat straw and lignin following analysis using the proposed enforcement method. The results of the study show that some of the radioactivity partially characterized as conjugates with DCA is picked up by the analytical method as base-releasable DCA.

Both the subject study and the original study show that the majority of propanil residues in wheat are found in the bound fractions. Although the fractionation of the bound residues into cellular components (conducted in the original study) indicated incorporation of the radiolabel, additional work to upgrade the study did not confirm this metabolic path. However, the registrant demonstrated that incorporation occurred in rice, and therefore it is likely that this occurred to some extent in the wheat.

CBRS concludes that the wheat metabolism study has been upgraded to fully adequate. Based on the results of the rice, wheat and animal metabolism studies, the HED Metabolism Committee will be asked to make a determination will be made regarding the residues to be regulated.

cc: CSwartz: SF: List A File: RF: Circulation
7509C:CSwartz:CBRS:CM2:RM804F:703 305 5877:11/9/95
RDI:RSQuick:11/14/95 RBPerfetti:11/14/95 EZager:11/14/95