

US EPA ARCHIVE DOCUMENT

3-14-95
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAR 14 1995

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Profenofos. Nature of the Residue in Cotton. Case No. 2540. Chemical No. 111401. MRID No. 43186801. DP Barcode: D201827. CBRS No. 13539. Radiovalidation Data. MRID No. 43203501. DP Barcode: D203218. CBRS No. 13725.

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BACKGROUND

Ciba Plant Protection has submitted data on the metabolism of profenofos in cotton, "¹⁴C-Profenofos: Nature of the Residue in Field-Grown Cotton" (MRID No. 43186801). These data are reviewed here. This data submission is in response to the Phase IV review of profenofos (C. Olinger, 11/29/90). In that Phase IV review, a new plant metabolism study was required because the previously submitted study identified only 4% of the TRR in cotton seed and 32% of the TRR in cotton foliage. A route of metabolism was not proposed in the original submission.

Radiovalidation data for the enforcement method (PAM Vol. II, Method I) have also been submitted along with the plant metabolism study and are reviewed here (MRID No. 43203501). Concerns noted in the Phase IV review regarding the use of toluene instead of benzene in the method have been addressed.



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Profenofos is an insecticide-miticide registered for control of mites in cotton. Tolerances have been established for combined residues of profenofos [O-(4-bromo-2-chlorophenyl)-O-S-propyl phosphorothioate and its metabolites converted to 4-bromo-2-chlorophenol and calculated as profenofos in or on cotton seed (3.0 ppm), eggs and meat (0.05 ppm), and milk (0.01 ppm) [40 CFR 180.404). Food/feed additive tolerances have been established for cottonseed hulls at 6.0 ppm and in soapstock at 15 ppm (21 CFR 561.53).

CONCLUSIONS

Nature of the Residue.

1. The nature of the residue of profenofos in plants (cotton) is adequately understood. Profenofos is metabolized in the cotton plant to 4-bromo-2-chlorophenol (2 to 6.2 %TRR), 4-bromo-2-chloroanisole (0.7 to 1.2 %TRR) and three sugar conjugates of 4-bromo-2-chlorophenol (0.4 to 33.8 %TRR). Parent profenofos was present at 6.5 to 33.8 %TRR.
2. The proposed metabolic pathway is supported by the data. (See Detailed Considerations.)
3. The existing tolerance expression has been established for profenofos and its metabolites converted to 4-bromo-2-chlorophenol and calculated as profenofos. The results of this metabolism study do not change the tolerance expression.

Analytical Method.

4. The existing enforcement method is PAM Vol. II, Method I, Pesticide Registration Section 180.404. It is a common moiety method and is acceptable for the determination of profenofos residues and its metabolites converted to 4-bromo-2-chlorophenol and calculated as profenofos equivalents. Method validation data were submitted for a comparison of the enforcement method with the radiolabel method used in the plant metabolism study. These data indicate the enforcement method is a valid method for determination of profenofos residues in/on treated cotton samples.

Storage Stability

5. Storage stability data exist for residues of profenofos in or on cottonseed and its processed commodities. Profenofos residues are stable in/on cottonseed for up to 9 months of frozen storage, but decline ca. 30% after 14 months of storage, and ca. 40% after 24 months of storage. Cottonseed samples stored for longer than 9 months and analyzed for profenofos residues should be corrected for decline during storage.

RECOMMENDATIONS

The registrant should be advised that the plant metabolism data are adequate to satisfy GLN 171-4(a) and the nature of the residue of profenofos in cotton plants is adequately understood.

DETAILED CONSIDERATIONS

Qualitative Nature of the Residue

A metabolic pathway for profenofos was proposed and is supported by the data. Parent compound, O-(4-bromo-2-chlorophenyl)-O-ethyl-S-propyl-phosphorothioate P(6), undergoes hydrolytic cleavage to form 4-bromo-2-chlorophenol. 4-Bromo-2-chlorophenol P(4) could then be transformed through reaction with a glucosyltransferase to form conjugates of 4-bromo-2-chlorophenol, such as, the glucose conjugate identified as P(3). This compound P(3) could then be further transformed to a sulfate conjugate, such as, glucosyl sulfate bromochlorophenol P(2) through reaction with a sulfotransferase. Enzymatic hydrolysis and subsequent methylation of bromochlorophenol could form bromochloroanisole P(5).

Test System and Material

Radiolabeled ¹⁴C-profenofos was supplied by Ciba Plant Protection (Ciba). The compound was synthesized with a radiopurity of 98.6% and a chemical purity of 98.7% by Ciba. Profenofos (O-(4-bromo-2-chlorophenyl)-O-ethyl-S-propyl-phosphorothioate) was synthesized with the radiolabel in the positioned in the phenyl ring. The chemical structure is shown in Figure 1.

Six foliar applications of ¹⁴C-profenofos were made to field-grown cotton weekly for a maximum application of 6.0 pounds a.i./acre (the maximum label use rate). The test site was located in Greenville, Mississippi. The soil was classified as a silt loam. Rainfall and irrigation records were kept.

Cotton was planted on April 30, 1992. The first application of profenofos was made on July 8, 1992 (10 weeks after planting) and weekly thereafter for five weeks. A portion of the cotton was harvested as immature plants on July 28, 1992 after the 4th application and again as mature plants on November 3, 1992 immediately after the final application of profenofos. Total time from planting to harvest was 6 months. Immature plants were used for identification of profenofos and its metabolites to ensure ample radiolabelled material for extraction and identification by GC/MS methods. They were stored frozen (-20°C) for 5 months prior to extraction. Mature plants were stored frozen for 1 to 23 days prior to shipment to the analytical laboratory facility. Samples were kept at the lab facility for 2 to 9.5 months in frozen storage prior to extraction. Harvested plants were separated into stalks, lint and seeds.

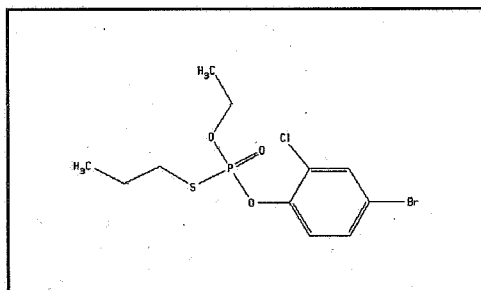


Figure 1

Total Radioactive Residue

¹⁴C-profenofos residues were determined by combustion and/or liquid scintillation counting (LSC) techniques. The limit of quantitation on the technique used was 0.0006 ug profenofos. Sample calculations were included. The Total Radioactive Residues (TRR) in the various cotton fractions were: 37.561 ppm in immature cotton stalks, 13.544 ppm in mature stalks, 0.655 ppm in cotton seeds, and 1.539 ppm in lint.

Table 1. Total Radioactive Residues in Cotton Fractions.*

Sample	TRR (ppm)	Hexane	CHCl ₃	Aqueous	PES	TRR %
Immature Stalk	37.56		17.51 (46.6)	20.36 (54.2)	2.2 (5.8)	106.6
Mature Stalk	13.54		4.4 (32.5)	6.58 (48.6)	1.86 (13.7)	94.8%
Seed	0.655	0.077 (11.8)	0.013 (1.9)	0.131 (19.9)	0.376 (57.4)	91.0%
Lint ^{a)}	1.54	NA	NA	NA	NA	NA

* Values in parentheses are percentage of TRR. a) Lint fraction was not extracted.

Extraction and Analysis of Residue

All plant fractions analyzed were extracted initially with MeOH/H₂O (9:1). The methanol was removed from the filtrate with N₂ and the filtrate was partitioned with chloroform (CHCl₃) yielding an organic and an aqueous phase that was subsequently analyzed by C-18 HPLC. In the case of the cotton seed, the seeds were refluxed with hexane prior to extraction with MeOH/H₂O to remove oils. In the case of the immature stalk, prior to partitioning with chloroform, the aqueous filtrate was partitioned with a 50/50 hexane/heptane solution to remove pigments prior to further partitioning and analysis. The post-extraction solids (PES) from the cotton seed fraction was subjected to enzymatic digestion and hydrolysis with strong acid and base solutions to liberate bound radioactivity. Specifically, the post-extraction solids containing bound residue were treated sequentially with cellulase, protease, 2% Triton X-114, 0.5 M HCL, 0.5 M NaOH, then 6.0 M HCL and finally with 6 M NaOH. The residue released after each step ranged from 0.004 to 0.08 ppm (0.7 to 12.3% of the TRR). See Table 3 for details.

Table 2. Distribution and Characterization of Radioactive Residues Detected in Extracts of Cotton (immature stalks, mature stalks, seed) treated with Profenofos.

Sample Fraction	%TRR	PPM	Characterization/Identification
Immature Stalks (TRR = 37.56 ppm)			
CHCl ₃	46.6	17.51	HPLC detected three peaks. P(4) 0.74 ppm, 2% TRR, p(5) 0.29 ppm, 0.8% TRR, and P(6) 12.68 ppm, 33.8% TRR.
Aqueous	54.2	20.36	HPLC detected three peaks. P(1) 2.34 ppm, 6.3% TRR, P(2) 12.68 ppm, 33.8% TRR and P(3) 2.78 ppm, 7.4% TRR.
PES	5.8	2.2	
Total Extracted	100.8	37.86	
Mature Stalks (TRR = 13.54 ppm)			
CHCl ₃	32.5	4.4	HPLC detected three peaks. P(4) 0.189 ppm, 1.4% TRR, P(5) 0.088 ppm, 0.7% TRR and P(6) 3.84 ppm, 28.4% TRR.
Aqueous	48.6	6.58	HPLC detected three peaks. P(1) 1.93 ppm, 14.2% TRR, P(2) 4.1 ppm, 30.3% TRR and 0.38 ppm, 2.8% TRR. One unknown was present at 0.125 ppm, 0.9% TRR.
PES	13.7	1.86	
Organic	6.2	0.844	HPLC detected one peak: P(4) 0.648 ppm, 4.8% TRR.
Aqueous	3.1	0.422	Not analyzed further.

Sample Fraction	%TRR	PPM	Characterization/Identification
PES	1.3	0.169	Not analyzed further.
Total Extracted	90.53	12.26	
Seed (TRR = 0.655 ppm)			
Hexane	10.8	0.071	HPLC detected three peaks: P(4) 0.009 ppm, 1.4% TRR, P(5) 0.008 ppm, 1.2% TRR and P(6) 0.034 ppm, 5.2% TRR. Unaccounted for radioactivity was 0.02 ppm, 3.0% TRR.
MeOH/H ₂ O	19.70	0.129	
CHCl ₃	2.3	0.015	HPLC detected two peaks: P(4) 0.005 ppm, 0.7% TRR and P(6) 0.008 ppm, 1.3% TRR. Unaccounted for radioactivity was 0.002 ppm, 0.4% TRR.
Aqueous	19.9	0.13	HPLC detected three peaks: P(1) 0.009 ppm, 1.4% TRR, P(2) 0.113 ppm, 17.3% TRR and P(3) 0.002 ppm, 0.4% TRR. Unaccounted for radioactivity was 0.027 ppm, 4.1% TRR.
PES	57.4	0.376	See Table 3.
Total Extracted	33.60	0.22	

P(1) = polysaccharide conjugate of bromochlorophenol, P(2) = glucosyl sulfate conjugate of bromochlorophenol, P(3) = glucose conjugate of bromochlorophenol, P(4) = bromochlorophenol, P(5) = bromochloroanisole and P(6) = profenofos.

Table 3. Fractionation of the Cotton PES.

Fractionation Step	Seed	
	%TRR	ppm
Initial TRR of PES = 0.376 ppm		
Cellulase	12.3	0.08
Protease	2.6	0.017
2% Triton X-114	3.6	0.023
0.5 M HCL	0.7	0.004
0.5 M NaOH	10.9	0.072
6 M HCL	1.1	0.007
6 M NaOH	8.6	0.056
Total Incorp.	39.80	0.26
Solids	2.0	0.013
Recovered	42.7	0.278

Characterization of Residues

In total, four analytical methods were used to identify radiolabeled material present in the immature stalk extracts: two-dimensional thin-layer chromatography (TLC), high pressure liquid chromatography (HPLC), gas-chromatography/mass spectrometry (GC/MS) and nuclear magnetic resonance (NMR). Complete confirmatory procedures were used on the immature stalk material to positively identify profenofos and its metabolites because of the high concentration of radiolabeled material present. TLC and HPLC were used to identify and confirm compounds present in extracts from cotton seed and mature stalks.

For the three compounds present in the organic extracts from immature stalks (profenofos P(6), bromochlorophenol P(4) and bromochloroanisole P(5)) TLC and HPLC were used to initially isolate and identify compounds. The retention times of radiolabeled standards of profenofos, bromochlorophenol and bromochloroanisole were compared against the retention times of peaks in the organic extracts. For positive confirmation, a GC/MS system equipped for selective ion monitoring (SIM) was used. The mass spectrum of each compound was identical to its standard confirming its identity.

For the three compounds in the aqueous extracts, identification procedures included a derivitization step, whereby the aqueous extracts were acetylated and then analyzed with HPLC, MS, -FAB/MS and/or NMR. Retention times and mass spectra of a standard for the acetylated glucose conjugate of bromochlorophenol were compared against those for acetylated-compounds detected in the aqueous extracts. Two of the compounds in the aqueous extracts were identified as glucose conjugates of bromochlorophenol P(2), and P(3), and one was characterized as a polysaccharide conjugate of bromochlorophenol P(1).

Table 4 provides the detailed findings of the residue characterization-identification process.

Table 4: Summary of ¹⁴C-residues identified/characterized in cotton treated with Profenofos.

Component	Imm. Stalk		Mature Stalk		Seed	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
Identified						
Profenofos P(6)	33.8	12.68	28.4	3.84	6.5	0.042
Bromochlorophenol P(4)	2.0	0.74	6.2	0.84	2.1	0.014
Bromochloroanisole P(5)	0.8	0.29	0.7	0.088	1.2	0.008
Glucosyl sulfate bromochlorophenol conj. P(2)	33.8	12.68	30.3	4.10	17.3	0.113
Glucose bromochlorophenol conj. P(3)	7.4	2.78	2.8	0.38	0.4	0.002
Total I.D.						
Unk./Char.						
Polysaccharide bromochlorophenol conj. P(1)	6.3	2.34	14.2	1.93	1.4	0.009
Total ID/Char.	84.1	31.51	82.6	11.18	28.9	0.188
Unk./Unchar.	--	--	0.9	0.125	--	--
Total	84.1	31.51	83.5	11.30	28.9	0.188

Immature Stalks. Six compounds were identified in immature cotton stalks by HPLC, TLC, GC/MS or NMR. The majority of the TRR was identified as two compounds: Profenofos and P(2) the glucosyl sulfate conjugate of bromochlorophenol. All other metabolites were present at <10% of the TRR. Approximately, 84% of the TRR was characterized/identified, and 6% of the TRR remained bound in the post-extraction solids (PES). Approximately 10% of the TRR was unaccounted for and may have been lost during sequential extraction and concentration steps and lyophilization.

Mature Stalks. As in the immature stalks, six compounds and one unknown were identified by HPLC, TLC, GC/MS or NMR. The majority of the TRR was detected as three compounds: Profenofos, P(1) and P(2) bromochlorophenol conjugates. Other metabolites are present at <10% of the TRR. One unknown characterized as polar was detected in the aqueous extract. The PES contained 1.3% of the TRR and was not analyzed further. Approximately 10-15% of the TRR was unaccounted for and may have been lost through extraction/concentration procedures used.

Cotton seed. The same six compounds found in immature and mature stalk fractions were identified by HPLC, TLC, GC/MS or NMR in the seed fraction. Additionally characterized was another 39.8% of the TRR remaining in the PES. The bound TRR in the PES was not identified, but characterized through its association with different plant matrices, i.e., pectin, cellulose, sugars, proteins, etc. Table 3 exhibits the details of the fractionation procedures used to liberate bound TRR. The majority of the released radioactivity was bound in the cellulose fraction, accounting for 0.08 ppm, 12.3% TRR. Overall, 68.7% of the TRR was characterized (39.8%)/identified (28.9%) in the seed fraction. A total of 76.3% of the TRR present in the cotton seed was extractable when the enzymatic digestion and acid/base hydrolysis procedures were used on the radioactivity remaining in the PES.

In summary, the same five compounds were identified and one compound characterized in all cotton fractions extracted and analyzed: stalks (mature and immature) and seeds. (Lint which contained 1.539 ppm TRR was not extracted or analyzed. No explanation was given.) These six compounds were: parent Profenofos P(6), bromochlorophenol P(4), bromochloroanisole P(5), and three conjugates of bromochlorophenol (a polysaccharide conjugate P(1), a glucosyl sulfate conjugate P(2) and a glucose conjugate P(3). Profenofos and the glucosyl sulfate conjugate of bromochlorophenol were present in the greatest amounts in all three plant matrices analyzed. All of these compounds can be converted to the common moiety 4-bromo-2-chlorophenol and determined by the enforcement method.

Analytical Method

Radiovalidation data were submitted to validate the existing enforcement methodology, PAM Vol. II, Method I, Pesticide Regulation Section 180.404 (referenced within the submission as AG-322A). The study entitled, "Validation of Analytical Method AG-322A for the Determination of Profenofos Residues as 4-Bromo-2-Chlorophenol in Cottonseed with Accountability Data", was submitted and reviewed. Control, fortified control and ¹⁴C-profenofos treated samples from the new plant metabolism study were analyzed by this method. As required in the Phase IV review, toluene was substituted for benzene in the method.

Residues of profenofos are extracted from cottonseed, cottonseed hulls, foliage and cottonseed meal by refluxing with methanol-water (9:1). An aliquot of the extract is refluxed for 30 minutes under aqueous basic conditions. The solution is acidified with 2N HCL and refluxed for an additional 2 hours in the presence of iso-octane. The layers are separated, and the aqueous phase is extracted with hexane, which is combined with the iso-octane. The iso-octane-hexane solution contains the hydrolysis product 4-bromo-2-chlorophenol. After further clean-up on a silica gel column, the hydrolysis product is determined by GC with electron capture

detection (ECD) on a column of 10% OV-101 on Gas-Chrom Q.

Accuracy. Recovery values from fortified control samples averaged 96% for all samples fortified and 84% for samples fortified at the 0.05 ppm limit of quantitation (standard deviation = 13, coefficient of variation = 13% and n = 4). Examples of the linearity of the method over a range of concentrations were provided.

Precision. Cottonseed samples treated with profenofos were extracted and analyzed with the GC method. The concentration of profenofos ranged from 0.23 to 0.27 ppm in one extract from the cottonseed samples from which aliquots were injected repeatedly to confirm reproducibility of the method. For aliquots from cotton stalk extract injected repeatedly, the range was 11.6 to 12.9 ppm.

Extractability. The enforcement method successfully extracted 86% of the cotton stalks, and only 43% of the ¹⁴C-profenofos from treated cottonseed. Other methods of extraction presented in the plant metabolism study successfully extracted only between 25 and 33% of the ¹⁴C-profenofos residues from cottonseed. Much of the residue remained bound and non-extractable without the aid of enzymatic digestion and severe acid/base hydrolysis.

Accountability. The total accountability for residues of profenofos in ¹⁴C-profenofos treated cottonseed was 37%, and 91% in treated cotton stalk. These values were determined by a comparison of the TRR in extracts of the crop samples, as determined by combustion and LSC, to the amounts of analytes determined by GC analysis. (See Table 5.)

Much of the residue in the cottonseed is bound and not extractable by the enforcement method. Separate analysis of the bound residues by enzymatic digestion and acid/base hydrolysis releases small amounts (0.004 to 0.08 ppm) of the residue from various fractions, i.e., cellulose, protein, pectin, etc. (See Table 3.) Use of the enforcement method to analyze the bound residue resulted in little detectable residue. This is probably because the bound residues could not be converted to 4-bromo-2-chlorophenol and/or were not released by the method.

Table 5. Comparison of %TRR and PPM Determined by the Radiolabel (Hot) and GC (Cold) Methods Used to Analyze Profenofos Residues.

Sample	"Radiolabel Method" (¹⁴ C-Hot)		"GC Method" (Cold)		Accountability	
	%TRR	PPM	%TRR	PPM	"Hot"	"Cold"
Seed	32.0	0.21	37.1	0.24	N/A	37.0%
Stalk	76.66	10.38	91.06	12.33	N/A	91.0%

Storage Stability

Storage stability data were submitted and reviewed (F. Suhre, 5/19/94, CBRS No.). The data were adequate and support a period of frozen storage for up to 9 months for weathered residues of profenofos in/on cottonseed. Residues of profenofos in/on cottonseed samples stored for more than 9 months should be corrected for decline. Residues decline ca. 30% after 14 months and 40% after 24 months.

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