MEMORANDUM

SUBJECT: Chlosulfuron (ID 118601). Residue Analytical Methods [171-4(c)&(d)] and Wheat Processing Data [171-4(l)]. Barcode: D194775, D195395, D195930, and D199193; CBRS No. 12481, 12603, 12680, and 13225; MRID No.:429006-01, -02, -03, 429266-01, 430510-01 and 431078-01: Case No. 0631.

FROM: Francis B. Suhre, Section Head
Special Review Section I
Chemistry Branch II--Reregistration Support
Health Effects Division (7509C)

THRU: Edward Zager, Chief
Chemistry Branch II--Reregistration Support
Health Effects Division (7509C)

TO: Jane Mitchell, PMT-71
Reregistration Branch
Special Review and Reregistration Division (7508W)

Attached is a review of registrant's response to Residue Chemistry Data Requirements for Chlorsulfuron Residue Analytical Methods [171-4(c)&(d)] and Wheat Processing data [171-4(l)]. This information was reviewed by Dynamac Corporation under the supervision of CBRS/HED. The data assessment has undergone secondary review in the Branch and has been revised to reflect Branch policies.

CBRS makes the following conclusions with respect to the submitted studies:

1. The Residue Analytical Method described in registrant report AMR 2341-92 (MRID No. 429006-03) appears adequate for data collection and tolerance enforcement for chlorsulfuron residues in/on plant commodities (wheat, barley, and oats). Provided that chlorsulfuron and 5-hydroxy chlorsulfuron are the only compounds that need to be regulated in/on plant commodities, an Agency method trial will be conducted on this method.

2. The Residue Analytical Method described in registrant report AMR 2715-93 (MRID No. 429266-01) appears adequate for data collection and tolerance enforcement for chlorsulfuron residues in animal commodities. Provided that chlorsulfuron is the only compound that needs to be regulated in animal commodities, an Agency method trial will be conducted on this method.

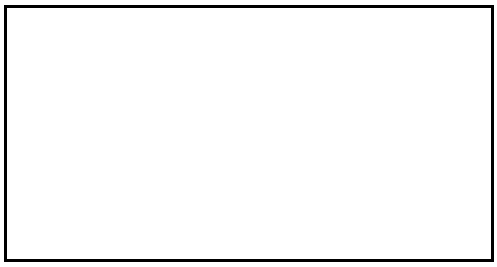
3. These proposed enforcement methods (AMR 2341-92, AMR 2715-93) should be subjected to radiovalidation studies using samples from the ongoing wheat and goat metabolism studies. The radiovalidation studies should include analyses for residues of chlorsulfuron, 5-hydroxy chlorsulfuron, and other potential residues of concern.

4. The submitted wheat processing study is adequate and will satisfy reregistration data requirement 171-4(l) for wheat upon submission of supporting storage stability data and provided that chlorsulfuron and 5-hydroxy chlorsulfuron are the only residues that need to be regulated in/on plant commodities. No food or feed additive tolerances are required. CBRS had previously waived the requirement for data on wheat grain dust (CBRS No. 9095, D172239, P. Deschamp, 12/27/91).

If you need additional information, please advise.

cc: RF, SF, List B Rereg. F., Circ., Dynamac.
RDI: MMetger:04/21/94;EZager:04/25/94;FBS:04/26/94.

CHLORSULFURON



Shaughnessy No. 118601; Case 0631

(CBRS No. 12481; DP Barcode D194775)

(CBRS No. 12603; DP Barcode D195395)

(CBRS No. 12680; DP Barcode D195930)

(CBRS No. 13225; DP Barcode D199193)

Task 4

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

In response to the Chlorsulfuron Reregistration Standard Update, dated 2/20/91, E.I. du Pont de Nemours and Co. has submitted the following: data pertaining to the magnitude of the residue in wheat processed commodities (1993; MRID 42900601); a description and validation data pertaining to a proposed enforcement method intended to replace the existing Pesticide Analytical Manual (PAM) Vol. II method for the determination of chlorsulfuron and its 5-hydroxy metabolite in/on plant commodities, along with data depicting radiolabel determination of the extraction efficiency of the submitted data collection method for wheat and the proposed enforcement method for plants (1993; MRIDs 42900602 and 42900603): data from an independent laboratory validation of the proposed enforcement method for plants (1993; MRID 43051001; note that MRID 43051001 replaces MRID 42948901, which was originally submitted under DP Barcode D195930); a proposed enforcement method for the determination of residues of chlorsulfuron, thifensulfuron methyl, and metsulfuron methyl in animal tissues and milk (1993; MRID 42926601); and data from an independent laboratory validation of the proposed enforcement method for chlorsulfuron residues in animal tissues and milk (1993; MRID 43107801). The submitted data are reviewed and evaluated in this document for their adequacy in fulfilling the outstanding residue chemistry data requirements. The Conclusions and Recommendations stated in this document pertain only to residue analytical methods and wheat processed commodities; other data requirements stated in the Chlorsulfuron Update are not addressed herein.

The qualitative nature of chlorsulfuron residues in plants and animals is not adequately understood. New metabolism studies on wheat, ruminants, and poultry are required to fulfill data requirements pertaining to the nature of the residue in plants and animals.

Tolerances for residues in/on the raw agricultural commodities of

barley, oats, and wheat are currently expressed in terms of the combined residues of chlorsulfuron [2-chloro-*N*-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]benzenesulfonamide] and its metabolite, 2-chloro-5-hydroxy-*N*-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]benzenesulfonamide [40 CFR §180.405(a)]. Tolerances for food items derived from animals are expressed in terms of chlorsulfuron *per se* [40 CFR §180.405(b)]. No food or feed additive tolerances have been established.

Adequate methods are available for the enforcement of tolerances for chlorsulfuron residues in/on plant and animal commodities. PAM Vol. II lists Methods I and II, HPLC methods with photoconductivity detection (PCD), for the determination of chlorsulfuron and its 5-hydroxy metabolite in plants, and for the determination of chlorsulfuron *per se* in animal tissues and milk.

No Codex MRLs have been established or proposed for chlorsulfuron; therefore, issues of compatibility between Codex MRLs and U.S. tolerances do not exist.

CONCLUSIONS AND RECOMMENDATIONS

Residue Analytical Methods

1. The submitted description and validation data pertaining to the proposed enforcement analytical method for plants are acceptable. Data pertaining to independent laboratory validation of the proposed enforcement method are adequate to satisfy the requirements of PR Notice 88-5 (independent laboratory validation) for Method AMR 2341-92. The data indicate that Method AMR 2341-92 adequately recovers residues of chlorsulfuron and its metabolite 5-hydroxy chlorsulfuron from wheat grain, forage, and straw, and confirm that the method is adequate for data collection and tolerance enforcement for residues in/on plant commodities (wheat, barley, and oats). The qualitative nature of chlorsulfuron residues in plants is not adequately understood; a new metabolism study on wheat is required. Provided that chlorsulfuron and 5-hydroxy chlorsulfuron are the only compounds that need to be regulated in/on plant commodities, an Agency method trial will be conducted on this method.

1b. The submitted description and validation data pertaining to the proposed enforcement analytical method for animal tissues and milk are acceptable. Data pertaining to independent laboratory validation of the proposed enforcement method are adequate to satisfy the requirements of PR Notice 88-5 (independent laboratory validation) for the proposed method. The data indicate that the method adequately recovers residues of chlorsulfuron from milk, ground beef, and liver, and confirm that the method is adequate for data collection and tolerance enforcement for residues in animal commodities. The qualitative nature of chlorsulfuron residues in animals is not adequately understood; new metabolism studies on ruminants and poultry are required. Provided that chlorsulfuron is the only

compound that needs to be regulated in animal commodities, an Agency method trial will be conducted on this method.

1c. The submitted extraction efficiency data indicate that the extraction procedures used in the proposed enforcement methods for plant and animal commodities are effective in liberating radioactive residues from wheat, oat, and goat matrices. However, these efforts fall short of fulfilling the requirements for radiovalidation since actual levels of chlorsulfuron and 5-hydroxy chlorsulfuron were not determined. CBRS recommends that these proposed enforcement methods be subjected to radiovalidation studies using samples from the ongoing wheat and goat metabolism studies. The radiovalidation studies should include analyses for residues of chlorsulfuron, 5-hydroxy chlorsulfuron, and other potential residues of concern.

Wheat Processed Commodities

2a. The submitted wheat processing study is adequate and will satisfy reregistration data requirements upon submission of supporting storage stability data; the registrant stated that a storage stability study with wheat processed commodities is in progress. The submitted wheat processing study indicates that residues of chlorsulfuron and 5-hydroxy chlorsulfuron were nondetectable (<0.05 ppm each) in/on one sample each of bran, low-grade and patent flour, middlings, shorts, and small screenings, and two samples each of grain dust (light impurities) and large screenings processed from wheat grain bearing nondetectable residues following treatment at 4x the maximum single application rate.

2b. The maximum theoretical concentration factor for wheat commodities is 9x (Maximum Theoretical Concentration Factor memorandum, 1/14/93, D. Edwards and E. Zager). Although a 5x exaggeration is the maximum the Agency considers realistic for foliar applications, given that chlorsulfuron is an herbicide, and that residues did not accumulate in the RAC or in processed commodities, CBRS concludes that the 4x application rate is sufficient to confirm that residues of chlorsulfuron and its 5-hydroxy metabolite are not likely to accumulate or concentrate in wheat processed commodities. No food or feed additive tolerances are required. CBRS had previously waived the requirement for data on wheat grain dust (CBRS No. 9095, D172239, P. Deschamp, 12/27/91).

DETAILED CONSIDERATIONS

Residue Analytical Methods

Proposed Enforcement Method for Residues in Plants (Method No. AMR 2341-92): Du Pont submitted (1993; MRID 42900603) a proposed enforcement method for the determination of residues of chlorsulfuron and its 5-hydroxy metabolite in/on wheat grain, forage, and straw. The method is intended to replace the existing HPLC/PCD method (Method I in PAM Vol. II) for the determination of chlorsulfuron and its 5-hydroxy metabolite in plants. The method involves use of an HPLC system capable of switching from one column to another to isolate chlorsulfuron and 5-hydroxy chlorsulfuron from matrix interferences. Detection is by UV at 240 nm.

In this method, wheat grain samples are extracted twice with 30 mM potassium phosphate. The supernatants are combined and adjusted to pH 3 by the addition of concentrated phosphoric acid. The mixture is centrifuged, and the resulting supernatant is filtered and passed through a C18 Empore™ extraction disk (the registrant noted that use of the extraction disk results in faster separation than can be achieved with conventional solid-phase extraction cartridges or columns). Chlorsulfuron and 5-hydroxy chlorsulfuron are then eluted from the disk with three aliquots of ethyl acetate. The resulting eluates are combined and evaporated to dryness under a stream of nitrogen at 40 C, then resuspended in acetonitrile:30 mM phosphate buffer, pH 3 (25:75; v,v). For wheat forage and straw, samples are extracted similarly, except that the ethyl acetate eluates collected following disk extraction are combined with 30 mM phosphate buffer, pH 3. Next sodium chloride is dissolved in the solution, and it is extracted twice with ethyl acetate. The resulting organic layers are combined and evaporated under a stream of nitrogen, then resuspended as for wheat grain.

Extracts are injected onto an HPLC system equipped with a Zorbax® SB-CN guard column (12.5 mm x 4.0 mm, 5-μ), two analytical columns, a switching valve, and a UV detector set at 240 nm. The first analytical column (Column I) is a Zorbax SB-Cyano column (150 mm x 4.6 mm, 5-μ for wheat grain, 250 mm x 4.6 mm, 5-μ for wheat forage and straw). Column II is a Zorbax® SB-C8 column (150 mm x 4.6 mm, 5-μ) for wheat grain, and a Zorbax ODS column (150 mm x 4.6 mm, 5-μ) for wheat forage and straw. The switching valve has two positions. Valve position 1 channels the eluent from Column I to the UV detector. Valve position 2 channels the eluent from Column I to Column II and then to the UV detector. The HPLC procedure involves injecting samples through the guard column and Column I to the detector with the valve in position 1. When chlorsulfuron or 5-hydroxy chlorsulfuron begins to elute, the valve is switched to position 2 and the desired peak is "trapped" on Column II. The valve is then switched back to position 1, and the remainder of the sample is run through Column I to the detector. After Column I has been cleaned and equilibrated, the valve is again switched to position 2, and chlorsulfuron or 5-hydroxy chlorsulfuron is eluted

to the detector. Two mobile phases are used. Acetonitrile:30 mM phosphate buffer, pH 3 (25:75; v,v) is used for most of the analysis; the mobile phase is switched to acetonitrile:30 mM phosphate buffer, pH 3 (80:20; v:v), and the flow rate is increased when the valve is switched back to Column I after trapping. Chlorsulfuron and 5-hydroxy chlorsulfuron are quantitated by peak height comparisons with standards. The limits of quantitation for each compound are 0.025 ppm for wheat grain, 0.25 ppm for wheat forage, and 0.10 ppm for wheat straw.

The registrant provided method validation data from wheat grain, forage, and straw samples separately fortified with chlorsulfuron and 5-hydroxy chlorsulfuron at 0.025-0.10 ppm (0.25-1x the tolerance for each component) for wheat grain, 0.25-0.50 ppm (0.0125-0.025x the tolerance) for wheat forage, and 0.10-0.50 ppm (0.2-1x the tolerance) for wheat straw. Apparent residues of chlorsulfuron were 0.039 ppm in/on one unfortified sample of wheat straw. Apparent residues of chlorsulfuron and 5-hydroxy chlorsulfuron were less than the limit of quantitation in/on three unfortified samples of wheat grain (<0.025 ppm each), six unfortified samples of wheat forage (<0.25 ppm each), and three unfortified samples of wheat straw (<0.10 ppm each). We note that apparent residues of chlorsulfuron were detectable (i.e., reported by the registrant as greater than 0) in/on one sample of unfortified wheat straw (0.039 ppm) and apparent residues of 5-hydroxy chlorsulfuron were detectable in/on one of five unfortified wheat forage samples (0.082 ppm), and in/on two of three unfortified wheat straw samples (0.059 and 0.089 ppm). Method validation data are presented in Table 1. Sample calculations, standard curves, and chromatograms were provided.

Table 1.Method recoveries of chlorsulfuron and its 5-hydroxy metabolite from fortified wheat matrices analyzed using du Pont Method AMR 2341-92.

Wheat Matrix	Fortification Level (ppm)	% Recovery (No. Samples)	
		Chlorsulfuron	5-OH Chlorsulfuron
Wheat grain	0.025	77-99 (3)	66-83 (3)
	0.050	84-89 (3)	80-95 (3)
	0.10	69-96 (3)	80-88 (3)
Wheat forage	0.25	72-84 (6)	66-87 (5)
	0.35	71-84 (6)	72-79 (4)
	0.50	69-88 (6)	72-87 (4)
Wheat straw	0.10	73-88 (3)	99-112 (3)
	0.25	72-82 (3)	89-103 (3)
	0.50	80-98 (5)	78-102 (3)

Extraction Efficiency of Current Analytical Methods for Plants: Du Pont also submitted (1993; MRID 42900602) data demonstrating the extraction efficiency of the HPLC/UV column-switching method proposed for enforcement and the LC/MS method used in the wheat processing study. A detailed discussion of the LC/MS method is presented with the wheat processing study.

For the study, [phenyl(U)-^{14}C]chlorsulfuron (radiochemical purity >95%, specific activity 11.5 μCi/mg) was formulated with inert ingredients to make the equivalent of the 75% FlC formulation (final specific activity unspecified). The test substance was applied using a CO_2 plot sprayer. Two treatments were examined. For the first treatment, the formulated test substance was applied at a field rate of 0.38 oz ai/A (1x the maximum single application rate) to wheat and oats at Feekes Stage 7. In the second treatment the test substance was applied at 0.38 oz ai/A at Feekes Stage 7 and again 3 days prior to harvest. Each test consisted of two control pots, four Treatment 1 pots and two Treatment 2 pots. Information pertaining to the maintenance schedule was included. The crops were fertilized, and watered as necessary. Wheat and oat forage samples were collected at 0, 3, and 14 days following Treatment 1. For both treatments, mature plants were harvested 63 days following the initial application, and were separated into grain, forage, and straw.

Samples were analyzed for total radioactive residues by LSS following combustion. Samples were then prepared for analysis by blending with liquid nitrogen. Samples were extracted twice with 30 mM potassium phosphate (used in the proposed enforcement method) or methanol:30 mM potassium phosphate (20:80; v:v; used in the processing study method). Following homogenization and centrifugation, the supernatants were collected and combined for each matrix. Supernatants were analyzed by liquid scintillation spectrometry (LSS). The registrant calculated the extraction efficiency of the two methods by comparing the average dpm detected in the supernatants with the average dpm detected in the samples. Extraction efficiencies are presented in Table 2. The submitted data indicate that the extraction procedures used in the proposed enforcement method and the data collection method for the wheat processing study are effective in liberating radioactive residues from wheat and oat matrices. However, these efforts fall short of fulfilling the requirements for radiovalidation since actual levels of chlorsulfuron and 5-hydroxy chlorsulfuron were not determined. CBRS recommends that this proposed enforcement method be subjected to a radiovalidation study using samples from the ongoing wheat metabolism study. The radiovalidation should include analyses for residues of chlorsulfuron, 5-hydroxy chlorsulfuron, and other potential residues of concern.

Table 2. Efficiency of extraction procedures for two analytical methods intended for the determination of chlorsulfuron in wheat.

Wheat Matrix	Post-treatment Interval	% Extraction Efficiency [a]	
		100% K_2HPO_4	80% K_2HPO_4:20% MeOH
Wheat forage	0	159	144
	3	116	99

	14	86	74
Wheat grain (Treatment 1)	63	153	128
(Treatment 2)	3 [b]	101	102
Wheat straw (Treatment 1)	63	81	72
(Treatment 2)	3	105	90
Oat forage	0	93	107
	3	108	98
	14	127	122
Oat grain (Treatment 1)	63	122	120
(Treatment 2)	3	102	101
Oat straw (Treatment 1)	63	87	101
(Treatment 2)	3	100	97

[a] % Extraction efficiency = 100 x (dpm in extract)/(dpm in unextracted sample).
[b] Treatment 2 plants received a second application of chlorsulfuron at 0.38 oz ai/A three days prior to harvest (63 days following the initial application).

Independent Laboratory Validation of Method AMR 2341-92: Du Pont submitted (1993; MRID 43051001) data pertaining to an independent laboratory validation of the proposed enforcement method (du Pont Method No. AMR 2341-92) for the determination of residues of chlorsulfuron and its 5-hydroxy metabolite in/on wheat grain, forage, and straw. The validation was conducted by Enviro-Test Laboratories (ETL, Edmonton, AB, Canada). One slight modification to the method, involving sample transfer between glassware, was made by the laboratory.

One extraction set of six wheat samples was analyzed for each matrix. Each extraction set consisted of two unfortified control samples, two samples fortified at the tolerance level (considering combined fortification levels), and two samples fortified at 3x the tolerance level. The laboratory stated that analysis of one set of six samples requires ca. 8-10 hours. Raw data and chromatograms, along with standard curves, were included in the submission.

Recoveries of chlorsulfuron and 5-hydroxy chlorsulfuron from samples fortified at the tolerance and at 3x the tolerance for each wheat matrix are presented in Table 3. Apparent residues of chlorsulfuron and 5-hydroxy chlorsulfuron were nondetectable in/on two unfortified samples each of wheat grain (<0.025 ppm each), wheat forage (<0.25 ppm each), and wheat straw (<0.10 ppm each).

Table 3. Independent laboratory validation recoveries of chlorsulfuron and 5-hydroxy chlorsulfuron from fortified wheat matrices analyzed using du Pont Method AMR 2341-92.

	Fortification Level (ppm)	% Recovery [a]

Wheat Matrix	Chlorsulfuron	5-OH Chlorsulfuron	Chlorsulfuron	5-OH Chlorsulfuron
Wheat grain	0.049	0.042	92, 110	74, 86
	0.15	0.13	93	92, 85
Wheat forage	9.9	8.5	70, 78	79, 86
	30	25	73, 93	92, 112
Wheat straw	0.26	0.22	69, 73	105, 114
	0.77	0.66	81, 83	85, 97

[a] Recovery values represent analysis of two samples at each fortification level. Values were not corrected for residues in unfortified samples.

The submitted data are adequate to satisfy the requirements for independent laboratory validation (PR Notice 88-5) of Method AMR 2341-92. The data indicate that Method AMR 2341-92 adequately recovers residues of chlorsulfuron and its metabolite 5-hydroxy chlorsulfuron from wheat grain, forage, and straw, and confirm that the method is adequate for data collection and tolerance enforcement for residues of chlorsulfuron and 5-hydroxy chlorsulfuron in/on plant commodities (wheat, barley, and oats). The qualitative nature of the residue in plants is not adequately understood; a new metabolism study on wheat is required. Provided that chlorsulfuron and 5-hydroxy chlorsulfuron are the only compounds that need to be regulated in/on plant commodities, an Agency method trial will be conducted on this method.

Proposed Enforcement Method for Residues in Animal Tissues and Milk: Du Pont submitted (1993; MRID 42926601) a proposed enforcement method for the determination of residues of chlorsulfuron and two other sulfonylurea herbicides (thifensulfuron methyl and metsulfuron methyl) in animal tissues and milk. The method involves reverse-phase HPLC analysis with UV detection.

Samples of liver, muscle, kidney, fat, and milk are extracted by matrix solid-phase extraction. A small amount of ground/homogenized sample is combined with a relatively large amount of C18 derivatized silica packing material, and is macerated with a mortar and pestle. The mixture is allowed to dry, then is packed into an empty column and washed with hexane; the hexane wash is discarded. The sulfonylurea pesticides are eluted from the column with ethyl acetate:methylene chloride (25:75; v:v) acidified with 0.1% glacial acetic acid. The ethyl acetate:methylene chloride eluate is evaporated to dryness under nitrogen (40-45 C) and reconstituted in ethyl acetate:hexane (50:50; v:v), then passed through a solid-phase silica extraction cartridge. The sulfonylurea pesticides are selectively eluted from the column with acetonitrile:ethyl acetate (25:75; v:v) acidified with 1% glacial acetic acid. The resulting eluate is evaporated to dryness under nitrogen and is reconstituted in acetonitrile:5 mM potassium phosphate buffer, pH 6 (25:75; v:v) for HPLC analysis.

The reconstituted extracts are injected onto an HPLC equipped with a Zorbax® SB-Phenyl

column (250 mm x 4.6 mm i.d.) and a UV detector set at 245 nm. A mobile phase of acetonitrile:5 mM potassium phosphate buffer, pH 2.7 (25:75; v:v) is initially used. The mobile phase ratio is switched to acetonitrile:5 mM potassium phosphate buffer, pH 6.0 (5:95; v:v) to elute the sulfonylurea pesticides, and then switched to acetonitrile:5 mM potassium phosphate buffer, pH 6.0 (75:25; v:v) to wash interfering peaks off the column. The sulfonylurea herbicides are quantitated by peak height comparisons with standards. Detection limits were calculated to be <0.003-0.004 ppm for all of the herbicides in whole milk, ground beef, and beef liver; the registrant stated that the overall detection limit is 0.004 ppm. The limit of quantitation is 0.02 ppm for whole milk, ground beef, and beef liver (based on the lowest fortification level used in this study). The registrant reports that six samples can be prepared during an eight-hour day, and that the 50 minutes of instrument analysis required can be run unattended overnight. Representative chromatograms and standard curves were included in the submission.

The registrant provided method validation data from the analysis of whole milk, ground beef, and beef liver samples fortified simultaneously with chlorsulfuron, thifensulfuron methyl, and metsulfuron methyl at 0.02 and 0.10 ppm (presumably commercially obtained; the registrant did not state where samples of whole milk, ground beef, and beef liver were obtained). Apparent residues of chlorsulfuron were 0.004 ppm in one unfortified sample of ground beef, and were nondetectable in one unfortified sample of ground beef (<0.003 ppm), two unfortified samples of milk (<0.003 ppm), and three unfortified samples of beef liver (<0.003 ppm). Apparent residues of thifensulfuron methyl were 0.007 and 0.014 ppm in two unfortified samples of milk, 0.005 ppm in one unfortified sample of ground beef, and nondetectable (<0.003 ppm) in one unfortified sample of ground beef and three unfortified samples of beef liver. Apparent residues of metsulfuron methyl were 0.006 ppm in one unfortified sample of ground beef, and were nondetectable in one unfortified sample of ground beef (<0.004 ppm), two unfortified samples of milk (<0.003 ppm), and three unfortified samples of beef liver (<0.003 ppm). Recoveries of sulfonylurea herbicides are presented in Table 4.

Table 4. Recoveries of chlorsulfuron, thifensulfuron methyl, and metsulfuron methyl from samples of milk, ground beef, and beef liver fortified simultaneously with each analyte and analyzed using a proposed enforcement method for residues in animal tissues and milk.

Matrix	Fortification Level (ppm)	% Recovery [a]		
		Chlorsulfuron	Thifensulfuron methyl	Metsulfuron methyl
Milk	0.020	82-103	99-121	96-107
	0.100	74-90	83-97	79-101
Ground beef	0.020	95-113	106-121	106-122
	0.100	70-85	99-104	94-102
Beef liver	0.020	88-102	80-98	94-106
	0.100	72-95	86-100	82-100

[a] Four samples were analyzed at each fortification level.

The extraction efficiency of the method was evaluated by the registrant using samples taken from goats, from an ongoing metabolism study, dosed with 25 ppm [^{14}C]chlorsulfuron, labeled in the triazine or phenyl ring, for three consecutive days. The projected completion date for the metabolism study is March 1994. Aliquots of milk, goat liver, and goat kidney extracts following the initial matrix solid-phase extraction with ethyl acetate:methylene chloride (25:75; v:v) acidified with 0.1% glacial acetic acid were analyzed by LSS. Radioactive residues of the extracts were compared to those obtained from combustion/LSS of unextracted matrices. Preliminary results indicate extraction efficiencies of 99% and 107%, respectively, for milk and kidney samples from goat(s) treated with [phenyl-^{14}C]chlorsulfuron, and an extraction efficiency of 33% for liver from goat(s) treated with [triazine-^{14}C]chlorsulfuron. The registrant indicated that further extraction of liver samples with acetonitrile and methanol demonstrated cumulative recoveries of 90%. The submitted data indicate that the extraction procedures used in the proposed enforcement method are effective in liberating radioactive residues from animal matrices. However, these efforts fall short of fulfilling the requirements for radiovalidation since actual levels of chlorsulfuron were not determined. CBRS recommends that this proposed enforcement method be subjected to a radiovalidation study using samples from the ongoing goat metabolism study. The radiovalidation should include analyses for residues of chlorsulfuron and other potential residues of concern.

Independent Laboratory Validation of the Proposed Enforcement Method for the Determination of Chlorsulfuron in Animal Tissues and Milk: Du Pont submitted (1994; MRID 43107801) data pertaining to an independent laboratory validation of the proposed enforcement method for the determination of residues of chlorsulfuron in animal tissues and milk. The validation was conducted by Du Pont Agricultural Products, Global Technology Division, Experimental Station (Wilmington, DE). The registrant stated that the laboratory was independent of the authors of the method, and that no communication occurred between the authors of the method and the laboratory. A minor modification to the method, involving standard preparation, was made by the laboratory.

Two samples of milk, ground beef and liver were fortified with chlorsulfuron at ca. 0.10 ppm and 0.30 ppm each for milk and at ca. 0.30 ppm and 0.90 ppm each for ground beef and

liver (1x and 3x the respective tolerances). The registrant did not state the source of the samples of whole milk, ground beef, and beef liver. Because a recovery of 51% was obtained from liver fortified at 0.90 ppm in the initial analysis, a second set of two liver samples fortified at 0.90 ppm were analyzed. The laboratory stated that analysis of eight samples (four controls and four fortified samples) requires ca. 8 hours, and that HPLC analyses can then be run unattended overnight. Raw data and chromatograms, along with standard curves were included in the submission.

Recoveries of chlorsulfuron from samples fortified at the tolerance and at 3x the tolerance for milk, ground beef, and liver are presented in Table 5. Apparent recoveries of chlorsulfuron were nondetectable (detection limit unspecified) in four unfortified samples each of milk and ground beef, and six unfortified samples of liver.

Table 5. Independent laboratory validation recoveries of chlorsulfuron from fortified samples of milk, ground beef, and beef liver analyzed using the proposed enforcement method for residues in animal tissues and milk.

Matrix	Fortification Level (ppm)	% Recovery [a]
Milk	0.010	69, 96
	0.30	91, 95
Ground beef	0.30	101, 106
	0.90	99, 110
Liver	0.30	85, 98
	0.90	51, 71-103

[a] Recovery values represent analysis of two samples at each fortification level for each matrix except that a second set of two liver samples fortified at 0.90 ppm was analyzed due to a low recovery in the first run. Values were not corrected for residues in unfortified samples.

The submitted data are adequate to satisfy the requirements for independent laboratory validation (PR Notice 88-5) of the proposed enforcement method for the determination of chlorsulfuron residues in animal tissues and milk. The data indicate that the method adequately recovers residues of chlorsulfuron from milk, ground beef, and liver, and confirm that the method is adequate for data collection and tolerance enforcement for residues in animal commodities. The qualitative nature of the residue in animals is not adequately understood; new metabolism studies with ruminants and poultry are required. Provided that chlorsulfuron is the only compound that needs to be regulated in animal commodities and that no tolerances are required for poultry commodities, an Agency method trial will be conducted on this method.

Magnitude of the Residue in Wheat Grain and Processed Commodities

Tolerances of 0.1, 20.0, and 0.5 ppm have been established for the combined residues of chlorsulfuron and its 5-hydroxy metabolite in/on wheat grain, forage, and straw, respectively [40 CFR §180.405(a)].

Directions for Use: The 75% DF and 75% FlC formulations are registered for: (i) a single broadcast preemergence application to winter wheat at 0.38 oz ai/A; and (ii) postemergence application to winter, spring, and durum wheat made after the two-leaf stage but before boot stage at 0.13-0.25 oz ai/A alone or in a tank mix with other herbicides; in the Pacific Northwest the 75% DF and 75% FlC may be applied at the rates specified above after the two-leaf stage through the second-joint stage. Application may be made using ground or aerial equipment. No pregrazing interval or PHI has been established. The following regional maximum seasonal rates are in effect: (i) 0.25 oz ai/A (18-month period) for CA, northern ID, OR, and WA; (ii) 0.13 oz ai/A (48-month period) for southern ID, MN, MT, ND, SD, UT, and northern WY; (iii) 0.25 oz ai/A (36-month period) for CO, western KS, western NE, eastern NM, OK Panhandle, TX Panhandle, and southeastern WY; (iv) 0.25 oz ai/A for central KS, central NE, and central OK; and (v) 0.38 oz ai/A for AR, LA, central and north central TX, and southern OK.

The 62.5% DF (MAI) formulation is registered for: (i) a single broadcast preemergence application to winter wheat at 0.13-0.25 oz ai/A; (ii) postemergence application to spring or winter wheat made after the two-leaf stage but before boot stage at 0.13-0.25 oz ai/A alone or in a tank mix with other herbicides; for spring wheat, the maximum application rate is 0.19 oz ai/A with application made after the two-leaf stage through the second joint stage. For specific weed problems, the 62.5% DF may be applied pre- or postemergence (when wheat has reached the 4-5-leaf stage), alone or in a tank mix with other herbicides at up to 0.31 oz ai/A. Application may be made using ground or aerial equipment. No pregrazing interval or PHI has been established. The following maximum seasonal rates are in effect: (i) 0.31 oz. ai/A (36-month period) for far western KS, the panhandles of OK and TX, and part of NE; (ii) 0.31 oz ai/A (24-month period) for western KS and western NE; (iii) 0.4 oz ai/A for central KS, NE, OK, and TX; (iv) 0.19 oz ai/A (24-month period) for eastern CO, southwestern SD, southeastern WY, and NE panhandle.

[These use directions were obtained from labels for the following chlorsulfuron end-use products registered to du Pont: 75% DF (EPA Reg. No. 352-404, dated 10/89); 75% FlC (EPA Reg. No. 352-522, dated 7/93); and 62.5% DF (EPA Reg. No. 352-445, dated 12/93)].

Detailed Considerations: Du Pont submitted data (1993; MRID 42900601) from a processing study depicting residues of chlorsulfuron and its 5-hydroxy metabolite in/on wheat grain and processed commodities. Wheat was harvested 66 days following a single broadcast application of the 75% DF formulation at 1.55 oz ai/A (4x the maximum single application rate of 0.38 oz ai/A). We note that the registrant incorrectly reported this application rate to be 5x the maximum rate. Applications were made using a CO_2 plot sprayer when the flag leaf was just visible. Wheat grain samples were shipped overnight at ambient temperature to the Engineering Biosciences Research Center (EBRC), where they were frozen on receipt (-18 C) and stored frozen for 13-18 days prior to processing.

Wheat samples were processed into bran, flour (low-grade and patent), middlings, shorts, small and large screenings, and grain dust (light impurities) by a procedure intended to simulate industrial practice as closely as possible. Samples were dried at 60-70 C for ca. 2-3 hours, then cleaned by aspiration and screening. The moisture content of the cleaned

grain was adjusted to 16% by soaking in water for 4-6 hours. The conditioned wheat was then milled on four corrugated roller mills. After the bran was separated, the sample was reduced to flour on a smooth roller mill. Processed samples were returned to the freezer and were shipped to the du Pont Experimental Station (Wilmington DE) where they were stored until they could shipped to Enviro-Test Laboratories (ETL, Edmonton, AB, Canada) for analysis. Processed samples were stored at <-20 C for up to 4 months prior to analysis. The total storage interval from harvest to analysis was 12.5 months.

Residues of chlorsulfuron and its 5-hydroxy metabolite were nondetectable (<0.05 ppm each) in/on one sample each of bran, low-grade and patent flour, middlings, shorts, and small screenings, and two samples each of grain dust (light impurities) and large screenings processed from wheat grain bearing nondetectable residues of chlorsulfuron and 5-hydroxy chlorsulfuron (<0.05 ppm each) following treatment at 4x the maximum single application rate. Apparent residues of chlorsulfuron and its 5-hydroxy metabolites were nondetectable (<0.05 ppm each) in/on one untreated sample of wheat grain and of each of the processed commodities. CBRS had previously waived the requirement for data on wheat grain dust (CBRS No. 9095, D172239, P. Deschamp, 12/27/91).

Residue Analytical Methods: Samples of wheat grain, bran, flour (low-grade and patent), middlings, shorts, small and large screenings, and grain dust (light impurities) were analyzed for residues of chlorsulfuron and its 5-hydroxy metabolite by Enviro-Test Laboratories (Edmonton, AB, Canada) using LC/MS with selected ion monitoring (SIM) at m/z 141 and at m/z 184 for analyte confirmation. Briefly, samples were macerated with dry ice, then homogenized with methanol:30 mM potassium phosphate buffer, pH 8-9 (20:80; v:v) and centrifuged. The supernatant was collected, then adjusted to pH 3-4 with 10% aqueous phosphoric acid, and partitioned twice with dichloromethane (DCM). The DCM phases were combined, then mixed with 30% aqueous acetonitrile, and DCM was evaporated off at 38 C. The remaining aqueous phase was brought to volume with 30% aqueous acetonitrile and injected onto an LC/MS system equipped with a C18 column. The limit of quantitation was 0.05 ppm for each analyte in each commodity.

The registrant provided concurrent method recoveries from untreated control samples of each of the commodities listed above fortified with chlorsulfuron and 5-hydroxy chlorsulfuron. Method recoveries are presented in Table 6. Sample calculations, representative chromatograms, and raw data were provided. The method recovery data indicate that the submitted analytical method is adequate for residue data collection in/on wheat grain and its processed commodities.

Table 6. Concurrent method recoveries of chlorsulfuron and its 5-hydroxy metabolite from untreated wheat commodities fortified with each of the analytes.

	Fortification Level (ppm)		% Recovery [a]	
Wheat Commodity	Chlorsulfuron	5-OH Chlorsulfuron	Chlorsulfuron	5-OH Chlorsulfuron
Wheat grain	0.049	0.042	82	90
Bran	0.049	0.042	96	74

Wheat Commodity	Fortification Level (ppm)		% Recovery [a]	
	Chlorsulfuron	5-OH Chlorsulfuron	Chlorsulfuron	5-OH Chlorsulfuron
Wheat grain	0.049	0.042	82	90
Flour - Low-grade	0.049	0.042	76	74
Patent	0.099	0.085	76	79
Middlings	0.099	0.085	71	81
Shorts	0.099	0.085	101	106
Small screenings	0.20	0.17	95	82
Large Screenings	0.049	0.042	102	83
Grain dust (light impurities)	0.049, 0.099	0.042, 0.085	86, 111	102, 80

[a] Recovery values represent analysis of one sample at each fortification level. Values were not corrected for residues in unfortified samples.

Storage Stability: Samples of wheat commodities from the submitted processing study were stored at <-18 C for up to 12.5 months prior to analysis. To support these storage conditions, the registrant cited previously reviewed storage stability data for chlorsulfuron in wheat grain (MRID 41976407; CBRS No. 8519, D167861, P. Deschamp, 1/8/92) which indicate that residues of the parent compound are stable in wheat grain and straw stored for up to 5 years at -24 C. The registrant also cited a storage stability study (MRID 42292501; CBRS No. 9871, D178278, J. Smith, 8/6/92; CBRS No. 10855, D184482, J. Abbotts, 3/30/93) which indicates that residues of 5-hydroxy chlorsulfuron in green forage are marginally stable following storage for up to 29 months at ca. -20 C. The registrant stated that results of a concurrent study depicting the freezer storage stability of chlorsulfuron and 5-hydroxy chlorsulfuron in/on the processed fractions of wheat will be submitted on completion. CBRS concludes that, pending receipt of the concurrent storage stability data, the wheat processing study is supported by adequate storage stability data.

The submitted wheat processing study is adequate and will satisfy reregistration data requirements upon submission of the outstanding concurrent storage stability study. The maximum theoretical concentration factor for wheat commodities is 9x (Maximum Theoretical Concentration Factor memorandum, 1/14/93, D. Edwards and E. Zager). Although a 5x exaggeration is the maximum the Agency considers realistic for foliar applications, given that chlorsulfuron is an herbicide, and that residues did not accumulate in the RAC or in processed commodities, CBRS concludes that the 4x application rate is sufficient to confirm that residues of chlorsulfuron and its 5-hydroxy metabolite are not likely to accumulate or concentrate in wheat processed commodities. No food or feed additive tolerances are required.

AGENCY MEMORANDA CITED IN THIS DOCUMENT

CBRS No.: 8519
DB Barcode: D167861
Subject: Reregistration of Chlorsulfuron. Storage Stability Data.
From: P. Deschamp, CB II, HED
To: T. Stowe, RD, SRRD
Date: 1/8/92
MRID(s): 41976407

CBRS No.: 9095
DB Barcode: D172239
Subject: Reregistration of Chlorsulfuron. Request for a Waiver from the Requirement of Data on Wheat Grain Dust
From: P. Deschamp, CB II, HED
To: T. Stowe, RD, SRRD
Date: 12/27/91
MRID(s): none

CBRS No.: 9871
DB Barcode: D178278
Subject: Chlorsulfuron. Storage Stability. Metabolite A1 in Green Wheat Forage.
From: J. Smith, CB II, HED
To: L. Rossi, RD, SRRD
Date: 8/6/92
MRID(s): 42292501

CBRS No.: 10855
DB Barcode: D184482
Subject: Reregistration of Chlorsulfuron (118601). Submission of Additional Storage Stability Data.
From: J. Abbotts, CB II, HED
To: T. Stowe, RB, SRRD
Date: 3/30/93
MRID(s): none

MASTER RECORD IDENTIFICATION NUMBERS

The citations for the MRID documents referred to in this review are presented below.

42900601 Klemens, A. and Tomic, D. (1993) Residues of Chlorsulfuron in Wheat Grain and Its Processed Fractions Following Application of Glean® Herbicide. Project ID: AMR 2221-92, EBRC Project ID: GLE-2221-92, Enviro-Test Laboratories Project ID: 92-P1510. Unpublished study prepared by E.I. du Pont de Nemours and Company. 109 p.

42900602 Klemens, F., Orangio, K. and Gesterak, V. (1993) Extraction Efficiency of Analytical Methods for the Determination of [Phenyl(U)-^{14}C]-Chlorsulfuron Derived Residues in Wheat and Oats. Project ID: AMR 2309-92. Unpublished study prepared by

E.I. du Pont de Nemours and Company. 119 p.

42900603 Klemens, F. and Orangio, K. (1993) Analytical Method (Column Switching/Heart cut) for the Determination of Residues of Chlorsulfuron (DPX-W4189) and 5-Hydroxychlorsulfuron (IN-N5754) in Wheat (Forage, Grain, and Straw). Project ID: AMR 2341-92. Unpublished study prepared by E.I. du Pont de Nemours and Company. 105 p.

42926601 de Bernard, P. and Powley, C. (1993) Enforcement Method for the Determination of Thifensulfuron Methyl, Metsulfuron Methyl, and Chlorsulfuron in Milk and Animal Tissues. Project ID: AMR 2715-93. Unpublished study prepared by E.I. du Pont de Nemours and Company. 41 p.

43051001 Tauber, R. and Bruns, G. (1993) Independent Laboratory Validation of DP-W4189 and IN-N5754 Residues in wheat (Grain, Forage, and Straw) Using Column Switching (Heart Cut) Liquid Chromatography (HPLC/UV). Revision No. 1. Project ID: AMR 2616-93. Laboratory Project ID: DUP64.REP. Unpublished study prepared by E.I. du Pont de Nemours and Company. 270 p.

43107801 Hill, S. and Nathan, E. (1994) Independent Laboratory Validation of the Analytical Enforcement Method for the Determination of Chlorsulfuron in Milk and Animal Tissues by Liquid Chromatography. Project ID: AMR 2805-93. Unpublished study prepared by E.I. du Pont de Nemours and Company. 42 p.

References not used:

[The following submission was replaced by MRID 43051001.]

42948901 Tauber, R. and Bruns, G. (1993) Independent Laboratory Validation of DP-W4189 and IN-N5754 Residues in wheat (Grain, Forage, and Straw) Using Column Switching (Heart Cut) Liquid Chromatography (HPLC/UV). Project ID: AMR 2616-93. Laboratory Project ID: DUP64.REP. Unpublished study prepared by E.I. du Pont de Nemours and Company. 270 p.