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

SEP 16 1994

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Sethoxydim. Case No. 2600. Metabolism in Sugar Beets. MRID No. 431445-01. CBRS No. 13401. DP Barcode:D200367.

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BASF Corporation has submitted a metabolism study of sethoxydim on sugar beet in satisfying GLN 171-4(a) for reregistration. Structures of sethoxydim {(±)-2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one} and its metabolites are shown in Attachment to this reivew.

The study title is "Metabolism and Distribution of ^{14}C-Sethoxydim in Sugar Beets," E. J. Panek, February 23, 1994, and a BASF Report No. M9405.

CONCLUSIONS AND RECOMMENDATION

1. Two-dimensional TLC's were submitted to support structural assignment of the residues extracted into the early dichloromethane fractions (DCM-I & II) for sugar beet top and root. Identification was through comparision of Rf values of various mixtures of known standards. The extracted samples were carried through 2-dimensional TLC and mixtures of certain standards and samples were analyzed in two different solvent systems. HPLC's for standards MSO2, MSO, M2SO2, M1SO2, and M2SO were submitted. However, mass specs of the MSO2 (a major component) fractions isolated from sugar beet tops and roots were not superimposable with those of the standards; there were either extraneous peaks (see p 206) or vastly

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different ion ratios (p 207). The registrant needs to explain these observations.

2. The residue analytical method which includes an oxidation step and an esterification step accounts for ca 50% of the TRR. The method is adequate for enforcement purpose.

The sugar beet metabolism study is upgradeable pending an explanation from the registrant as stated in Conclusion 1.

DETAILED CONSIDERATIONS

The in-life portion of the study was conducted at Battelle Columbus Operations, Columbus, Ohio. In a greenhouse sugar beets were grown in sandy loam soil in 20 inch diameter by 17 inch deep plastic containers at temperatures that ranged from 68-77 F. Sethoxydim was labeled with carbon-14 at the C-4 of the cylcohexene ring (94% radiopurity and a specific activity of 60.97 μCi/mg). A spray solution was prepared in crop oil concentrate and water in proportions similar to a typical spray tank mixture. One hundred and 13 days after planting, 6 sugar beet plants were sprayed until the foliage was thoroughly covered with formulated sethoxydim at a rate equivalent to 1 lb ai/A. Six plants also served as control.

Samples were collected at 0, 60, and 100 days after treatment. The plants were separated into tops and roots. The samples were stored frozen at -20 C at Battelle and shipped to Nippon Soda Ltd, Japan for residue characterization. Chemical analysis lasted 18 months (6/92 to 12/93).

Crop samples were extracted with methanol and aqueous methanol that yielded ca 90-93% total radioactive residue (TRR). The fibrous residue left was then extracted with methanol:chloroform and acetone that yielded 0.4-0.5% TRR. The methanol and aqueous methanol fractions were combined, reduced in volume, diluted with water, and extracted at pH 2 with dichloromethane. The organosoluble residues were separated into neutral and acidic compounds by partitioning with aqueous sodium hydroxide followed by back extraction into hexane and then dichloromethane. The aqueous fractions were combined, passed through Amberlite XAD-4 columns, treated with pectinase, and the released exocons were extracted into dichloromethane. Residues remaining in the aqueous solution were methylated and then extracted into dichloromethane. The final aqueous phase was subjected to the residue analytical method to determine any sethoxydim related compounds left. The non-extractable residues (9% TRR for tops and 6% for roots) were reacted with polysaccharide hydrolyzing enzymes (cellulase, pectolyase, driselase).

One- and/or two-dimensional TLC analyses were used for the separation and detection of sethoxydim metabolites (through Rf's of

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known standards). Regions of silica gel corresponding to individual metabolites were scraped off and the radioactivity was determined by a liquid scintillation counter. Aliquots of liquid or TLC silica gel samples were mixed with a scintillation cocktail and assayed directly and fibrous residues were oxidized and the trapped carbon dioxide was mixed with a scintillation cocktail and then assayed. A list of known standards is attached at the end of this review. DME, DME-OH, MSO, and MSO2 were detected by UV 365 nm after spraying with 0.01% purimulin in 80% acetone, and the others by 254 nm directly without spraying. The identity of many metabolites was confirmed by HPLC retention time and/or LC-MS.

Table 1. Distribution and characterization of TRR in sugar beet top treated with sethoxydim at 1x rate (60-day PHI).

Fractions	% TRR	ppm	Characterization/identification
			22.22 ppm
CH2Cl2 (DCM-I) (DCM-II)	44.7 (39.7) (4.8)	9.92	parent, 0.3% (0.06 ppm); MSO, 4.5% (0.98 ppm); MSO2, 21.4% (4.71 ppm); 5-OH-MSO, 6.4% (1.42 ppm); 5-OH-MSO2, 6.1% (1.34 ppm); M1S, 0.5% (0.11 ppm); M1SO, 0.3% (0.07 ppm); M1SO2, 0.5% (0.11 ppm); M2S, 0.1% (0.03 ppm); M2SO, 0.4% (0.08 ppm); M2SO2, 0.6% (0.13 ppm); 6-OH-M2SO2, 0.4% (0.09 ppm); 6 unknowns, others and origin (all <1.5% each)
XAD-4	5.5	1.21	MSO, 1.0% (0.22 ppm); MSO2, 1.9% (0.42 ppm); 5-OH-MSO2, 0.3% (0.06 ppm); M2SO2, M1S, M1SO, M2S (all <0.01 ppm); M2SO, 0.3% (0.06 ppm); M2SO2, 0.3% (0.07 ppm); 6-OH-M2SO2, 0.1% (0.02 ppm); others (all <0.9% each)
pectinase	9.3	2.05	MSO, 0.3% (0.06 ppm); MSO2, 0.4% (0.09 ppm); 5-OH-MSO2, 0.1% (0.02 ppm); M1 & M2 metabolites (all <0.1% each); others (all <2.2% each)
methy-lation	18.8	4.17	DME, 4.1% (0.9 ppm); DME-OH, 0.6% (0.13 ppm); MGSO, 3.4% (0.75 ppm); others (all <5.5% each)
oxidation & methy-lation	6.3	1.39	DME, 1.8% (0.41 ppm); MGSO, 0.6% (0.13 ppm); others (all <1.5% each)
Aqueous, final	5.5		
Residue	9.0		

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Table 2. Distribution and characterization of TRR in sugar beet root treated with sethoxydim at 1x rate (60-day PHI).

Fractions	% TRR	ppm	Characterization/identification
1.42 ppm			
CH2Cl2 (DCM-I) (DCM-II)	67.1 (57.5) (9.6)	0.95	parent, 0.5% (<0.01 ppm); MSO, 4.0% (0.06 ppm); MSO2, 28.7% (0.4 ppm); 5-OH-MSO, 9.3% (0.13 ppm); 5-OH-MSO2, 12.4% (0.17 ppm); M1S, 0.4% (<0.01 ppm); M1SO, 0.2% (<0.01 ppm); M1SO2, 0.7% (0.01 ppm); M2S, 0.1% (<0.01 ppm); M2SO, 2.5% (0.03 ppm); M2SO2, 2.5% (0.03 ppm); 6-OH-M2SO2, 1.1% (0.02 ppm); 6 unknowns, others and origin (all <1.2% each)
pectinase	5.9	0.08	MSO2, 0.7% (<0.01 ppm); M2SO, 0.7% (<0.01 ppm); M2SO2, 1.1% (0.01 ppm); others (all <0.7% each)
3N HCl	1.0	0.01	MSO2, M2SO2, 5-OH-MSO and others (all <0.2% each)
oxidation & methy-lation	11.9	0.17	DME, 5.9% (0.08 ppm); DME-OH, 0.8% (0.01 ppm); MGSO, 0.5% (<0.01 ppm); others (all <1.7% each)
Aqueous	4.8		
Residue	6.2		

As can be seen from the above two tables, initial dichloromethane extractions (DCM I: acidic M type, & DCM II: neutral M1, M2 type) removed about half or more of the TRR. Other fractions that released any significant amount of TRR (>10% TRR) were from the methylation and oxidation/methylation step.

The metabolites identified and characterized included sethoxydim, MSO2, 5-OH-SO2, 5-OH-SO, MSO, M2SO2, M2SO, 6-OH-M2SO2, M1SO2, M1S, M1SO, M2S, and 6-OH-M2SO.

The registrant submitted photographs of sample TLC's, HPLC's, and LC-MS to support metabolite characterization. Two-dimensional TLC's were submitted to support structural assignment of the residues extracted into the early dichloromethane fractions (DCM-I & II) for sugar beet top and root. Identification was through comparision of Rf values of various mixtures of known standards. The extracted samples were carried through 2-dimensional TLC and mixtures of certain standards and samples were analyzed in two different solvent systems. HPLC's for standards MSO2, MSO, M2SO2, M1SO2, and M2SO were submitted. However, mass specs of the MSO2 (a major component) fractions isolated from sugar beet tops and roots were not superimposable with those of the standards; there were either extraneous peaks (see p 206) or vastly different ion ratios (p 207). The registrant needs to explain these observations.

Table 3. Percent TRR Distribution of Sethoxydim Metabolites in Sugar Beet Top and Root.

	Sugar beet top (22.2 ppm)			Sugar beet root (1.42 ppm)		
	CH2Cl2	amberlite	add'l frns	CH2Cl2	pectinase	add'l frns
Parent	0.3			0.5		
MSO	4.5	1.0	0.3	4.0	0.2	
MSO2	21.4	1.9	0.4	28.7	0.7	0.1
M1S	0.5	<0.1	<0.1	0.4		
M1SO	0.3	<0.1	<0.1	0.2		
M1SO2	0.5			0.7	0.2	
M2S	0.1	<0.1	<0.1	0.1		
M2SO	0.4	0.3	<0.1	2.5	0.7	
M2SO2	0.6	0.3	0.2	2.5	1.1	0.1
5-OH-MSO	6.4			9.3		0.1
5-OH-MSO2	6.1	0.3	0.1	12.4		
6-OH-M2SO*	0.2			0.3		
6-OH-M2SO2	0.4	0.1	<0.1	1.1	0.3	
MGSO			3.4			
MGSO2			4.1			
OH-MGSO2			0.6			
DME			1.8			5.9
DME-OH						0.8
DM ester MGSO			0.6			0.5
Unknowns	1.2	0.1	9.5	3.2	2.1	4.3

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	Sugar beet top (22.2 ppm)			Sugar beet root (1.42 ppm)		
	CH2Cl2	amberlite	add'l frns	CH2Cl2	pectinase	add'l frns
Others	0.2	1.2	5.8	0.3		0.5
Origin	1.5	0.2	7.3	0.9	0.6	0.6
Residue	9.0			6.2		

* assignment tentative

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Appendix 3 in the submission also reported stability data under frozen conditions (-16 C) for sethoxydim residues. Aliquots (10-50 g sugar beet top or 25-50 g root) of the 60-day PHI sample from the metabolism study were removed at various intervals, extracted, and analyzed by TLC. TLC profiles showed that levels of sethoxydim, MSO2, 5-OH-SO2, and M1 and M2 type of metabolites in the top and root remained relatively constant for 20 months. Additional experiments were conducted in which the sugar beet top and root samples after extraction were oxidized and methylated. This process converted sethoxydim residues to DME and DME-OH. Amounts of DME and DME-OH, expressed in %TRR, were quantitated by TLC.

Table 4. Stability of Sethoxydim and Metabolites in Sugar Beet Top and Root

Days stored frozen	Sugar beet top	Sugar beet root	Days stored frozen
	DME + DME-OH	DME + DME-OH	
99	43.7% TRR	59.2% TRR	101
381	44.7%	52.5%	393
426	38.7%		

The 426-day sugar beet top sample and the 393-day sugar beet root sample were also quantitated by GC residue method (Method 30B) to check the accountability of sethoxydim residues in plant commodities. Method 30B converts sethoxydim residues to DME and DME-OH through oxidation and methylation. It accounted for 46% of TRR in sugar beet top and root. Concurrent recoveries ranged 68-102% for sethoxydim and 5-OH-MSO2 in sugar beet top and root. The stability data support the validity of the metabolism results obtained from samples that were stored frozen for 18 months.

Attachment - Structures of sethoxydim and metabolites (one page)

cc (with Attachment):Circ, SF, RF, List B File, Cheng
RDI:ARRathman:9/15/94:MSMetzger:9/15/94:EZager:9/15/94
7509C:CBRS:LCheng:CM#2:RM810D:9/15/94:03:SETHOXYDIM\BEETMETA

ATTACHMENT

CBRS 13401

INFORMATION ON STANDARDS

Structure	Abbreviation(s)	Lot Number	Purity (%)
	BAS 562 H, BAS 9052 H, NP 55, or MS STM	KN-9952 EC-5-13 (11.7 mCi/mmol)	97.0 >99
	MSO (X = H)	31-2145(1)HH	97.2
	5-OH MSO (MU-2) (X = H)	P-15	96.4
	MSO_2 (X = H)	12-0724	>99
	5-OH MSO_2 (MU-1) (X = OH)	5814023	96.6
	M1S	6312919	>99
	M1SO	551373	>99
	$M1SO_2$	6314919	98.4
	M2S	12-0725	98.5
	M2SO	31-2145(3)HH	98.1
	$M2SO_2$ (X = H)	2283401-1	>99
	6-OH $M2SO_2$ (MU) (X = OH)	31-5160-MH	>99
	MGSO (X = SO)	31-84173-HI	93.1
	$MGSO_2$ (X = SO_2)	31-8182-B-SY	96.2
	DME (X = H)	31-7265-MH	97.6
	DME-OH (X = OH)	31-8257-TS	99

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