



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

JUN 26 1985

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: PP#3F2904 (RCB No.941); Poast (sethoxydim) on soybeans
and alfalfa. Amendment of 3/12/85.
Accession Numbers: 073398, 073399

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The petitioner, BASF Wyandotte Corporation, has submitted this amendment in response to RCB's original review (memo of 1/12/84, J. Onley) in which several deficiencies were noted. The deficiencies are stated below, followed by the petitioner's response and RCB's comments.

Deficiency 1a

Since a 90-day PHI is needed in order for field residues to decline to levels less than 10 ppm on soybeans, the petitioner should reconsider his request for the establishment of a 20 ppm BAS 9052H tolerance on soybean forage, i.e., the grazing and feeding restrictions on soybean forage and ensilage should be returned to the present proposed label. For example, soybeans will grow to maturity in 126-158 days; it requires about 53 days for soybeans to grow from planting to the third node stage. Therefore, when considering the necessary growth time and the necessary 90 day PHI, the proposed tolerance on soybean forage becomes impractical. However, the establishment of an appropriate tolerance on soybean hay would be practical in conjunction with the required 90-day PHI.

Petitioner's Response to Deficiency 1a

The petitioner has revised Section B to include grazing and foraging restrictions. Cutting hay is not restricted, and a tolerance of 10 ppm is proposed for hay.

RCB's Comment

Deficiency 1a is resolved. The proposed tolerance for soybean hay is discussed under No. 6b, below.

Deficiency 1b

Under "Restrictions and Limitations" of the proposed alfalfa label we suggest that the following be used:

"Do not apply Poast within 7 days of feeding, grazing or harvesting when used alone. Do not apply Poast and 2,4-DB as a tank mix unless the 60-day feeding, grazing, and harvesting restrictions on the 2,4-DB label can be observed."

Petitioner's Response to 1b.

The petitioner has submitted the requested statement in a revised Section B.

RCB's Comment

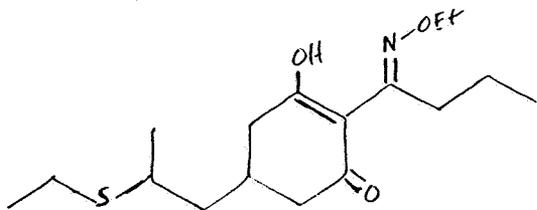
Deficiency 1b is resolved.

Deficiency 2a

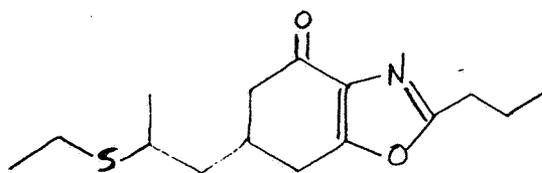
The nature of the residue in plants is not adequately understood. The Laboratory Report No. PM-39 (Metabolism and Distribution of [4-¹⁴C]-BAS 9052 H in Soybean Plants), submitted in the present petition, showed that about 60% of the applied activity was not characterized in soybean plants harvested 7 days after application. The quantity of uncharacterized activity increased with time. The petitioner should further attempt to release more of the bound or polar residues and identify the aglycones.

Petitioner's Response to Deficiency 2a

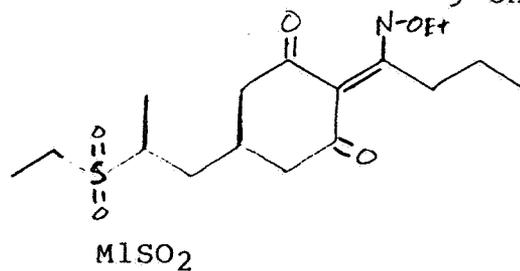
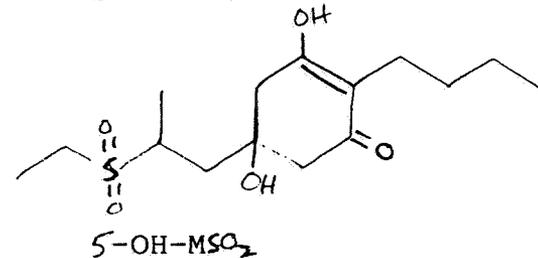
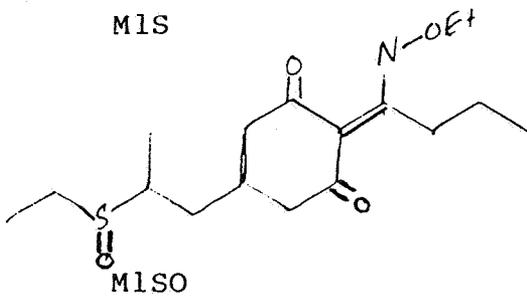
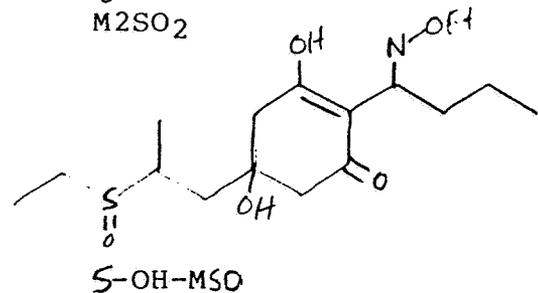
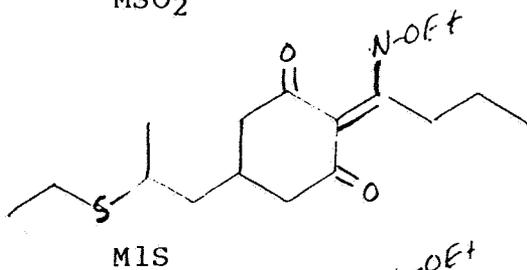
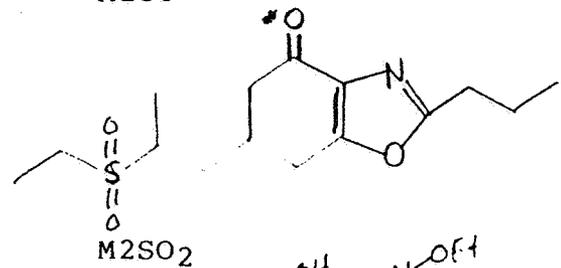
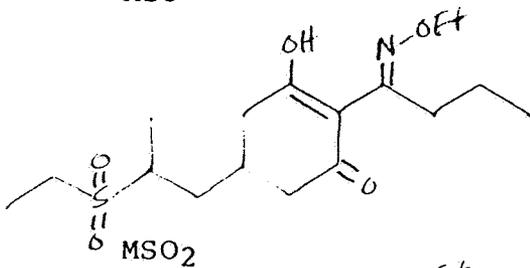
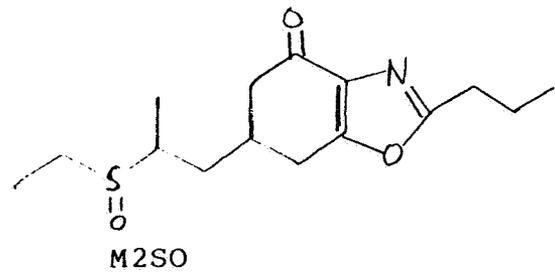
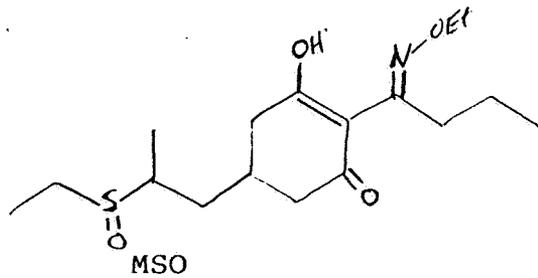
Two plant metabolism studies using ¹⁴C-ring labeled BAS 9052 H (sethoxydim) are submitted, one on alfalfa and one on soybeans. The following standards, representing several expected metabolites, have been synthesized:



MS (parent)



M2S



The two studies are summarized below.

Alfalfa

Alfalfa was treated with ¹⁴C ring-labeled BAS 9052 H at a rate equivalent to 0.5 lb a.i./A. The plants were harvested at a PHI of 14 days. The total activity, in BAS 9052 H equivalents, amounted to 16.8 ppm.

A 20 g sample was macerated, then extracted with 50% aqueous methanol; 94% of the activity went into solution. Very little partitioned into hexane (0.02%). The pH was adjusted to 2, and the solution was partitioned against methylene chloride. The

methylene chloride phase was extracted with 0.1N NaOH, which took up 33% of the total activity; 9% remained in the methylene chloride, and this was examined by TLC. Identified metabolites include M2SO (3% of the total activity), M2SO₂ (0.9), M2S (0.06), M1SO (1.9), M1SO₂ (1.3), M1S (0.16), and 6-OH-M2SO₂ (0.16). The identity of most metabolites was confirmed by co-chromatography and/or HPLC.

The basic layer was treated with HCl until the pH was 2; extraction of this with methylene chloride removed 26% of the total activity, which was examined by TLC. Identified metabolites include MSO₂ (1.9% of the total activity), M2SO₂ (0.9), MSO (11), 5-OH-MSO₂ (0.7), M2SO (2.0), 5-OH-MSO (1.8). The activity remaining in the aqueous phase was added back to the original aqueous methanol phase.

The aqueous methanol phase now contained 50% of the total activity. The methanol was evaporated, and the remaining aqueous solution was passed through a XAD-4 column. The column was rinsed with 100 ml water (this fraction contained 6% of the total activity) and was then eluted with 560 ml methanol. The methanol was evaporated and the residue dissolved in ethanol. The ethanol solution was passed through a membrane filter, the residue on the filter was dissolved in water, the water was removed, and the residue was again dissolved in ethanol. This solution was again passed through a membrane filter.

Three portions of the alcohol solution were separately treated with pectinase, papain, or beta-glucosidase. By doing this it was determined that pectinase was most effective in releasing conjugated activity.

A portion of the alcohol solution was then subjected to TLC which resulted in eleven separate bands. Bands 1-5 were subjected to TLC co-chromatography with standards. MSO and M2SO were identified in band 1, but activity in other bands was too low for identification.

Bands 6-11 were separately treated with pectinase, then acidified before partitioning against methylene chloride. Most (68%; i.e., 34% of the total activity) of the activity in the aqueous phase went into methylene chloride. The combined methylene chloride phases were subjected to TLC, and the ten bands that resulted were subjected to two dimensional TLC for identification of metabolites. The identities of several metabolites were confirmed by co-chromatography, HPLC, or mass spectroscopy after purification by HPLC. The most significant metabolite released by pectinase was MSO (8.3% of the total activity). Several other metabolites in this phase were identified, but none amounted to more than 3% of the total activity.

Of the total activity in alfalfa, 54% was identified by HPLC, TLC, or mass spectroscopy. The most significant metabolite was the sulfoxide (MSO) which comprised 26% of the total activity.

The next most significant metabolite was M2SO which comprised about 9 percent of the total activity. Nine additional identified metabolites comprised less than 5% of the total activity each. The largest single fraction of unidentified activity was the water soluble pass fraction from the XAD-4 column which accounted for 6% of the total activity.

In a separate experiment entitled "GLC Accountability of Radioactive Residues in Alfalfa Hay and Soybean Hay Resulting from Treatment with BAS 9052 H-[-¹⁴C]," samples of alfalfa hay carrying weathered residues of ¹⁴C BAS 9052 H were subjected to analytical method 30H (discussed below under deficiency 3a). The hay carried radioactive residues equivalent to 59.6 ppm BAS 9052 H; the method determined 30.8 ppm or 52% of the initial residue. The average recovery of fortified hay samples was 81%; thus, correcting for procedural losses, the GLC method accounts for 64% of the total radioactivity. This study does not establish the structure of any metabolites, but it does establish that most of the activity can be converted to one of the compounds determined by the analytical method.

Soybeans

A soybean metabolism study has also been submitted. Soybeans were treated with ¹⁴C labeled BAS 9052 H at a rate equivalent to 0.5 lb. a.i./A and harvested 14 days later. The total radioactive residue in soybean forage was equivalent to 4.5 ppm BAS 9052 H equivalents.

The soybean plants were macerated in methanol, which took up 90% of the total activity. This solution was partitioned against hexane, which took up very little of the activity (0.04% of the total). Partitioning against methylene chloride took up about 25% of the total activity; the methylene chloride phase was partitioned against 0.1 N NaOH, which took up most of the activity, leaving 9% in the methylene chloride. This latter solution was subjected to two dimensional TLC, and most of this fraction was identified as M2SO (4.3% of the total activity), M2SO₂ (1.5), M1SO₂ (1.4), and M1SO (1.2).

The basic layer was made acidic, then partitioned against methylene chloride; this released 16% of the total activity into the organic phase. (The aqueous portion was added to the original MeOH/aqueous phase from above.) Two dimensional TLC was used for identification of metabolites in the methylene chloride with the following results: MSO and an MSO isomer (10% of the total activity), and MSO₂ and an MSO₂ isomer (4% of the total activity).

The MeOH/aqueous phase, which now contained 65% of the total activity, was absorbed onto an XAD-4 column. Eluting with 100 ml of water removed 6.4% of the total activity, and eluting with methanol removed 59.4% of the total activity. The methanol was evaporated and the residue dissolved in ethanol, which took up 54% of the total activity. The ethanol soluble fraction was

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cleaned up by TLC which resulted in ten different bands; these were separately extracted with chloroform/methanol. Metabolites in band 1 (2.8% of the total activity) were identified as MSO, M2SO, and M1SO by TLC co-chromatography. Activity in band 2 was not identified because of low activity (1.2 % of the total) and the activity in bands 3-10 were separately subjected to enzymatic hydrolysis with pectinase. The pH was brought to 2 and the resulting solution partitioned against methylene chloride, which took 37% of the total activity.

The methylene chloride solutions representing bands 3-10 were subjected to TLC. Six metabolites were identified, the most important of which were MSO (14% of the total activity) and M2SO (9% of the total activity). No other metabolite in this fraction comprised greater than 5% of the total activity. The identity of MSO was confirmed by MS and HPLC.

Of the total activity in soybeans, about 61% was positively identified as nine different compounds. The most significant single unidentified portion was the 6% from elution of the XAD-4 column with water.

In a separate experiment entitled "GLC Accountability of Radioactive Residues in Alfalfa Hay and Soybean Hay Resulting from Treatment with BAS 9052 H-[4-¹⁴C], samples of soybean hay carrying weathered residues of ¹⁴C BAS 9052 H were subjected to analytical method 30H (discussed below under deficiency 3a). The hay carried radioactive residues equivalent to 1.82 ppm BAS 9052 H; the method determined 0.88 ppm or 48% of the initial residue. The average recovery of fortified hay samples was 81%; thus, correcting for procedural losses, the GLC method accounts for 60% of the total radioactivity. This study does not establish the structure of any metabolites, but it does establish that most of the activity can be converted to one of the compounds determined by the analytical method.

RCB's Comment

Considering the complexity of metabolism demonstrated by the submitted plant metabolism studies and the lack of any single fraction that contains a significant amount of unidentified activity, it is unlikely that an important metabolite remains unidentified.

Based on the above studies, RCB concludes that the nature of the residue in soybeans and alfalfa is adequately understood. The residue of concern consists of parent plus metabolites containing the 2-cyclohexene moiety (calculated as parent). Deficiency 2a is resolved.

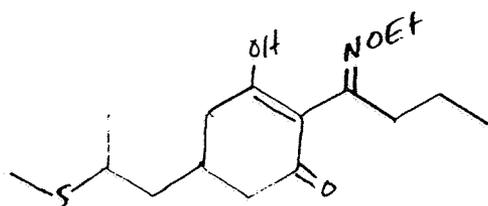
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Deficiency 2b

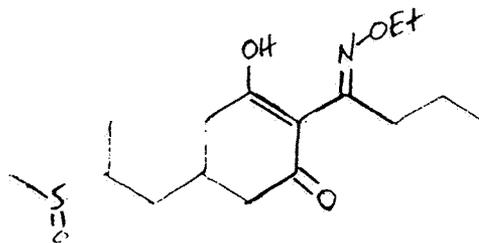
At this time, we conclude that the nature of the residue in animals is not adequately understood for the establishment of more tolerances on crops involving feed items. The petitioner will need to carry out a new lactating animal study at a feeding level between 50-100 ppm ^{14}C BAS 9052 H (parent compound). The petitioner should also submit results from a poultry metabolism study carried out at a feeding level between 10-20 ppm ^{14}C BAS 9052H.

Petitioner's Response to Deficiency 2b

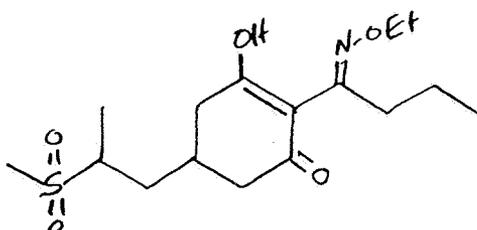
The petitioner has submitted goat and poultry metabolism studies, discussed below. The following compounds were available as reference standards: MS, MSO, MSO₂, M1SO, M1SO₂, M2SO, M2SO₂, and 5-OH-M2SO₂ (structures for the preceding are given under 2a, above). In addition to these, the following "nor" metabolite standards were available:



Nor-MS



Nor-MSO

Nor-MSO₂Goats

A lactating goat was fed ^{14}C -labeled BAS 9052 H sulfoxide (^{14}C MSO) at a level equivalent to 100 ppm in the dry diet for five days. Excreta and milk were collected during the dosing period, and the goat was sacrificed 24 hours after the final dose. Fat, liver, kidney, and muscle were sampled for residue characterization.

Urine and milk samples were treated similarly. Each was brought to pH 10, then extracted with methylene chloride, which took up 8.5% of the urine activity and 8.2% of the milk activity. The samples were brought to pH 3 and again extracted with methylene chloride. This dissolved 67% of the activity for both samples. Thus, for both urine and milk, about 25% of the activity remained in the aqueous phase.

Two samples of the pH 3 dichloromethane extract of urine activity were subjected to HPLC. One was spiked with a reference mixture of MSO and nor-MSO, and the second was spiked with MSO₂ and nor-MSO₂. Activity eluting with the reference compounds was noted. In this way the following metabolites were identified: MSO (24% of the urine activity), nor-MSO (16%), MSO₂ (3.6%), and nor-MSO₂ (1.8%). Reference compounds M1SO₂, M2SO, M2SO₂, 5-OH-MSO₂, MS, and nor-MS did not chromatograph with any detectable radioactive component.

In a similar manner, the pH 3 dichloromethane extract of milk was characterized. The following metabolites were identified: MSO (25% of milk activity), nor-MSO (5%), MSO₂ (10%), and nor-MSO₂ (<3%). Thus 43% of the milk activity was positively identified. Some of the activity was lost (10-15%), and no unidentified portion of the organosoluble activity accounted for greater than 8% of the total activity.

A separate experiment compared the results of the LSC counting with results obtained when the samples were subjected to the proposed analytical methodology. Milk carrying radioactivity equivalent to 0.25 ppm BAS 9052 H equivalents (as determined by LSC) was found by GC to carry 0.17 ppm residues. Correction for an average recovery from milk of 80% brings the percent determined by the cold method to about 80% of that determined by LSC. This is evidence that a great majority of the metabolites convert to DME or OH-DME (compounds determined by the analytical method) on treatment with hydrogen peroxide.

Residues in liver amounted to 2.2 ppm sulfoxide equivalents. Extraction with methanol solubilized 76% of this activity. The following metabolites were identified by TLC: nor-MSO (3% of the total liver activity), MSO (8%), nor-MSO₂ (4%), MSO₂ (21%), nor-MS (7%), and MS (12%). Thus 55% of the total liver activity was identified, 24% was not extracted, and the remainder of the activity (21%) consisted of several unidentified components.

Residues in kidney were 6.3 ppm sulfoxide equivalents. Methanol extraction dissolved 87% of the activity, and the following metabolites were identified by TLC: nor-MSO (12% of total kidney activity), MSO (21%), nor-MSO₂ (5%), MSO₂ (16%), nor-MS (<2%), and MS (2.5%). Thus 58% of the total activity in kidney was identified, 13% was bound, and the remainder of the activity consisted of several unidentified components.

Radioactive residues in muscle amounted to about 0.4 ppm sulfoxide equivalents, and residues in fat were 0.4-1.0 ppm sulfoxide equivalents. Fat and muscle were not further characterized. However most of the activity (70-80% after correction for recovery) was determined by the proposed analytical methodology.

RCB's Comments Concerning the Goat Metabolism Study

The petitioner has demonstrated that in goats the following

metabolic routes take place:

1. The sulfur atom is oxidized to the sulfoxide and sulfone.
2. Demethylation to form a "nor" series of metabolites occurs.
3. The ring is hydroxylated in the 5-position.
4. The imino group is de-ethoxylated.
5. An oxazole is formed as a result of a Beckman rearrangement.

Metabolites formed by these processes are determined by the proposed analytical methodology, including the demethylated "nor" series of metabolites, which were not uncovered in earlier metabolism studies. Additionally, the petitioner has demonstrated that the proposed methodology will determine an average of 60% (not corrected for recovery) of the total radioactive residues in goat tissues. A majority of the residues in liver and kidney were identified, but residues in fat and muscle were not, apparently because little activity was present. Even though activity in fat and muscle wasn't examined, its nature is partly known based on the validation studies that showed that the proposed analytical methodology determines most of this activity.

RCB considers the nature of the residues in ruminants to be adequately understood, provided the petitioner will submit additional raw data. Specifically, we require reproductions of TLC's that were used to identify metabolites in liver and kidney. If these do not support the petitioner's identification of metabolites, further studies may be needed.

Poultry

Four groups of five laying hens each were daily dosed at 5 mg/bird (equivalent to about 50 ppm in the diet) for seven days; a fifth group served as controls. Birds were sacrificed 6, 24, or 48 hours after the final dose. Eggs and excreta were collected throughout the study.

Most of the activity (85-90%) was uncovered in the excreta, and a small amount 0.4-0.9% was recovered in eggs. Residues in eggs ranged from 0.1-2.1 ppm and levels were quite erratic. Methanol dissolved 91% of the egg activity. TLC analysis showed two significant components: MSO (49% of the total egg activity) and MSO₂ (27%). Five other fractions of the TLC constituted 2-9% each.

Radioactive residues in pooled liver samples from chickens that had been sacrificed 6 hours after the final dose were characterized. Most (83%) of this activity dissolved in methanol. TLC analysis uncovered four major (<10% of the total activity) components: MSO (24%), MSO₂ (17%), and two unidentified compounds (11 and 14%). Several minor (0.3-4.6%) components were uncovered.

The proposed methodology determined 31% of the liver activity (not corrected for recovery).

Residues in liver declined to 0.52-0.71 ppm 24 hours after the final dose, and to 0.17-0.36 ppm after 48 hours.

Activity in fat and muscle was low compared to that in liver. No characterization was attempted. The following table summarizes the level of activity in tissues at the various sacrifice intervals:

Radioactive residues in tissues (ppm Sulfoxide equivalents)

Tissue	Hours after the final dose		
	6	24	48
Muscle	0.57-0.74	0.12-0.16	0.02-0.13
Fat	0.01-0.41	0.07-0.10	<0.02-0.04
Liver	1.46-2.0	0.52-0.71	0.17-0.36

A method validation study discussed below under 3b, below, shows that the best analytical method available determines 43-68% of the activity (corrected for recovery) in kidney, fat, and muscle; the liver sample was too messy for this method (the direct oxidation method). Method 30 determined 44% of the liver residues (corrected for recovery).

RCB's Comments Concerning Poultry Metabolism

The nature of the residue in poultry is not well understood. Of edible tissues, characterization was attempted only for liver, resulting in identification of 41% of this activity. In addition, available methodology is capable of determining only 44% of the liver activity, 43% of the fat activity, and 64% of the muscle activity.

RCB requires that additional attempts be made to characterize activity in poultry fat, muscle, and liver. The extracted activity from fat and muscle should be subjected to TLC, and the methanol soluble liver activity should be subjected to hydrolysis to release any polar conjugates. Any released activity should be characterized. The nature of the residue in eggs is adequately understood.

Deficiency 2b is not resolved.

Deficiency 3a

In the plant metabolism studies, methanol did not prove to be an effective solvent for the extraction of weathered residues from soybean plants. We now have some concern as to whether or not methanol is suitable for use as the extraction solvent in the analytical procedure already approved for analytical purposes. In view of the preceding, we conclude that the analytical methodology for the determination of BAS 9052 H residues in plant commodities needs to be further investigated on samples carrying weathered residues. For example, the petitioner may want to carry out his

initial extraction of plant samples with a solvent mixture of methanol plus acid, or he may want to apply ultrasonic treatments, enzymatic hydrolyses, etc., during any step of the sample preparation in an effort to release more of the bound residues from the plant matrices. In brief, we require the petitioner to validate the analytical methodology as part of the ^{14}C metabolism studies.

Petitioner's Response to Deficiency 3a

In conjunction with the soybean and alfalfa metabolism studies discussed above the petitioner has submitted three studies that compare the recovery of residues by GC methods to the results found by counting radioactivity. The studies are summarized below:

1. "A Comparison of BWC Agricultural Methods 30B and 30G in Determining the GLC Accountability of Radioactive Residues in Soybean Forage and Soybean Hay Resulting From Treatment with BAS 9052 H [4- ^{14}C]."

Method 30B involves extraction of forage or hay with methanol, precipitation with calcium hydroxide, dichloromethane extraction, oxidation with hydrogen peroxide to form substituted pentanedioic acids, methylation, dichloromethane partitioning, silica gel column chromatography, followed by determination by GC equipped with a sulfur specific FPD detector.

Method 30G is a variation of 30B, modified to determine residues in alfalfa and soybean forage. The most important modification is the deletion of a dichloromethane partitioning step that, in Method 30B, occurs just before the oxidation step.

The petitioners have analyzed soybean forage samples taken 14, 35, and 98 days after the last application of radiolabel test material. The radioactive residues in these samples were 4.48, 5.11, and 1.74 ppm BAS 9052 H equivalents, respectively. The radioactivity remaining in the sample was determined at various steps of the analytical procedure as outlined in the following table:

Step	Description	Percent ¹⁴ C Remaining in Sample			
		Method 30B	Method 30G		
		14 day	14 day	35 day	98 day
A	Initial conc. Soybean forage or hay	100 (4.48 ppm)	100 (4.48 ppm)	100 (5.11 ppm)	100 (1.74 ppm)
B	Methanol extract	86	86	85	63
C	Filtrate after precipitation	80	83	83	61
D	Combined dichloro- methane fractions (30B only)	52	--	--	--
E	Combined dichloro- methane fractions (after methylation)	46	76	74	53
F	Silica Gel column eluate				
	By LSC	41	66	--	--
	By GC	34(43) ¹	49(62) ¹	53(68) ¹	51(65) ¹

¹-numbers in parentheses are corrected for average recovery of 78.5%

2. "A Comparison of BWC Agricultural Chemicals Methods 30 and 30G in Determining GLC Accountability of Radioactive Residues in Soybean Seeds from Soybean Plants treated with BAS 9052 H [4-¹⁴C]."

Treated soybean seeds carrying 1.61 ppm radioactive equivalents of BAS 9052 H were subjected to methods 30 and 30G. The loss of radioactivity for several steps of the method was monitored by LSC as tabulated below:

<u>Step</u>	<u>Percent ¹⁴C Remaining in Sample</u>	
	<u>Method 30</u>	<u>Method 30G</u>
Initial concentration in Soybean Seed	100	100
Methanol-water extract	91	93
Filtrate after precipitation	89	89
Combined dichloromethane phases after pH 2 extraction	80	--
Combined dichloromethane phases after methylation	73	72
Silica gel column eluate		
By LSC	64	65
By GC	65(88) ¹	69(83) ²

1-corrected for average recovery of 74% for method 30
 2-corrected for average recovery of 83% for method 30G

3. "GLC Accountability of Radioactive Residues in Alfalfa Hay and Soybean Hay from Plants Treated with BAS 9052 H [¹⁴C]."

Alfalfa hay carrying radioactive residues equivalent to 59.6 ppm BAS 9052 H (PHI=14 days) and soybean hay carrying radioactive residues equivalent to 1.8 ppm BAS 9052 H (PHI=77 days) were subjected to BWC Method 30H as well as LSC. Method 30H, like method 30G, is a variation of Method 30. Method 30G and 30H are very similar; the dichloromethane partitioning step, included in Method 30, is omitted from both. Method 30G is designed for forage samples, and method 30H is designed for hay samples. Loss of radioactivity during the procedure was monitored by LSC with the following results:

<u>Step</u>	<u>Percent ¹⁴C Remaining in Sample</u>	
	<u>Alfalfa</u>	<u>Soybean</u>
Initial Concentration in hay	100	100
Methanol extract	91	66
Filtrate after precipitation	87	61
Combined dichloromethane extracts	71	52
Silica gel column eluate	60	46
Post column partition		
by LSC	--	46
by GC	52(64) ¹	48(59) ¹

¹-corrected for average recovery of 81% for method 30 H

RCB' Comments

The petitioner has demonstrated that methanol or methanol/water will extract 63-93% (average=83%) of the total radioactivity in soybeans, soybean hay and forage, and alfalfa hay and forage. Extraction of residues from weathered samples appears to be more difficult, but when the figures are corrected for procedural losses, the recovery of radioactivity is 43-88% (average = 67%). RCB considers this an acceptable level of recovery. Deficiency 3a is resolved.

Deficiency 3b

Methanol is also used in the proposed analytical methodology for extracting metabolized residues from animal commodities; the methodology should be further investigated.

Petitioners Response to Deficiency 3b

Two method validation studies for animal tissues have been submitted, one for poultry and one for goats. These are discussed below.

1. "Method Validation for BAS 9052 H Residues in Chicken Tissues and Eggs after Feeding with ¹⁴C-BAS 9052 H Sulfoxide."

Chicken tissues and eggs carrying radioactive residues as a result of a metabolism study (discussed above) were subjected to BWC Method 30 and a variation of this method (designated D.O.) in which the sample extract is oxidized directly without prior dichloromethane partitioning. These methods determine DME, OH-DME, and nor-DME. The results are tabulated below:

Tissue	total activity (ppm)	percent extracted by methanol	percent determined by analytical method	
			BWC 30	D.O.
muscle	0.614	95	18(26) ¹	43(64) ¹
fat	0.314	98	22(31) ¹	39(43) ¹
kidney	2.52	97	30(43) ¹	52(72) ¹
eggs	1.03	98	46(66) ¹	53(68) ¹
liver	0.66	79	31(44) ¹	-- 2

¹-the numbers in parentheses corrected for recovery.

²-liver sample was too messy for analysis by the direct oxidation method.

The loss of activity through these procedures was followed by counting the remaining activity at various points. The most significant losses for Method 30 were due to water soluble activity (12-45%, depending on the tissue) and losses as a result of HPLC (1-26%). In general the direct oxidation method recovered a greater percentage of the activity, and the greatest losses for this method occurred as a result of methanol/bicarbonate partitioning (5-13%) and HPLC (2-30%).

2. "Method Validation for BAS 9052 H Residues in Goat Tissues and Milk after Feeding with ¹⁴C-BAS 9052 Sulfoxide."

Goat tissues and milk carrying radioactive residues as a result of a metabolism study (discussed above) were subjected to BWC Method 30 and the direct oxidation method (see above). The results are tabulated below:

Tissue	total activity (ppm)	percent extracted by methanol	percent determined by analytical method	
			BWC 30	D.O.
leg muscle	0.59	95	55	53(60) ¹
fat	0.58	96	46	44
kidney	5.82	98	65	80(73)
liver	2.23	85	53	--2
milk	0.25	99	65	68(76) ¹

¹-the numbers in parentheses are corrected for recovery.

²-liver sample was too messy for analysis by the direct oxidation method.

As with the chicken study discussed above, the loss of activity through these procedures was followed by counting the remaining activity at various points. The most significant losses for method 30 were due to water soluble activity (6-32%, depending on the tissue). The difference between method 30 and the direct oxidation method were not great. The greatest losses as a result of the direct oxidation method occurred as a result of methanol/bicarbonate partitioning (1-37%).

RCB's Comments

The petitioner has demonstrated that methanol is a suitable solvent for extracting residues from animal tissues, milk, and eggs. Deficiency 3b is resolved.

Deficiency 4

Storage Stability. The recoveries of BAS 9052 H and its metabolites from stored samples are surprisingly much higher than the recoveries of standards/residues that were fortified at the time of analysis; the petitioner should explain this difference.

Petitioner's Response to Deficiency 4

This deficiency was resolved in the conference of 4/4/84 (see memo of 4/9/84, D. Griffith). The values for the stored samples were corrected for recovery and are therefore higher than those for the unstored samples which were not corrected for recovery.

RCB's Comment

This deficiency is resolved.

Deficiency 5a

We reserve our conclusion on the adequacy of the proposed 20 ppm tolerance on alfalfa forage and hay until those questions raised in the Nature of the Residue and Analytical Methods sections of this review have been resolved.

Petitioner's Response to Deficiency 5a

The petitioner has resolved questions concerning plant metabolism and analytical methodology (see 2a and 3a, above).

The petitioner has also reanalyzed treated alfalfa samples using method 30G (for forage) and method 30H (for hay). These samples had earlier been analyzed by method 30B, the results of which are discussed in our 1/12/85 memo (J. Onley).

Twenty-four alfalfa hay samples from five states were re-analyzed. Residues in alfalfa hay from treatments approximating the proposed use (total seasonal application of 1 lb. a.i./A,

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21 day PHI) ranged up to 28 ppm. This same sample, when earlier analyzed by method 30B, was found to carry residues of 12.7 ppm. The above values are not corrected for recovery. The residue data submitted earlier for alfalfa hay are generally lower.

For alfalfa forage, no grazing or foraging is allowed until 7 days after application. Residue data (reanalyzed by Method 30) reflecting two applications totaling 1 lb a.i./ A and PHI's of less than ten days are few, and all are from Connecticut. Re-analysis of alfalfa forage samples uncovered residues of up to 11.4 ppm from treatments approximating the proposed use. Earlier results were as high as 14 ppm (this particular sample was not reanalyzed).

The validation studies discussed earlier (see 3a, above) suggest that higher values would be uncovered if samples were analyzed by method 30H or 30G. The reanalysis of alfalfa forage and hay samples bears out this expectation.

RCB's Comments

We are unable to reach a conclusion as to the appropriateness of the proposed tolerances for alfalfa forage and hay. For further consideration the petitioner should indicate the type of application (ground or aerial) that was used in the already submitted residue data. The petitioner should be informed that we require both ground and aerial data reflective of the proposed use. We also require additional residue data for soybean forage that represents the maximum proposed rate and minimum PHI. This latter requirement is because too few data reflect the proposed PHI.

Deficiency 5a is not resolved.

Deficiency 5b

An alfalfa forage sample coded 503 (Illinois) had a residue of 38 ppm at the proposed PHI of 7 days; the petitioner states that this sample had been allowed to dry prior to analysis. Since alfalfa hay could be considered dried alfalfa forage, the sample would appear to be one for alfalfa hay. The petitioner should be able to submit a further explanation on the residue level in this sample.

Petitioner's Response to 5b

With the original submission the petitioner requested that sample 503 be disregarded because it consisted of 95-100% grass (i.e., no alfalfa) and that this grass had been allowed to dry. These samples were taken from a field that had a poor stand of alfalfa. The petitioner therefore requested that these samples should not be used for tolerance setting.

RCB's comment

We agree. The sample in question will not be used for tolerance setting.

Deficiency 5c

We need to know the mode of application (aerial and/or ground) on alfalfa plots.

Petitioner's response to 5c

The petitioner has not responded to this deficiency.

RCB's comment

This deficiency remains outstanding. See 5a, above, for further discussion concerning residue data for alfalfa.

Deficiency 6a

The petitioner should submit some residue data on his reserve soybean hay samples after those questions raised on plant metabolism and analytical methodology have been resolved. At this time we reserve our conclusion on the adequacy of the proposed BAS 9052 H tolerances on soybean hay and forage. In view of the questions raised on the adequacy of the methodology for determining BAS 9052 H residues in soybean forage and alfalfa forage and hay, we now question the adequacy of the established tolerance on soybean seeds. The petitioner should address this issue also. After the methodology question is resolved, we will also re-evaluate the residue data in light of the revised use pattern for soybeans.

Petitioner's Response to 6a

Plant metabolism and analytical methodology (plants) deficiencies have been resolved (see 2a and 3a, above). Also, the validation studies discussed earlier (see 3a, above) demonstrate that Method 30, used to analyze soybeans for the original submission, is adequate. Grazing and foraging restrictions have been included in a revised Section B; therefore no tolerance is required for soybean forage.

For this amendment 54 soybean hay samples from 8 states were reanalyzed by method 30H. Samples taken 75-100 days (the proposed PHI is 90 days) after the last of two applications at 0.5 lb a.i. per acre or after one application at 0.75 lb ai.i per acre carried residues of 1.2-6.7 ppm (not corrected for recovery).

RCB's Comment

The residue data are adequate to support the proposed tolerance on soybean hay (10 ppm) provided that aerial application has been

A

adequately represented. The petitioner should indicate the type of application for the submitted field trials. If none of the residue trials reflect aerial application, then additional studies will be needed. Deficiency 6a is not resolved.

Deficiency 6b

We need to know the mode of application (aerial and/or ground) on soybean plots.

Petitioner's Response to 6b

The petitioner has not responded to this deficiency.

RCB's comment

This deficiency remains outstanding. See 6a, above.

Deficiency 7

After reviewing the submitted residue data for supporting the new BAS 9052 H uses on alfalfa and soybeans (with no forage, ensilage, and hay restrictions), we are unable to draw conclusions on the adequacy of the established meat, milk, poultry, and egg tolerances until those questions raised in the Animal Metabolism and Analytical Methods sections of this review have been resolved. After resolution of the preceding questions, the petitioner may need to analyze some of his reserve meat, milk, poultry, and egg samples from his previous feeding studies.

Petitioner's Response

The petitioner has submitted two feeding studies, one for goats and one for poultry. In addition, the petitioner has reanalyzed stored samples from a previous cow feeding study; the original analysis had not included determination of the "nor" series of metabolites, which had not yet been uncovered in animal metabolism studies. These studies are discussed below.

1. "Residues of BAS 9052 H Metabolite MSO in Milk and Tissues of Lactating Goats."

Three groups of goats were dosed daily with the most pre-dominant plant metabolite of BAS 9052 H, its sulfoxide (MSO), at levels equivalent to about 30, 100, or 300 ppm in the diet for 28 days. A fourth group was fed hay carrying weathered residues of BAS 9052 H at a level equivalent to about 75 ppm in the diet, and a fifth group served as a control.

The animals were milked twice daily, and urine and feces were collected from one goat in each group three or four times during the study.

Residues were determined by Method 30, which has been discussed earlier in this review.

Residue levels uncovered in milk are summarized in the following table.

<u>Feeding level</u>	<u>PPM in Milk</u>
30 ppm	<0.01-0.03
100 ppm	<0.01-0.07
300 ppm	<0.01-0.11
75 ppm (hay)	<0.01-0.18

Residues uncovered in milk are summarized below:

<u>Feeding level</u>	<u>Fat</u>	<u>Kidney</u>	<u>Liver</u>	<u>Muscle</u>
30 ppm	<0.05	<0.05	<0.05	<0.05
100 ppm	<0.05	<0.31	<0.05	<0.05
300 ppm	0.07	<0.45	0.17	<0.05
75 ppm (hay)	<0.05	<0.44	0.14	<0.05

2. "Determination of Residues in the Eggs and Tissues of the Laying Hen Following Dosing with MSO."

Chickens were dosed with either 25, 80, or 250 ppm MSO in the diet for 29 days. A fourth group served as controls. Eggs were collected daily, and the chickens were sacrificed on the day of the last dose, two days after the last dose, or seven days after the last dose.

Residues in eggs were 0.31-1.08 ppm as a result of the lowest feeding level, 1.04-3.9 ppm as a result of the 80 ppm feeding level, and 2.15-11.9 ppm as a result of the 250 ppm feeding level. Levels in eggs tended to increase until about seven days into the dosing period, after which levels were quite erratic, with no significant trends. Levels fell fairly quickly after dosing was discontinued, but were still detectable seven days after the final dose for the two higher feeding levels (0.21 and 0.45 ppm in eggs from chickens fed 80 and 250 ppm, respectively).

Residues uncovered in tissues are summarized in the following table:

<u>Feeding level</u>	<u>Tissue Residue in PPM</u>				
	<u>Fat</u>	<u>Kidney</u>	<u>Liver</u>	<u>Muscle</u>	<u>Skin</u>
25 ppm	<0.05	0.75	0.4	0.06	0.10
80 ppm	0.05	1.04	0.42	0.10	0.23
250 ppm	0.17	1.46	1.26	0.21	0.60

3. "Determination of Sethoxydim and its Metabolite Residues in Beef Tissues (Muscle, Liver, and Kidney) and Milk Samples Obtained from Cows Exposed to Thirty Consecutive Daily Doses of Sethoxydim."

This is not a new feeding study, but reanalysis of samples from an earlier study to include determination of the "nor" series of metabolites as nor-DME. In a 1980 study, cows had been dosed with BAS 9052 H at a level equivalent to 50 ppm in the diet for 30 days. Milk had been collected daily, and the animals were sacrificed within one day of the final dose.

The tissues and milk were initially analyzed for DME only (see PP#0G2396, memo of 12/4/80, E. Zager); later the stored samples were reanalyzed to include determinations for OH-DME (see PP#2F2670, memo of 7/23/82, M. Nelson). Residue levels were found to be essentially the same as previously reported; nor-DME was uncovered in only one tissue sample: liver, at 0.06 ppm. The following table summarizes the total residue (DME plus OH-DME plus nor-DME) found in milk and tissues:

<u>Tissue</u>	<u>Residue (ppm)</u>
muscle	<0.03
liver	<0.15-0.20
kidney	<0.15-0.16
milk	<0.05-0.06

Fat was apparently not sampled.

RCB's Comments

Until metabolism questions are resolved, we cannot make any conclusions concerning secondary residues in meat, milk, poultry, and eggs. Additional methodology and feeding studies could be required for poultry depending on the outcome of the additional studies RCB has requested.

Deficiency 7 is not resolved.

Recommendations

RCB recommends against the proposed tolerances. For further consideration we require:

1. The petitioner should submit reproductions of TLC's that were used to identify metabolites in liver and kidney of goats. If these are satisfactory, nothing further will be required in conjunction with the goat metabolism study. See deficiency 2a, above.
2. RCB requires that additional attempts be made to characterize activity in poultry fat, muscle, and liver. The extracted activity from fat and muscle should be subjected to TLC, and the methanol soluble liver activity should be subjected to hydrolysis to

release any polar conjugates. Any released activity should be characterized. See deficiency 2b, above.

3. The petitioner should indicate the type of application (ground or aerial) that was used in the already submitted residue experiments for both soybeans and alfalfa. The petitioner should be informed that we require both ground and aerial data reflective of the proposed use. In addition, we require additional residue data for soybean forage that represents the maximum proposed rate and minimum PHI. This latter requirement is because too few available data reflect the proposed PHI. See deficiencies 5a, 5c, 6a and 6b, above.

4. Depending on the outcome of the requested further studies concerning poultry metabolism, additional methodology and feeding studies may be required. See deficiency 7, above.

Other Considerations

An International Residue Limit Status sheet is attached. No Codex, Mexican, or Canadian tolerances are established for sethoxydim on the subject crops.

A method trial may be required to evaluate the method used to determine the "nor" series of metabolites depending on the outcome of the additional studies on animal metabolism.

TS-769:RCB:KHArne:kha:CM-2:Rm810:557-7377:6/25/85
CC: TOX, PM, PP#3F2904, K. Arne, RF, Circ., Thompson, FDA, EEB,
EAB, PMSD/ISB
RDI: JHO, 6/24/85; RDS, 6/25/85

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Arne

1, JWS
6/21/85

INTERNATIONAL RESIDUE LIMIT STATUS

2-[1-(ethoxyimino)butyl]-5-(2-ethylthio)propyl]-3-hydroxy-2-cyclohexene-1-one

CHEMICAL Sethoxydim (Poast)

PETITION NO. 3F2904

CCPR NO. none

Codex Status

Proposed U.S. Tolerances

No Codex Proposal
Step 6 or above

Residue (if Step 9): _____

Residue: parent plus
metabolites containing
2-cyclohexene-1-one moiety

Crop(s) Limit (mg/kg)

Crop(s) Tol. (ppm)
soybean, hay 10 ppm
alfalfa hay 40 ppm
alfalfa forage 40 ppm
milk 0.05 ppm

CANADIAN LIMIT

MEXICAN TOLERANCIA

Residue: _____

Residue: _____

Crop Limit (ppm)
none (on given commodities) ^{1/}

Crop Tolerancia (ppm)

NOTES:

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1/ There is a 0.1 ppm ^{Canadian} negligible residue type limit on soybean, but not specifically on soybean hay.