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

MAY - 9 1991

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP# 0F3860 Sulfosate (Touchdown™) in or on soybean seed, forage and hay. Evaluation of analytical methods and residue data.

MRID No. 414621-02,03,04,05,06
412099-19
CBTS No. 6814, 6815, 6816
HED# 0-1513

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Health Effects Division (H7509C)

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and

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Health Effects Division (H7509C)

THRU: Richard D. Schmitt, Ph.D., Chief
Chemistry Branch I: Tolerance Support
Health Effects Division (H7509C)

ICI Americas Inc., Agricultural Products has proposed the establishment of tolerances for the combined residues of the herbicide sulfosate (Touchdown™), N-phosphonomethylglycine, (carboxymethylamino methyl phosphonate) and its metabolite, AMPA (aminomethylphosphonic acid) (calculated as the herbicide) in or on: soybean seed @ 2.0 ppm, soybean forage @ 1.0 ppm and soybean hay @ 3.0 ppm.

Sulfosate is a NEW herbicide; there are no established tolerances for the trimethylsulfonium salt of glyphosate. An earlier petition (PP# 9F3796, S. Koepke) for tolerances for corn grain, forage and fodder is pending resolution of deficiencies. Tolerances have been established for the isopropylamine (Roundup® herbicide) and sodium sesqui salts of glyphosate under 40 CFR 180.364. Sulfosate is similar in chemical structure and proposed use to glyphosate.

CONCLUSIONS/DEFICIENCIES

1. The product chemistry and manufacture of sulfosate was previously reviewed and found to be adequate by Registration Division when sulfosate was first submitted as a non-food use chemical.

2a. The proposed labels recommends the use of ammonium sulfate as an adjuvant. This recommendation must either be deleted from the labels and a revised Section B be submitted or residue data must be generated supporting the use of this adjuvant with sulfosate.

2b. The proposed labels are unclear as to the number and type of applications allowed. The labels state that only one application is allowed. Does this mean one pre-emergent or one spot application or one of each is allowed as long as one does not exceed the 4 lbs a.i./A/yr? A revised Section B is required.

3a. Contingent upon the successful review of the required raw data, CBTS finds the submitted metabolism studies for the cationic moiety of sulfosate on soybeans to be adequate for the purposes of this petition only. The hydroponic study seems to indicate that virtually no metabolism takes place within the plant and is considered adequate for this purpose. The preemergent study leaves 21% of low level residues uncharacterized. Should a different use pattern be proposed that may result in higher levels of residues, further characterization of the nature of the residue in the uncharacterized fractions would be required. Additional raw data detailing the quantitation of the radioactivity (such as sample weights, raw counts in each sample, etc.) are required for all cation metabolism studies. The reviewer should have sufficient detailed data to reproduce all calculations used to generate the final tabulated data.

3b. CBTS finds the submitted metabolism studies for the anionic moiety of sulfosate on soybeans to be inadequate. The hydroponic study seems to indicate that virtually no metabolism takes place within the plant, but no quantitation data were submitted. In the preemergent study, it is required that the metabolism study

be repeated separating the plants sufficiently that control and sample background levels generated from $^{14}CO_2$ from soil will be kept at a minimum. CBTS requires that the nature of at least 90% of the residue in the plant be determined. Additional raw data detailing the quantitation of the radioactivity (such as sample weights, raw counts in each sample, etc.) are required for all studies. The reviewer should have sufficient detailed data to reproduce all calculations used to generate the final tabulated data.

4. CBTS concluded that sufficient detail was provided to submit the methods for method trials. ACL has requests for clarifications on each of these methods. A copy of their preliminary findings is attached. A copy should be forwarded to the registrant. Contingent upon these clarifications and the successful completion of these method trials, CBTS would consider the methods to be adequate for both the anionic (carboxymethylamino methyl phosphonate [CMP] and its metabolite, aminomethylphosphonic acid [AMPA]) and cationic (trimethylsulfonium ion [TMS]) moieties of sulfosate. The milk, egg and edible tissue method for the cationic moiety of sulfosate requires further development prior to the initiation of its method trial. There are interferences in milk and cow liver that need to addressed. Both methods for milk, eggs and edible tissues were received after August 1, 1989 and must be subjected to an independent method validation trial prior to an Agency method trial (PR Notice 88-5, 7/15/88).

5. Fifteen field trials were carried out in eleven different states (Mississippi, Alabama, Iowa, Illinois, Arkansas, Minnesota, Missouri, Tennessee, Nebraska, Virginia and Indiana) in 1988 using sulfosate on soybeans. CBTS considers geographical representation of the residue data to be adequate. However, as noted in conclusion 8, additional data from another crop year are required.

6. Storage stability data were submitted for TMS, CMP and AMPA in soybean seed and soybean straw. All three analytes were stable for up to two years in these matrices. These data were reviewed previously (PP#9F3796, S.Koepke, 1/4/91) and found to be adequate pending method trials. CBTS considers the storage stability data to be adequate for soybean seed, forage and hay pending the successful completion of method trials.

7. Tolerances already exist for glyphosate on soybean seed @ 6 ppm, soybean forage @ 15 ppm and soybean hay @ 15 ppm. The existing regulatory method and the proposed analytical methods for sulfosate cannot differentiate the source of the anionic moieties and such a method cannot be developed. Therefore, the tolerances requested would require a revision of 40 CFR 180.3 to include sulfosate as a related herbicide to glyphosate and that

the enforcement tolerance would be based on the higher of the two approved tolerances.

8. Since soybean is a major crop additional residue data are required. Fifteen values all from the same crop year for the magnitude of the residue in soybean are insufficient. At least ten additional geographically relevant field trials are required.

9. CBTS does not consider the processing study to be adequate since the processor's sample did not contain any measurable residue. It is required that the processing study be repeated using a seed sample that has measurable residues at or near the proposed tolerance level in order to determine if the residues concentrated in any processed fraction.

10. CBTS finds the magnitude of the residue data for milk and meat to be adequate. At the feeding level equivalent to that present in the crop (<= 3 ppm), no measurable residues from parent were detected. CBTS concludes that sulfosate belongs to category 2 of 40 CFR 180.6(a), it is not possible to establish with certainty whether finite residues will be incurred, but there is a reasonable expectation of finite residues. Therefore, a revised Section F proposing tolerances of 0.04 ppm in milk, 0.1 ppm in meat, fat and meat byproducts (except liver) of cattle, goats, hogs, horses and sheep and 0.4 ppm in liver of cattle, goats, hogs, horses and sheep is required. These values would be expressed as parent sulfosate calculated as either the sum of the magnitudes of the residues of AMPA and CMP or the magnitude of the TMS residue, whichever is greater.

11. CBTS finds the magnitude of the residue data for poultry and eggs to be adequate. At the feeding level equivalent to that present in the crop (<= 3 ppm), no measurable residues from parent were detected except in chicken kidney. CBTS concludes that sulfosate belongs to category 2 of 40 CFR 180.6(a), it is not possible to establish with certainty whether finite residues will be incurred, but there is a reasonable expectation of finite residues. Therefore, a revised Section F proposing tolerances of 0.1 ppm in poultry meat, fat and meat byproducts and 0.03 ppm in eggs is required. These values would be expressed as parent sulfosate calculated as either the sum of the magnitudes of the residues of AMPA and CMP or the magnitude of the TMS residue, whichever is greater.

12. The previously submitted laying hen metabolism studies for both the anion and cation were found to be inadequate (PP#9F3796, S.Koepke, 1/4/91). The nature of the residue in eggs and poultry is not defined. All conclusions in this review are contingent upon the residues of concern in eggs and poultry being TMS, CMP and AMPA.

13. The previously submitted goat metabolism studies for both

the anion and cation were found to be inadequate (PP#9F3796, S.Koepke, 1/4/91). The nature of the residue in milk and ruminant edible tissues is not defined. All conclusions in this review are contingent upon the residues of concern in milk and ruminant edible tissues being TMS, CMP and AMPA.

14. An "International Residue Status" sheet is attached. There are no Canadian, Mexican or Codex tolerances for Sulfosate on or in soybean. There are Codex limits of 5.0 ppm for soybean (dry), 0.2 ppm for soybean (immature seeds), 20 ppm for soybean fodder and 5 ppm for soybean forage (green) for glyphosate per se. There is also a Canadian negligible residue type limit of 0.1 ppm for glyphosate per se for "all food crops". There are no compatibility problems associated with this petition.

RECOMMENDATIONS

CBTS recommends against the requested tolerances because of conclusions 2a, 2b, 3a, 3b, 3c, 4, 7, 8, 9, 10, 11, 12 and 13.

Note: All conclusions are subject to successful completion of all method trials and resolution of the nature of the residues of concern.

DETAILED CONSIDERATIONS

MANUFACTURE AND FORMULATION (MRID# 409430-00,01,02,03)

The product chemistry and manufacture was previously reviewed and found to be adequate (memo, 3/17/87, K. Leifer) by Registration Division when sulfosate was first submitted as a non-food use chemical. A letter stating that no additional product chemistry data was necessary was sent (2/15/89, R. Taylor) to the petitioner.

Two formulations are proposed for use:

Touchdown® 4-LC, a 40% a.i. liquid concentrate. Each gallon contains 4 lbs a.i.

Touchdown™ Concentrate a 52.2% a.i. liquid concentrate. Each gallon contains 5.5 lbs a.i.

PROPOSED USE (MRID# 409430-00)

Touchdown® is a nonselective systemic herbicide proposed for pre-

emergent and spot weed control on soybean fields. This formulation is currently approved for use in noncrop areas to control unwanted vegetation.

Touchdown® requires a six hour rain free period after application. Rain occurring within six hours of application may reduce weed control.

Do not apply by aircraft. Do not apply this product through any type of irrigation system.

Touchdown® should be applied to actively growing emerged weeds when they are small. Weeds 6" in height are easiest to control.

A surfactant or wetting agent (approved for crop usage) is required to improve coverage of weed foliage. All surfactants or wetting agents should contain at least 50% active ingredient.

The use of ammonium sulfate is proposed to improve control of weeds. The petition for a tolerance is for the trimethylsulfonium salt and not the ammonium salt. Use of different salts would require additional residue data. When sulfosate is premixed with ammonium sulfate, the ammonium salt will result. CBTS requires this use be removed from the label or that residue data be generated using ammonium sulfate mixed with sulfosate.

Broadcast application of Touchdown® should be made in 10 to 30 gallons of water per acre with conventional spray equipment or in 1 to 10 gallons of water per acre with low volume equipment. Increase volumes if foliage is dense.

Spot applications should use a 1 to 3% a.i. solution of Touchdown® in water. Spray the solution on actively growing foliage until uniformly wet, but not to the point of runoff. Re-treat if necessary. A PHI of at least 8 weeks is proposed for this use.

The labels state that wiper applications may be used by mixing 2 gallons Touchdown®-4LC or 2.5 quarts of Touchdown® Concentrate in 2 gallons of water. Apply this mixture to weeds while avoiding contact with desirable vegetation. For improved control, make two applications in opposite directions.

The following statement appears on the proposed labels in various places for soybean:

> Do not make more than one application or exceed a total of 4 lbs Touchdown® (4 quarts for 4-LC and 5.8 pints for Concentrate) per acre per year.

It is required for the label to be clarified as to whether this refers to one spot treatment or one pre-emergent treatment or one of each. In addition, this should also read 4 lbs a.i./A per year not 4 lbs of Touchdown® per acre per year. A PHI of 8 weeks for spot treatments is specified for soybeans on the label. No PHI is given or required for pre-emergent use.

Do not plant rotational crops other than corn within one year in a field treated with Touchdown® 4-LC.

CBTS finds the label to be inadequate. The use of ammonium sulfate is required to be deleted or residue data generated using ammonium sulfate. Additionally, the number of proposed possible types and number of applications per year is unclear. A revised Section B is required.

NATURE OF THE RESIDUE (MRID# 412099-19, 414621-02)

One plant metabolism study was submitted with this petition. This was on the nature of the residue from the cation in soybeans. A previous submission (9F3796) contained the corresponding study on the anionic portion of sulfosate. For purposes of clarity and simplicity, both are being reviewed with this petition.

The metabolism of the trimethylsulfonium moiety (cation) of sulfosate was carried out in three parts, a root absorption study, a foliar absorption study and a preemergent study. In all three cases, sulfosate labeled in trimethylsulfonium moiety with ^{14}C at a specific activity of 20 mCi/mmol was used. The labeled compound was prepared at the Western Research Center, ICI Americas, Inc. Its purity was determined to be 94.4% using two different thin layer chromatography systems. Combustion samples were analyzed in triplicate and compared to standard samples of tri[1-^{14}C]palmitate to insure accuracy.

Hydroponic or root absorption studies were carried out in 100ml beakers with glass marbles and 400-600 ml Hyponex solution added. At least seven soybean seedlings were placed on top of the marbles and grown under greenhouse conditions for two weeks. At the end of two weeks, the seedlings were transferred to fresh beakers containing 400 ml of Hyponex solution, one of which also contained 8.5 mg unlabeled and 0.125 mg of labeled sulfosate. This corresponds to treatment with 3.9 ppm TMS equivalents with a specific activity of 14,400 dpm/µg TMS. The hydroponics solutions were checked daily and kept at the original volume of 400 ml with distilled water.

After nine days, five plants in the treated flask were severely desiccated and only two healthy plants remained. One of the healthy plants was used for autoradiography to determine the distribution of the radiolabel. The remaining healthy plant was divided into two parts: roots and leaves/stems. Each sample was weighed, ground in liquid nitrogen and subsamples combusted. The remaining sample portions were stored frozen until extraction.

The root and leaf/stem tissue was extracted first with methanol followed by 2M HCl and each fraction assayed with liquid scintillation counting. The methanol extraction removed 85% and 99% of the ^{14}C residue from the leaves/stems, respectively. The 2M Hcl extraction removed the remaining 15% of the residue from the leaves/stems fraction. The hydroponic solution and the methanol fractions were characterized by 2-dimensional thin layer chromatography.

Table I.

Root Absorption of Sulfosate Cation

Sample	dpm	% of Applied	TMS Equivalents (ppm)
Leaves/Stems	0.295×10^6	1.3	2.5 ± 0.82
Roots	6.84×10^6	30.3	65.4 ± 13.2
Solution	15.9×10^6	70.4	---
Total	23.0×10^6	102	

Table II.

Foliar Absorption of Sulfosate Cation

	Hours After Treatment (Conc in ppm)						
Plant Parts	0	6	24	48	96	168	336
Treated Leaf	8.80	16.7	15.1	12.3	8.2	11.3	13.8
Other Leaves	0.014	0.050	0.063	0.060	0.066	0.066	0.110
Above Treatment	0.004	0.025	0.022	0.148	0.038	0.015	0.009
Below Treatment	0.003	0.031	0.035	0.750	0.035	0.038	0.053
Roots	0.005	0.002	0.009	0.009	0.020	0.016	0.014

The autoradiogram corresponded to the numbers depicted in Table I. Most of the radioactivity remained in the roots with only 4% of that absorbed by the plant translocated to the leaves. Thus,

images of the leaves are absent from the autoradiogram. The methanol extracts and the hydroponic solution were characterized by 2-D thin layer chromatography. Ninety to ninety-five percent of each of these samples cochromatographed with the TMS cation of sulfosate. These data seem to indicate that virtually no metabolism takes place within the plant. The hydroponic study is considered adequate to show that little metabolism of the cation takes place in the plant.

For the foliar absorption studies, soybean plants were grown hydroponically in a manner similar to the root absorption study. After two weeks, the middle leaf of each first trifoliate was lightly coated with a 1000:1 dilution of water:Ethoquad C/12 surfactant using a cotton swab. The leaves were then each treated with 2.58 μg ^{14}C-TMS sulfosate (1.49×10^6 dpm). The plants were then sampled at various time intervals for combustion analysis and autoradiography. Autoradiography indicated that most of the absorbed ^{14}C appears to have moved to the roots or to the newly developing plant foliage. This experiment indicates that there is rapid foliar uptake and transport of the cation portion of sulfosate. The combustion results are in agreement with those from the autoradiography (Table II). Although each sample was weighed according to the report, no weights were submitted.

A preemergence study was carried out using ^{14}C-TMS sulfosate at the highest anticipated single application rate (4.28 lbs a.i./A) in order to simulate the actual field use. Six clay pots were planted with soybean seeds and filter paper discs were placed evenly on the surface of the soil. The ^{14}C-TMS sulfosate (0.971 mg or 79.3μCi) was applied in a 4LC formulation at a volume equivalent rate of 25 gal/A. The spray coverage was estimated by removing the discs after spraying and analyzing them by liquid scintillation counting. The plants were harvested at the beginning of senescence. Plants were divided into three parts, leaves/stems, beans and pods. The combustion analysis is depicted in Table III. Residues were highest in the pods (3.05 ppm) and lowest in the beans (0.103 ppm). The data indicate that soybeans absorb some of the cation of sulfosate from treated soil.

Each plant part was subsampled and extracted using methanol, water and 1 M NH_4Cl. The extracts were separated from plant material by centrifugation and analyzed by liquid scintillation counting. The results are listed in Table IV. A large variability was reported for the combustion analyses. As a result, the percentage figures in the Table are based on the totals from liquid scintillation counting of the fractions. The petitioner argues that this is a more accurate representation of

the results. No raw data depicting the variability were submitted, only the compiled averages with error limits. Copies of the raw data are required. The reviewer should have enough raw data to reconstruct all the calculations.

Table III.

Preemergent Application of Sulfosate Cation

Plant Parts	Weight (g)	Total dpm	% of Total Applied	ppm TMS Equivalents
Leaves/Stems	145.5	1.5×10^6	1.1	1.47
Beans	37.1	0.0268×10^6	0.02	0.103
Pods	44.9	0.964×10^6	0.7	3.95
Hay	227.5	2.49×10^6	1.82	1.56

Table IV.

Fractionation of Preemergent Treated Samples

	Leaves/Stems			Pods			Beans		
Fraction	dpm	% Dist	ppm	dpm	% Dist	ppm	dpm	% Dist	ppm
Available	773,000		1.47	536,000		3.05	14,000		0.103
Methanol Extract	377,000	72	1.06	561,000	94	2.89	1,800	19	0.02
Water Extract	63,000	12	0.18	24,000	4	0.12	2,500	26	0.03
NH_4Cl Extract	48,000	9	0.13	ND	ND	ND	1,600	16	0.02
Non-extracted ^{14}C	35,000	7	0.10	9,000	2	0.06	3,800	39	0.04
Recovered ^{14}C	523,000			594,000			9,700		
% Recovered ^{14}C	68			110			67		

The methanol extracts of the leaves/stems and pods were further purified by column followed by thin layer chromatography. The radioactivity in these two fractions consisted of approximately 97% sulfosate cation. No other radioactive peak was present in the chromatograms. The total identified would correspond to approximately 78% of the radioactivity in the plant. The remaining 22% was present in the NH_4Cl and water extracts and no attempts to characterize these fractions were attempted.

Contingent upon the successful review of the required raw data, CBTS considers that the nature of the residue is adequately known for the purposes of this petition only. Should a different use pattern be proposed that may result in higher levels of residues, further characterization of the nature of the residue in the

uncharacterized fractions would be required.

A second preemergence study was carried out using ^{14}C-TMS sulfosate at the highest anticipated single application rate (4.28 lbs a.i./A) in order to simulate the actual field use in a similar manner to the first study. The ^{14}C-TMS sulfosate (6.03 mg or 492.5μCi) was applied in a 4LC formulation at a volume equivalent rate of 25 gal/A. The specific activity of the sulfosate was five times higher in this second study, but the magnitude of the residue was also five times less. As a result, no further characterization of the nature of the residues was considered possible.

Contingent upon the successful review of the required raw data, CBTS finds the submitted metabolism studies for the cationic moiety of sulfosate on soybeans to be adequate for the purposes of this petition only. The hydroponic study seems to indicate that virtually no metabolism takes place within the plant and is considered adequate for this purpose. The preemergent study leaves 21% of low level residues uncharacterized. Should a different use pattern be proposed that may result in higher levels of residues, further characterization of the nature of the residue in the uncharacterized fractions would be required. Additional raw data detailing the quantitation of the radioactivity (such as sample weights, raw counts in each sample, etc.) are required for all cation metabolism studies. The reviewer should have sufficient detailed data to reproduce all calculations used to generate the final tabulated data.

Table V.

Foliar Absorption Study Sulfosate Anion

Plant Parts	Time After Treatment (hours) 0	6	24	48	120	168	336
Treated leaf	1,885,000 (76.5%)	2,284,000 (92.8%)	2,524,000 (102.6%)	2,062,000 (83.8%)	2,553,000 (104%)	1,970,000 (80.1%)	1,540,000 (62.6%)
Leaves	2,000 (0.09%)	55,000 (2.20%)	67,000 (2.70%)	133,000 (5.40%)	89,000 (3.60%)	186,000 (7.6%)	129,000 (5.2%)
Roots	9,000 (0.37%)	31,000 (1.30%)	102,000 (4.20%)	143,000 (5.80%)	88,000 (3.60%)	275,000 (11.2%)	105,000 (4.30%)
Stems	tr	97,000 (3.90%)	120,000 (4.90%)	197,000 (8.00%)	52,000 (2.10%)	90,000 (3.70%)	54,000 (2.20%)
Cotyledon	tr	tr	tr	2,000 (0.08%)	1,000 (0.04%)	5,000 (0.20%)	----- -----
Total	77.0%	100%	114%	103%	113%	102%	74%

The metabolism of the glyphosate portion (anion) of sulfosate was carried out in three parts, a root absorption study, a foliar absorption study and a preemergent study. In all three cases, sulfosate labeled in anionic portion with ^{14}C at a specific activity of 30 mCi/mmol was used. The labeled compound was prepared at the Western Research Center, ICI Americas, Inc. Its purity was determined to be 95% using two different thin layer chromatography systems. Combustion samples were analyzed in triplicate and compared to standard samples of tri[1-^{14}C]palmitate to insure accuracy.

The foliar absorption studies were carried out in 100ml beakers with glass marbles and 220 ml Hyponex solution added. Single soybean seedlings were placed on top of the marbles and grown under greenhouse conditions for two weeks. After two weeks, the middle leaf of each first trifoliate was lightly coated with a 205:1 dilution of water:Ethoquad C/12 surfactant using a cotton swab. The leaves were then each treated with 5μl of the stock solution (concentration not given) of [^{14}C-anion]sulfosate. The plants were then sampled at various time intervals for combustion analysis and autoradiography. Autoradiography indicated that most of the absorbed ^{14}C appears to have moved to the roots or to the newly developing plant foliage. This experiment indicates that there is rapid foliar uptake and transport of the anion portion of sulfosate. The combustion results are in agreement with those from the autoradiography (Table V.). Although each sample was weighed according to the report, no weights were submitted. This study is considered adequate to demonstrate the rapid foliar uptake and distribution of the anionic moiety of sulfosate.

A preemergence study was carried out using [^{14}C-anion]sulfosate at the highest anticipated single application rate (4.375 lbs a.i./A) in order to simulate the actual field use. Six clay pots were planted with six soybean seeds and filter paper discs were placed evenly on the surface of the soil. The [^{14}C-anion]sulfosate (8 mg or 32 x 10^6) was applied in a 4LC formulation at a volume equivalent rate of 25 gal/A. Treated controls were treated with an equivalent amount of unlabeled sulfosate. Untreated controls were not treated with sulfosate. The spray coverage was estimated by removing the discs after spraying and analyzing them by liquid scintillation counting. The plants were harvested at the beginning of senescence. Plants were divided into three parts, leaves/stems, beans and pods. The combustion analysis is depicted in Table VI. Residues were highest in the pods (3.8 ppm) and lowest in the beans (0.395 ppm). The data indicate that soybeans absorb some of the anion of sulfosate from treated soil.

It is disturbing that the control values for this experiment are

extremely high. The petitioner explains this by incorporation from $^{14}CO_2$ generated from the soil of the treated plants. This may in fact be true. Lichtenstein has been documented that CO_2 from fonofos on soil was incorporated into the aerial portion of plants (J.Ag.and Food Chem., 33, 1985, pp. 160-167). However, the purpose of these experiments is to determine the nature of the residue in soybeans from preemergent treatment. Two gram subsamples were separately extracted with methanol, water, 10% ammonium hydroxide or 1N Hcl. The results are listed in Table VII. It is evident that there are quantifiable differences in the distribution of the radiolabel in the various fractions. In addition, the petitioner calculated net ppm by subtracting the amount of ^{14}C-label found in the treated controls. This is not acceptable. As presented, the data indicate 3.8 ppm present in the pod. The petitioner did not attempt to further evaluate the nature of the residue present in any of the fractions.

Table VI.

Preemergent Application of Sulfosate Anion

Plant Part	Untreated Control (ppm)	Treated Control (ppm)	^{14}C-CMPA Treated (ppm)	Net ppm ^{14}C-CMPA treated
Leaves/Stems	1.12	0.65	1.18	0.28
Beans	0.76	0.12	0.395	0.30
Pods	1.28	1.17	3.8	2.57

Table VII.

Fractionation of Preemergent Treated Samples

	Treated Control		^{14}C-CMPA Treated	
	Extracted	Not Extracted	Extracted	Not Extracted
MeOH	2,900 (14%)	18,000 (86%)	2,100 (14%)	12,500 (86%)
H_2O	3,800 (55%)	3,100 (45%)	7,000 (32%)	14,700 (68%)
10% NH_4OH	6,900 (51%)	6,600 (49%)	2,900 (29%)	7,200 (71%)
1 M Hcl	5,900 (61%)	3,800 (39%)	3,400 (27%)	9,000 (72%)

The preemergence metabolism study is inadequate. No attempts were made to determine the nature of the residue. It is required that the metabolism study be repeated separating the plants sufficiently that control and sample background levels generated from $^{14}CO_2$ from soil will be kept at a minimum. CBTS considers

the nature of the residue from the anionic moiety of sulfosate in soybeans to be undefined

Hydroponic or root absorption studies were carried out in 1000ml beakers with glass marbles and 500 ml Hyponex solution added. At least seven soybean seedlings were placed on top of the marbles and grown under greenhouse conditions for two weeks. At the end of two weeks, the seedlings were transferred fresh beakers containing 500 ml of Hyponex solution, one of which also contained a combination of ^{13}C with 20% ^{14}C labeled sulfosate at a concentration of 4.4 ppm. The hydroponics solutions were checked daily and kept at the original volume of 500 ml with distilled water.

After nine days, one of the plants was used for autoradiography to determine the distribution of the radiolabel. The remaining plants were divided into two parts: roots and leaves/stems. Each sample was weighed, ground in liquid nitrogen and subsamples combusted. The remaining sample portions were stored frozen until extraction.

The root and leaf stem tissue was extracted first with methanol followed by 2M HCl and each fraction assayed with liquid scintillation counting. The methanol extraction removed 85% and 99% of the ^{14}C residue from the leaves/stems, respectively. The 2M HCl extraction removed the remaining 15% of the residue from the leaves/stems fraction. The hydroponic solution and the methanol fractions were characterized by 2-dimensional thin layer chromatography.

Table VIII.

Root Absorption of Sulfosate Anion (CMPA)

Sample	dpm	% of Applied	CMPA (ppm)
Leaves/Stems	1.72×10^6	1.7	1.2
Roots	5.60×10^6	5.6	16.7
Solution	73.8×10^6	74.0	2.46
Total (with cleaning rinses)	89.1×10^6	89.1	

The autoradiogram corresponded to the numbers depicted in Table VIII. Most of the radioactivity in the plant remained in the roots with some translocated to the leaves. Thus, the image of the entire plant was visible in the autoradiogram with the roots as the darkest area.

The leaves/stems and the root samples were homogenized and extracted with distilled water and the extract analyzed by thin layer chromatography. No data are given for the analysis. The petitioner states that the residue was primarily CMPA with less than 1% being AMPA. These data are required. CBTS requires that 90% of the residue be identified.

CBTS finds the submitted metabolism studies for the anionic moiety of sulfosate on soybeans to be inadequate. The hydroponic study seems to indicate that virtually no metabolism takes place within the plant, but no quantitation data were submitted. In the preemergent study, it is required that the metabolism study be repeated separating the plants sufficiently that control and sample background levels generated from $^{14}CO_2$ from soil will be kept at a minimum. CBTS requires that the nature of at least 90% of the residue in the plant be determined. Additional raw data detailing the quantitation of the radioactivity (such as sample weights, raw counts in each sample, etc.) are required for all studies. The reviewer should have sufficient detailed data to reproduce all calculations used to generate the final tabulated data.

ANALYTICAL METHOD (MRID# 412099-19 414621-02)

Two analytical methods were submitted for the analysis of the residues of sulfosate in soybeans, one each for the cationic and anionic portions of the compound. Both were reviewed previously (PP#9F3796, S.Koepke, 1/4/91) and found to be adequate for corn pending method trials. Both were developed at ICI's Western Regional Center and are report numbers: WRC 85-33 (cation) and WRC 85-34R (anion). Both methods have been submitted for a method trial and all subsequent conclusions are subject to a successful outcome of these trials.

The method used to obtain residue data on the trimethylsulfonium cation was ICI's WRC 85-33R: Determination of SC-0224 Cation Residues in Crops, Water and Soil by Gas Chromatography. The detection limits are listed as 0.05 ppm in water, soil and crops and 0.1 ppm for animal feed and forage products.

Briefly, crops are extracted with water with conditions dependent on the water and oil content of that crop. Soil samples are extracted with aqueous potassium hydroxide. The aqueous extracts of all samples may be acidified as in the determination of the anions. Crop samples that have an apparent measurable background level of trimethylsulfonium (TMS) or dimethylsulfide (DMS) are eluted through a weak cation exchange resin to lower the background. A secondary clean-up step involving elution through a strong cation exchange resin is necessary for those crops that

still have an apparent high background level.

The eluate is concentrated to almost dryness by heating and then dealkylated with base at 100°C for 1 hour. The samples are then ready for gas chromatographic analysis. Samples that still have an apparent high background level (>0.05 ppm) are eluted through CC-4 adsorbent prior to chromatography.

Various gas chromatography conditions can be used depending on the columns and type of chromatograph (capillary or packed column) available. Detection is by a flame photometric detector operated in the sulfur mode.

Preliminary findings in the method trials indicate that certain minor problems with the cation methods remain to be resolved (ACL memo, 1/30/91, H. Hunley). The method is required to be re-written addressing these points prior to method trials. A summary of ACL's findings follows:

This method is written as a general method for the cation of sulfosate and requires clarification in the fresh fruits and vegetable extraction procedure. The method refers to the anion analytical method for the acidification procedure rather than delineating the procedure. A regulatory method should have all necessary steps delineated in the directions, it should not be necessary to refer to another procedure. In addition, no criteria are given as to when the strong cation resin clean-up procedure is necessary. The catalog number of the vials is required. A table of commodities requiring the post dealkylation CC-4 column clean-up is required to be provided. The analyst should not be required to run controls to determine if this procedure is necessary. Finally, the materials and methods section lists two additional gas chromatography columns but no operating parameters are given. These are required. An explanation as to why trimethylsulfonium iodide is used for the calibration rather than dimethylsulfide as used in the milk, eggs and edible tissues method is required. A copy of these preliminary findings by ACL is attached.

The method used to obtain residue data on the sulfosate anion was ICI's WRC 85-34R: Determination of SC-0224 Anion Residues in Crops, Water and Soil by Liquid Chromatography. The detection limits are listed for CMP and AMPA as 0.01 ppm in water, 0.05 ppm in soil and crops and 0.1 ppm for animal feed and forage products.

Briefly, CMP and AMPA are extracted from crop samples with water under conditions dependent on the water and oil content of the crop and from soil samples with NH_4OH. After extraction, the samples are acidified and crop samples are eluted through a

cation exchange resin column. All samples are then evaporated to dryness at 50°C.

Residues are derivatized with 9-fluorenylmethyl chloroformate in borate buffer, giving fluorescent derivatives. The excess reagent is extracted with ethyl acetate. The residues are chromatographed on hplc using an anion exchange column and fluorescence detection. Regular washings of the hplc column are recommended.

Preliminary findings in the method trials indicate that certain minor problems with the cation methods remain to be resolved (ACL memo, 1/30/91, H. Hunley). The method is required to be re-written addressing these points prior to method trials. A summary of ACL's findings follows:

ACL requests the catalog number for the hplc in line filter used in the analyses. Further detail as to when gravity elution or 1 to 2 psi pressure in the cation exchange cleanup is required. In addition, a table of commodities requiring this cleanup is required. The analyst should not be required to determine this. Further detail as to how the FMCL derivative is isolated for analysis is required. Further detail on the hplc conditions in the crop analysis method, in particular the mobile phase conditions for each analyte, is required. In the milk, eggs and edible tissues method, details as to how the aqueous phases are transferred are required. A copy of these preliminary findings by ACL is attached.

Method validations for soybean forage, hay and seed were accomplished by fortification with either TMS, CMP or AMPA in each crop fraction and measuring recoveries. Recoveries for TMS ranged from 72 to 120% with an average of 95.2% for soybean seed when fortified at 0.05, from 70 to 126% with an average of 86.7% for soybean forage when fortified at 0.10 and 0.70 ppm and from 70 to 127% with an average of 86.5% for soybean hay when fortified at 0.10 and 0.70 ppm. Recoveries for CMP ranged from 70 to 120% with an average of 90.0% for soybean seed when fortified at 0.10 and 0.20 ppm, from 74 to 108% with an average of 87.3% for soybean forage when fortified at 0.10 and 0.20 ppm and from 73 to 106% with an average of 92.9% for soybean hay when fortified at 0.10 and 0.20 ppm. Recoveries for AMPA ranged from 66 to 100% with an average of 86.0% for soybean seed when fortified at 0.10 and 0.20 ppm, from 71 to 92% with an average of 83.0% for soybean forage when fortified at 0.10 ppm and from 71 to 100% with an average of 84.2% for soybean hay when fortified at 0.10 ppm.

In the TMS analyses, there is a considerable background of TMS in control samples even after weak cation column cleanup. TMS is a

naturally occurring compound. Untreated control levels ranged from 0 to 0.053 ppm in soybean straw, from 0 to 0.051 ppm in soybean seed. In the CMP analyses, untreated control levels ranged from 0 to 0.14 ppm in soybean straw, from 0 to 0.09 ppm in soybean seed. In the AMPA analyses, untreated control levels ranged from 0 to 0.03 ppm in soybean straw, from 0 to 0.11 ppm in soybean seed.

Both the analytical methods (WRC 85-33 (cation) and WRC 85-34R (anion)) can also be applied to soybean processed products. Method validation for hulls, meal, soapstock, crude and refined oil was accomplished by fortification with either TMS, CMP or AMPA in each crop fraction and measuring recoveries. Recovery for TMS when fortified at 0.05 ppm was 73% for soybean hulls, 76% for soybean meal, 72% for soybean soapstock, 70% for crude soybean oil and 72% for refined soybean oil. Recovery for CMPA when fortified at 0.10 ppm was 101% for soybean hulls, 82% for soybean meal, 80% for soybean soapstock and when fortified at 0.05 ppm, 98% for crude soybean oil and 80% for refined soybean oil. Recovery for AMPA when fortified at 0.10 ppm was 73% for soybean hulls, 87% for soybean meal, 65% for soybean soapstock and when fortified at 0.05 ppm 98% for crude soybean cil and 84% for refined soybean oil.

In the TMS analyses of processed commodities, there is a considerable background of TMS in control samples even after weak cation column cleanup. TMS is a naturally occurring compound that appears to concentrate in soybean meal (0.16 ppm background). Untreated control levels were below the limit of detection for soybean stalks, crude oil, refined oil and soapstock. Soybean hulls have a background level of 0.022 ppm. For CMP untreated control levels were below the limits of detection for soybean crude oil, refined oil and soapstock. Untreated control levels were 0.25 ppm in soybean stalks, 0.15 ppm in soybean hulls and 0.05 ppm in soybean meal. For AMPA untreated control levels were below the limits of detection for soybean stalks, crude oil and refined oil. Untreated control levels were 0.13 ppm in soybean soapstock, 0.09 ppm in soybean hulls and 0.10 ppm in soybean meal.

Two analytical methods were submitted for the analysis of the residues of sulfosate in milk, eggs and edible tissues, one each for the cationic and anionic portions of the compound. Both were developed at ICI's Western Regional Center and are report numbers: RRC 87-42 (cation) and RRC 87-41 (anion). These methods were received after August 1, 1989 and must be subjected to an independent method validation trial prior to an Agency method trial (PR Notice 88-5, 7/15/88).

The method used to obtain residue data on the trimethylsulfonium cation was ICI's RRC 87-42: Determination of Sulfosate Cation

Residues in Milk, Eggs and Edible Tissues by Gas Chromatography. The detection limits are listed as 0.01 ppm in cow fat, 0.02 ppm in milk, eggs and cow kidney, cow fat, 0.04 ppm in cow muscle, 0.05 ppm in chicken muscle, kidney, fat and liver and 0.08 ppm in cow liver.

Samples are extracted with water/acetone with conditions dependent on the water and oil content of that sample. Milk is blended with acetone and the liquid portion decanted and centrifuged. The liquid portion is evaporated and dissolved in water for dealkylation. Eggs are treated in the same manner as milk. Cow muscle, liver or fat are blended with a mixture of water and acetone (6:54 v/v) and the liquid portion decanted and centrifuged. The liquid portion is evaporated, redissolved in water and heated to 100°C for 10 min. The sample is ready for dealkylation. Cow kidney is blended in the same manner as cow muscle. The liquid portion is absorbed into a charcoal-diatomaceous earth column. The column is eluted with aqueous acetone (10:90 v/v) and the eluant evaporated to dryness. The sample is dissolved in water in preparation for dealkylation. Chicken muscle, kidney and fat is blended with water and centrifuged. For chicken fat the sample is ready for dealkylation as below. For chicken muscle and kidney, the liquid portion is heated at 100°C for 10 min and the sample filtered. These samples are dealkylated as below. Chicken liver is blended with a methanol water mixture (1:1 v/v), centrifuged and the methanol evaporated on a hot plate. The sample is ready to be dealkylated. The liquid portions of all samples may require filtration prior to dealkylation.

The samples are dealkylated with base at 100°C for 1 hour. The samples are then ready for gas chromatographic analysis.

Various gas chromatography conditions can be used depending on the columns and type of chromatograph (capillary or packed column) available. Detection is by a flame photometric detector operated in the sulfur mode.

Preliminary findings in the method trials indicate that certain minor problems with the cation methods remain to be resolved (ACL memo, 1/30/91, H. Hunley). The method is required to be re-written addressing these points prior to method trials. A summary of ACL's findings follows:

This method is written as a general method for the cation of sulfosate in eggs , milk and edible tissues and requires clarification in the sample concentration step for all the relevant commodities. No specific directions or temperatures are given for evaporating the samples to dryness. In addition, there is an interfering peak in the cow liver and milk controls. Modification of the method is required to eliminate these

interferences. A copy of these preliminary findings by ACL is attached.

The method used to obtain residue data on the carboxymethylaminomethylphosphonate anion (CMPA) and its metabolite, aminophosphonic acid (AMPA) was ICI's RRC 87-41: Determination of Sulfosate Anion Residues in Milk, Eggs and Edible Tissues by Liquid Chromatography. The detection limits for both CMP and AMPA are listed as 0.02 ppm in milk, 0.05 ppm in cow and chicken muscle, kidney, fat, and chicken liver, 0.2 ppm in cow liver and 0.01 ppm CMP and 0.02 ppm AMPA in eggs.

Briefly, CMP and AMPA are extracted from samples with water under conditions dependent on the water and oil content of the sample. Milk is shaken with acetic acid-water (6:94 v/v) and the liquid portion decanted and centrifuged. The aqueous portion is taken for clean-up. Eggs, muscle or fat are blended with water and the liquid portion decanted and centrifuged. The liquid portion is acidified prior to clean-up. Liver or kidney are blended in the same manner as eggs, muscle or fat. After centrifugation, the liquid portion washed with chloroform and acidified for clean-up. After acidification, the samples are eluted through a cation exchange resin column. All samples are then evaporated to dryness at 50°C.

Residues are derivatized with 9-fluorenylmethyl chloroformate in borate buffer, giving fluorescent derivatives. The excess reagent is extracted with ethyl acetate. The residues are chromatographed on hplc using an anion exchange column and fluorescence detection. Regular washings of the hplc column are recommended.

Method validations for eggs, chicken kidney, liver, fat and muscle were accomplished by fortification with either TMS, CMP or AMPA in each sample type and measuring recoveries. Recoveries for TMS ranged from 82 to 110% with an average of 97% for eggs fortified at the 0.05 ppm level, from 70 to 83% with an average of 76% for chicken kidney fortified at the 0.092, and 0.23 ppm levels, from 71 to 117% with an average of 91.7% for chicken liver fortified at the 0.092 and 0.46 ppm levels, from 75 to 116% with an average of 93.3% for chicken fat fortified at the 0.092 and 0.23 ppm levels and from 75 to 109% with an average of 87.3% for chicken muscle fortified at the 0.092 and 0.23 ppm levels. Recoveries for CMP ranged from 71 to 108% with an average of 82.2% for eggs fortified at the 0.01, 0.02, 0.03, 0.04 and 0.40 ppm levels, from 66 to 108% with an average of 85% for chicken kidney fortified at the 0.2, 0.4 and 0.5 ppm levels, from 64 to 75% with an average of 70.7% for chicken liver fortified at the 0.2, 0.5 and 1.0 ppm levels, from 90 to 114% with an average of 102% for chicken fat fortified at the 0.2 and 0.5 ppm levels and

from 71 to 78% with an average of 74% for chicken muscle fortified at the 0.2 and 0.5 ppm levels. Recoveries for AMPA ranged from 60 to 100% with an average of 77.5% for eggs fortified at the 0.01, 0.02, 0.03, 0.05 and 0.4 ppm levels, from 58 to 71% with an average of 66.3% for chicken kidney fortified at the 0.2, 0.4 and 0.5 ppm levels, from 66 to 93% with an average of 80.3% for chicken liver fortified at the 0.2, 0.5 and 1.0 ppm levels, from 85 to 86% with an average of 85.5% for chicken fat fortified at the 0.2 and 0.5 ppm levels and from 66 to 87% with an average of 73.7% for chicken muscle fortified at the 0.2 and 0.5 ppm levels.

Method validations for milk, cow kidney, liver, fat and muscle were accomplished by fortification with either TMS, CMP or AMPA in each sample type and measuring recoveries. Recoveries for TMS ranged from 70 to 120% with an average of 100.5% for milk fortified at the 0.02, 0.207 and 2.07 ppm levels, from 41 to 102% with an average of 72.6% for cow kidney fortified at the 0.03, 0.207 and 2.07 ppm levels, from 40 to 90% with an average of 72.1% for cow liver fortified at the 0.08, 0.41 and 4.14 ppm levels, from 75 to 100% with an average of 86.3% for cow fat fortified at the 0.04, 0.207 and 2.07 ppm levels and from 78 to 119% with an average of 93.7% for cow muscle fortified at the 0.04, 0.08, 0.207 and 2.07 ppm levels. Recoveries for CMP ranged from 83 to 107% with an average of 95.4% for milk fortified at the 0.02, 0.05, 0.1, 0.2 and 0.5 ppm levels, from 81 to 87% with an average of 84% for cow kidney fortified at the 0.5, 1.0 and 2.0 ppm levels, from 67 to 75% with an average of 71.7% for cow liver fortified at the 0.5 and 1.0 ppm levels, from 65 to 84% with an average of 77.3% for cow fat fortified at the 0.2 and 0.5 ppm levels and from 69 to 96% with an average of 79.3% for cow muscle fortified at the 0.2 and 0.5 ppm levels. Recoveries for AMPA ranged from 73 to 121% with an average of 93.9% for milk fortified at the 0.02, 0.05, 0.1, 0.2 and 0.5 ppm levels, from 64 to 91% with an average of 73.3% for cow kidney fortified at the 0.5, 1.0 and 2.0 ppm levels, from 46 to 66% with an average of 56.7% for cow liver fortified at the 0.5 and 1.0 ppm levels, from 83 to 100% with an average of 89% for cow fat fortified at the 0.2 and 0.5 ppm levels and from 73 to 86% with an average of 80.7% for cow muscle fortified at the 0.2 and 0.5 ppm levels.

CBTS concluded that sufficient detail was provided to submit the methods for method trials. ACL has requests for clarifications on each of these methods. A copy of their preliminary findings is attached. Contingent upon these clarifications and the successful completion of these method trials, CBTS would consider the methods to be adequate for the both anionic and cationic moieties of sulfosate. The milk, egg and edible tissue method for the cationic moiety of sulfosate requires further development prior to the initiation of its method trial. There are interferences in milk and cow liver that need to addressed. Both

methods for milk, eggs and edible tissues were received after August 1, 1989 and must be subjected to an independent method validation trial prior to an Agency method trial (PR Notice 88-5, 7/15/88).

RESIDUE DATA (MRID# 412099-17,18)

Fifteen field trials were carried out in eleven different states (Mississippi, Alabama, Iowa[3], Illinois[3], Arkansas, Minnesota, Missouri, Tennessee, Nebraska, Virginia and Indiana) in 1988 using sulfosate on soybeans (See figure 1). These states correspond to 76.6% of all soybean production for in the United States (See Figure 2 for areas of major soybean production) and represent the major climatic regions where soybeans are grown (Agricultural Statistics 1986). CBTS considers geographical representation to be adequate.

Eleven field trials were protocolled as magnitude of the residue and four as processing studies. Each trial received a broadcast pre-emergent application of 8 lbs a.i./A (except two process trials which received 24 lbs a.i./A), a spot treatment of 2 lbs a.i./A and a wick application using a 33% (v/v) solution. The soybeans were grown to maturity in each trial following the agronomic practices for the region.

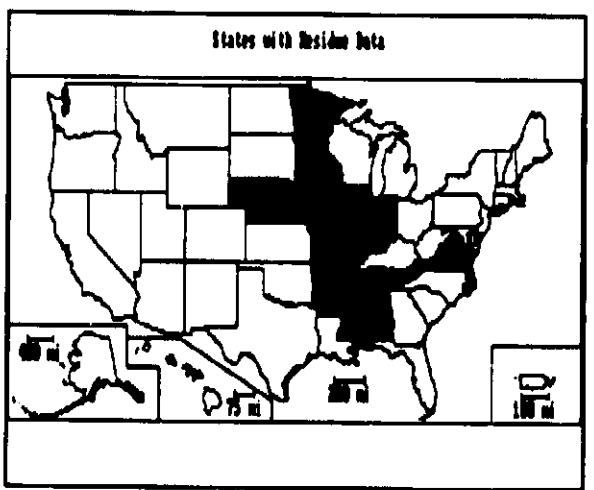

Figure 1. States for Which Residue Data are Available.

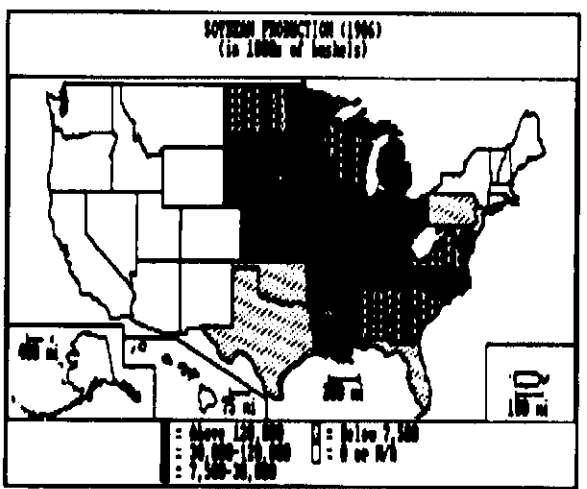

Figure 2. Soybean Production in the United States (1986).

Forage samples were collected at the beginning pod stage. Each sample consisted of at least 2.5 lbs of forage collected from at least twelve separate plants. Eight of the eleven forage samples received only the pre-emergence broadcast application. Three samples (Missouri, Nebraska and Virginia) received a non-protocolled spot application in addition to the pre-emergence

TABLE IX.
Residue Data in Soybeans

Location	PHI* (days)	Rate (lb ai/A)	Sample Type	TMS (ppm)	CMP (ppm)	AMPA (ppm)
Mississippi	65	10.0	Forage	0.10	<0.10	<0.10
	51		Hay	<0.10	<0.10	<0.10
	7		Seed	0.14	<0.10	<0.10
Alabama	79	10.0	Forage	<0.10	<0.10	<0.10
	61		Hay	<0.10	<0.10	<0.10
	61		Seed	<0.05	<0.20	<0.20
Iowa	63	10.0	Forage	<0.10	<0.10	<0.10
	55		Hay	<0.10	<0.10	<0.10
	6		Seed	0.10	0.35	0.17
Illinois	78	10.0	Forage	0.73	0.60	<0.10
	56		Hay	<0.10	<0.10	<0.10
	7		Seed	0.05	<0.10	<0.10
Arkansas	0	10.0	Forage	<0.10	<0.10	<0.10
	56		Hay	0.25	0.10	<0.10
	6		Seed	0.07	<0.10	<0.10
Minnesota	0	10.0	Forage	<0.10	<0.10	<0.10
	55		Hay	0.61	<0.10	<0.10
	6		Seed	0.73	<0.20	0.10
Missouri	16	10.0	Forage	0.78	0.38	<0.10
	16		Hay	0.88	<0.10	<0.10
	7		Seed	0.11	<0.10	<0.10
Tennessee	0	10.0	Forage	<0.10	<0.20	<0.10
	67		Hay	0.10	<0.20	<0.10
	14		Seed	0.19	<0.20	<0.20
Nebraska	26	10.0	Forage	<0.10	<0.10	<0.10
	34		Hay	<0.10	<0.10	<0.10
	11		Seed	<0.05	<0.10	<0.10
Virginia	6	10.0	Forage	<0.10	<0.10	<0.10
	69		Hay	<0.10	<0.10	<0.10
	5		Seed	0.05	<0.20	<0.20
Indiana	89	10.0	Forage	<0.10	<0.10	<0.10
	142		Hay	1.19	2.7	0.38
	15		Seed	0.22	1.7	<0.20
Illinois	7	10.0	Seed	<0.05	0.11	<0.10
	7	26.0	Seed	0.16	0.29	<0.10
Iowa	11	10.0	Seed	0.09	<0.20	<0.10
	11	26.0	Seed	0.40	1.04	0.50

* Time from last application: For forage, time from pre-emergent application; For hay, time from spot treatment; for seed, time from wiper application.

treatment. Hay samples are defined as whole plant including seeds and pods. Each hay sample consisted of at least 1.0 lb of hay from at least twelve separate plants. All hay samples received a pre-emergent application and a spot treatment. Hay samples were collected approximately eight weeks following the spot treatment. Exceptions were Missouri (2 weeks), Tennessee (9 weeks), Nebraska (4 weeks) and Virginia (10 weeks). Seed samples were collected from plots receiving a wick application in addition to broadcast and spot applications. Each seed sample consisted of at least 2.5 lbs of seed collected from at least twelve separate areas of the test plot. Two samples (Arkansas and Tennessee) received an additional wick application since the seeds were not mature enough within seven days after the first wick application. All seed samples were harvested 5 to 15 days following the wick application. All samples were frozen immediately and stored at -20°C until analysis. Samples wereextracted for analysis anywhere from 240 to 421 days after harvest.

TABLE X.

Storage Stability

	Percent Recovery			
Matrix/Analyte	0-Day	6-Mo	1-Yr	2-Yr
Soybeans				
TMS fortified at 0.46 ppm	86	69	90	102
CMP fortified at 1.0 ppm	100	108	75	104
AMPA fortified at 1.0 ppm	90	86	106	81
Soybean Straw				
TMS fortified at 0.46 ppm	66	74	76	64
CMP fortified at 1.0 ppm	100	102	84	106
AMPA fortified at 1.0 ppm	74	70	93	82

Residue data are presented in Table IX. For the TMS residue, the maximum values found were: 0.73 ppm in soybean seed (Minnesota), 1.19 ppm in soybean hay (Indiana) and 0.78 ppm in soybean forage (Missouri). For the CMP residue, the maximum values found were: 1.7 ppm in soybean seed (Indiana), 2.7 ppm in soybean hay (Indiana) and 0.60 ppm in soybean forage (Illinois). For the

AMPA residue, the maximum values found were: 0.0.50 ppm in soybean seed (Iowa processing study), 0.38 ppm in soybean hay (Indiana) and no detectable residues above background in any soybean forage sample.

Storage stability data were submitted for all three analytes in soybean seed and soybean straw (Table X.). Separate samples were spiked with each analyte and stored at -20°C for the requisite time period before extraction and analysis. All three analytes were stable for up to two years in these matrices. These data were reviewed previously (PP#9F3796, S.Koepke, 10//90) and found to be adequate pending method trials. CBTS considers the storage stability data to be adequate for soybean seed, forage and hay.

The combined residues (CMP and AMPA) in the Indiana field trial exceed the requested tolerance of 3.0 ppm for soybean hay by 0.08 ppm at the exaggerated rate. The other requested tolerances would be adequate (soybean seed @ 2.0 ppm and soybean forage @ 1.0 ppm) even at the exaggerated rate. However, tolerances already exist for glyphosate on soybean seed @ 6 ppm, soybean forage @ 15 ppm and soybean hay @ 15 ppm. The existing regulatory method and the proposed analytical methods for sulfosate cannot differentiate the source of the anionic moiety Therefore, the tolerances requested would require a revision of 40 CFR 180.3 to include sulfosate as a related herbicide to glyphosate and that the enforcement tolerance would be based on the higher of the two approved tolerances.

Since soybean is a major crop additional residue data are required. Fifteen values all from the same crop year for the magnitude of the residue in soybean are insufficient. At least ten additional geographically relevant field trials are required.

The seed samples from the Illinois field trial were submitted for a soybean processing trial. Two samples, both a 1x and 3x were processed by the Food Protein Research and Development Center, The Texas A&M University System, College Station, Texas 77843-2476. The processing consisted of a dry-milling procedure similar to large-scale commercial operations.

The seeds are first dried to achieve a moisture content of 10% or less. Following drying, the soybeans are cracked to produce hulls, flaked and extracted with hexane. The flakes are dried to become meal and the solvent is removed from the crude oil by evaporation at 75-85 °C. This crude product is degummed by refining with water and the crude lecithin separated out leaving the crude oil. The oil is further refined with sodium hydroxide yielding the refined oil and soapstock. Samples of all fractions were frozen and shipped to ICI's Western Research Center for analysis.

Results of the residue processing study are listed in Table XI. An extremely disconcerting piece of data is the differences between the levels of residue in the seed samples submitted by the processor and that submitted by the field trialist. There

Table XI.

Residue Levels in Processing Study

Commodity	Rate (lbs a.i./A)	Residue (ppm) TMS	CMP	AMPA
Seed[a]	10.0	<0.05	0.11	<0.10
Seed[a]	26.0	0.16	0.29	<0.10
Seed[b]	10.0	<0.05	<0.10	<0.10
Seed[b]	26.0	<0.05	<0.10	<0.10
Hulls	10.0	<0.05	<0.10	<0.10
Hulls	26.0	<0.05	<0.10	<0.10
Meal	10.0	<0.05	<0.10	<0.10
Meal	26.0	<0.05	<0.10	<0.10
Soapstock	10.0	<0.05	<0.10	<0.10
Soapstock	26.0	<0.05	<0.10	<0.10
Crude oil	10.0	<0.05	<0.05	<0.05
Crude oil	26.0	<0.05	<0.05	<0.05
Refined oil	10.0	<0.05	<0.05	<0.05
Refined oil	26.0	<0.05	<0.05	<0.05

[a] Samples submitted directly from the field trialist.
[b] Samples submitted from the processor.

are measurable levels in the field trialist's sample, but no measurable residues in the processor's sample. Both measurements were made on subsamples of the original field sampling. The measured levels should be not necessarily be identical, but

certainly similar in value. CBTS does not consider the processing study to be adequate since the processor's sample did not contain any measurable residue. It is required that the processing study be repeated using a seed sample that has measurable residues at or near the proposed tolerance level in order to determine if the residues concentrated in any processed fraction.

MEAT, MILK, POULTRY AND EGGS (MRID# 412099-11,12,13,14)

No new metabolism data were submitted with this petition. Magnitude of the residue studies were submitted for meat, milk, poultry and eggs. All conclusions are subject to successful completion of the analytical method trials.

Feeding studies were carried out on six groups of three lactating dairy cattle were dosed daily for 28 days with sulfosate equivalent to 0, 0.5, 5, 50, 300 and 1000 ppm by Hazelton Laboratories, America, Inc.. The doses were prepared in gelatin capsules and given by gavage. The animals were observed for general condition and activity. Body weight, feed consumption and milk production were monitored. Milk samples were collected at intervals for analysis. After 28 days, dosing was discontinued and two animals from each group were sacrificed. After 7 days of withdrawal, the remaining animals were sacrificed. At sacrifice, samples of liver, kidney, muscle and fat were collected for residue analysis. All sample analyses were performed at ICI's Western Research Center. Results are presented in Tables XII-XXI. All samples were analyzed between 11 and 66 days after collection. Storage stability data were submitted for each analyte and sample type and indicate that residues of CMP are stable up to 671 days in milk, 687 days in muscle and fat, 1311 days in liver and 1304 days in kidney. Similar data indicate that residues of AMPA are stable up to 671 days in milk, 687 days in muscle and fat, 691 days in liver and 1311 days in kidney. Similar data indicate that residues of TMS are stable up to 683 days in milk, 687 days in muscle and fat, 1311 days in liver and 1325 days in kidney.

Maximum residue levels in milk for TMS were below the limit of detection for the 0.5 and 5 ppm feeding studies and range from 4.0 ppm at 1000 ppm to 0.93 ppm at the 300 ppm feeding level and 0.18 ppm at the 50 ppm feeding level. Residue levels were at or below the detection limits in milk of 0.02 ppm for both CMP and AMPA. The maximum value for CMP was 0.04 ppm at the 1000 ppm feeding level.

Of the 3 analytes, CMP was present in the highest concentrations in the kidney at each dosage level. The highest values were 7.6 ppm at 1000 ppm, 2.6 ppm at 300 ppm, 0.44 ppm at 50 ppm, and less

Table XII.
Dosage Level 1000 ppm/day
MILK

Time Interval (days)	TMS (ppm)	CMP (ppm)	AMPA (ppm)
-1	<0.02	<0.02	<0.02
1	1.3 - 1.9	<0.02	<0.02
2	1.4 - 4.0	0.02	<0.02
4	1.3 - 3.3	0.02 - 0.03	<0.02
7	1.2 - 2.4	0.03 - 0.04	<0.02
14	1.5 - 2.5	0.02	<0.02
21	1.0 - 3.0	<0.02 - 0.02	<0.02
28	1.2 - 1.8	<0.02 - 0.03	<0.02
35	0.48[a]	<0.02	<0.02

[a] Feeding of active ingredient was stopped after 28 days.

Table XIII.
Dosage Level 1000 ppm/day
Tissues

	TMS (ppm)	CMP (ppm)	AMPA (ppm)
Kidneys	0.05 - 4.5	0.18 - 7.6	0.24 - 1.7
Liver	0.14 - 2.0	<0.2 - 0.51	<0.2
Fat	0.05 - 0.08	0.06 - 0.10	<0.05
Muscle	<0.04 - 1.6	<0.05 - 0.08	<0.05

Table XIV.
Dosage Level 300 ppm/day
MILK

Time Interval (days)	TMS (ppm)	CMP (ppm)	AMPA (ppm)
-1	<0.02	<0.02	<0.02
1	0.37 - 0.48	<0.02 - 0.02	<0.02
2	0.47 - 0.67	<0.02	<0.02
4	0.43 - 0.93	<0.02 - 0.02	<0.02
7	0.46 - 0.79	<0.02	<0.02
14	0.67 - 0.86	<0.02	<0.02
21	0.41 - 0.62	*	*
28	0.40 - 0.54	*	*
35	0.02 - 0.05[a]	<0.02	<0.02

*= not analyzed
[a] Feeding of active ingredient was stopped after 28 days.

Table XV.
Dosage Level 300 ppm/day
Tissues

	TMS (ppm)	CMP (ppm)	AMPA (ppm)
Kidneys	0.04 - 1.9	0.12 - 2.6	<0.05 - 0.58
Liver	0.15 - 0.69	<0.2	<0.2
Fat	<0.01 - 0.10	0.06	<0.05
Muscle	0.05 - 0.63	<0.05	<0.05

Table XVI.
Dosage Level 50 ppm/day
MILK

Time Interval (days)	TMS (ppm)	CMP (ppm)	AMPA (ppm)
-1	<0.02	*	*
1	0.05 - 0.11	<0.02	<0.02
2	0.07 - 0.16	<0.02	<0.02
4	0.07 - 0.13	<0.02	<0.02
7	0.06 - 0.14	<0.02	<0.02
14	0.06 - 0.18	<0.02	<0.02
21	0.06 - 0.13	<0.02	<0.02
28	0.06 - 0.13	<0.02	<0.02
35	0.03 - 0.05[a]	<0.02	<0.02

[a] Feeding of active ingredient was stopped after 28 days.

Table XVII.
Dosage Level 50 ppm/day
Tissues

	TMS (ppm)	CMP (ppm)	AMPA (ppm)
Kidneys	0.02 - 0.18	<0.05 - 0.44	<0.05 - 0.08
Liver	0.12 - 0.32	<0.2	<0.2
Fat	0.01	<0.05 - 0.06	<0.05
Muscle	0.05 - 0.11	<0.05	<0.05

Table XVIII.
Dosage Level 5 ppm/day
MILK

Time Interval (days)	TMS (ppm)	CMP (ppm)	AMPA (ppm)
-1	<0.02 - 0.02	*	*
1	<0.02 - 0.03	*	*
2	0.02 - 0.03	*	*
4	0.02 - 0.03	*	*
7	0.02	*	*
14	<0.02 - 0.03	*	*
21	<0.02 - 0.02	*	*
28	0.02	<0.02	<0.02
35	<0.02 - 0.02[a]	*	*

*= not analyzed
[a] Feeding of active ingredient was stopped after 28 days.

Table XIX.
Dosage Level 5 ppm/day
Tissues

	TMS (ppm)	CMP (ppm)	AMPA (ppm)
Kidneys	<0.02 - 0.03	<0.05	<0.05
Liver	<0.08	<0.2	<0.2
Fat	<0.01	<0.05	<0.05
Muscle	0.04 - 0.05	<0.05	<0.05

Table XX.
Dosage Level 0.5 ppm/day
MILK

Time Interval (days)	TMS (ppm)	CMP (ppm)	AMPA (ppm)
-1	<0.02	*	*
1	<0.02	<0.02	<0.02
2	<0.02 - 0.03	<0.02	<0.02
4	<0.02 - 0.02	<0.02	<0.02
7	<0.02	<0.02	<0.02
14	<0.02 - 0.02	*	*
21	<0.02 - 0.03	*	*
28	<0.02	*	*
35	<0.02 - 0.02[a]	*	*

*= not analyzed
[a] Feeding of active ingredient was stopped after 28 days.

Table XXI.
Dosage Level 0.5 ppm/day
Tissues

	TMS (ppm)	CMP (ppm)	AMPA (ppm)
Kidneys	<0.02 - 0.02	<0.05	<0.05
Liver	<0.08 - 0.08	<0.2	<0.2
Fat	<0.01 - 0.01	<0.05	<0.05
Muscle	<0.04 - 0.04	<0.05	<0.05

than 0.05 ppm at the other doses.

In the liver, TMS was present in the highest concentrations at each dosage level. The highest values were 2.0 ppm at 1000 ppm,0.69 ppm at 300 ppm, 0.32 ppm at 50 ppm, and less than 0.08 ppm at the other doses. CMP was detectable only in the 1000 ppm study at 0.51 ppm.

Of the 3 analytes, CMP was present in the highest concentrations in the fat at each dosage level. The highest values were 0.10 ppm at 1000 ppm, 0.06 ppm at 300 ppm, 0.06 ppm at 50 ppm, and less than 0.05 ppm at the other doses. TMS was detectable only in feeding studies at levels above 5 ppm and the highest value was 0.15 ppm.

In the muscle, TMS was present in the highest concentrations at each dosage level. The highest values were 1.6 ppm at 1000 ppm,0.63 ppm at 300 ppm, 0.11 ppm at 50 ppm, and less than 0.05 ppm at the other doses. CMP was detectable only in the 1000 ppm study at 0.08 ppm.

CBTS finds the magnitude of the residue data for milk and meat to be adequate. At the feeding level equivalent to that present in the crop (<= 3 ppm), no measurable residues from parent were detected. CBTS concludes that sulfosate belongs to category 2 of 40 CFR 180.6(a), it is not possible to establish with certainty whether finite residues will be incurred, but there is a reasonable expectation of finite residues. Therefore, a revised Section F proposing tolerances of 0.04 ppm in milk, 0.1 ppm in cow muscle, kidney and fat and 0.4 ppm in cow liver is required. These values would be expressed as parent sulfosate calculated as either the sum of the magnitudes of the residues of AMPA and CMP or the magnitude of the TMS residue, whichever is greater.

Poultry residue studies were conducted by dosing by intubation daily four groups of 10 single-comb White Leghorn laying hens with 0, 0.5, 5 or 50 ppm of sulfosate for 28 days. The birds were observed for general condition and activity. Body weight,

feed consumption and egg production were monitored. Egg samples were collected at intervals for analysis. After 28 days, dosing was discontinued and seven birds from each group were sacrificed. After 7 days of withdrawal, the remaining birds were sacrificed.

TABLE XXII.

EGGS: 50 ppm Dose

Time Interval (Days)	CMP (ppm)	AMPA (ppm)	TMS (ppm)
-1	*	*	*
1	<0.010	<0.02	<0.02
2	<0.010	<0.02	<0.02
4	<0.010	<0.02	<0.02
7	0.010	<0.02	<0.02
14	0.011	<0.02	<0.02
21	0.015	<0.02	<0.02
28	0.014	<0.02	<0.02
35	<0.010	<0.02	<0.02

At sacrifice, samples of liver, kidney, muscle and fat were collected for residue analysis. All sample analyses were performed at ICI's Western Research Center. Results are presented in Tables XXII and XXIII. All samples were analyzed between 30 and 163 days after collection. Storage stability data were submitted for each analyte and sample type and indicate that residues of CMP are stable up to 683 days in egg, 687 days in muscle and fat, 1311 days in liver and 1304 days in kidney. Similar data indicate that residues of AMPA are stable up to 683 days in eggs, 687 days in muscle and fat, 691 days in liver and 1311 days in kidney. Similar data indicate that residues of TMS are stable up to 683 days in eggs, 687 days in muscle and fat, 1311 days in liver and 1325 days in kidney.

Except for a value of 0.072 ppm of CMP found in liver of the 5 ppm dose, no detectable levels of residues of any analyte were found in either the 0.5 or 5 ppm doses.

At the highest dose of 50 ppm sulfosate, maximum levels of residues plateau after 21 days in eggs at 0.015 ppm for CMP. No detectable levels of residues of the other analytes were present in eggs. Only three tissue samples exhibited measurable levels of residues at the high dose: 0.31 ppm of CMP in kidneys, 0.18 ppm of TMS in kidneys and 0.13 ppm of TMS in liver. No detectable levels of residues of any analyte were found in the lower doses.

CBTS finds the magnitude of the residue data for poultry and eggs to be adequate. At the feeding level equivalent to that present in the crop (<= 3 ppm), no measurable residues from parent were detected except in chicken kidney. CBTS concludes that sulfosate belongs to category 2 of 40 CFR 180.6(a), it is not possible to establish with certainty whether finite residues will be incurred, but there is a reasonable expectation of finite residues. Therefore, a revised Section F proposing tolerances of 0.1 ppm in chicken muscle, fat, and liver, and 0.03 ppm in eggs is required. These values would be expressed as parent sulfosate calculated as either the sum of the magnitudes of the residues of AMPA and CMP or the magnitude of the TMS residue, whichever is greater.

TABLE XXIII.

TISSUES: 50 ppm Dose

	CMP		AMPA		TMS	
	28 Days	35 Days	28 Days	35 Days	28 Days	35 Days
Kidneys	0.31 (0.29)	0.11	<0.05	<0.05	0.18 (0.18)	<0.05
Liver	<0.05	<0.05	<0.05	<0.05	0.13 (0.12)	0.065
Fat	<0.05 (<0.05)	<0.05 (<0.05)	<0.05	<0.05	<0.05	<0.05
Muscle	<0.05	<0.05	<0.05	<0.05	0.05	<0.05

The previously submitted laying hen metabolism studies for both the anion and cation were found to be inadequate (PP#9F3796, S.Koepke, 1/4/91). The nature of the residue in eggs and poultry is not defined. All conclusions in this review are contingent upon the residues of concern in eggs and poultry being TMS, CMP and AMPA.

The previously submitted goat metabolism studies for both the anion and cation were found to be inadequate (PP#9F3796, S.Koepke, 1/4/91). The nature of the residue in milk and ruminant edible tissues is not defined. All conclusions in this review are contingent upon the residues of concern in milk and ruminant edible tissues being TMS, CMP and AMPA.

OTHER CONSIDERATIONS

An "International Residue Status" sheet is attached. There are

no Canadian, Mexican or Codex tolerances for Sulfosate on or in soybean. There are Codex limits of 5.0 ppm for soybean (dry), 0.2 ppm for soybean (immature seeds), 20 ppm for soybean fodder and 5 ppm for soybean forage (green) for glyphosate per se. There is also a Canadian negligible residue type limit of 0.1 ppm for glyphosate per se for "all food crops". There are no compatibility problems associated with this petition.

Attachments: International Residue Limit Status Sheet.
ACL preliminary comments.

CC: S. Koepke (CBTS),PP0F3860, PIB/FOB (C. Furlow), Circulation(7),RF,SF

H7509C:CBTS:Reviewer(SK):CM#2:Rm810:557-4380:Typist(SK):4/19/91.
RDI:Section Head: R.S. Quick:4/19/91: Br.Sr.Scientist:R.A. Loranger:4/24/91.

Attachment: 1

Page 1 of 1

INTERNATIONAL RESIDUE LIMIT STATUS

CHEMICAL Sulfosate (trimethylsulfonium salt of glyphosate)

CODEX NO. NA

CODEX STATUS:

[✓] No Codex Proposal Step 6 or Above *

Residue (if Step 8): _____

Glyphosate per se for glyphosate MRLs

Crop(s)	Limit (mg/kg)

PROPOSED U.S. TOLERANCES:

Petition No. OF3860

DEB Reviewer S Koepke

Residue: parent, carboxymethylamino, phosphonic acid as combined residue

Crop(s)	Limit (mg/kg)
soybean seed	2 ppm
soybean forage	1 ppm
soybean hay	3 ppm

CANADIAN LIMITS:

[✓] No Canadian Limit **

Residue: glyphosate

Crop(s)	Limit (mg/kg)

MEXICAN LIMITS:

[✓] No Mexican Limit

Residue: _____

Crop(s)	Limit (mg/kg)

NOTES

* No Codex soybean limits for sulfosate per se. There are Codex Step 8 limits of 5, 0.2, 20 and 5 for soybean (dry), soybean (immature seeds), soybean fodder and soybean forage (green) respectively.

** No Canadian soybean limit for glyphosate per se. There is a 0.1 ppm negligible residue type Canadian limit for all food crops.

Form Revised 1989

ATTACHMENT 2

WRC-95-33R

TMV Pre-review of Touchdown
Cation Residues in Crops, Water, and Soil

Reviewed by: E.S. Greer
Date: 1-13-91
Analyte: TMS
Commodities: Corn grain and forage
Petitioner: ICI Americas INC.
Method: Determination of SC-0244 Cation Residues in Crops, water, and Soil by Gas chromatography

Section III. Apparatus And Reagents

A. Apparatus

B. Glass vials

No catalog number is given for vials.

Section IV. Procedure

A. Sample Extraction

1. Crops

Second paragraph for the extraction of fresh fruits and vegetables states that "aqueous extract may be acidified as described for the determination of SC-0224 anion". No criteria are given as to when acidifying is necessary, and no procedure is given for doing this. A reference to another method is sited for acidifying the extracts, but a regulatory method should describe all the steps needed to determine the residue. In the second paragraph on page 4 for the extraction of forage crops, grasses, and animal feeds the analyst is told to "acidify as above". It is unclear if this acidification step also applies to the extraction of grains, seeds, and nuts.

B. Pre-Dealkylation Clean-up

2. Strong Cation Resin

Method states that this is an optional clean-up that "may be used if necessary", but no criteria are given as to when this is the case.

D. Post Dealkylation CC-4 Mini Column Clean-up

No examples are given for the types of commodities that require this step. Also the analyst would have to run controls to determine if the background values exceeded the 0.05 PPM level that the method says requires this clean-up. A regulatory method precludes the use of controls as part of the procedure.

E. Gas Chromatography

A Megabore capillary column is listed in the apparatus section, but no operating conditions are given. Also no parameters are listed for the Super O confirmation column.

F Calibration

An analyst should not have to determine the dealkylation efficiency of a regulatory method. No specific directions are given for dealkylating the TMS calibration solutions. The question arises as to why trimethylsulfonium iodide is dealkylated to DMS and used as a reference Std. instead of using a DMS Std. for a referemce as is done in the tissue method. Would this indicate that the dealkylation step is inefficient? The last sentence is this section states " as described in III-B-10; this should be III-B-9 instead.

Comments

1. No recovery data is given for corn grain at the requested levels of 0.05 and 0.1 PPM. Data for corn stover is supplied for the 0.092 and .23 PPM levels.

2. No example chromatograms are included for corn grain.

3. The deficiences listed above by section will have to be addressed by the petitioner before this TMV can be initiated.

RRC-87-42

TMV Pre-review of Touchdown
Cation Residues in Milk, Eggs and Edible Tissue

Reviewed by: E.S. Greer
Date: 1-13-91
Analyte: TMS
Commodities: Milk and cow liver
Petitioner: ICI Americas INC.
Method: Determination of Sulfosate Cation Residues in Milk, Eggs, and Edible Tissues BY Gas chromatography

Section V. Procedure

A. Preparation of Control and Fortified Samples

The use of contols and fortified samples cannot be required in a regulatory method.

B. extraction

1. Milk

No specific directions or temperature are given for evaporating the acetone-water extract to dryness.

2. Eggs

No specific directions or temperature are given for evaporating the extract to 0.5ml.

3. Cow Muscle, Liver, Fat

No specific directions or temperature are given for evaporating the extract to dryness.

4. Cow Kidney

No specific directions or temperature are given for evaporating the clean-up column elute to dryness.

C. Alternative Clean-up

The alternative clean-up apparently does not apply to the commodities requested by DEB.

Comments

1. This method uses DMS as a calibration Std. instead of an in-house dealkylated TMS solution as is used for the crop method.

2. Recovery data is supplied for the commodities and levels requested.

3. Although the representitive chromatograms for milk and cow

liver samples show sharp highly resolved peaks, the untreated milk control(Figure 1.) has an interfering peak at the R.I. of the DMS with a peak height Approx. 44% of that from a 0.02 PPM spike. Cow liver controls(Figure 4) have an interfering peak with a peak height Approx. 68% of that from a 0.08 PPM spike. These chromatograms are not acceptable for a regulatory method.

4. These problems must be addressed by the petitioner before this TMV can be initiated.

WRC-85-34R

TMV Pre-review of Touchdown
Anion Residues In Crops, Soil, And Water

Reviewed by: E.S. Greer
Date: 1-7-91
Analytes: CMP, AMPA
Commodities: Corn grain and forage
Petitioner: ICI Americas INC.
Method: Determination of SC-0244 Anion Residues in Crops, Soil, and Water by Liquid Chromatography

Section III. Apparatus And Reagents

A. Apparatus

3. Filter

No catalog number is given for the HPLC inline filter.

12. Water Evaporator

See comments section.

Section IV. Procedure

D. Cation Exchange Cleanup

This section states "or use 1 to 2 psi pressure to hasten the resin washing or the sample elution", but just prior to eluting the CMP the statement, "Use gravity elution only." appears. Does gravity elution only apply to eliminating the "sugars and other interfering materials" or does it also apply to the elution of the analytes? Method states that the elution pattern of each lot of ion exchange resin should be checked with "real samples" prior to use. A residue method precludes the use of samples or controls for checking recoveries. The method also does not give any specific directions for calibrating the resin.

E.FMCL Derivative (Crops and Soil)

It is stated that the derivatives remain in the aqueous phase after the extraction with ethyl acetate, but no directions are given for isolating this phase before HPLC analysis.

H. HPLC Aanlysis

It is unclear as to what mobile phase is to be used with each analyte. For CMP analysis the analyst is told to refer to Table I, but this table shows that both the CMP and AMPA elute under the various conditions listed. A statement is made to

"Adjust the retention time between 15 and 40 minutes as may ocassionally be required for different sample types. In the next papagraph conditions are given for AMPA analysis, but a statement is made that the CMF does not elute under these conditions. The chromatograms included in the addendum don't make any references to the mobile phase and they show only one analyte per chromatogram. These problems are addressed satisfactorilly in the milk, egg and tissue method for these analytes(Method RRC 87-41).

Comments

1. Since ACL does not have an Evapo-Mix on hand, the lab's rotary evaporators will need adapters so they can be used with the 45ml centrifuge tubes required by the method.

2. ACL has been requested to analyze corn grain at 0.05PPM and corn forage at 0.1PPM, but no recovery data has been included. Chromatograms at all the levels requested for both commodities have been presented in the method appendix.

3. The method requires that the Stds. to be derivatized by the analyst.

4. The deficiencies list above by section number will have to be addressed by the petitioner before this TMV can be initiated.

RRC-87-41

TMV Pre-review of Touchdown
Anion Residues In Meat And Milk

Reviewed by: E.S. Greer
Date: 1-7-91
Analytes: CMP, AMPA
Commodities: Milk and cow liver
Petitioner: ICI Americas INC.
Method: Determination of Sulfosate Anion and Metabolite Residues in Milk, Eggs, and Edible Tissues by Liquid Chromatography

Section IV. Apparatus And Reagents

A. Apparatus

3. Filter

No catalog number given for the HPLC inline filter.

8. Evaporator

See comments section

Section IV. Procedure

A. Preparation of Control and Fortification Samples

This section states that untreated and fortified controls must be used with every set of samples. A regulatory method cannot require the use of controls as a check on the efficiency or reliability of the procedure.

B. Extraction

1. Milk

No directions are given for transferring the "clear aqueous phase" to the 2-oz narow-mouth bottle.

2. Egg, Fat, Muscle

Same comment as above.

3. Liver, Kidney

No directions are given for transferring "a 20-ml aliquot of the clear aqueous phase to a 60ml separatory funnel".

D. FMCL Derivatization

It is stated that the derivatives remain in the aqueous phase after the extraction with ethyl acetate, but no directions

are given for isolating this phase before HPLC analysis.

Comments

1. Since ACL does not have an Evapo-Mix on hand, the lab's rotary evaporators will need adapters so they can be used with the 45ml centrifuge tubes required by the method.

2. No chromatograms are included for CMP in milk at the .02PPM level as requested by DEB. No recovery data are presented for either CMP or AMPA in cow liver at 0.2PPM. Cow liver chromatograms at 0.05PPM for both analytes are included.

3. The "typical" chromatogram for CMP in milk at 0.05 PPM shows a very poorly resolved peak for the analyte. It is represented by a small peak on the tail of a large interfering peak. This chromatogram could not be considered reliable for detecting or quantitating this residue.

4. The method requires that the Stds. must be derivatized by the analyst.

5. A cutoff filter in the range of 300 to 315 nanometers may have to be purchased for ACL's fluoresence detector.

6. The deficiencies listed above by section number will have to be addressed by the petitioner before this TMV can be initiated.