UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: April 17, 2013

Subject: **Dicamba**. New Use of Dicamba on Dicamba-Tolerant Soybean. Petition for Establishment of New Tolerances for Soybean Forage and Soybean Hay. Residue Chemistry Summary.

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Summary of Submitted Residue Chemistry Studies

OCSPP 860 Series Guideline	MRID Number	Monograph Annex B Reference	Title	Primary Review	Secondary Review
860.1340	47899501	B.5.2	Method for the Determination of Dicamba and Its Major Metabolites in Soy Matrices by LC/MS/MS.	US EPA	PMRA
860.1300	47899523	B.7.1	Metabolism of Dicamba in Dicamba-Tolerant Soybeans.	US EPA	PMRA
860.1500 860.1380	47899524	B.7.6 B.7.7	Magnitude of Residues of Dicamba in Soybean Raw Agricultural and Processed Commodities after Application to MON 87708	PMRA US EPA	US EPA PMRA

ED_005172C_00001681-00001

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1.0 Executive Summary

Monsanto has submitted petition PP# 0F7725 requesting Section 3 registration for the use of dicamba on dicamba-tolerant soybean. Monsanto is also requesting to establish new tolerances for dicamba tolerant soybean forage at 45 ppm and for dicamba tolerant soybean hay at 70 ppm. The proposed application is for pre- and post-emergence uses of dicamba on dicamba-tolerant soybeans.

Dicamba (benzoic acid, 3,6-dichloro-2-methoxy-, aka 3,6-dichloro-*o*-anisic acid) is a selective systemic herbicide belonging to the benzoic acid chemical family and is currently registered for use on soybeans as pre-plant applications and not as post emergence applications because crop injury could occur if it were to come in contact with roots, stems, or foliage. Dicamba is available for use in either acid or salt forms with registered uses being maintained on a wide variety of crop and livestock commodities. Permanent tolerances are established under 40 CFR §180.227(a)(1) for dicamba and its 3,6-dichloro-5-hydroxybenzoic acid (5-hydroxydicamba) metabolite. Additional tolerances are established under 40 CFR §180.227(a)(2) for dicamba and its 3,6-dichloro-2-hydroxybenzoic acid (aka 3,6-diclorosalicyclic acid or DCSA) metabolite, as well as under 40 CFR §180.227(a)(3) for dicamba, 5-hydroxydicamba, and the DCSA metabolite.

Monsanto submitted a metabolism study which shows that dicamba applied to dicamba-tolerant soybean is converted to the metabolite 3,6-dichlorosalicylic acid (DCSA) and its glycosidic conjugates, which are the main metabolites formed. As an alternative less favorable pathway, DCSA is hydroxylated at the 5-position, to form 2,5-dichloro-3,6-dihydroxybenzoic acid (DCGA) and its glycosidic conjugates, which are found in amounts less than 10% of the total radioactive residue (TRR). The dicamba metabolite 5-hydroxydicamba was not identified in the TRR.

The nature of residues for dicamba-tolerant soybean is understood. The residues of concern (ROC) for monitoring the tolerance under 40 CFR §180.227(a)(3) includes parent dicamba, and the metabolites 5-hydroxydicamba, and DCSA. Data from the newly submitted metabolism and field trial studies support including residues of DCGA to the ROC in the risk assessment of dicamba.

The nature of dicamba residues in animals and in rotational crops were previously determined based on acceptable studies. The establishment of a tolerance on soybean forage and hay will not increase livestock dietary burden; therefore, no new revised tolerances on livestock commodities are required to support this petition.

Field residue trials were conducted to quantify the residues of dicamba and its metabolites in/on dicamba tolerant soybeans. The trials comply with OCSPP Harmonized Test Guideline 860.1500 both in terms of number and geographic representation (Appendix E). A dicamba formulation using the monoethanolamine salt (MON 11955) was applied once pre-emergence (1 lb ae/A), and twice post emergence (each at 0.5 lb ae/A). Soybean RACs were harvested and maintained frozen until analysis. Residues were analyzed by a validated analytical method and are supported by adequate storage stability data. DCSA was the major residue found in soybean

commodities and its amount ranged from 8.92 ppm to 51.3 ppm in forage, from 12.2 ppm to 61.1 ppm in hay and from 0.010 ppm to 0.440 ppm in seed.

Based on the Agency's previous recommendation to provide supplemental label information to include a plant back interval of 120 day for rotational crops, the registrant fulfilled this requirement in the submitted supplemental label. Therefore, additional rotational crop data are not needed at this time.

An adequate processing study was submitted. Residue data was generated using a validated data collection method and are supported by adequate storage stability data. Residues of dicamba, 5-OH dicamba, and DCSA slightly concentrated in soybean hulls, flour and meal from 1.2 to 1.5 fold and were diluted in protein concentrate, protein isolate, soymilk and tofu.

The residue values obtained from the field trial studies were evaluated using the Organization for Economic Cooperation and Development (OECD) calculation procedures for estimating tolerances/Maximum Residue Limits (MRLs). Using the OECD calculation procedures, and inputting the total residue, which includes the sum of the parent compound, and its metabolites 5-OH dicamba, and DCSA, expressed as parent equivalents, tolerances of 60 ppm for soybean forage and 100 ppm for soybean hay are recommended. The current tolerances of 10 ppm in soybean seed and 30 ppm in soybean hull are adequate. The US EPA and PMRA (Canada) established a harmonized tolerance (MRL) for soybean on seed at 10 ppm. There are currently no Mexican, Canadian or Codex MRLs established for soybean forage and hay.

2.0 Regulatory Recommendations

Provided a revised Section F reflecting the tolerance levels listed in Table 2.2.2, is provided, there are no residue chemistry considerations that would preclude granting the requested registration and establishing tolerances for soybean forage and hay.

2.1 Data Deficiencies/Data Needs

There are no residue chemistry deficiencies that preclude establishing permanent tolerances on soybean raw agricultural commodities (RACs) for dicamba. A revised human health risk assessment to support the requested use on dicamba tolerant soybean is underway.

2.2 Tolerance Considerations

2.2.1 Enforcement Analytical Method

The current enforcement method AM-0691B-0593-4, which has been subjected to successful ILV and EPA method validations, is available for the determination of dicamba and its metabolite 5-hydroxydicamba in soybean forage and seed. Another enforcement method, AM-0941-1094-0 is available for the determination of dicamba and its metabolites 5-hydroxy dicamba and 3,6 dichloro 2-hydroxybenzoic acid (DCSA) in soybean seed. Since the principle and procedures in both methods are similar, the Agency expects that the method AM-0691B-0593-4 may be used for the determination of dicamba, 5-hydroxy dicamba and DCSA in soybean

forage and hay and that the current analytical methods are suitable for enforcement.

2.2.2 Recommended Tolerances

The current tolerance expression is compliant with HED's Interim Guidance on Tolerance Expressions (05/27/2009, S. Knizner). The newly proposed tolerances should be established under 40 CFR §180.227(a)(3) as follows:

Table 2.2.2. Tolerance Summary for Dicamba.

Commodity	Proposed Tolerance (ppm)	HED-Recommended Tolerance (ppm)	Comments (*correct commodity definition*)
Soybean, forage	45	60	
Soybean, hay	75	100	

2.2.3 Revisions to Petitioned-For Tolerances

Tolerances proposed by the petitioner were estimated using the NAFTA MRL calculator. EPA's recommended tolerances, which differ from the proposed tolerances, were derived using the OECD MRL calculation procedures, which is the Agency's current standard for determination of tolerances.

2.2.4 International Harmonization

There are currently no Mexican, Canadian or Codex MRLs established for soybean forage and hay. This is a global joint review of parent dicamba and metabolites in dicamba-tolerant soybeans led by U.S. with Canada and Japan as participating reviewers. US EPA and PMRA (Canada) established a harmonized tolerance expression and tolerance residue level for soybean on seed at 10 ppm. Therefore, there are no issues with respect to international harmonization associated with establishing the recommended tolerances. The International Residue Limits (IRL) table is found in Appendix F.

2.3 Label Recommendations

The submitted label is acceptable and no revisions are required.

3.0 Introduction

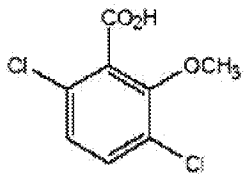
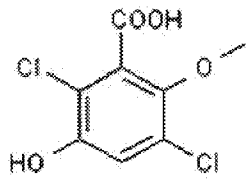
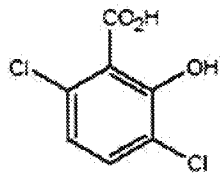
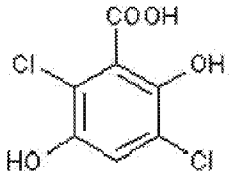
Dicamba is a selective systemic herbicide and belongs to the benzoic acid chemical family. Dicamba mimics the natural plant hormone indole-3-acetic acid (aka synthetic auxin) and produces an "auxin overload thereby causing susceptible plants to be injured and controlled. Dicamba end-use products are available as either acid or salt formulations with registered uses being maintained on a wide variety of crop and livestock commodities.

Monsanto has developed a dicamba-tolerant variety of soybean seed (MON 87708) capable of receiving dicamba treatments up to seven days before harvest. A dicamba mono-oxygenase (DMO) gene is introduced into dicamba-tolerant soybean seed to encode the enzyme dicamba *O*-demethylase to convert dicamba into the non-herbicide metabolite 3,6-dichlorosalicylic acid

(DCSA, and thus causing the soybean plant to tolerate the herbicidal effect of dicamba.

The chemical structure and nomenclature of dicamba and its metabolites 5-OH dicamba, DSCA and DCGA are presented in Table 3.1.1. The physicochemical properties of the technical grade of dicamba acid are presented in Table 3.2.1. The chemical names and structures of dicamba and its metabolites are shown in Appendix B.

3.1 Chemical Identity

Table 3.1. 1 **Test Compound Nomenclature: Dicamba and its Residues of Concern.**	
Compound	Chemical Structure 
Common name	Dicamba
IUPAC name	3,6-Dichloro-*o*-anisic acid
CAS name	Benzoic acid, 3,6-dichloro-2-methoxy-
CAS #	1918-00-9
End-use product/EP	M1691 herbicide on Roundup Ready® 2 Xtend Soybean Flowable Concentrate
Compound	
Common name	5-Hydroxy-dicamba
IUPAC name	2,5-dichloro-3-hydroxy-6-methoxybenzoic acid
CAS name	Benzoic acid, 2,5-dichloro-3-hydroxy-6-methoxy-
CAS registry number	7600-50-2
Compound	
Common name	DCSA; 3,6-dichlorosalicylic acid
IUPAC name	3,6-dichloro-2-hydroxybenzoic acid
CAS name	Benzoic acid, 3,6-dichloro-2-hydroxy-
CAS registry number	3401-80-7
Compound	
Common name	DCGA; 3,6-dichlorogentisic acid

IUPAC name	2,5-dichloro-3,6-dihydroxybenzoic acid
CAS name	Benzoic acid, 2,5-dichloro-3,6-dihydroxy-
CAS registry number	18688-01-2

3.2 Physical/Chemical Characteristics

Table 3.2.1. Physicochemical Properties of the dicamba technical grade		
Parameter	Value	Reference
Molecular weight (g/mole)	2.19.9699	
Melting point/range (°C)	114-116 °C (PAI) 90-100 °C (87% TGAI)	Residue Chemistry Chapter of the Dicamba RED, DP# 317699, 12/20/05, C. Olinger
pH	2.5-3.0 (87% TGAI)	
Density (g/cm^3)	1.57 g/mL at 25 °C (87% TGAI)	
Water solubility (mg/L at 25°C) (PAI)	500 mg/100 mL	
Solvent solubility (g/L at 25°C) (PAI)	dioxane 118.0 ethanol 92.2 isopropyl alcohol 76.0 methylene chloride 26.0 acetone 17.0 toluene 13.0 xylene 7.8 heavy aromatic naphthalene 5.2	
Vapor pressure at 25°C (PAI)	3.4 x 10^{-5} mm Hg	
Dissociation constant (pK_a)	1.97 (PAI)	
Octanol/water partition coefficient Log(K_{OW})	0.1 (PAI)	
	neutral: 511 (275 nm) acidic (pH 0-1): 1053 (281 nm) basic (pH 13-14): 469 (274 nm)	

3.3 Pesticide Use Pattern/Directions for Use (860.1200)

The dicamba product used for treating dicamba-tolerant soybean proposed for registration is the M1691 Herbicide (EPA Reg. No. 524-582) which is a soluble (flowable) concentrate formulation. This end–use product contains 56.8% active ingredient in the form of the diglycolamine salt of dicamba (equivalent to 4.0 lb ae/gal). A summary of the proposed directions for use taken directly from the supplemental M1691 herbicide label provided by the registrant are presented below in Table 3.3.1.

Table 3.3.1. Summary of Proposed Directions for Use of Dicamba.

Applic. Timing, Type, and Equip.	Formulation [EPA Reg. No.]	Applic. Rate (lb ae/A)	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb ae/A)	PHI (days)	Use Directions and Limitations
Soybean						
Broadcast spray application pre, at-planting or pre-emergence and post emergence foliar broadcast spray applications	524-582	1.0 pre-emergence and 0.5 post-emergence	Not specified	2.0	7 for forage 14 for hay 83 for seed	The maximum rate for any single, in-crop (post-emergence) application must not exceed 0.5 lb dicamba a.e. per acre. A second post-emergence application may follow up to the R1 reproductive stage

Conclusions.

The submitted Supplemental M1691 Herbicide Supplemental labeling is adequate and supported by adequate field trial data.

4.0 Metabolite/Degradate Residue Profile

4.1 Nature of the Residue

4.1.1 Summary of Plant Metabolism (860.1300)

Tier II summary review section 6.2.1 "Metabolism, distribution and expression of residues in plants"
Reregistration Eligibility Decision Memo, D317699, 12/20/2005, C. L. Olinger

The nature of the residue for non-dicamba resistant plants was previously determined to be understood (D317699, C. L. Olinger, 12/20/2005). Prior plant metabolism studies were reviewed which demonstrate that dicamba is rapidly absorbed and translocated in soybeans. The residues of concern that were concluded for the tolerance expression and risk assessment in soybean were dicamba parent and the metabolites 5-hydroxydicamba and DCSA.

The metabolism study submitted by the registrant in/on dicamba-tolerant soybean used a simulated formulation consisting of a mixture of unlabeled dicamba and dicamba uniformly labeled with ^{14}C- in the ring carbons was formulated as an aqueous solution of the diglycolamine salt. A single application of the test material, either as pre-emergence or post emergence, was applied at 2.55 and 2.52 lb ae/acre (2.86 and 2.82 kg/ha). The application rates represent slightly exaggerated rates relative to the maximum intended seasonal use rate in the US of 2.0 lb ae/acre (2.24 kg/ha). Radioactivity analyses (TRR determinations), extraction efficiencies and HPLC profiles were compared during various phases of the study and found that dicamba and its metabolites were stable for longer than two years. The levels of ^{14}C-dicamba-derived residues found in soybean RAC were quantifiable with the analytical method used. Acceptable extractabilities were obtained for all matrices. The identified dicamba metabolites were DCSA glucoside (60.32-74.48% of TRR), which was the major component in dicamba-tolerant soybean, DCSA HMGglucoside (1.14-7.62% of TRR), DCGA glucoside (0.75-4.32%), DCGA malonylglucoside (0.73-5.46% of TRR), DCSA (1.54-4.08% of TRR), in addition to two minor un-identified metabolites characterized as mixtures of unknown DCSA and DCGA conjugates, each constituted less than 2.0% of the TRR. The metabolite 5-hydroxy dicamba, which is part of the current tolerance expression, has not been detected. The identified and characterized radioactive residues in dicamba tolerant soybean are listed in Appendix A.

Based on the identified metabolites of dicamba in dicamba tolerant soybean the metabolic pathway has been concluded and proceeds as follows (see diagram in Appendix C):
The dicamba mono oxygenase gene (DMO), introduced to the seed, encodes the enzyme dicamba *O* demethylase to convert dicamba into the non-herbicide metabolite 3,6-dichlorosalicylic acid (DCSA), which is formed by demethylation of the aromatic methoxyl moiety of dicamba. DCSA is then conjugated to DCSA glucoside, some of which is further modified by esterification with 3-hydroxy-3-methylglutaric acid (HMGA) to form DCSA HMGglucoside. As an alternative less favorable pathway, DCSA is hydroxylated at the 5-position, to form 2,5-dichloro-3,6-dihydroxybenzoic acid (DCGA), which is converted to the

glucose conjugate DCGA glucoside and by malonylation of the glucose to DCGA malonylglucoside. .

4.1.2 Summary of Livestock Metabolism (860.1300)

Reregistration Eligibility Decision Memo, D317699, 12/20/2005, C. L. Olinger

The nature of dicamba residues in animals was previously determined based on acceptable metabolism studies conducted on ruminants and poultry (MRID 43245201-2). The residues of concern in meat, milk, poultry and eggs consisted of dicamba and 3,6-dichloro-2-hydroxybenzoic acid (DCSA) [40 CFR §180.227 (a)(2)].

4.1.3 Summary of Confined Rotational Crops (860.1850)

Reregistration Eligibility Decision Memo, D317699, 12/20/2005, C. L. Olinger

The nature of the dicamba residue in rotational crops was previously reviewed. It has been concluded that limited and/or extensive field accumulation studies with dicamba were not necessary and rotational crop tolerances need not be established provided the registrants amended all dicamba labels to specify a 120-day plant-back interval (PBI) when dicamba is applied at a maximum seasonal rate of 0.75 lb ae/A or less and that the label specifies that only crops with established tolerances can be rotated. The proposed supplemental label includes the necessary 120-day PBI.

4.1.4 Summary of Metabolites and Degradates

A table of the metabolites of dicamba in dicamba tolerant soybean is found in Appendix A. A diagram of the metabolic pathway is found in Appendix C.

4.2 Comparison of Metabolic Pathways

The metabolism of dicamba is qualitatively similar in all plants. Dicamba is metabolized in plants mainly by demethylation and hydroxylation. The main metabolites are 5-hydroxydicamba and DCSA. The metabolite 2,5-dichloro-3,6-dihydroxybenzoic acid (DCGA) has been recently identified in dicamba-tolerant plants. DCGA is formed by the hydroxylation of DCSA. In dicamba tolerant plants, the amounts of the metabolites DCSA, 5-hydroxy dicamba and DCGA vary significantly than in the corresponding dicamba non-tolerant plants. Metabolism in ruminants was similar to poultry, the metabolism proceeds in a similar fashion to that seen in plants described above, however, an additional metabolite, 2-amino-3,6-dichlorophenol has been identified in low amounts only in hen liver. In rat, dicamba is rapidly absorbed and excreted. The compound is not metabolized or accumulated by the tissues.

4.3 Residues of Concern Summary and Rationale

D410934 Memo of Conference 3-18-2013

Based on the results obtained from the metabolism and field trial studies, the residues present in both soybean and dicamba-tolerant soybean were comprised of dicamba, 5-hydroxydicamba, DCSA and DCGA. A consultation with the co-chairs of HED Residues of Concern Knowledgebase Subcommittee (ROCKS) was held on March 18, 2013, with participation of

Canadian PMRA on April 10,2013, to determine the residues of concern (ROC) for both tolerance setting and risk assessment purposes. HED and PMRA evaluated both the exposure and hazard profiles for dicamba, 5-OH dicamba, DCSA and DCGA. Based on available toxicity studies and structural similarities, HED and PMRA consider the parent and the metabolites 5-hydroxydicamba and DCGA to be of comparable toxicity. The metabolite DCSA is considered to be of potentially greater toxicity, based on the rat developmental study (D381800, K, Farwell, 12/17/2012). The submitted data supported the existing tolerance expression for dicamba on soybean which includes parent dicamba, the DCSA metabolite and the 5-OH dicamba metabolite found in non-resistant varieties as the residues monitored in the tolerance expression. Since dicamba, 5-OH dicamba, and DCSA account for the majority of residues in both tolerant and non-tolerant soybean, this tolerance expression provides sufficient residues to monitor for misuse for both tolerant and non-tolerant soybean; therefore the ROC for tolerance setting purposes is dicamba, 5-OH dicamba and DCSA. DCGA was not considered a significant contributor for determining misuse; and thus was not added to the tolerance expression. The ROCKS subcommittee co-chairs however supported the recommendation of adding the metabolite "conjugates of" DCGA for risk assessment in addition to the parent and metabolites included in the tolerance expression. The DCGA metabolite was included because it is present in appreciable concentrations up to 7.6 ppm (mean = 2.66 ppm) in feed items and in quantifiable amounts up to 0.14 ppm (mean = 0.032 ppm) in the seed in the registrant's submitted field trial data. In addition, amounts of DCGA found in soybean processed seed fractions were comparable to the amounts of DCSA. Appendix D summarizes the residues of concern from the metabolism and field trial studies and Table 4.3.1 summarizes the residues of concern to be included in the tolerance expression and for risk assessment.

Table 4.3.1. Dicamba Residues of Concern to be included in the Risk Assessment and Tolerance Expression.

Matrix	Tolerance Expression	Residues for Risk Assessment
Barley, corn, grasses, oats, proso millet, sorghum, teff, sugarcane, and wheat	Dicamba + 5-OH dicamba	Dicamba + 5-OH dicamba
Asparagus	Dicamba + DCSA	Dicamba + DCSA
Cotton	Dicamba + 5-OH dicamba + DCSA	Dicamba + 5-OH dicamba + DCSA
Soybean and aspirated grain fractions	Dicamba + 5-OH dicamba + DCSA	Dicamba + 5-OH dicamba + DCSA+ DCGA

5.0 Residue Profile

5.1 Residue Analytical Methods (860.1340)

5.1.1 Data Collection Methods

OECD Tier II summary-Section 4.3 Residues in and/or on plants, plant products, foodstuffs (of plant and animal origin), feeding stuffs

The analytical method AG-ME-1321-01 "Analytical Method for the Determination of Dicamba

and Its Major Metabolites in Soy Matrices by LC/MS/MS" was used for data collection. The method incorporates an acid hydrolysis step which converts conjugates of 3,6-dichlorosalicylic acid (DCSA) and 3,6-dichlorogentisic acid (DCGA),to their respective chemophores (non-glycosidic hydrolysis products) DCSA and DCGA. These analytes along with dicamba and 5-hydroxydicamba are detected and quantitated by ESI LC/MS/MS in the negative ion mode. Analyte-specific ^{13}C-labeled internal standards were utilized in the method to compensate for matrix effects in the LC/MS/MS analysis and method procedural losses. The residues are calculated as µg/g, ppm and expressed in terms of dicamba equivalent.

Soybean matrices are extracted using 40:60 (volume/volume) acetonitrile:water. Soybean seed is first defatted by extraction with hexane, then the residues of concern were extracted with 40:60 acetonitrile:water. An aliquot of the extract is hydrolyzed in 1N HCl at 95 °C in a water bath. The hydrolysate is partitioned with 40:60 (volume/volume) ethyl acetate:isooctane and the organic phase is partially concentrated under vacuum. Water is then added to the organic phase, and the sample is further concentrated under vacuum until only the aqueous solution remains. The aqueous solution is filtered, acidified and analyzed by LC/MS/MS with turbo ion-spray ionization in the negative ion mode to quantitate dicamba, 5-hydroxydicamba, DCSA and DCGA. The LC-MS/MS run was 12 minutes. Only one ion transition for each analyte was monitored. The monitored ion transitions (precursor/product ions) are listed below.

Analyte	Precursor ion Q1 (amu)	Product ion Q3 (amu)
Dicamba	219	175
5-hydroxydicamba	235	191
DCSA	205	161
DCGA	221	177
$^{13}C_6$-dicamba	225	181
$^{13}C_6$-5- hydroxydicamba	241	197
$^{13}C_6$-DCSA	211	167
$^{13}C_6$-DCGA	227	183

The method was validated at four fortification levels ranging from 0.005 to 2 ppm for dicamba tolerant soybean forage, hay and seed including 7 replicates at each level except the 2.0 ppm fortification level which was done in duplicate. Processed fractions were fortified at three concentration levels ranging from 0.01 to 2 ppm including a different number of replicates at each level, mostly 1 or 2 replicates and 4 replicates in a few cases. In general, recoveries fell within the acceptable range of 70-120% with few exceptions for 5-hydroxydicamba and DCGA at or slightly above the limit of quantitation (LOQ). Poor recoveries of DCGA were obtained when it was spiked directly to the matrix. The registrant proposed to overcome this issue by spiking DCGA and its IS into the extract which is not in accordance with the OCSPP Harmonized Test Guidelines 860.1340. The lowest level of method validation (LLMV) was 0.005 ppm for soybean forage, hay and seed and 0.01 for soybean processed fractions. The LLMV was considered the LOQ of the method.

Conclusions.

The method has been adequately validated for dicamba, 5-hydroxydicamba and DCSA in dicamba tolerant soybean RAC and processed fractions. The determination of DCGA is not suitable using this method because the validation data are unacceptable Since residues of this metabolite are included for risk assessment only, residue values will be estimated using the metabolism study, which has been adequately validated and will provide a conservative estimate of exposure.

5.1.2 Multi-Residue Methods (MRM) (860.1360)

D375578, A. Kamel, 6/2013

Appendix II of PAM Volume I specifies that dicamba is completely recovered using Section 402 E2 of Protocol B but is only partially recovered using Section 402 E1 of Protocol B.

BASF Corporation has recently submitted multi-residue method data for the dicamba metabolites 5-OH dicamba and 3,6 dichloro salicylic acid (DCSA) (MRID 48001304). The metabolites were screened through multi-residue methods described in the United States Food and Drug Administration (FDA) Pesticide Analytical Manual Volume I (PAM Vol. I). Testing through Protocol B revealed that partial recoveries were obtained for DCSA only on non-fatty food, but Protocol B was not suitable for 5-OH dicamba. Testing through Protocols A, C, D, E, and F showed that the protocols were not applicable to 5-OH dicamba because it was either not fully recoverable, or due to the lack of a fluorescence response. DCSA was not tested under Protocol D, E, or F because it did not show acceptable chromatographic separation under Protocol C. 5-OH dicamba and DCSA were not tested under Protocol G because the compounds are not substituted ureas.

5.1.3 Tolerance Enforcement Methods

The current enforcement method AM-0691B-0593-4, which has been subjected to a successful ILV as well as EPA method validation is available for the determination of dicamba and its metabolite 5-hydroxydicamba in soybean forage and seed. Another enforcement method, AM-0941-1094-0 is available for the determination of dicamba and its metabolites 5-hydroxy dicamba and 3,6 dichloro 2-hydroxybenzoic acid (DCSA) in seed. For animal commodities, the analytical method AM 0685 "Determination of dicamba and and 3,6 dichloro 2-hydroxybenzoic acid (DCSA) in liver, kidney, skeletal muscle, adipose tissue and milk" is available. The Agency expects the analytical method AM-0691B-0593-4 to be suitable for the determination of all residues of interest in all matrices since the method is similar to AM-0941-1094-0 in its steps and principle.

Conclusions.

The enforcements methods described above are expected to be suitable for the determination all the residues of concern in all matrices.

5.1.4 Submittal of Analytical Reference Standards (860.1650)

Analytical standards for dicamba and its metabolites of concern are currently available in the EPA National Pesticide Standards Repository (personal communication of Peter Savoia, HED, with Theresa Cole, BEAD, 01/30/2013). The current stock of standards is set to expire on 11/30/2015.

5.2 Storage Stability (860.1380)

OECD Tier II summary section 6.3.1.2 "Residues resulting from supervised trials in soybean" and Table 6.3.1.2-5. "Storage Period of Soybean Samples"
Reregistration Eligibility Decision Memo, D317699, 12/20/2005, C. L. Olinger

The storage stability study was previously evaluated for residues of dicamba in soybeans and soybean processed fractions from field and processing studies in which samples were stored for 4 to 10 months prior to analysis (D317699 C. Olinger, 2005). These storage stability data indicate that residues of dicamba and its metabolites are reasonably stable under frozen storage conditions in/on soybean forage for up to 4 months, 6 months in the seed RAC (dicamba only) and in refined oil for up to 3 months (MRID 43814101, MRID 43814102).

Conclusions

Adequate data have been provided to demonstrate that residues of dicamba and its metabolites were stable in soybean matrices during freezer storage.

5.3 Residue Data

5.3.1 Crop Field Trials (860.1500)

OECD Tier II summary section 6.3.1.2 "Residues resulting from supervised trials in soybean"

Monsanto submitted crop field trials which were conducted in 2008 at twenty-two locations in the United States to quantify the residues of dicamba and its metabolites in/on soybeans. The location of the North American soybean field trials are summarized in Appendix D. The distribution of the soybean field trials included 2 trials in Region 2 (GA and SC), 3 trials in Region 4 (LA, AR and MO) and 17 trials in Region 5 (2 trials each in IL, IA, KS, MN, NE, SD and WI; and 1 trial each in IN, MI and ND). At each field site, treatment and control plots were established using dicamba-tolerant soybeans MON 87708. In addition to the dicamba tolerance trait, these seeds are also stacked with a glyphosate-tolerance trait.

A formulation using the monoethanolamine salt of dicamba.(MON 11955) was applied once pre-emergence, and once each at the V3 growth stage and at the R1/R2 growth stage, at nominal application rates of 1.00 lb a.e./A (1.12 kg a.e./ha), 0.500 lb a.e./A (0.560 kg a.e./ha) and 0.5 lb a.e./A (0.560 kg a.e./ha), respectively according to the proposed new supplemental label for the registered formulation M1691 (EPA Reg. No. 524-582). Soybean RACs were harvested at the typical commercial harvest stages, processed or ground and were maintained frozen at -20°C for intervals ranging from 31-293 days to study the stability of the residues of interest (some samples were stored for a maximum of 400 days to resolve discrepancies between field

replicates). Residues were analyzed by an analytical method which converts the DCSA and DCGA conjugates into their respective acid hydrolysis products.

The average of 44 field samples for each soybean matrix showed that DCSA (sum of hydrolysis products of all DCSA conjugates) was the major residue found and ranged from 8.92 ppm to 51.3 ppm in forage, from 12.2 ppm to 61.1 ppm in hay and from 0.010 ppm to 0.440 ppm in seed. Residues of DCGA ranged from 0.356 ppm to 5.90 ppm in forage, from 0.167 ppm to 7.26 ppm in hay, and from <0.011 ppm (<LOQ) to 0.135 ppm in seed. A surface residue of un-changed dicamba ranged from <0.021 ppm (<LOQ) to 2.62 ppm in forage, from <0.014 ppm (<LOQ) to 1.16 ppm in hay, and were non-quantifiable (<LOQ; <0.013 ppm) in seed. Residues of 5-hydroxydicamba was generally not detected above the LOQ in soybean commodities, except in forage at an average of 0.006 ppm. The summary of dicamba and its metabolites residues found in soybean matrices treated with the maximum application rate are shown in Table 5.3.1.2.

The residue data from trials conducted to bridge the two SC formulations MON 11955 (monethanolamine salt of dicamba) and MON 54140 (diglycolamine (DGA) salt of dicamba) show that the residue levels in soybean forage, hay and seed are similar following treatment with the two different formulations of dicamba.

Residue data from the two decline trials show that in general residues of dicamba, DCSA, DCGA and 5-hydroxy dicamba decreased over the sampling time from day 3 to day 100. Residues (mean) of dicamba and 5-hydroxy were low in all matrices at 3 days after the last application and decreased to <LOQ at subsequent intervals. Therefore, no conclusion can be made regarding residue decline of these two analytes. Residues (mean) of DCGA and DCSA in seed remained constant, though low, over the sampling period at both sites.

Table 5.3.1.2. Summary of Residues from Field Trials with Dicamba.

Commodity	Total Applic. Rate lb a.e./A (kg a.e./ha)	PHI (days)	Residue Levels[a, b] (ppm)						
			n	Min.	Max.	HAFT	Median (STMdR)	Mean (STMR)	Std. Dev.
DCGA[c]									
Forage	1.96-2.04 (2.19-2.28)	7-10	44	0.356	5.90	5.27	1.93	2.02	1.02
Hay		13-15	44	0.167	7.26	7.19	2.00	2.66	1.91
Seed		73-98	44	<0.011	0.135	0.131	0.017	0.032	0.029
DCSA									
Forage	1.96-2.04 (2.19-2.28)	7-10	44	8.92	51.3	50.4	15.0	17.0	8.00
Hay		13-15	44	12.2	61.1	60.7	31.9	32.2	11.2
Seed		73-98	44	0.010	0.440	0.439	0.033	0.059	0.089
Dicamba									
Forage	1.96-2.04 (2.19-2.28)	7-10	44	<LOQ	2.62	2.47	0.068	0.374	0.603
Hay		13-15	44	<LOQ	1.16	1.01	0.051	0.130	0.216
Seed		73-98	44	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
5-OH Dicamba									
Forage	1.96-2.04 (2.19-2.28)	7-10	44	<LOQ	0.009	0.009	0.005	0.006	<LOQ
Hay		13-15	44	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Seed		73-98	44	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

[a]Concentrations of the individual analytes are reported as dicamba equivalents
[b]Values < LOQ are assumed to be at the LOQ.
[c] DCGA residues were quantitated by a non-validated method

Conclusion:

Residue data for dicamba, DCSA, and 5-OH dicamba were generated using a validated data collection method, supported by adequate storage stability data and are acceptable with respect to the number of trials and geographic locations (Appendix E). The analytical method used to quantify residues of DCGA has not been adequately validated. However, since residues of this metabolite are not included in the tolerance expression no additional data for the DCGA metabolite is required at this time. DCSA was the major residue found and ranged from 8.92 ppm to 51.3 ppm in forage, from 12.2 ppm to 61.1 ppm in hay and from 0.010 ppm to 0.440 ppm in seed.

5.3.2 Field Rotational Crops (860.1900)

Reregistration Eligibility Decision Memo, D317699, 12/20/2005, C. L. Olinger

The December 2005 Reregistration Eligibility Decision (RED) for dicamba requires additional rotational crop studies in order to satisfy data requirements. However, these data are not needed if a 120-day plantback interval is specified when dicamba is applied at the maximum seasonal rate of 0.75 lb ae/A. For greater seasonal application rates of 0.75-2.0 lb ae/A, only crops with

established tolerances can be rotated for planting. Because no new rotational crop data have been submitted, the plantback restrictions noted in the 2005 RED are required and have been appropriately reflected on the proposed product label for dicamba-tolerant soybean.

5.3.3 Processed Food and Feed (860.1520)

DER Reference List

OECD Tier II summary section 4.3 "Residues in and/or on plants, plant products, foodstuffs (of plant and animal origin), feedingstuffs"

OECD Tier II summary section 6.5 "Effects of Industrial Processing and/or Household Preparation"

Monsanto submitted data for processed fractions of dicamba-tolerant soybean seed. Samples of seed from two sites were generated using a rate 50% higher than the suggested maximum yearly application of dicamba. Processed soybean fractions of hulls, defatted flour, toasted defatted meal, protein isolate, protein concentrate, crude lecithin, degummed oil, refined bleached deodorized (RBD) oil, soymilk and tofu were prepared and stored frozen until analyzed. The analytical method was validated at three fortification levels to test its suitability for analyzing each processed fraction. Recoveries of residues were generally within acceptable range (70-120%), except for defatted flour at levels of 0.01 and 0.02 ppm for 5-hydroxydicamba. Since 5-hydroxydicamba has not been detected in any of the seed or processed fractions, poor recoveries at low spiking levels is not of concern. Dicamba and 5-hydroxydicamba residues in the whole seed and processed fractions were below the LOQ; therefore processing factors could not be calculated. Concentration factors were determined separately for DCSA and DCGA and calculated by obtaining the ratio of the residue in seed to that of the corresponding fraction. Residues were found to concentrate up to 1.5× in soybean hulls, toasted defatted meal and defatted flour. Residues were diluted in protein concentrate, protein isolate, soymilk and tofu. Residues in other fractions were below LOQ. Amounts of DCGA and DCSA metabolites were almost identical in most fractions, indicating further transformation of DCSA into DCGA during processing. Table 5.3.3.1 shows the residue data obtained from seed processed fractions in dicamba tolerant soybean.

Conclusions:

The analytical method used for the determination of dicamba and its metabolites in processed seed fractions was adequate. The current tolerance level of 10 ppm for soybean seed and 30 ppm for soybean hulls are adequate and cover all processed fractions.

Table 5.3.3.1. Summary of Residue Data from Soybean processed seed fractions.						
RAC	Processed Commodity	Total Rate lb ai/A (g ai/ha)	Residues (ppm)		Processing Factor	
			DCSA	DCGA	DCSA	DCGA
Soybean from first site	Seed	50% exaggerated rate (~3.0 lb ae/A)	0.065	0.055	---	---
	Hulls		0.082	0.054	1.27	0.99
	Toasted defatted meal		0.076	0.069	1.18	1.26
	Degummed oil		<0.010	<0.010	<0.16	<0.18
	RBD oil		<0.010	<0.010	<0.16	<0.18
	Crude lecithin		<0.010	<0.010	<0.16	<0.18
	Defatted fluor		0.071	0.067	1.1	1.23
	Protein isolate		<0.010	<0.010	<0.16	<0.18
	Protein concentrate		<0.010	<0.010	<0.16	<0.18
	Soymilk		<0.010	<0.010	<0.16	<0.18
	Tofu		<0.010	<0.010	<0.16	<0.18
Soybean from second site	Seed	50% exaggerated rate (~3.0 lb ae/A)	0.173	0.142	---	---
	Hulls		0.263	0.142	1.52	1
	Toasted defatted meal		0.26	0.191	1.51	1.34
	Degummed oil		<0.010	<0.010	<0.06	<0.07
	RBD oil		<0.010	<0.010	<0.06	<0.07
	Crude lecithin		0.037	<0.010	0.21	<0.07
	Defatted fluor		0.244	0.182	1.41	1.28
	Protein isolate		0.028	0.017	0.16	0.12
	Protein concentrate		0.015	0.015	0.08	0.11
	Soymilk		0.016	0.01	0.09	0.07
	Tofu		0.015	<0.010	0.09	<0.07

5.3.4 Meat, Milk, Poultry and Eggs (860.1480)

Reregistration Eligibility Decision Memo, D317699, 12/20/2005, C. L. Olinger

A bovine feeding study conducted at a feeding level of 1000 ppm (MRID 44891303) has been previously reviewed. Data showed that the highest levels of dicamba residues accumulated in kidney and liver tissues and were 46.6 and 5.06 ppm respectively, while significantly lower levels accumulated in fat and muscle tissue. Tolerances for livestock commodities ranging from a level of 0.2 ppm for fat to 25 ppm for kidney were recommended

A poultry feeding study with dicamba was previously determined not to be required based on the results of the submitted poultry metabolism study. In the poultry study, the TRR levels in eggs, liver, muscle, and fat were all <0.004 ppm following dosing at 10 ppm (~1.9× the maximum dietary burden of 5.2 ppm) in the diet for four consecutive days. Residues in eggs plateaued after the first day of dosing (i.e., there was no accumulation with increasing days of dosing). HED did not anticipate the occurrence of quantifiable residues of dicamba or DCSA in poultry eggs and meat as a result of treating crops with poultry feed items at the maximum use patterns. Therefore, HED concluded that tolerances are not needed in poultry eggs and meat at that time but may be required if additional uses are registered in the future.

For this action, a tolerance of 60 ppm in soybean forage and 100 ppm in soybean hay is recommended. Soybean forage and hay are considered minor sources of livestock feed and the

proposed tolerances are significantly lower than other feed commodity established tolerances for more predominant dietary sources such as grass forage, fodder, hay crop group 17, forage at 125 ppm and hay at 200 ppm. The worst case estimated livestock dietary burden will therefore be unaffected by the new recommended tolerances.

5.3.5. Food Handling (860.1460)

This guideline requirement is not relevant to the current action

5.3.6 Water, Fish, and Irrigated Crops (860.1400)

This guideline requirement is not relevant to the current action.

5.4 Food Residue Profile

Data on the metabolism of dicamba in dicamba-tolerant soybean demonstrate that dicamba is rapidly absorbed and translocated in soybeans. The highest residues are generally found in the foliage or forage and hay portions of plants used as feedstuffs, while lower residues are found in seeds of soybean and its processed fractions used as food items. In the field trial data, quantifiable residues were found in seed, while residues appear to decline over time, there is the potential for exposure from consuming the edible portion of soybean. The highest levels of dicamba residues in beef accumulated in kidney and liver tissues. The occurrence of quantifiable residues of dicamba or DCSA in poultry eggs and meat as a result of treating crops with poultry feed items at the maximum use patterns are not anticipated.

6.0 Tolerance Derivation

Reregistration Eligibility Decision Memo, D317699, 12/20/2005, C. L. Olinger

Permanent tolerances are established under 40 CFR §180.227(a)(3) for dicamba parent, 5-OH dicamba and DSCA metabolites. The tolerance expression established under 40 CFR §180.227(a)(3) for soybean seed (10 ppm) and soybean hull (30 ppm) will not change based on the new metabolism data provided in support of this action. Tolerances for hull and seed were previously raised from 13 ppm to 30 ppm based on calculations of maximum HAFT combined residue level (7.44 ppm) of the RAC and the observed concentration factor for soybean hulls (3.8×). In seed, the highest total residues were 8.13 ppm in/on samples of soybean seed harvested 6-8 days following treatments at 1.25× the maximum rate (D317699, 12/20/2005, C. Olinger).

The International Residue Limit (IRL) summary is shown in Appendix F of this memorandum. There are currently no Mexican, Canadian or Codex MRLs established for soybean forage and hay.

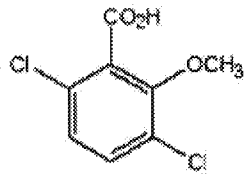
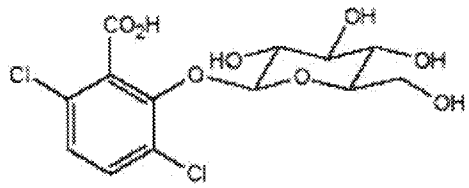
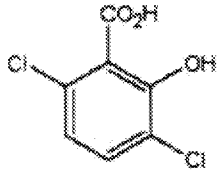
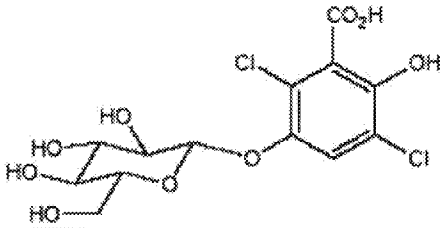
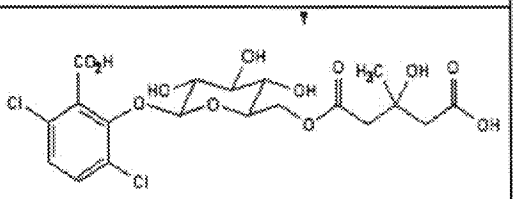
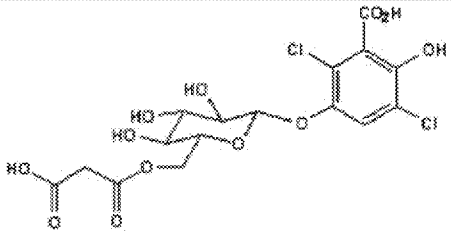
Recommended tolerances for soybean forage and soybean hay were obtained using the Organization of Economic Cooperation and Development (OECD) calculation procedure. Residue data from the 22 field trial locations with the average of duplicate samples at each location were entered (total 22 data points) by adding residues of DCSA, dicamba and 5-

hydroxydicamba from each location into the OECD MRL calculator. There were no data censored since all amounts (total of dicamba + DCSA + 5-hydroxy dicamba) were above the LOQ. LOQ amounts were entered for 5 hydroxydicamba if the detected amount is <LOQ. Recommended MRLs (Tolerances) were found to be 60 ppm for soybean forage and 100 ppm for soybean hay. The calculation inputs/outputs are found in Appendix G. Tolerances proposed by the petitioner were estimated using the NAFTA MRL calculator, which were different than the recommended tolerances above. The Agency is currently applying tolerance calculations based on the OECD calculator.

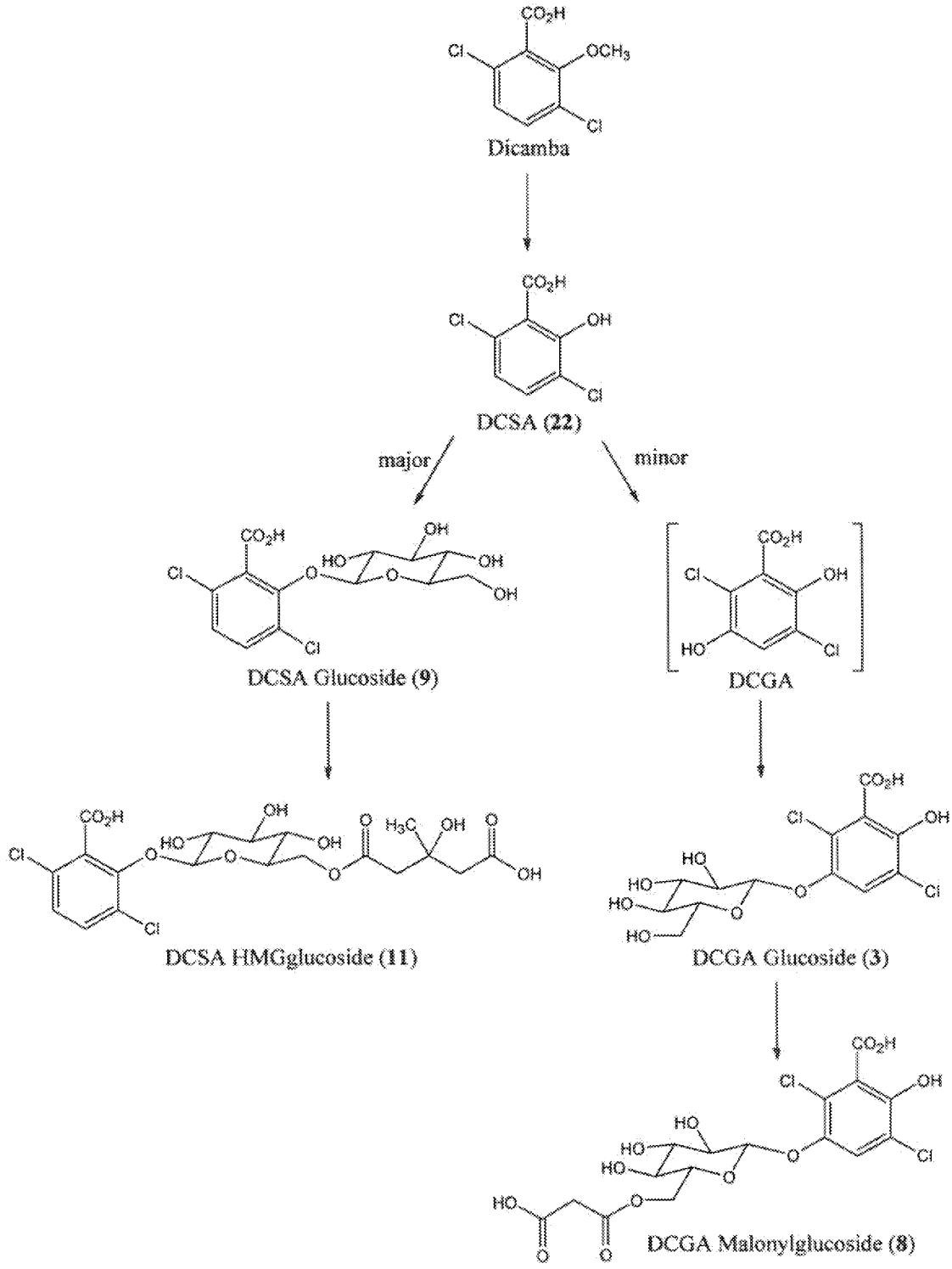
Appendix A. Summary of Characterization and Identification of Radioactive Residues in Dicamba-Tolerant Soybean Matrices Following Application of [Phenyl-U-^{14}C]dicamba

	Pre-emergence treatment[a]				Post-emergence treatment[a]		
	Pre-Forage	Forage	Hay	Seed	Forage	Hay	Seed
Identification	Percent of Matrix TRR (mg/kg, ppm)[b]						
Sugars	1.47(0.048)	0.96 (0.014)	1.08 (0.011)	8.42 (0.025)	-	0.49 (0.190)	9.15 (0.036)
Unknown	0.26 (0.008)	0.40 (0.006)	-	1.22 (0.004)	-	0.18 (0.071)	-
DCGA Glucoside	2.77 (0.09)	1.14 (0.016)	3.45 (0.036)	1.60 (0.005)	0.75 (1.007)	4.32 (1.690)	2.07 (0.008)
Unknown	0.64 (0.021)	0.58 (0.008)	0.53 (0.006)	0.33 (0.001)	0.36 (0.478)	-	0.31 (0.001)
Unknown	0.2 (0.007)	-	0.50 (0.005)	0.72 (0.002)	-	0.71 (0.280)	0.62 (0.002)
Unknown	-	-	-	-	-	-	0.57 (0.002)
DCGA Malonylglucoside	5.46 (0.177)	1.40 (0.020)	0.73 (0.008)	4.73 (0.014)	1.11 (1.485)	1.61 (0.631)	4.64 (0.018)
DCSA Glucoside	68.96 (2.24)	74.48 (1.067)	70.8 (0.748)	11.55 (0.034)	60.32 (80.913)	67.26 (26.333)	15.27 (0.059)
Unknown	-	-	-	1.26 (0.004)	0.14 (0.189)	-	0.84 (0.003)
DCSA HMGglucoside	7.62 (0.247)	5.21 (0.075)	6.67 (0.070)	8.73 (0.025)	1.14 (1.535)	2.48 (0.970)	9.61 (0.037)
Unknown	-	-	0.74 (0.008)	0.38 (0.001)	0.18 (0.239)	-	0.61 (0.002)
Unknown	-	-	-	-	0.18 (0.239)	-	0.48 (0.002)
Unk DCSA/DCGA Conj.	0.29 (0.009)	1.26 (0.018)	1.64 (0.017)	0.75 (0.002)	0.38 (0.503)	1.75 (0.686)	0.62 (0.002)
Unknown	-	-	0.75 (0.008)	-	0.40 (0.541)	0.95 (0.373)	0.36 (0.001)
Unknown	0.62 (0.02)	0.41 (0.006)	1.07 (0.011)	0.18 (0.001)	0.26 (0.352)	0.91 (0.354)	0.33 (0.001)
Unk DCSA/DCGA Gluc.	-	0.55 (0.008)	0.51 (0.005)	-	0.12 (0.164)	-	-
Unknown	-	-	-	-	0.18 (0.239)	0.38 (0.149)	-
DCSA	1.46 (0.047)	3.19 (0.046)	1.54 (0.016)	0.37 (0.001)	4.08 (5.473)	1.93 (0.757)	0.46 (0.002)
Dicamba	0.8 (0.026)	1.61 (0.023)	0.85 (0.009)	0.20 (0.001)	24.21 (32.473)	12.33 (4.828)	0.64 (0.003)
Unknown	-	-	-	-	-	-	0.52 (0.002)
Unknown	-	-	-	-	-	-	0.11 (0.0004)
Triglycerides	na	na	na	13.87 (0.040)	na	na	10.76 (0.042)
Total ACN/H_2O Extractable Residues	**90.55 (2.94)**	**91.19 (1.307)**	**90.87 (0.9580)**	**40.44 (0.12)**	**93.81 (125.83)**	**95.30 (37.312)**	**47.21 (0.1814)**
Total Identified or Characterized Metabolites	**88.83 (2.88)**	**89.8 (1.287)**	**87.28 (0.92)**	**36.35.21 (0.107)**	**92.11 (123.553)**	**92.17 36.085**	**43.09(0.1374)**

[a] The application rates achieved were 2.55 and 2.52 lb ae/acre (2.86 and 2.82 kg/ha), for the pre- and post emergence applications, respectively
[b] All mg/kg (ppm) values are expressed as dicamba equivalents.

Appendix B. Summary of Chemical Names and Structures of Dicamba and its Metabolites.		
Common name/code	Chemical name	Chemical structure
Dicamba	Benzoic acid- 3,6-dichloro, 2-methoxy	
DCSA Glucoside	Benzoic acid- 3,6-dichloro-2-β-D-glucopyranoside	
DCSA	Benzoic acid 3,6-dichloro, 2-hydroxy	
DCGA Glucoside	Benzoic acid- 2,5-dichloro-3-(β-D-glucopyranosyloxy)-6-hydroxy	
DCSA HMGglucoside	Benzoic acid- 3,6-dichloro-2-(β-D-glucopyranosyloxy 3-hydroxy-3-methylglutaryl	
DCGA malonylglucoside	Benzoic acid- 2,5-dichloro-3-(β-D-glucopyranosyloxy malonyl)-6-hydroxy	

Appendix C. Metabolic Pathways



Appendix D Summary of Metabolites and Degradates of Dicamba on Dicamba Tolerant Soybean						
Metabolism						
Soybean %TRR (ppm)						
Parent/ Metabolite	Forage		Hay		Seed	
	PRE-T	POE-T	PRE-T	POE-T	PRE-T	POE-T
Dicamba	1.205 (0.0245)	24.21 (32.473)	0.85 (0.009)	12.33 (4.828)	0.20 (0.001)	0.64 (0.003)
DCSA	81.51 (1.878)	66.04 (88.59)	79.63 (0.856)	73.42 (55.073)	21.03 (0.062)	25.96 (0.1)
DCGA	6.58 (0.202)	2.36 (3.159)	6.33 (0.066)	7.68 (3.007)	7.08 (0.021)	7.33 (0.028)
5-OHdicamba	ND	ND	ND	ND	ND	ND

Field Trials			
Mean Residues, ppm (range)			
Parent/Metabolite	Forage	Hay	Seed
Dicamba	0.374 (<0.021-2.62)	0.13 (<0.014-1.16)	<0.013
DCSA	17.0 (8.92-51.3)	32.2 (12.2-61.1)	0.059 (0.01-0.44)
DCGA	2.02 (0.356-5.9)	2.66 (0.167-7.26)	0.032 (<0.011-0.135)
5-OHdicamba	0.006 (<0.005-0.009)	<0.014	<0.021

Appendix E. Field Trial Geographic Distribution

Appendix E.Trial Numbers and Geographical Locations.			
NAFTA Growing Zones	Soybean		
	Submitted	Requested	
		Canada[1]	U.S.[2]
Region 2 (GA-1, SC-1)	2	-	2
Region 4 (AR-1, LA-1, MO-1)	3	-	3
Region 5 (IA-1, 2, IL-1, 2, IN-1, KS-1, 2, MI-1, MN-1, 2, ND-1, NE-1, 2, SD-1, 2, WI-1, 2)	17	12	15

[1] As per DIR 98-02, Residue Chemistry Guidelines
[2] As per OCSPP 860.1500, Table 5 for field soybean.

Appendix F. International Residue Limits Table

Dicamba (PC Code 128931; 03/21/2013)

Summary of US and Internati on al Tolerances and Maximum Residue Limits			
Residue Definition			
US	Canada	Mexico[2]	Codex[3]
40 CFR 180.227: residues of dicamba, 3,6-dichloro-0-anisic acid, and its metabolites, 3,6-dichloro-5-hydroxy-0-anisic acid, and 3,6-dichloro-2-hydroxybenzoic acid, calculated as the stoichiometric equivalent of dicamba	Plants except dry soybeans: benzoic acid, 3,6-dichloro-2-methoxy-, including the metabolite benzoic acid, 2,5-dichloro-3-hydroxy-6-methoxy- Dry soybeans: benzoic acid, 3,6-dichloro-2-methoxy-, including the metabolites benzoic acid, 3,6-dichloro-2-hydroxy- and benzoic acid, 2,5-dichloro-3-hydroxy-6-methoxy-		Plant: dicamba Animal: sum of dicamba and 3,6-dichlorosalicylic acid (DCSA) expressed as dicamba. The residue is not fat-soluble.

Commodity[1]	*Tolerance (ppm) /Maximum Residue Limit (mg/kg)*			
	US	Canada	Mexico[2]	Codex[3]
Soybean, forage	60			
Soybean, hay	100			
Completed: M. Negussie; 03/25/2013				

[1] Includes only commodities of interest for this action. Tolerance values should be the HED recommendations and not those proposed by the applicant.
[2] Mexico adopts US tolerances and/or Codex MRLs for its export purposes.
[3] * = absent at the limit of quantitation; Po = postharvest treatment, such as treatment of stored grains. PoP = processed postharvest treated commodity, such as processing of treated stored wheat. (fat) = to be measured on the fat portion of the sample. MRLs indicated as proposed have not been finalized by the CCPR and the CAC.

Appendix G. OECD MRL Calculation Procedure Inputs/Outputs

1. Outputs

Compound	dicamba+DCSA+5-OH	dicamba+DCSA+5-OH
Crop	Soybean	Soybean
Region / Country	USA	USA
GAP	Forage	Hay
Total number of data (n)	22	22
Percentage of censored data	0%	0%
Number of non-censored data	22	22
Lowest residue	10.183	12.828
Highest residue	50.378	60.900
Median residue	15.716	32.287
Mean	17.330	32.341
Standard deviation (SD)	7.961	11.127
Correction factor for censoring (CF)	1.000	1.000
Proposed MRL estimate		
- Highest residue	50.378	60.900
- Mean + 4 SD	49.174	76.848
- CF x 3 Mean	51.989	97.024
Unrounded MRL	51.989	97.024
Rounded MRL	60	100

2. **Inputs: Average of duplicate samples from 22 field trials**

Residues (mg/kg)	
20.162	
50.378	
14.176	
10.183	
13.680	
13.826	
17.264	
19.326	
21.026	
19.502	
10.574	
14.976	
14.927	
17.319	
12.700	
12.811	
13.958	
19.624	
16.376	
17.036	
15.914	
15.519	

Residues (mg/kg)	
35.013	
12.828	
34.078	
23.934	
21.194	
14.978	
29.954	
46.733	
34.338	
46.714	
20.923	
31.982	
44.126	
22.028	
26.577	
35.751	
38.176	
32.375	
36.252	
30.463	
32.199	
60.900	

6.5 Effects of Industrial Processing and/or Household Preparation

Report: IIA 6.5/01 Moran, S.J. and Foster, J.E. 2010, Magnitude of Residues of Dicamba in Soybean Raw Agricultural and Processed Commodities after Application to MON 87708, Monsanto Report No. MSL0022660. MRID 47899524. PMRA No. 1894534.

Guidelines:

EPA Pesticide Assessment Guidelines, OCSPP 860.1500 Crop Field Trials, OCSPP 860.1520 Processed Food/Feed, Canada Pest Management Regulatory Agency Directive DIR98-02, Residue Chemistry Guidelines, Section 9 – Crop Field Trials

GLP: Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided.

Acceptability: Under the conditions and parameters used in the study, the industrial processing of soybean seed fractions is acceptable.

Materials and Methods:

Active substance (common name):	Dicamba
Crop/crop group:	Soybean/MON 87708
Content of active substance (g/kg or g/L)	480 g/L
Formulation:	MON 54140
Formulation Type:	Soluble concentrate
Application Rate and Timing:	1.0 lb/a (1.12 kg/ha) pre-emergence 2.0 lb/a (2.24 kg/ha) at R1/R2

Plots were established for the purpose of generating seed for processing at two sites of the field residue trial program conducted in 2008 in the United States to quantify the residues of dicamba in dicamba-tolerant soybean MON 87708 (section 6.3.1.2). In the plots intended to generate seed for processing, dicamba, formulated as MON 54140 was applied at an exaggerated total rate of 3.36 kg a.e./ha (3.0 lb a.e./a, 1.5 ×) in split applications, with the intent that the seed residue would be high enough to provide reliable data to determine whether residues concentrated in the commodities generated during processing, as shown in Table 6.5.1.

Table 6.5.1 Formulation and rate used for application to plots for generation of seed for processing

			Application Rate (expressed as dicamba acid equivalents)	
Treatment	**Number of Sites**	**Formulation code, type and content of the a.s. as a.e.**	**Pre-emergence**	**R1/R2**
8[a]	2	MON 54140 SL 480 g a.e./L	1.0 lb/a (1.12 kg/ha)	2.0 lb/a (2.24 kg/ha)

[a] An exaggerated rate (1.5×) was used at the two sites to produce seed for processing.

Seed for processing was shipped frozen to a small-scale processing facility which simulated commercial processing and was stored frozen until thawed for processing. Processed fractions were shipped to the analytical laboratory on dry ice and were placed into freezer storage and maintained frozen until analysis. All extractions of soybean processed fractions were completed within thirty days from the day they were processed; thus, a storage stability study for the processed fractions was not needed.

The processed fractions were analyzed to determine the residues of dicamba and 5-hydroxydicamba and the residues hydrolyzed to DCSA and DCGA using the same method "AG-ME-1321-01" used for the analysis of soybean forage, hay and seed (section 4.3). Minor preparation modifications were used for the processed tofu and lecithin such as specific dilution patterns. Results obtained from method validation for dicamba, 5-hydroxy dicamba, and DCSA in soybean processed fractions (soybean hulls, defatted flour, toasted defatted meal, protein isolate, protein concentrate, crude lecithin, degummed oil, refined bleached deodorized (RBD) oil, soymilk and tofu) were generally within the acceptable range of 70-120% at different fortification levels ranging from 0.01 to 3 μg/g , except recoveries of 5-hydroxydicamba in defatted flour, which were 31% and 64% at fortification levels of 0.01 and 0.02 ppm respectively. The limit of quantitation (LOQ), defined as the lowest concentration at which an acceptable recovery is obtained (lowest level of method validation (LLMV)) was 0.01 ppm in soybean processed samples.

Since residues of dicamba and 5-hydroxydicamba were below LOQ in the seed from the 1× treatment (Treatment 4, section 6.3.1.2), the seed used for processing (1.5× treatment, Treatment 8) and in all the processed fractions; therefore the processing factor for dicamba and 5-hydroxydicamba were not calculated. The processing factors were separately calculated based on the residues of DCSA and DCGA. LOQ (0.01 ppm) values were used in calculations when residues were below the LOQ. Table 6.5-2 shows the results of the calculation of the processing factors for both sites, and Table 6.5-3 shows the average concentration factors. A slight concentration was observed in hulls, toasted defatted meal and defatted flour in which the average concentration factors based on DCSA were 1.40, 1.35 and 1.26, respectively, and based on DCGA were 1.3 and 1.25 in toasted defatted meal and defatted flour, respectively. The processing factors for hulls and toasted defatted meal were below the EPA default processing factors of 11.3 and 2.2 for hulls and meal, respectively (US EPA OCSPP Test Guideline 860.1520 Processed Food/Feed and PMRA Regulatory Directive Dir98-02, Table 3 in Section 10- Processed Food/Feed).

Table 6.5-2 Residues and Concentration Factors from Processing of Soybean Seed

Processed Fraction	Average Residue[a] (mg/kg)		Concentration Factor[b]	
	DCSA	DCGA	DCSA	DCGA
York NE site				
Seed for processing	0.065	0.055	NA	NA
Hulls	0.082	0.054	1.27	0.99
Toasted defatted meal	0.076	0.069	1.18	1.26
Degummed oil	<0.010	<0.010	<0.16	<0.18
RBD[c] oil	<0.010	<0.010	<0.16	<0.18
Crude lecithin	<0.010	<0.010	<0.16	<0.18
Defatted flour	0.071	0.067	1.10	1.23
Protein isolate	<0.010	<0.010	<0.16	<0.18
Protein concentrate	<0.010	<0.010	<0.16	<0.18
Soymilk	<0.010	<0.010	<0.16	<0.18
Tofu	<0.010	<0.010	<0.16	<0.18
Delavan WI site				
Seed for processing	0.173	0.142	NA	NA
Hulls	0.263	0.142	1.52	1.00
Toasted defatted meal	0.260	0.191	1.51	1.34
Degummed oil	<0.010	<0.010	<0.06	<0.07
RBD[c] oil	<0.010	<0.010	<0.06	<0.07
Crude lecithin	0.037	<0.010	0.21	<0.07
Defatted flour	0.244	0.182	1.41	1.28
Protein isolate	0.028	0.017	0.16	0.12
Protein concentrate	0.015	0.015	0.08	0.11
Soymilk	0.016	0.010	0.09	0.07
Tofu	0.015	<0.010	0.09	<0.07

[a] Residues are expressed as each analyte per se
[b] Concentration factor = residue in processed fraction/ residue in corresponding seed
[c] RBD oil = refined, bleached, deodorized oil

Table 6.5.3. Overall Average Concentration Factors for Each Processed Fraction

Fraction	Average Concentration Factor[a]	
	DCSA	DCGA
Hulls	1.40	0.99
Toasted Defatted Meal	1.35	1.30
Degummed Oil	<0.11	<0.13
RBD Oil	<0.11	<0.13
Crude Lecithin	<0.18	<0.13
Defatted Flour	1.26	1.25
Protein Isolate	<0.16	<0.15
Protein Concentrate	<0.12	<0.14
Soymilk	<0.12	<0.13
Tofu	<0.12	<0.13

[a] Average Concentration factor = average of the site average concentration factor for each fraction. The LOQ was not determined for processed fractions; concentration factors were calculated based on the LLMV of 0.01 ppm.

Conclusions

Processing of soybean seed from plots at two locations treated with an exaggerated seasonal rate of 3 lb a.e./a (3.36 kg a.e./ha) of dicamba showed that DCSA concentration was observed in soybean hulls, toasted defatted meal and defatted flour upon processing and the concentration factors were 1.40, 1.35 and 1.26, respectively. DCGA residues concentrated in toasted defatted meal and defatted flour fractions, with factors of 1.30 and 1.25, respectively.

6. Residues in or on Treated Products, Food and Feed

6.3 Residues resulting from supervised trials on crops

Dicamba is a selective systemic herbicide belonging to the benzoic acid chemical family and is classified as a Group 4 herbicide. In the US, dicamba is registered for use on soybeans as pre-plant and pre-harvest applications. Dicamba is not registered for use on soybeans in Canada. The current application is for pre- and post-emergence uses of dicamba on dicamba-tolerant soybeans MON 87708.

Results from the supervised residue trials conducted in the US in/on dicamba tolerant soybeans during the 2008 growing season are reported below in support of the proposed uses. The US and Canadian use patterns are summarized below in Section 6.3.1.1.

Abbreviations contained in the tables are indicated in the legend below in order to avoid repetition in each table. Dicamba is formulated as different salts; therefore, the content of the active substance is expressed as acid equivalents.
A = acre
a.s. = active substance
a.e. = acid equivalents
DALA = days after the last application
DCSA = 3,6-dichloro-2-hydroxybenzoic acid
DCGA = 2,5-dichloro-3,6-dihydroxybenzoic acid
GPA = gallons per acre
HAFT = highest average field trial
5-OH dicamba = 2,5-dichloro-3-hydroxy-6-methoxybenzoic acid
ha = hectare
LOD = limit of detection
LOQ = limit of quantitation
na = not applicable
PHI = pre-harvest interval
RAC = raw agricultural commodity
SN = solution

6.3.1 Residue Trials (pre-harvest use on major crops)

6.3.1.1 Good Agricultural Practices (GAP) Relevant to the Highest Residues Likely to Occur

Crop	Country	Formulation type and content of a.s. as a.e.	Application				PHI
			Method	Rate lb a.e./A (kg a.e./ha)	Spray Volume GPA (L/ha)	No.	
Soybean	United States	SN 480	Broadcast Foliar Foliar	1.00 (1.12) 0.500 (0.560) 0.500 (0.560)	20.0 (187) 20.0 (187) 21.2 (198)	1 1 1	73-95
Soybean	Canada	SN 480	Broadcast Foliar	0.257-0.536 (0.288-0.600) 0.536 (0.600)	≥11.8 (≥110) ≥11.8 (≥110)	1 1*	73-95

*single or split application through R1 growth stage; maximum single application 0.536 lb a.e./A(0.600 kg a.e./ha); maximum yearly application 1.07 lb a.e./A (1.20 kg a.e./ha).

6.3.1.2 Residues resulting from supervised trials in soybean

Report:

IIA 6.3.1.2/1 MRID No. 47899524. PMRA No.1894534. Moran, S.J. and Foster, J.E., 2010. Magnitude of Residues of Dicamba in Soybean Raw Agricultural and Processed Commodities after Application to MON 87708. Monsanto Report No. MSL0022660.

Guidelines:

EPA Pesticide Assessment Guidelines, OPPTS 860.1500 Crop Field Trials, Canada Pest Management Regulatory Agency Directive DIR98-02, Residue Chemistry Guidelines, Section 9 – Crop Field Trials PMRA

GLP: Yes

Acceptability: The study is considered to be acceptable.

Materials and Methods:

The field program was conducted in 2008 at twenty-two locations in the United States to quantify the residues of dicamba and its metabolites in/on soybeans. The location of the North American soybean field trials are summarized in Table 6.3.1.2-1. The distribution of the soybean field trials included 2 trials in Region 2 (GA and SC), 3 trials in Region 4 (LA, AR and MO) and 17 trials in Region 5 (2 trials each in IL, IA, KS, MN, NE, SD and WI; and 1 trial each in IN, MI and ND). At each field site, treatment and control plots were established using dicamba-tolerant soybeans MON 87708. In addition to the dicamba tolerance trait, these seeds are also stacked with a glyphosate-tolerance trait. An untreated control plot (Treatment 1) and the Treatment 4 plot were established at all twenty-two sites. Residue declines trials were conducted at the IA-1 and MN-1 sites (Treatment 5). Side-by-side plots were established at the AR-1, IL-2, KS-1 and MN-2 sites in order to bridge the residue data between the MON 54140 (Treatment 6) and MON 11955 (Treatment 5) formulations of dicamba.

For each trial, the actual climatic conditions (average minimum and maximum air temperature, monthly rainfall and irrigation) were reported together with the historic climatic conditions (average minimum and maximum air temperature and monthly rainfall). The actual temperature recordings were generally within normal historical ranges for the study period. The monthly rainfall recordings were generally comparable to historic values. Irrigation was used as needed. Adverse weather conditions at the SD-2 site caused all V3 applications to be made at the V4 growth stage.

The different treatments used during the trials are summarized in Table 6.3.1.2-2. Two different soluble concentrate formulations of dicamba were used: MON 54140 and MON 11955. MON 54140 is formulated using the diglycolamine salt of dicamba. MON 11955 is formulated using the monoethanolamine salt of dicamba. During Treatment 4, the MON 11955 formulation of dicamba was applied using a broadcast spray once pre-emergence, and once each at the V3 growth stage (V4 at the SD-2 site) and at the R1/R2 growth stage, at nominal application rates of 1.00 lb a.e./A (1.12 kg a.e./ha), 0.500 lb a.e./A (0.560 kg a.e./ha) and 0.5 lb a.e./A (0.560 kg a.e./ha), respectively. Using either Treatment 5 (MON 11955 formulation) or Treatment 6 (MON 54140 formulation), dicamba was applied as a broadcast spray twice, once at the V3 growth stage and once at the R1/R2 growth stage, each at the nominal rate of 1.0 lb a.e./A (1.12 kg a.e./ha). All applications were made within ±5% of the target rate, except at the IA-1 (Treatment 4, foliar application at 0.451 lb a.e./A or 0.505 kg a.e./ha) and WI-1(Treatment 4, foliar application at 0.462 lb a.e./A or 0.517 kg a.e./ha) sites where application rates exceeded the ±5% target range. The pre-emergence application was made after planting but prior to crop emergence. The V3 post-emergence applications were made when at least 50% of the soybean plants

contained trifoliate leaves at three nodes. The R1/R2 post-emergent applications were made when at least 50% of the soybean plants were between flower (open flower at any node on the main stem) and full flower (open flowers at one of the two uppermost nodes on the main stem). All spray application were made within ±5% of the target spray volume of 19-21 GPA (178-196 L/ha), except at the LA-1, KS-1, IN-1 and WI-2 sites. A non-ionic surfactant (NIS) (80% minimum active) was included at a rate of 1 pint per 100 gallons of spray solution, except at the IA-1 and IA-2 sites where NIS was added to all pre-emergence treatments at 0.5 pint per 100 gallons, and at the WI-2 site where NIS was added to all pre-emergence applications at 2.0 pints per 100 gallons. "Spray grade" ammonium sulfate (AMS) was included for all applications at 17 lb per 100 gallons of spray solution. The actual application rates and spray volumes used at each site are reported in Tables 6.3.1.2-6, 6.3.1.2-7 and 6.3.1.2-8.

Soybean RACs were harvested at the typical commercial harvest stage. Samples of soybean seed were harvested at maturity without the pod, at 73-95 days after the last application (DALA). The whole aerial portion of the soybean plant was cut at 7-8 DALA for forage (10 DALA at the WI-2 site) and at 13-15 DALA for hay. Samples of soybean hay were dried in the field or on racks at the field facility, for 1 to 10 days to a moisture content of 10 to 20%. In addition, at the IA-1 and MN-1 sites, forage samples from Treatment 5 were harvested 3-, 7-, 10-, and 14-days after the final application and samples of soybean seed were harvested 7-days before normal seed harvest, at normal seed harvest and at 7- and 14- days after normal seed harvest, in order to evaluate residue decline. The "normal" seed harvest at the MN-1 site was collected 10 days after the first seed sampling and only four days before the third seed sampling.

At each site, two replicate samples were harvested from each treated plot and one sample from each control plot. A minimum of 1 kg RAC sample was collected for forage and seed, and 0.5 kg for hay from at least twelve separate areas of the plot, except at the NE-1 and KS-1 sites where hay samples weighed less than the minimum 0.5 kg requirement. Samples were collected first from the untreated control plots, and then from the treated plots. Each sample was composited from at least twelve separate sub-samples collected from different locations in the plot. Samples of forage and hay were harvested using hand pruners, shears, sickles and hedge trimmers. Samples of seed were harvested using a combine. Control and treated plots harvested at the same site were harvested on the same day and placed into sample bags with bar-coded labels. The only exception is that during the decline trials only one control sample was harvested for forage and seed at the sites, although samples were harvested on four separate days. The treated plots were sampled in the order: Treatment 4, Treatment 5 and Treatment 6. Control and treated samples were transported from the field in coolers with blue ice, dry ice or wet ice (encased in plastic), or in transport freezers. Samples remained frozen prior to and during shipment to the analytical facility at Monsanto Company. The RAC samples were stored frozen at the field until shipping by ACDS freezer truck to the Monsanto analytical facility in St. Louis, Missouri. All RAC samples were received frozen and in good condition at the sponsor facility. Samples of soybean RAC were processed or ground using dry ice and were maintained frozen at ~-20°C, except when a sample was removed for analysis.

Table 6.3.1.2-1 Location of the Soybean Trials Conducted in North America

Crop/Crop Group	NAFTA Region	No. of Trials Submitted	No. of Trials Required	
			Canada[a]	USA[b]
Soybean/6- Legume Vegetables (Succulent or Dried)	2	2	-	2
	4	3	-	3
	5	17	12	15

[a] Regulatory Directive Dir98-02.
[b] OPPTS 860.1500 Crop Field Trials.

Table 6.3.1.2-2 Treatments Conducted in Residue Trials with Dicamba on Dicamba-Tolerant Soybeans

			Nominal Application Rate (expressed as dicamba acid equivalents)		
Treatment	**Number of Sites**	**Formulation**	**Pre-emergence**	**V3**	**R1/R2**
1 (Control)	22	-	-	-	-
4	22	MON 11955	1.00 lb a.e./A (1.12 kg a.e./ha)	0.500 lb a.e./A (0.560 kg a.e./ha)	0.500 lb a.e./A (0.560 kg a.e./ha)
5	6[a]	MON 11955	-	1.00 lb a.e./A (1.12 kg a.e./ha)	1.00 lb a.e./A (1.12 kg a.e./ha)
6	4[b]	MON 54140	-	1.00 lb a.e./A (1.12 kg a.e./ha)	1.00 lb a.e./A (1.12 kg a.e./ha)

[a] AR-1, IL-2, IA-1, KS-1, MN-1, MN-2.
[b] AR-1, IL-2, KS-1, MN-2.

Analytical method AG-ME-1321-01 was used for the determination of dicamba and its metabolites 5-OH dicamba, DCGA and DCSA in soybean seed, forage and hay. The full description of this method and the results of the method validation are presented in Section 4.3.

Soybean matrices were extracted with 40:60 (v:v) acetonitrile:water. An aliquot of the extract was hydrolyzed in 1N HCl at 95°C in a water bath, converting the glycoside conjugates to DCGA and DCSA. The hydrolyzate was partitioned with 40:60 (v:v) ethyl acetate:isooctane. The organic phase was partially concentrated, after which water was added. Each sample was concentrated to the aqueous remainder and then was filtered, acidified and quantified by liquid chromatography with tandem mass spectrometry (LC-MS/MS) with turbo ion spray ionization in the negative ion mode. This method uses analyte specific ^{13}C-labeled internal standards to compensate for matrix effects and procedural recovery. When the concentration of samples exceeded the concentration of the highest calibration standard, the samples were re-extracted and diluted prior to analysis. Due to the considerable difference in the concentration of DCGA and DCSA in forage and hay compared to dicamba and 5-OH dicamba, these analytes were often quantitated in separate analyses.

Method AG-ME-1321-01 was validated concurrently for all four analytes during each analysis set at spiking levels ranging from 0.01 to 5.0 ppm in seed and from 0.01 to 150 ppm in forage. In hay, DCSA and DCGA were spiked at levels ranging from 0.01 to 150 ppm, and dicamba and 5-OH dicamba were spiked at 0.01 to 50 ppm and 0.01 to 0.4 ppm, respectively. A standard solution containing DCSA, dicamba and 5-OH dicamba and their corresponding ^{13}C-analogs was spiked directly into the matrix prior to extraction. The DCGA standard solution and its ^{13}C-analog were spiked into the extraction aliquot given that poor recoveries were obtained with spiking directly to the matrix. This approach is not in accordance with OCSPP Residue Chemistry Guidelines 860.1340 or with PMRA Regulatory Directive Dir98-02 (Section 3.3.2).

In order to determine the LOD and LOQ statistically in soybean matrices, additional samples of seed (n = 4-14 per spiking level/analyte combination), forage (n = 8 per spiking level/analyte combination) and hay (n = 8 per analyte/spiking level combination) were each spiked at 0.0005 ppm and 0.001 ppm. This analysis was conducted at the Statistics Technology Center of Monsanto Regulatory using SAS Software (Version 9.2). The LOD and LOQ for soybean seed, forage and hay, as determined by statistical analysis, are reported in Table 6.3.1.2-3.

Table 6.3.1.2-3 Calculated LOD and LOQ for Dicamba Residues in Soybean Forage, Hay and Seed

		LOD and LOQ[a] (ppm[b])			
Matrix	**Parameter**	**DCGA**	**DCSA**	**Dicamba**	**5-OH Dicamba**
Forage	**LOD**	0.004	0.012	0.015	0.004
	LOQ	0.006	0.013	0.021	0.005
Hay	**LOD**	0.006	0.006	0.011	0.010
	LOQ	0.013	0.007	0.014	0.014
Seed	**LOD**	0.006	0.004	0.010	0.014
	LOQ	0.011	0.005	0.013	0.021

[a] The LOQ is the $LOQ_{20\%}$, corresponding to a coefficient of variation of 20%.
[b] The LOD and LOQ are expressed as each analyte *per se*.

The residue levels for DCGA, DCSA and 5-OH dicamba, reported as the analyte *per se*, were each converted to dicamba equivalents using a molecular weight conversion factor as outlined below:

DCGA:(MW Dicamba 221.04 g/mol) ÷ (MW DCGA 223.01 g/mol) = 0.991

DCSA: (MW Dicamba 221.04 g/mol) ÷ (MW DCSA 207.01 g/mol) = 1.07

5-OH dicamba: (MW Dicamba 221.04 g/mol) ÷ (MW 5-OH 237.04 g/mol) = 0.933

Results

The concurrent recoveries of DCGA, DCSA, dicamba and 5-OH dicamba from soybean matrices are summarized in Table 6.3.1.2-4. Recoveries of dicamba and 5-OH dicamba from soybean forage, hay and seed were not consistently within the acceptable 70-120% range at the 0.010 ppm spiking level. As such, the LOQ and LOD for dicamba, 5-OH dicamba, DCSA and DCGA were determined statistically. All the residue data were assessed based on the calculated LOQs in soybean matrices. This approach is considered acceptable for the purposes of this study.

Table 6.3.1.2-4 Concurrent Recoveries from Analysis of Fortified Control Samples of Soybean RACs.

Matrix	Spike Level (ppm)	Sample Size (n)	Corrected Recoveries (%)[a]	Mean ± Standard Deviation (%)[b]
DCGA[c]				
Soybean Forage	0.010	15	89.2, 112, 80.8, 82.8, 83.9, 79.6, 83.7, 79.4, 80.0, 82.8, 82.0, 74.8, 78.9, 80.4, 87.3	83.8 ± 8.5
	0.020	15	98.5, 83.1, 89.3, 95.4, 96.9, 92.6, 93.6, 107.0, 103.5, 101.2, 81.0, 95.5, 84.0, 87.5, 88.5	93.2 ± 7.6
	0.050	7	91.8, 91.9, 87.7, 99.5, **123**, 102, 82.2	96.9 ± 13.3
	0.100	5	86.6, 108.0, 85.1, 87.2, 92.3	91.8 ± 9.4
	0.200	11	94.5, 112, **69.2**, 70.8, 99.0, 96.7, 97.5, 71.5, 88.4, 101, 78.0	89.0 ± 14.4
	0.400	4	96.5, 94.3, 95.0, 86.8	93.2 ± 4.3

Matrix	Spike Level (ppm)	Sample Size (n)	Corrected Recoveries (%)[a]	Mean ± Standard Deviation (%)[b]
	5.00	1	111	111
	10.0	1	108	108
	25.0	2	96.0, 99.2	97.6
	50.0	4	99.6, 103, 117, 118	109 ± 9.5
	150	2	**125, 125**	**125**
Soybean Hay	0.010	12	**66.7**, 73.7, 78.3, 79.3, **64.7**, 78.8, 83.2, 74.4, 90.2, 89.1, 96.4, 100	81.2 ± 11.0
	0.020	13	76.5, 79.0, 81.0, 90.5, 82.3, 91.5, 91.0, 105, 82.5, 105, 101, 83.6, 90.4	89.2 ± 9.6
	0.050	10	81.9, 101, 97.4, 85.2, 80.2, 104, 93.6, 106, 92.1, 101	94.2 ± 9.3
	0.100	5	82.4, 84.3, 86.0, 75.4, **69.0**	79.4 ± 7.1
	0.200	7	110, 70.0, 91.6, 79.5, 84.0, 88.8, 104	89.7 ± 13.8
	0.400	6	94.1, 96.8, 70.8, 79.0, 83.0, 80.0	84.0 ± 9.8
	0.800	1	115	115
	10.0	2	78.0, 77.2	77.6
	50.0	3	92.8, 99.6, 106	99.5 ± 6.6
	100	2	92.2, 93.2	92.7
	150	2	96.0, 100	98.0
Soybean Seed	0.010	12	77.8, 95.3, 83.0, 78.1, 71.1, 88.0, 89.7, 74.4, 74.0, 99.7, 93.2, 75.9	83.4 ± 9.5
	0.020	14	105, 72.4, 82.5, 88.0, 91.5, 115.4, 95.5, 94.0, 90.1, 82.5, 106, 99.0, 103, 99.6	94.6 ± 11.3
	0.050	13	92.4, 92.1, 88.8, 99.2, 122, **64.4**, 95.4, 97.8, 71.1, 107, 101, 102, 94.4	94.4 ± 14.6
	0.100	4	86.9, 87.1, 90.1, 98.5	90.7 ± 5.4
	0.200	5	91.5, 99.5, **66.5**, 115, 117	97.9 ± **20.5**
	0.400	4	88.8, 86.8, 99.9, 119	98.6 ± 14.8
	1.00	2	106, 137	122
	5.00	2	**128, 135**	132
DCSA				
Soybean Forage	0.010	13	73.8, 84.5, 91.6, 110, 89.4, 85.3, 86.7, 82.5, 89.9, 88.6, 96.7, 98.7, 104	90.9 ± 9.5

Matrix	Spike Level (ppm)	Sample Size (n)	Corrected Recoveries (%)[a]	Mean ± Standard Deviation (%)[b]
	0.020	16	99.0, 97.2, 96.5, 84.4, 101, 97.0, 95.0, 104, 83.9, 82.0, 94.8, 84.3, 105, 107, 108, 93.7	95.8 ± 8.4
	0.050	11	99.3, 101, 99.4, 80.4, 97.5, 100, 90.8, 105, 103, 97.9, 102	97.8 ± 6.8
	0.100	7	99.4, 114, 84.5, 107, 111, 113, 114	106 ± 10.9
	0.200	11	108, 105, 102, 104, 99.7, 104, 105, 102, 104, 98.5, 106	103 ± 2.8
	0.400	4	108, 97.6, 105, 107	104 ± 4.7
	5.00	1	85.8	85.8
	10.0	1	89.2	89.2
	25.0	2	88.0, 89.6	88.8
	50.0	4	91.8, 91.8, 92.2, 99.6	93.9 ± 3.8
	150	2	101, 105	103
Soybean Hay	0.010	11	99.1, 105, 108, **124**, 100, 72.3, 76.4, 93.2, 81.3, 80.3, 99.2	94.4 ± 15.6
	0.020	13	108, 111, 112, 91.0, 102, 90.6, 85.2, 82.3, 84.3, 102, 98.1, 90.7, 104	97.0 ± 10.3
	0.050	10	108, 109, 105, 103, 95, 98, 99, 96, 95, 109	102 ± 5.8
	0.100	5	112, 114, 115, 114, 103	112 ± 4.9
	0.200	8	109, 102, 107, 112, 110, 97.2, 98.4, 99.6	104 ± 5.8
	0.400	8	101, 107, 110, 101, 108, 112, 99.4, 108	106 ± 4.7
	5.00	1	92.0	92.0
	10.00	2	101, 96.5	98.8
	50.0	2	105, 108	107
	100	2	106, 108	107
	150	2	90, 92	91
Soybean Seed	0.010	12	93.9, 102, 81.2, 95.7, 110, 114, 116, 105, 120, 98.4, 93.7, 102	103 ± 11.1
	0.020	14	113, 94.5, 104, 114, 118, 94.5, 95.8, 98.5, 110, 112, 119, 97.5, 93.0, 95.5	104 ± 9.7
	0.050	14	115, 108, 101, 101, 96.4, 109, 101, 101, 104, 108, 99, 106, 104, 116	105 ± 5.8
	0.100	4	110, 113, 115, 112	113 ± 2.1

Matrix	Spike Level (ppm)	Sample Size (n)	Corrected Recoveries (%)[a]	Mean ± Standard Deviation (%)[b]
	0.200	5	116, 104, 99.0, 102, 105	105 ± 6.5
	0.400	4	118, 107, 99, 103	107 ± 8.2
	1.00	2	99.9, 111	105
	5.00	2	96.4, 103	99.7
Dicamba				
Soybean Forage	0.010	13	**35.7**, **63.2**, **137.0**, 87.7, **63.9**, 80.8, **65.7**, 92.7, 85.9, 95.2, 110, **121**, **57.4**	84.3 ± **27.8**
	0.020	15	106, 91.5, 76.5, 99.5, 107, 81.2, 101, 71.6, 77.0, 86.0, 75.0, 79.4, 102, **126**, 118	93.2 ± 16.8
	0.050	10	108, 113, 93.2, 89.4, 84.7, 105, 97.2, 100, 81.2, 108	98.0 ± 10.7
	0.100	7	112, 113, 82.5, 103, 107, 112, 118	107 ± 11.7
	0.200	10	91.5, 102, 88.5, 112, 90.0, 99.5, 106, 92.7, 111, 104	99.7 ± 8.7
	0.400	4	99.4, 95.5, 117, **126**	109 ± 14.5
	5.00	1	89.2	89.2
	10.0	1	90.3	90.3
	25.0	2	88.0, 90.8	89.4
	50.0	4	88.0, 92.6, 98.2, 119	99.5 ± 13.7
	150	2	117, **122**	120
Soybean Hay	0.010	12	76.1, 90.8, 115, **131**, 102, 106, 92.8, 119, 70.5, **35.9**, 75.3, **123**	94.8 ± **27.1**
	0.020	13	96.0, 102, **123**, 100, 99.9, 93.1, 101, 72.5, 85.1, 102, 77.0, 83.5, 97.3	94.8 ± 13.0
	0.050	10	115, 110, 116, 92.5, 97.7, 95.0, 95.6, 106, 91.4, 119	104 ± 10.6
	0.100	5	104, 106, 109, 98.4, 97.4	103 + 5.0
	0.200	3	109, **125**, 100	111 ± 12.6
	0.400	3	113, 117, 118	116 ± 2.6
	50.0	2	108, 117	113
Soybean Seed	0.010	12	73.8, 94.8, **53.3**, **66.2**, **68.1**, 86.9, 104, **60.8**, 90.2, 85.5, 94.5, **126**	83.7 ± 20.4
	0.020	14	82.0, 81.0, 93.0, 101 102, 84.0, 79.5, 76.5, 111, 94.9, 108, 100, 100, 105	94.1+ 11.5

Matrix	Spike Level (ppm)	Sample Size (n)	Corrected Recoveries (%)[a]	Mean ± Standard Deviation (%)[b]
	0.050	14	91.8, 105, 112, 94.2, 108, 97.1, 97.8, 97.9, 80.2, 88.6, 91.8, 95.0, 104, 114	98.4 ± 9.4
	0.100	4	101, 103, 109, 96.8	102 ± 5.1
	0.200	5	90.5, 106, 100, 101, 99.0	99.3 ± 5.6
	0.400	4	91.0, 103, 101, 93.5	97.1 ± 5.8
	1.00	2	102, 112	107
	5.00	2	96.6, 101	98.8
5-OH Dicamba				
Soybean Forage	0.010	17	96.6, 75.5, **68.7**, 82.1, 76.2, 81.3, 88.2, 75.4, 87.3, 73.7, **61.0**, 81.3, 104, 107, 85.6, 93.0, 97.9	84.4 ± 12.5
	0.020	19	87.5, 90.7, 71.3, 89.0, 92.5, 98.5, 87.5, 94.5, 106, 91.0, 93.5, 94.0, 96.0, 98.0, 92.9, 101, 103, 105, 90.0	93.8 ± 7.8
	0.050	10	107, 113, 101, 88.0, 99.6, 99.0, 112, 92.8, 95.8, 97.0	101 ± 8.1
	0.100	7	93.9, 101, 105, 99.6, 108, 108, 109	104 ± 5.6
	0.200	5	95.0, 103, 116, 91.0, 96.0	100 ± 9.8
	0.400	2	98.3, 112	105
	50.0	2	105, 113	109
	150	2	113, 123	118
Soybean Hay	0.010	13	**63.6**, 73.5, 78.5, 81.9, **69.9**, 81.3, **60.3**, **13.1**, 73.4, 86.5, **49.7**, **54.7**, **52.5**	**64.5** ± 19.5
	0.020	14	89.0, 92.5, 95.0, 78.5, 89.5, **54.0**, 94.0, 94.5, 80.0, 77.3, 94.5, 92.0, 82.0, 92.0	86.1 ± 11.2
	0.050	11	95.8, 104, 98.2, 101, 103, 103, 99.0, 93.0, 105, 104, 86.6	99.3 ± 5.7
	0.100	6	102, 103, 104, 107, 100, 104	103 ± 2.3
	0.200	2	111, 97.5	104
	0.400	1	102	102
Soybean Seed	0.010	13	70.1, 88.6, 61.2, **57.2**, 72.9, 93.4, **121**, **137**, **46.2**, **52.6**, **54.2**, **13.9**, 71.6	72.3 ± **32.2**
	0.020	15	72.0, 82.5, **62.5**, 91.0, 117, 118, **126**, 99.0, 92.7, 83.0, 88.0, 90.1, 70.0, 101, 92.0	92.3 ± 18.0

Matrix	Spike Level (ppm)	Sample Size (n)	Corrected Recoveries (%)[a]	Mean ± Standard Deviation (%)[b]
	0.050	15	92.8, 101, 99.3, 96.0, 117, 97.0, 86.0, 94.6, 74.9, 75.1, 87.4, 99.8, 96.4, 98.0, 86.6	93.5 ± 10.5
	0.100	4	109, 113, 116, 104	111 ± 5.2
	0.200	6	88.5, 101, 91.5, 117, 99.0, 99.0	99.3 ± 9.9
	0.400	4	97.5, 104, 93.0, 94.8	97.3 ± 4.8
	1.00	1	104	104
	5.00	2	109, 112	111

[a]Recoveries outside the 70-120% range are indicated in bold text.
[b]Mean recoveries outside the 70-120% and standard deviations >20% are indicated in bold text.
[c]DCGA and its labeled internal standard were not spiked directly to the matrix

The storage period of soybean samples during the field trial study are summarized in Table 6.3.1.2-5. Soybean samples were stored frozen for a maximum interval of 293 days (forage), 284 days (hay) and 177 days (seed) for DCGA; 280 days (forage), 284 days (hay) and 177 days (seed) for DCSA; 279 days (forage), 284 days (hay) and 177 days (seed) for dicamba; and 279 days (forage), 284 days (hay) and 177 days (seed) for 5-OH dicamba. Selected forage samples from the Proctor, Arkansas and Montezuma, Georgia sites were re-extracted and analyzed for residues of DCGA, DCSA, dicamba and 5-OH dicamba in August 2009 to resolve discrepancies between field replicates. The storage interval for those samples was a maximum of 400 days. Residues of dicamba have been demonstrated concurrently during a previously reviewed soybean field trial study (PMRA No. 1640670; MRID No. 43814101) and a processing study (PMRA No. 1640657; MRID 43814102) to be relatively stable during freezer storage for approximately 4.1 months in forage extracts (dicamba, DCA and 5-OH dicamba), 3 months in seed extracts (dicamba, DCSA and 5-OH dicamba) and 6 months in the seed RAC (dicamba). Given that samples were stored frozen throughout the study and the available data demonstrating the stability of dicamba residues in soybean matrices, there is reasonable expectation that residues of dicamba were stable in soybean matrices during freezer storage.

Table 6.3.1.2-5 Storage Period of Soybean Samples

Matrix	Storage Temperature (°C)	Actual Storage Duration from Harvest to Analysis[a] (Days)			
		DCGA	DCSA	Dicamba	5-OH Dicamba
Forage	~-20	121 to 293[b]	121-280[b]	121-279[b]	146-279[b]
Hay		144-284	144-284	144-284	144-284
Seed		31-177	31-177	31-177	31-177

[a] The analysis of samples was conducted within three days of extraction.
[b] Selected forage samples from the Proctor, Arkansas and Montezuma, Georgia sites were re-extracted and analyzed in August 2009 to resolve discrepancies between field replicates. The storage interval for those samples was a maximum of 400 days.

The residue data from the trials conducted using Treatment 4 are reported in Table 6.3.1.2-6 and are summarized in Table 6.3.1.2-9. Residues of DCGA ranged from 0.356 ppm to 5.90 ppm in forage, from 0.167 ppm to 7.26 ppm in hay, and from <0.011 ppm (<LOQ) to 0.135 ppm in seed. Residues of DCSA ranged from 8.92 ppm to 51.3 ppm in forage, from 12.2 ppm to 61.1 ppm in hay and from 0.010 ppm to 0.440 ppm in seed. Residues of dicamba ranged from <0.021 ppm (<LOQ) to 2.62 ppm in forage, from <0.014 ppm (<LOQ) to 1.16 ppm in hay, and were non-quantifiable (<LOQ; <0.013 ppm) in seed. Residues of 5-OH dicamba ranged from <0.005 ppm (<LOQ) to 0.009 ppm in forage, and were <LOQ in hay (<0.014 ppm) and seed (<0.021 ppm).

The residue data from the trials conducted to bridge the two SC formulations MON 11955 (monethanolamine salt of dicamba; Treatment 5) and MON 54140 (DGA salt of dicamba; Treatment 6) are reported in Table 6.3.1.2-7. The residue profile in soybean forage, hay and seed is similar following treatment with the two different formulations of dicamba.

Residue data from the two decline trials are reported in Table 6.3.1.2-8. In forage, residues (mean) of DCGA and DCSA decreased over the sampling period. Residues (mean) of dicamba in forage from the IA-1 site were low at 3 days after the last application (0.082 ppm) and were <LOQ (<0.021 ppm) at all subsequent intervals. At the MN-1 site, residues of dicamba (mean) in forage decreased over the sampling period. Residues (mean) of 5-OH dicamba in forage, although low, decreased over the sampling period from 0.007-0.008 ppm at the 3-day PHI to <0.005 ppm at the 10-day and 14-day PHIs. In seed, residues (mean) of dicamba and 5-OH dicamba were each <LOQ at each sampling period. Therefore, no conclusion can be made regarding residue decline of these 2 analytes in seed. Residues (mean) of DCGA and DCSA in seed at the IA-1 site did not change over the sampling period. At the MN-1 site, residues (mean) of DCGA and DCSA in seed decreased slightly by the end of the sampling period.

Residues of DCGA, DCSA, dicamba and 5-OH dicamba were generally not quantified (i.e., <LOQ) in untreated control samples of soybean forage, hay and seed, except for residues of DCSA in one forage sample (0.0165 ppm; sample # REG08096-00327, KS-2 site), residues of DCSA in two hay samples (0.0128 ppm; sample No. REG08096-00674, WI-2 site and 0.0113 ppm; sample # REG08096-00584, MN-1 site) and residues of dicamba in one forage sample (0.0441 ppm; sample # REG08096-00426, SD-2 site). Residues of DCGA were detected in one forage sample (0.0425 ppm; sample # REG08096-00426, SD-2 site) and residues of dicamba were detected in one forage sample (0.0168; sample #REG08096-00381, ND-1 site).

Table 6.3.1.2-6 Residues in Soybean Raw Agricultural Commodities Following Three Applications of the SC Formulation MON 11955 (Monoethanolamine Salt of Dicamba) from Trials Conducted in the United States during the 2008 Growing Season (Treatment No. 4). A Non-Ionic Surfactant (NIS) and Ammonium Sulfate (AMS) were included in all Spray Applications

City, State and NAFTA Region (Trial No.)	Formulation	Application No./Method	Rate/Application				Growth Stage at Each Application	Matrix	Residues (ppm)[a]				PHI (Days)
			lb a.e/A	GPA	kg a.e.ha	L/ha			DCGA[b]	DCSA	Dicamba	5-OH Dicama	
Proctor, Arkansas, Region 4 (AR-1)	MON 11955	1. Broadcast	1.00	20.2	1.12	189	Pre-emergence	Forage	1.95 1.76	21.6 18.6	0.051 0.058	0.007 0.008	7
		2. Foliar	0.499	20.3	0.559	190	V3						
		3. Foliar	0.500	20.3	0.560	190	R1	Hay	1.93 2.46	30.2 39.7	0.049 0.052	<0.014; <0.014 (0.010)	14
								Seed	<0.011 (0.008); <0.011 (0.006)	0.034 0.047	<0.013 <0.013	<0.021 <0.021	89
Montezuma, Georgia Region 2 (GA-1)	MON 11955	1. Broadcast	0.988	20.4	1.11	191	Pre-emergence	Forage	4.63 5.90	51.3 49.4	<0.021 <0.021 (0.015)	0.008 0.006	8
								Hay	0.428 0.374	12.2 13.4	<0.014 <0.014	<0.014 <0.014	15
		2. Foliar	0.498	20.0	0.558	187	V3	Seed	0.126 0.135	0.437 0.440	<0.013 <0.013	<0.021 <0.021	77
		3. Foliar	0.498	19.9	0.558	186	R1/R2						
Richland, Iowa Region 5 (IA-1)	MON 11955	1. Broadcast	1.01	19.7	1.13	184	Pre-emergence	Forage	1.54 1.94	14.1 14.2	<0.021 <0.021	<0.005 <0.005 (0.004)	7
								Hay	3.74 3.62	36.0 32.1	<0.014 0.014	<0.014 <0.014	14
		2. Foliar	0.451	20.2	0.505	189	V3	Seed	0.012 0.013	0.012 0.014	<0.013 <0.013	<0.021 <0.021	80
		3. Foliar	0.507	19.8	0.568	185	R1						
Hedrick, Iowa Region 5 (IA-2)	MON 11955	1. Broadcast	0.995	20.0	1.11	187	pre-emergence	Forage	1.61 1.72	10.1 10.2	0.025 0.030	<0.005 <0.005	8

City, State and NAFTA Region (Trial No.)	Formulation	Application No./Method	Rate/Application				Growth Stage at Each Application	Matrix	Residues (ppm)[a]				PHI (Days)
			lb a.e/A	GPA	kg a.e.ha	L/ha			DCGA[b]	DCSA	Dicamba	5-OH Dicama	
		2. Foliar	0.516	20.2	0.578	189	V3	Hay	6.20 5.78	23.5 24.3	0.026 0.021	<0.014 (0.011); <0.014 (0.010)	14
		3. Foliar	0.501	19.6	0.561	183	R1	Seed	0.018 0.014	0.012 0.011	<0.013 <0.013	<0.021 <0.021	95
Wyoming, Illinois Region 5 (IL-1)	MON 11955	1. Broadcast	1.00	20.3	1.12	190	Pre-emergence	Forage	1.76 1.79	13.9 13.4	<0.021 (0.020); 0.029	<0.005 <0.005	7
		2. Foliar	0.501	20.1	0.561	188	V3						
		3. Foliar	0.499	19.7	0.559	184	R1/R2	Hay	0.416 0.761	20.5 21.8	0.027 0.032	<0.014 <0.014	14
								Seed	0.018; <0.011 (0.010)	0.017 0.011	<0.013 <0.013	<0.021 <0.021	95
Carlyle, Illinois Region 5 (IL-2)	MON 11955	1. Broadcast	1.03	20.3	1.15	190	Pre-emergence	Forage	1.95 2.19	13.5 14.1	<0.021 <0.021	<0.005 <0.005	8
		2. Foliar	0.510	20.9	0.571	195	V3	Hay	1.33 1.41	14.0 15.9	<0.014 <0.014	<0.014 <0.014	15
		3. Foliar	0.500	19.8	0.560	185	R1	Seed	0.026 0.024	0.048 0.050	<0.013 <0.013	<0.021 <0.021	74
Rockville, Indiana Region 5 (IN-1)	MON 11955	1. Broadcast	1.00	20.2	1.12	189	Pre-emergence	Forage	2.20 2.22	17.1 15.0	1.11 1.30	0.008 0.009	7
								Hay	6.41 4.62	32.4 26.9	0.283 0.296	<0.014 <0.014	15
		2. Foliar	0.496	19.9	0.556	186	V3	Seed	0.038 0.051	0.045 0.042	<0.013 <0.013	<0.021 <0.021	73
		3. Foliar	0.491	18.8	0.550	176	R1						
Cunningham, Kansas Region 5 (KS-1)	MON 11955	1. Broadcast	0.987	20.0	1.11	187	Pre-emergence	Forage	1.21 1.09	19.0 19.6	<0.021 <0.021	<0.005 0.005	8
								Hay	1.11 1.48	48.5 44.9	0.021 0.017	<0.014 <0.014	14

City, State and NAFTA Region (Trial No.)	Formulation	Application No./Method	Rate/Application				Growth Stage at Each Application	Matrix	Residues (ppm)[a]				PHI (Days)
			lb a.e/A	GPA	kg a.e.ha	L/ha			DCGA[b]	DCSA	Dicamba	5-OH Dicama	
		2. Foliar	0.489	20.4	0.548	191	V3	Seed	0.011; <0.011 (0.009)	0.024 0.020	<0.013 <0.013	<0.021 <0.021	95
		3. Foliar	0.508	21.2	0.569	198	R1						
Hudson, Kansas Region 5 (KS-2)	MON 11955	1. Broadcast	1.02	20.4	1.14	191	Pre-emergence	Forage	2.18 2.16	20.7 21.3	<0.021 <0.021	<0.005 <0.005	7
								Hay	1.40 1.31	31.7 36.9	0.021 0.027	<0.014 <0.014	13
		2. Foliar	0.493	19.7	0.552	184	V3	Seed	0.016 0.016	0.013 0.016	<0.013 <0.013	<0.021 <0.021	77
		3. Foliar	0.518	20.7	0.580	194	R1/R2						
Washington, Louisiana Region 4 (LA-1)	MON 11955	1. Broadcast	0.994	20.1	1.11	188	pre-emergence	Forage	2.21 2.09	19.2 19.6	0.095 0.097	<0.005 0.006	7
		2. Foliar	0.492	21.3	0.551	199	V3	Hay	2.00 1.93	45.7 47.5	0.092 0.108	<0.014 <0.014	14
		3. Foliar	0.507	20.0	0.568	187	R1	Seed	<0.011 (0.006); <0.011 (0.006)	0.02 0.017	<0.013 <0.013	<0.021 <0.021	85
Conklin, Michigan Region 5 (MI-1)	MON 11955	1. Broadcast	1.00	19.7	1.12	184	Pre-emergence	Forage	1.29 1.36	10.2 8.92	0.925 1.09	0.006 0.007	7
		2. Foliar	0.500	19.9	0.560	186	V3						
		3. Foliar	0.496	19.9	0.556	186	R1/R2	Hay	3.46 2.39	21.7 18.1	1.16 0.858	<0.014 (0.013); <0.014	13
								Seed	0.061 0.055	0.095 0.090	<0.013 <0.013	<0.021; <0.021 (0.014)	88
Campbell, Minnesota	MON 11955	1. Broadcast	0.999	20.0	1.12	187	pre-emergence	Forage	1.94 1.95	13.8 14.3	0.282 1.56	<0.005	7

City, State and NAFTA Region (Trial No.)	Formulation	Application No./Method	Rate/Application				Growth Stage at Each Application	Matrix	Residues (ppm)[a]				PHI (Days)
			lb a.e/A	GPA	kg a.e.ha	L/ha			DCGA[b]	DCSA	Dicamba	5-OH Dicama	
Region 5 (MN-1)												<0.005	
		2. Foliar	0.501	20.0	0.561	187	V3	Hay	2.31 1.96	34.3 29.6	0.021 <0.014 (0.0136)	<0.014 <0.014	14
		3. Foliar	0.501	20.0	0.561	187	R1	Seed	0.043 0.045	0.054 0.056	<0.013 <0.013	<0.021 <0.021	78
Fergus Falls, Minnesota Region 5 (MN-2)	MON 11955	1. Broadcast	1.00	20.1	1.12	188	pre-emergence	Forage	2.56 2.43	14.9 14.3	0.275 0.368	<0.005 <0.005	7
								Hay	2.00 4.11	40.7 47.3	0.096 0.128	<0.014 <0.014	15
		2. Foliar	0.502	20.1	0.562	188	V3	Seed	0.048 0.054	0.071 0.074	<0.013 <0.013	<0.021 <0.021	78
		3. Foliar	0.501	20.0	0.561	187	R2						
Fisk, Missouri Region 4 (MO-1)	MON 11955	1. Broadcast	0.995	19.9	1.11	186	Pre-emergence	Forage	0.356 0.381	18.2 16.3	0.063 0.063	0.006 <0.005	7
								Hay	0.765 0.700	21.6 22.4	<0.014 <0.014	<0.014 <0.014	15
		2. Foliar	0.504	20.2	0.564	189	V3	Seed	0.014 0.016	0.020 0.026	<0.013 <0.013	<0.021 <0.021	81
		3. Foliar	0.506	20.2	0.567	189	R1/R2						
Carrington, North Dakota Region 5 (ND-1)	MON 11955	1. Broadcast	1.00	20.1	1.12	188	Pre-emergence	Forage	0.615 0.844	12.1 12.7	0.302 0.288	<0.005 <0.005	7
		2. Foliar	0.500	20.0	0.560	187	V2-V3	Hay	4.72 4.70	26.6 26.2	0.201 0.125	<0.014 <0.014	14
		3. Foliar	0.505	20.1	0.566	188	R1	Seed	0.048 0.041	0.059 0.051	<0.013 <0.013	<0.021 <0.021	87

City, State and NAFTA Region (Trial No.)	Formulation	Application No./Method	Rate/Application				Growth Stage at Each Application	Matrix	Residues (ppm)[a]				PHI (Days)
			lb a.e/A	GPA	kg a.e.ha	L/ha			DCGA[b]	DCSA	Dicamba	5-OH Dicama	
York, Nebraska Region 5 (NE-1)	MON 11955	1. Broadcast	1.01	20.1	1.13	188	Pre-emergence	Forage	1.52 1.39	12.7 12.3	0.350 0.262	0.006 <0.005 (0.004)	7
		2. Foliar	0.502	20.0	0.562	187	V3						
		3. Foliar	0.500	20.0	0.560	187	R2	Hay	1.31 1.49	33.4 37.5	0.285 0.288	<0.014 <0.014	14
								Seed	0.030 0.029	0.029 0.026	<0.013 <0.013	<0.021 <0.021	87
Osceola, Nebraska Region 5 (NE-2)	MON 11955	1. Broadcast	0.979	19.5	1.10	182	Pre-emergence	Forage	1.53 1.65	13.9 13.9	0.043 0.059	0.007 0.006	8
		2. Foliar	0.500	20.0	0.560	187	V3	Hay	3.34 3.61	37.8 38.5	<0.014 0.014	<0.014 <0.014 (0.010)	14
		3. Foliar	0.505	20.1	0.566	188	R1/R2	Seed	0.015 0.016	0.029 0.032	<0.013 <0.013	<0.021 <0.021	86
Elko, South Carolina Region 2 (SC-1)	MON 11955	1. Broadcast	0.999	20.2	1.12	189	Pre-emergence	Forage	2.29 2.09	19.8 19.3	0.068 0.068	0.006 <0.005	7
								Hay	0.167 0.179	29.4 35.2	0.057 0.065	<0.014 <0.014	14
		2. Foliar	0.496	20.6	0.556	193	V3	Seed	<0.011 <0.011	0.019 0.021	<0.013 <0.013	<0.021 <0.021	88
		3. Foliar	0.503	19.9	0.563	186	R1/R2						
Centerville, South Dakota Region 5 (SD-1)	MON 11955	1. Broadcast	0.973	19.3	1.09	181	pre-emergence	Forage	1.64 2.91	12.8 15.0	2.32 2.62	0.006 0.006	7
		2. Foliar	0.495	19.7	0.554	184	V3	Hay	4.43 3.74	36.9 35.2	0.180 0.196	<0.014 <0.014	14
		3. Foliar	0.496	19.8	0.556	185	R2	Seed	0.056 0.051	0.122 0.116	<0.013 <0.013	<0.021 <0.021	76

City, State and NAFTA Region (Trial No.)	Formulation	Application No./Method	Rate/Application				Growth Stage at Each Application	Matrix	Residues (ppm)[a]				PHI (Days)
			lb a.e/A	GPA	kg a.e.ha	L/ha			DCGA[b]	DCSA	Dicamba	5-OH Dicama	
Britton, South Dakota Region 5 (SD-2)	MON 11955	1. Broadcast	0.996	19.9	1.12	186	Pre-emergence	Forage	2.74 1.83	16.9 16.3	0.423 0.437	0.006 0.006	7
		2. Foliar	0.502	20.0	0.562	187	V4	Hay	1.98 1.67	30.9 29.9	0.063 0.034	<0.014 <0.014	14
		3. Foliar	0.501	20.0	0.561	187	R1	Seed	<0.011 <0.011	0.046 0.050	<0.013 <0.013	<0.021 <0.021	88
Delavan, Wisconsin Region 5 (WI-1)	MON 11955	1. Broadcast	0.995	19.6	1.11	183	Pre-emergence	Forage	3.97 3.94	14.7 15.7	0.555 0.864	<0.005 (0.004) <0.005 (0.004)	7
		2. Foliar	0.462	18.5	0.517	173	V3	Hay	2.37 2.43	31.5 32.5	0.156 0.214	<0.014 <0.014	14
		3. Foliar	0.498	20.1	0.558	188	R1/R2	Seed	0.071 0.064	0.079 0.075	<0.013 <0.013	<0.021 <0.021	85
Fitchburg, Wisconsin Region 5 (WI-2)	MON 11955	1. Broadcast	0.503 , 0.507	20.9, 19.9	0.563, 0.568	195, 186	Pre-emergence	Forage	1.68 1.92	15.7 15.2	0.070 0.057	<0.005 (0.0047) 0.006	10
		2. Foliar	0.511	20.1	0.572	188	V3-V4	Hay	7.12 7.26	60.3 61.1	0.218 0.153	<0.014 <0.014	15
		3. Foliar	0.498	20.3	0.558	190	R1/R2	Seed	<0.011 <0.011	0.013 0.010	<0.013 <0.013	<0.021 <0.021	98

[a]Concentrations of the individual analytes are reported as dicamba equivalents using the molecular weight conversion factors 0.991 (DCGA), 1.07 (DCSA) and 0.933 (5-OH dicamba). For residue values <LOQ, the values detected are included in brackets.

[b] The analytical method used for quantitation was not validated for DCGA as per current regulatory guidelines.

Table 6.3.1.2-7 Residues in Soybean Commodities from Trials Conducted to Bridge between the Two SC Formulations MON 11955 (Monethanolamine Salt of Dicamba; Treatment 5) and MON 54140 (DGA Salt of Dicamba; Treatment 6). A Non-Ionic Surfactant and Ammonium Sulfate (AMS) were included in all Spray Applications

City, State and NAFTA Region (Trial No.)	Formulation	Application No./Method	Rate/Application				Growth Stage at Each Application	Matrix	Residues (ppm)[a]				PHI (Days)
			lb a.e./A	GPA	kg a.e./ha	L/ha			DCGA[b]	DCSA	Dicamba	5-OH Dicama	
Proctor, Arkansas, Region 4 (AR-1)	MON 11955	1. Foliar	0.999	20.3	1.12	190	V3	Forage	2.24 2.88	43.4 38.7	0.174 0.118	0.018 0.016	7
		2. Foliar	0.998	20.3	1.12	190	R1	Hay	5.25 4.29	89.9 76.3	0.153 0.112	0.027 0.031	15
								Seed	0.015 0.018	0.076 0.062	<0.013 <0.013	<0.021 <0.021	89
	MON 54140	1Foliar	1.00	20.3	1.12	190	V3	Forage	3.01 2.76	42.3 39.5	0.158 0.133	0.021 0.017	7
		2. Foliar	0.999	20.3	1.12	190	R1	Hay	4.05 5.37	76.4 99.2	0.138 0.170	0.023 0.033	15
								Seed	0.023 0.022	0.077 0.073	<0.013 <0.013	<0.021 <0.021	89
Carlyle, Illinois Region 5 (IL-2)	MON 11955	1. Foliar	0.992	20.4	1.11	191	V3	Forage	1.32 1.36	33.1 33.3	0.030 0.032	0.007 0.007	8
		2. Foliar	0.986	19.5	1.10	182	R1	Hay	1.95 2.31	36.6 36.4	0.018 0.016	<0.014 <0.014	15
								Seed	0.062 0.066	0.113 0.117	<0.013 <0.013	<0.021 <0.021	74
	MON 54140	1. Foliar	1.01	20.7	1.13	194	V3	Forage	0.889 0.967	20.4 26.1	0.027 <0.021 (0.0209)	<0.005 (0.004); 0.006	8
		2. Foliar	1.01	20.1	1.13	188	R1	Hay	4.36 2.17	25.0 33.9	<0.014 (0.009); <0.014 (0.0137)	<0.014 <0.014	15
								Seed	0.057 0.063	0.100 0.110	<0.013 <0.013	<0.021 <0.021	74

City, State and NAFTA Region (Trial No.)	Formulation	Application No./Method	Rate/Application				Growth Stage at Each Application	Matrix	Residues (ppm)[a]				PHI (Days)
			lb a.e./A	GPA	kg a.e./ha	L/ha			DCGA[b]	DCSA	Dicamba	5-OH Dicama	
Cunning-ham, Kansas Region 5 (KS-1)	MON 11955	1. Foliar	0.985	20.6	1.10	193	V3	Forage	1.11 0.904	53.1 57.9	0.024; <0.021 (0.0205)	<0.005 (0.004); 0.007	8
		2. Foliar	1.02	21.4	1.14	200	R1	Hay	2.78 3.08	137 117	0.040 0.038	<0.014 <0.014	14
								Seed	0.017 0.020	0.024 0.045	<0.013 <0.013	<0.021 <0.021	95
	MON 54140	1. Foliar	0.988	20.6	1.11	193	V3	Forage	0.961 1.00	52.8 45.9	<0.021 (0.019); <0.021 (0.016)	<0.005 (0.004); <0.005 (0.004)	8
		2. Foliar	1.05	21.9	1.18	205	R1	Hay	3.64 3.66	143 136	0.042 0.037	<0.014 (0.010); <0.014 (0.010)	14
								Seed	0.021 0.021	0.049 0.052	<0.013 <0.013	<0.021 <0.021	95
Fergus Falls, Minnesota Region 5 (MN-2)	NON 119555	1. Foliar	1.00	20.0	1.12	187	V3	Forage	4.00 4.04	27.9 30.9	0.544 0.799	<0.005 (0.004); 0.005	7
		2. Foliar	1.00	20.1	1.12	188	R2	Hay	5.89 5.92	91.2 96.8	0.250 0.291	<0.014 <0.014	15
								Seed	0.116 0.122	0.163 0.164	<0.013 <0.013	<0.021 <0.021	78
	MON 54140	1. Foliar	1.00	20.1	1.12	188	V3	Forage	4.78 5.21	35.6 37.2	1.15 1.11	0.007 0.006	7
		2. Foliar	1.00	20.0	1.12	187	R2	Hay	6.34 6.75	102 101	0.309 0.322	<0.014 (0.013); <0.014	15
								Seed	0.138 0.117	0.204 0.179	<0.013 <0.013	<0.021 <0.021	78

[a]Concentrations of the individual analytes are reported as dicamba equivalents using the molecular weight conversion factors 0.991 (DCGA), 1.07 (DCSA) and 0.933 (5-OH dicamba). For residue values <LOQ, the values detected are included in brackets.

[b] The analytical method used for quantitation was not validated for DCGA as per current regulatory guidelines.

Table 6.3.1.2-8 Evaluation of Residue Decline in Soybean Forage and Seed Using Treatment 5. A Non-Ionic Surfactant and Ammonium Sulfate (AMS) were included in all Spray Applications.

City, State and NAFTA Region (Trial No.)	Formulation	Application No./Method	Rate/Application				Growth Stage at Each Application	Matrix	Residues (ppm)[a]				PHI (Days)
			lb a.e./A	GPA	kg a.e./ha	L/ha			DCGA[b]	DCSA	Dicamba	5-OH Dicama	
Richland, Iowa Region 5 (IA-1)	MON 11955	1. Foliar	0.979	20.2	1.10	189	V3	Forage	5.88 4.90 (mean =5.39)	49.1 45.7 (mean =47.4)	0.091 0.073 (mean = 0.082)	0.007 0.007 (mean = 0.007)	3
									2.83 2.83 (mean = 2.83)	27.7 28.0 (mean = 27.9)	<0.021 (0.014); <0.021 (0.015) (mean = <0.021)	0.005; <0.005 (0.004) (mean = 0.005)	7
									2.56 2.56 (mean = 2.56)	23.9 27.8 (mean = 25.9)	<0.021 (0.020); <0.021 (0.010) (mean = <0.021)	<0.005 <0.005 (mean = <0.005)	10
									1.86 1.85 (mean = 1.86)	14.3 15.0 (mean =14.7)	<0.021 (0.003); <0.021 (0.004) (mean = <0.021)	<0.005 <0.005 (mean = <0.005)	14
		2. Foliar	0.998	20.1	1.12	188	R1	Seed	0.024 0.023 (mean = 0.024)	0.020 0.019 (mean =0.020)	<0.013 (0.003); <0.013 (0.003) (mean = <0.013)	<0.021 <0.021 (mean = <0.021)	73
									0.017 0.017	0.019 0.020 (mean = 0.020)	<0.013 <0.013 (mean = <0.013)	<0.021 <0.021 (mean =	80

City, State and NAFTA Region (Trial No.)	Formulation	Application No./Method	Rate/Application				Growth Stage at Each Application	Matrix	Residues (ppm)[a]				
			lb a.e./A	GPA	kg a.e./ha	L/ha			DCGA[b]	DCSA	Dicamba	5-OH Dicama	PHI (Days)
									(mean = 0.017			<0.021)	
									0.022 0.023 (mean = 0.023)	0.019 0.020 (mean = 0.020)	<0.013 (0.005); <0.013 (mean = <0.013)	<0.021 <0.021 (mean = <0.021)	87
									0.020 0.026 (mean = 0.023)	0.017 0.020 (mean = 0.019)	<0.013 (0.002); <0.013 (0.005) (mean = <0.013)	<0.021 <0.021 (mean = <0.021)	94
Campbell, Minnesota Region 5 (MN-1)	MON 19555	1. Foliar	0.998	20.1	1.12	188	V3	Forage	2.91 2.92 (mean = 2.92)	36.8 32.1 (mean = 34.5)	1.08 0.758 (mean = 0.919)	0.007 0.008 (mean = 0.008)	3
									3.41 2.77 (mean = 3.09)	26.5 24.3 (mean = 25.4)	0.448 0.373 (mean = 0.411)	0.007; <0.005 (0.004) (mean = 0.006)	7
									2.01 1.58 (mean =1.80)	22.4 21.9 (mean = 22.2)	0.045 0.046 (mean = 0.046)	<0.005 <0.005 (mean = <0.005)	10
									1.54 1.75 (mean = 1.65)	16.5 19.6 (mean = 18.1)	<0.021 (0.011); <0.021 (0.016) (mean = <0.021)	<0.005 <0.005 (mean = <0.005)	14
								Seed	0.081 0.079	0.082 0.079 (mean =	<0.013 (0.004); <0.013	<0.021 <0.021 (mean =	78

City, State and NAFTA Region (Trial No.)	Formulation	Application No./Method	Rate/Application				Growth Stage at Each Application	Matrix	Residues (ppm)[a]				
			lb a.e./A	GPA	kg a.e./ha	L/ha			DCGA[b]	DCSA	Dicamba	5-OH Dicama	PHI (Days)
									(mean = 0.080)	0.081)	(0.002) (mean = <0.013)	<0.021)	
									0.060 0.055 (mean = 0.058)	0.076 0.074 (mean = 0.075)	<0.013 <0.013 (mean = <0.013)	<0.021 <0.021 (mean = <0.021)	88
		2. Foliar	1.01	20.1	1.13	188	R1		0.081 0.080 (mean = 0.081)	0.082 0.076 (mean = 0.079)	<0.013 (0.005); <0.013 (0.002) (mean = <0.013)	<0.021 <0.021 (mean = <0.021)	92
									0.072 0.071 (mean = 0.072)	0.073 0.071 (mean =0.072)	<0.013 (0.001) <0.013 (0.004) (mean = <0.013)	<0.021 <0.021 (mean = <0.021)	100

[a]Concentrations of the individual analytes are reported as dicamba equivalents using the molecular weight conversion factors 0.991 (DCGA), 1.07 (DCSA) and 0.933 (5-OH dicamba). For residue values <LOQ, the values detected are included in brackets.

[b] The analytical method used for quantitation was not validated for DCGA as per current regulatory guidelines.

TABLE C.3.1.2-9 Summary of Residue Data from Crop Field Trials (Treatment 4) with Dicamba

Commodity	Total Applic. Rate lb a.e./A (kg a.e./ha)	PHI (days)	Residue Levels[a] (ppm)						
			n	Min.	Max.	HAFT	Median (STMdR)	Mean (STMR)	Std. Dev.
DCGA[b]									
Forage	1.96-2.04 (2.19-2.28)	7-10	44	0.356	5.90	5.27	1.93	2.02	1.02
Hay		13-15	44	0.167	7.26	7.19	2.00	2.66	1.91
Seed		73-98	44	<0.011	0.135	0.131	0.017	0.032	0.029
DCSA									
Forage	1.96-2.04 (2.19-2.28)	7-10	44	8.92	51.3	50.4	15.0	17.0	8.00
Hay		13-15	44	12.2	61.1	60.7	31.9	32.2	11.2
Seed		73-98	44	0.010	0.440	0.439	0.033	0.059	0.089
Dicamba									
Forage	1.96-2.04 (2.19-2.28)	7-10	44	<0.021	2.62	2.47	0.068	0.374	0.603
Hay		13-15	44	<0.014	1.16	1.01	0.051	0.130	0.216
Seed		73-98	44	<0.013	<0.013	<0.013	<0.013	<0.013	0
5-OH Dicamba									
Forage	1.96-2.04 (2.19-2.28)	7-10	44	<0.005	0.009	0.009	0.005	0.006	0.001
Hay		13-15	44	<0.014	<0.014	<0.014	<0.014	<0.014	<0.014
Seed		73-98	44	<0.021	<0.021	<0.021	<0.021	<0.021	<0.021

[a]Concentrations of the individual analytes are reported as dicamba equivalents using the molecular weight conversion factors 0.991 (DCGA), 1.07 (DCSA) and 0.933 (5-OH dicamba). For residue values <LOQ, the respective LOQ was used for all statistical calculations.
[b] The analytical method used for quantitation was not validated for DCGA as per current regulatory guidelines.

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Conclusions

A total of 22 trials were conducted in the United States in order to quantify the residues of dicamba and its metabolites in/on dicamba tolerant soybeans. The maximum residue in soybean RACs following Treatment 4 was 5.90 ppm (forage), 7.26 ppm (hay) and 0.135 ppm (seed) for DCGA; 51.3ppm (forage), 61.1 ppm (hay) and 0.440 ppm (seed) for DCSA; 2.62 ppm (forage), 1.16 ppm (hay) and <0.013 ppm (<LOQ) (seed) for dicamba; and 0.009 ppm (forage); <0.014 ppm (<LOQ) (hay) and <0.021 ppm (<LOQ) (seed) for 5-OH dicamba. The results of the trials to bridge the MON 11955 (monethanolamine) and MON 54140 (DGA salt) SC formulations of dicamba indicate that the residue profile was similar for both treatments. The results of the two decline trials indicate that residues of DCGA and DCSA did not change significantly in seed over the sampling period. No conclusion could be made regarding the decline of dicamba and 5-OH dicamba residues in seed as residues were <LOQ at each sampling interval. In forage, residues of DCGA, DCSA, dicamba and 5-OH dicamba decreased over the sampling period.

References

PMRA No. 1640670. MRID 43814101. Jimenez, N.C. (1995) Crop Residue Study with Dicamba Formulations on Soybeans. BASF Reg. Doc. ID No. 1995/5298. Unpublished study prepared by Sandoz Agro, Inc. 1468 pages.

PMRA No. 1640657. MRID No. 43814102. Formanski, L.J. (1995) Dicamba Residue Study on Soybean Grain and Soybean Processed Fractions. BASF Reg. Doc. No. 1995/5299. Unpublished study prepared by Sandoz Agro, Inc. 513 pages.

6 Residues in or on Treated Products, Food and Feed

6.2 Metabolism, distribution and expression of residues

6.2.1 Metabolism, distribution and expression of residues in plants

Abbreviations contained in the text and tables are indicated in the legend below.

ae	Acid equivalents
ACN	Acetonitrile
amu	Atomic mass units
conj.	Conjugate/conjugates
DAT	Days after treatment
DCGA	3,6-dichlorogentisic acid
DCSA	3,6-dichlorosalicylic acid
dpm	Disintegrations per minute
GC/EI/MS	Gas chromatography electron ionization mass spectrometry
gluc.	Glucoside/glucosides
HMG	3-hydroxy-3-methylglutaryl
HMGA	3-hydroxy-3-methylglutaric acid
HPLC/LSC	HPLC with liquid scintillation counting of fractions collected
HPLC/RAD	HPLC with radioactivity flow detection
LC/ESI/MS	Liquid chromatography electrospray ionization mass spectrometry
LSC	Liquid scintillation counting
MBq	Mega-Becquerel
MCi	Millicurie
mmol	Millimole
m/z	mass-to-charge ratio
na	Not applicable
nf	Not found
POE-C	Control soybean plants grown among the post emergence treated plants
POE-T	Post emergence treated soybean plants
PRE-C	Control soybean plants grown among the pre-emergence treated plants
PRE-T	Pre-emergence treated soybean plants
TRR	Total radioactive residue
Unk	Unknown
UNT-C	Untreated control soybean plants grown in a greenhouse enclosure separate from the treated plants
v/v	Volume to volume

6.2.1.1 Metabolism, distribution and expression of residues in soybean

Report: IIA 6.2.1.1/1 MRID 47899523. PMRA No. 1894536. Miller, M.J. and Mierkowski, M.J. (2010). Metabolism of Dicamba in Dicamba-Tolerant Soybeans. Monsanto Report No. MSL0022659.

Guidelines: EPA Pesticide Assessment Guidelines, Nature of the Residue – Plants, Livestock, US EPA OCSPP 860.1300; Metabolism in Crops, OECD Guideline for the Testing of Chemicals No. 501, PMRA DACO 6.3

GLP: Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality were provided.

Acceptability: Under the conditions and parameters used in the study, the plant metabolism data are classified as scientifically acceptable.

Executive Summary

In a metabolism study of dicamba in/on dicamba resistant soybean, a simulated formulation consisting of a mixture of unlabeled dicamba and dicamba uniformly labeled with ^{14}C in the ring carbons was formulated as an aqueous solution of the diglycolamine salt. The test material was applied, each with a different Lot No., at 2.55 and 2.52 lb ae/acre (2.86 and 2.82 kg/ha) for the pre emergence (PRE-T) and post emergence (POE-T) applications, respectively. The radiochemical purities of both lots were 99.2% and 99.5%. The application rates represent slightly exaggerated rates relative to the maximum intended seasonal use rate in the US of 2.0 lb ae/acre (2.24 kg/ha).

A single application, either as pre-emergence or post emergence, was applied to two groups of pots. Each group included control untreated pods (PRE-C and POT-C) interspersed among the treated group. An additional control group (UNT-C) was planted in a separate greenhouse. The pre-emergence application was made directly to the soil of the PRE-T group pots on the day of planting after the soybean seeds were planted. The post emergence application was made to the foliage of the plants of the POE-T group 29 days after planting at the R1 growth stage (first flower) using a hand held sprayer. Immature foliage (pre-forage) samples were collected as thinnings 14 days after planting and the pre-emergence application. Forage samples were collected 7 days after the post emergence application and 36 days after the pre-emergence application. Hay samples were collected 27 days after the post emergence treatment and 56 days after the pre-emergence treatment. Seed was collected 83 days after post emergence treatment and112 days after planting and pre-emergence treatment. Samples were processed by grinding and were stored frozen. The processed (ground) soybean forage, hay and seed samples were stored frozen at *ca.* -20 °C for approximately two years during the analysis phase of the study. The stability of dicamba soybean residues was assessed by comparing the radioactivity analyses (TRR determinations), extraction efficiencies and HPLC profiles determined during various phases of the study and found to be stable for longer than two years.

The levels of [^{14}C]dicamba-derived residues found in soybean forage, hay and seed were quantifiable with the analytical method used. Total radioactive residues (TRR) in the samples were determined by combustion analysis. Forage and hay samples were each extracted four times with 40:60 (v/v) acetonitrile:water. Seed samples were each extracted three times with hexane to remove oils, then once with acetonitrile followed by four extractions with 40:60 (v/v) acetonitrile:water. Extractabilities were greater than 90% for forage and hay matrices. For the PRE-T seed and POE-T seed, extraction efficiencies were 59.4% and 63.7%, respectively, of which 10.4% and 8.4% of the TRR was extracted in the oil (hexane) fraction, respectively.

Five discrete metabolites of dicamba and non-metabolized parent dicamba were identified. In addition, two metabolites of dicamba in the range of 1-2% of TRR were isolated and characterized. The identified and characterized radioactive compounds in pre-forage, forage and hay constituted 87.28-92.17% of the TRR. For seed, the identified and characterized radioactivity comprised 36.35-43.09% of the TRR. The dicamba metabolite DCSA glucoside was found to be the major component in dicamba-tolerant soybean foliage (pre-forage, forage and hay) from the pre- or post emergence treatments constituting 60.32-74.48% of TRR. DCSA HMGglucoside constituted 5.21-7.62% of TRR in PRE-T pre-forage, forage and hay, and 1.14 and 2.48% of TRR in the POE-T forage and hay, respectively. Surface residues of unchanged

dicamba was a significant component of the residue in the POE-T forage and hay (24.21% of TRR and 12.33% of TRR, respectively). DCGA glucoside constituted 0.75-4.32% of TRR in pre-forage, forage and hay, with larger amounts present in the hay compared to the forage. The DCGA malonylglucoside represented 5.46% of TRR in the PRE-T pre-forage, but only 0.73-1.61% of TRR in forage and hay. DCSA represented only 1.54-1.93% of TRR in hay, but larger amounts were observed in forage (3.19-4.08% of TRR). Metabolite fractions **14** and **18**, characterized as mixtures of unknown DCSA and DCGA conjugates, each constituted less than 2.0% of the TRR in soybean foliage.

The metabolism of dicamba in dicamba-tolerant soybean proceeds by initial demethylation of dicamba to form 3,6-dichlorosalicylic acid (DCSA) through the action of the dicamba O-demethylase enzyme resulting from introduction of the dicamba mono-oxygenase (DMO) gene into dicamba-tolerant soybean. Only small amounts of free DCSA are observed in soybean matrices; rather, the DCSA exists largely as its conjugate DCSA glucoside, some of which is further modified by esterification with 3-hydroxy-3-methylglutaric acid (HMGA) to form DCSA HMGglucoside. As a minor pathway, DCSA is hydroxylated at the 5-position to form 2,5-dichloro-3,6-dihydroxybenzoic acid (DCGA). DCGA is not observed as a free metabolite in soybean matrices but is converted to the glucose conjugate DCGA glucoside in which the glucose moiety is attached to the 5-hydroxyl of DCGA. The glucose conjugate is further converted by malonylation of the glucose moiety to DCGA malonylglucoside. The sum of DCGA glycosides and other characterized conjugates in PRE-T pre-forage, forage, hay and seed accounted for 8.52%, 4.35%, 6.33% and 7.08% of the TRR, respectively, while they accounted for 2.36%, 7.68% and 7.33% in POE-T forage, hay and seed, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material

Two separate lots of dicamba test substances were used for this study, one for the pre-emergence treatment and one for the post emergence treatment. Each test substance was prepared by mixing unlabeled dicamba with sufficient [^{14}C]dicamba to give a target specific activity of approximately 5.4 mCi/mmol (0.90 MBq/mg). The [^{14}C]dicamba (specific activity of 45.0 mCi/mmol, 7.53 MBq/mg) used for mixing was uniformly labeled with carbon-14 in the ring carbons, and was synthesized and purified by GE Healthcare (Amersham).

Preemergence treatment:

Description:	Dicamba-phenyl-U-^{14}C
CAS No. (unlabeled compound):	1918-00-9
Lot No.:	6103-01A
Specific Activity:	5.39 mCi/mmol (54190 dpm/µg, 0.903 MBq/mg)
Radiochemical Purity:	99.2% by reverse phase HPLC
Chemical Purity:	99.4%
Stability:	2 years

Postemergence treatment:

Description:	Dicamba-phenyl-U-^{14}C
CAS No. (unlabeled compound):	1918-00-9
Lot No.:	6103-01C

Specific Activity:	5.43 mCi/mmol (54560 dpm/µg), 0.909 MBq/mg
Radiochemical Purity:	99.5% by reverse phase HPLC
Chemical Purity:	99.1%
Stability:	2 years

2. Test Soil

The test soil was obtained from the Research For Hire Experimental Farm, Plot P-104. The soil properties determined by Agvise Laboratories are as follows:

Table 6.2.1.1-1 Soil Physicochemical Properties

Textural class:	Loamy sand
Sand:	80%
Silt:	12%
Clay:	8%
pH (H_2O):	7.1
Bulk density:	1.39 g/cc
Cation exchange capacity:	4.9 meq/100g
Percent organic matter:	0.4
Percent moisture at 1/3 bar:	7.7

3. Test Commodity

Crop:	Soybean
Variety:	Dicamba-tolerant soybean event GM_A90617
Lot No.:	GLP-0604-17294-S
Botanical name:	*Glycine max* L.
Commodities sampled:	Immature foliage (pre-forage), forage, hay and mature seed

Dicamba-tolerant soybean event GM_A90617 expresses a modified dicamba mono-oxygenase (DMO) gene derived from the soil bacterium *Stenotrophomonas maltophilia*. The GM_A90617 soybean event used in this study contained the same DMO gene expression cassette as dicamba-tolerant soybean event MON 87708.

B. STUDY DESIGN

The experimental portion of the study was conducted during the period from June 2006 to September 2009. Monsanto Company was responsible for technical oversight of the in-life portion of the study. The in-life portion of the study was contracted to PTRL West Inc., which was responsible for the preparation and application of the spray solutions. Definitive extractions of all matrices, quantitative HPLC analyses for metabolite quantitation, and isolation, purification and identification of metabolites was conducted at Monsanto Company.

1. Experimental Conditions

Dicamba tolerant soybean crop was grown in two greenhouses at the "Research For Hire" field site in Porterville, California, USA in 12-inch diameter pots containing a loamy sand soil. The study consisted of five groups of soybean plants: untreated control plants (designated UNT-C), plants treated pre-emergence at planting (designated PRE-T), untreated control plants (designated PRE-C) grown among the pre-emergence treated plants, post

emergence treated plants (designated POE-T) and untreated control plants (designated POE-C) grown among the post emergence treated plants. Untreated control (UNT-C) plants, and plants intended for the POE-C and POE-T groups (prior to the post emergence treatment), were grown in a separate greenhouse from that housing the treated plants. Four seeds were planted per pot. At 14 days after planting, the PRE-C and PRE-T pots were thinned to two plants per pot, and the POE-C and POE-T pots were thinned to one plant per pot.

2. Test Substance Preparation

For each of the two treatments, the dicamba test substance was formulated in water as the diglycolamine salt by dissolving the solid test substance in water containing approximately 1.05 molar equivalents of diglycolamine [2-(2-aminoethoxy)ethanol]. Aliquots were taken for determination of the concentration and homogeneity, and an additional aliquot was taken for the pre-application purity determination. The application solution for each treatment was dispensed into bottles containing equal volumes for application to individual pots or plants, as appropriate. Each bottle for the pre-emergence application contained 10.40 mL (0.5070 mCi) of test solution. For the post emergence application, each bottle contained 9.90 mL (0.5025 mCi) of test solution.

3. Test Substance Application

Applications of the test substance (separate pre- and post emergence applications) were conducted using a handheld sprayer that fit directly on the spray bottles, using a separate bottle for each pot. The pre-emergence application was made directly to the soil of the pots of the pre-emergence group (29 pots) on the day of planting after the soybean seeds were planted. The post emergence application was made to the foliage of the plants of the post emergence group (32 plants) 29 days later at the R1 (first flower) growth stage. Each pot/plant received a single application (either pre- or post emergence). The average achieved application rates were 20.87 mg ae per pot (2.86 kg ae/ha, 2.55 lb ae/acre) for the pre-emergence treatment and 20.63 mg ae per pot (2.82 kg ae/ha, 2.52 lb ae/acre) for the post emergence treatment. These rates represent slightly exaggerated rates relative to the intended maximum seasonal use rate in the US of 2.24 kg ae/ha (2.0 lb ae/acre). Aliquots of the spray solutions were analyzed by HPLC to determine the purity of the test substance after application. For both applications, the pre- and post application test substance purities were virtually identical indicating that the test substances were stable during formulation, transport to the field site and application.

4. Sampling

The thinnings collected from the untreated and pre-emergence group plants 14 days after planting were collected as immature foliage (pre-forage). Forage samples were collected from all pots of all treatments 7 days after the post emergence application and 36 days after the pre-emergence application. Hay was collected from all groups 27 days after the post emergence treatment and 56 days after the pre-emergence treatment. Seed was collected from all groups 83 days after the post emergence treatment (112 days after planting and the pre-emergence treatment).

At the field site at the time of harvest, forage collected from two individual plants from the post emergence group was rinsed separately in three consecutive vessels each containing 1 L of HPLC grade water. Aliquots of the rinses were collected for LSC analysis and an aliquot of the first rinse was also reserved for HPLC analysis. Pre-forage, forage, hay and seed samples were frozen at the field site and were shipped on dry ice to PTRL West, Inc.

where they were processed and where combustion analysis was conducted to determine the TRR. The processed samples were shipped on dry ice to Monsanto Company. At Monsanto, the samples were stored at approximately -20 °C and were thawed only for short periods of time necessary to obtain samples for analysis.

5. Sample Extraction and Analyses

Definitive combustion analyses of all processed (ground) soybean matrices for total radioactive residue (TRR) determination were conducted at PTRL West, Inc. Initial extractions and HPLC analyses of the PRE-T and POE-T forage and seed were conducted at PTRL West, Inc. to establish an initial baseline for storage stability through the course of the study. Definitive extraction and HPLC analyses for the determination of the distribution and isolation of metabolites were conducted at Monsanto Company.
A weighed amount (4-5 g) of forage, hay or seed was extracted with 20-35 mL of 40:60 (v/v) acetonitrile:water by shaking on a wrist-action shaker for about twenty minutes. After centrifugation, and removal of the supernatant, the extraction procedure was repeated three times for a total of four extractions. Seed samples were first extracted three times with hexane to extract oils, followed by an acetonitrile wash, and finally extraction (four times) with 40:60 (v/v) acetonitrile:water. The extraction pellets were dried prior to combustion of aliquots to determine the amount of un-extracted radioactivity.

Analyses to determine the distribution of the radioactivity in the extracts were conducted by reverse phase HPLC using either fraction collection with LSC determination of the radioactivity (HPLC/LSC) or HPLC/RAD using a radioactivity flow monitor.

Metabolites were isolated primarily from the POE-T forage extracts using HPLC. In the case of the isolations from seed extracts, solid phase extraction was used in some cases to clean up and clarify the extracts prior to HPLC analysis.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRR)

Following the application of [^{14}-C]dicamba to soybean at target doses of 2.5 lbs ae/acre (2.80 kg/ha) for both pre- and post emergence treatments, levels of radioactive residues (expressed as parent [^{14}C]dicamba equivalents) were measured in pre- forage, forage, hay and seeds as shown in Table 6.2.1.1-2.

The results indicated that ^{14}C residues occur at higher levels in forage and hay samples following post emergence treatment. The application of a slightly exaggerated recommended rate (target dose of of 2.5 lbs ae/acre (2.80 kg/ha) resulted in quantifiable amounts of ^{14}C dicamba in seeds in both the pre- and post emergence treatments.

Levels of radioactive residues (expressed as parent dicamba equivalents) in soybean foliage for the pre-emergence (at planting) treatment were relatively low and declined with time. The TRR in the pre-forage collected 14 days after the pre-emergence application was 3.248 mg/kg, while the TRR of forage collected at 36 days after the application was 1.433 mg/kg. The TRR of hay collected 56 days after application was 1.056 mg/kg.

Levels of radioactive residues in soybean foliage from the post emergence treatment were relatively high, decreasing from 134.147 mg/kg in the post emergence forage collected 7 days after application to 39.149 mg/kg in post emergence hay collected 27 days after treatment. A considerable amount (*ca.* 33%) of the residues in the post emergence forage was due to

surface residues comprised of non-metabolized parent dicamba, as determined by analysis of water washes of two selected post emergence forage plants. The TRR in the foliage of one of these forage plants (plant #28) after washing was 81.786 mg/kg. Radioactivity in the combined surface washes of this plant corresponded to an additional TRR of 41.298 mg/kg. It is likely that the non-metabolized dicamba in the post emergence hay was also largely due to dicamba surface residues. Residues in the seed were low, indicating limited translocation of dicamba foliage residues to the seed. Residue levels in the seed collected from the pre-emergence treatment at 112 DAT were 0.291 mg/kg, compared to residues of 0.389 mg/kg in seed collected at 83 DAT from the post emergence treatment.

Residues in the untreated controls were low, but significant, in the forage and seed harvested from PRE-C and POE-C untreated plants that were interspersed amongst the PRE-T and POE-T plants, respectively. This is likely due both to uptake of dicamba evaporating from the soil and/or plant surfaces and to uptake of $^{14}CO_2$ produced by mineralization of dicamba in the soil, and possibly the plants, of the treated pots. Residues in untreated control plants housed in a greenhouse separate from but adjoining that housed the treated plants were very low, although a detectable level of radioactivity was found in the untreated control hay and seed (0.001 and 0.013 mg/kg, respectively). It is conceivable that a very small amount of $^{14}CO_2$ released by mineralization of dicamba in the treated soil or ^{14}C-dicamba due to volatility was drawn into the greenhouse containing the untreated plants.

B. EXTRACTION AND CHARACTERIZATION OF RESIDUES

1. Extraction and Characterization of Residues in Soybean Forage, Hay and Seeds

The highest extractability of ^{14}C- residue was achieved using 40:60 (v/v) acetonitrile:water for forage and hay. Seed samples were first extracted three times with hexane to extract oils, followed by an intermediate acetonitrile extraction to remove the excess hexane, and then by four 40:60 acetonitrile:water extractions.

A summary of the extractions is provided in Table 6.2.1.1-3. Extractabilities (normalized percent extracted) for foliage (pre-forage, forage and hay) were all above 90%, except for the PRE-C forage which likely contained significant residues from uptake and incorporation of liberated $^{14}CO_2$ into natural products. Extractabilities for the POE-T forage and hay were somewhat higher than for the PRE-T forage and hay, likely a reflection of additional easily-extracted surface residues of unchanged dicamba in the POE-T forage and hay. Extractabilities (including hexane extraction) ranged from approximately 60-64% for seed from the PRE-T and POE-T groups, and were approximately 44% for seed from the PRE-C and POE-C groups. The amount of the extracted radioactivity in the hexane extracts (oil fraction) ranged from 8.4% of TRR in POE-T seed to 17.8% of TRR in the PRE-C seed. Relative to the seed of the treated groups, the larger proportion of residues in the oil fraction of the PRE-C seed, and the lower extractabilities for the PRE-C and POE-C seed, likely reflects a higher proportion of residues produced by uptake of $^{14}CO_2$ formed by mineralization of ^{14}C-dicamba in the treated soil of the PRE-T pots. The PRE-T pots were in close proximity to the PRE-C pots (by design) and were in the same greenhouse that housed the POE-C plants (as well as the POE-T plants).

Table 6.2.1.1-2 Total Radioactive Residues (TRRs) in Soybean following pre-emergence and post emergence application of ^{14}C-dicamba

TRRs* (mg/kg, ppm)** in treated forage, hay and seeds at target doses of 2.5 lbs ae/acre (2.80 kg/ha)***		
Commodity	Sampling Time, Days after Planting (Days after Treatment)	TRR (mg/kg, ppm)
UNT-C Pre-forage	14	0.000
UNT-C Forage	36	0.000
UNT-C Hay	56	0.001
UNT-C Seed	112	0.013
PRE-C Forage	36	0.080
PRE-C Seed	112	0.170
POE-C Forage	36	0.280
POE-C Seed	112	0.138
PRE-T Pre-forage	14	3.248
PRE-T Forage	36	1.433
PRE-T Hay	56	1.056
PRE-T Seed	112	0.291
POE-T Forage	36	134.147
POE-T Hay	56	39.149
POE-T Seed	112	0.389
POE-T Washed Forage Plant #28****	36	81.786

*Limit of quantification (LOQ) was 2x background. Background levels ranged from 36-71 dpm
** Dicamba equivalents
** The actual rate achieved were 2.55 and 2.52 lb ae/acre (2.86 and 2.82 kg/ha), for the pre emergence (PRE-T) and post emergence (POE-T) applications, respectively
**** Foliage of plant after washing. The combined water washes of the plant contained radioactivity representing additional residues of 41.298 mg/kg (ppm)

Table 6.2.1.1-3 Extraction Efficiency Summary for Residues of ^{14}C-Dicamba in Soybean Pre-Forage, Forage, Hay and Seed

Matrix	% ^{14}C extracted (mg/kg, ppm)	% ^{14}C not extracted (mg/kg, ppm)	Account-ability	Normalized* % Extracted	Normalized% not extracted
PRE-T Pre-forage	92.8 (2.959)	9.1 (0.289)	101.8	91.1	8.9
PRE-T Forage	105.7 (1.307)	10.2 (0.126)	115.9	91.2	8.8
PRE-T Hay	92.0 (0.960)	9.2 (0.096)	101.2	90.9	9.1
PRE-T Seed**	62.7 (0.173)	43.0 (0.118)	105.7	59.4	40.7
Hexane	10.9			10.4	
ACN,ACN/H_2O***	51.8			49.0	
POE-T Forage	91.7 (125.811)	6.1 (8.336)	97.8	93.8	6.2
POE-T Hay	92.9 (37.308)	4.6 (1.841)	97.5	95.3	4.7
POE-T Seed**	62.8 (0.248)	35.8 (0.141)	98.6	63.7	36.3
Hexane*	8.3			8.4	
ACN, ACN/H_2O***	54.5			55.3	
Washed Forage Plant #28	89.2 (72.964)	10.8 (8.822)	na****	89.2	10.8
PRE-C Forage	96.8 (0.061)	31.2 (0.019)	128.0	75.6	24.4
POE-C Forage	98.9 (0.262)	6.9 (0.018)	105.8	93.5	6.5
PRE-C Seed**	44.2 (0.075)	56.5 (0.095)	100.8	43.9	56.1
Hexane	18.0			17.8	
ACN, ACN/H_2O***	26.3			26.1	
POE-C Seed**	46.6 (0.061)	59.0 (0.077)	105.6	44.2	55.8
Hexane	12.4			11.7	
ACN, ACN/H_2O***	34.3			32.5	

*Normalized to 100% accountability

** Seed samples were extracted with hexane to extract oils, followed by an intermediate acetonitrile extraction, and then by 40:60 ACN/H_2O extractions.

***The ACN and ACN/H_2O extracts were combined, concentrated and analyzed by HPLC.

****The pellet was not combusted.

2. Chemical and Enzymatic Digestion of Un-extracted Seed Residues

The un-extracted residues from the seed extraction pellets (post-extraction solids) from the PRE-T and POE-T seed comprised 40.7% (0.118 mg/kg) and 36.3% (0.141 mg/kg) of the TRR, respectively. Chemical or enzyme digestions designed to release various classes of biomolecules (e.g., starch, protein, cellulose) into the soluble phase showed to be more favorable than dilute acid and base extractions, which resulted in release of less than 2% of the TRR in any individual extraction.

The chemical and enzymatic digestions of the pre-emergence and post emergence seed resulted in release of small amounts of residues (0.25-3.75% of TRR) in each of the phosphate rinse, starch, pectin and cellulose fractions, as shown in Table 6.2.1.1-4. Larger amounts of residues were released in the protein fraction (10.06% and 8.18% of TRR for the PRE-T and POE-T seed, respectively). The largest portion of un-extracted residues was released in the hemicellulose fraction (13.92% and 12.90% of TRR for the PRE-T and POE-T seed, respectively).

Protein, pectin, cellulose and hemicellulose fractions from the chemical and enzymatic digestion of seed un-extracted residues contained residues greater than 0.01 mg/kg. These were acidified and partitioned with ethyl acetate to characterize the nature of the released residues. Virtually none of the residues from the protein, pectin and cellulose fractions partitioned into the organic phase, demonstrating that the residues were highly water-soluble, were unlikely to be dicamba-related, and were likely due to extensive metabolism of dicamba and incorporation of resulting small molecules, e.g., $^{14}CO_2$, into plant constituents. A significant portion (*ca.* 19% and 13%, respectively) of the residues released in the PRE-T and POE-T hemicellulose fractions partitioned into the respective organic phase. However, this represented less than 0.01 mg/kg in both cases, and additional experiments demonstrated that these residues were primarily not dicamba-related.

3. Storage Stability

The processed (ground) soybean forage, hay and seed samples received from PTRL West, Inc. were stored frozen at *ca.* -20 °C for approximately two years during the analysis phase of the study at Monsanto Company. The stability of dicamba soybean residues was assessed by comparing the radioactivity analyses (TRR determinations), extraction efficiencies and HPLC profiles determined during various phases of the study. As an initial stability baseline, extractions and HPLC profiles were conducted within 30 days of harvest for forage, hay and seed. On completion of the analytical phase of the study, four matrices (PRE-T forage, PRE-T and POE-T hay, and POE-T seed) were reanalyzed for assessment of stability over the two-year storage period. The combustion (TRR) values, extraction efficiencies and chromatographic profiles were very similar to those obtained in the initial baseline analyses, thereby demonstrating stability of the residues during frozen storage. There were two minor differences in the appearance of the chromatographic profiles resulting from incomplete resolution of two components due to column performance deterioration or sample size, but in both cases, it was determined that the profiles were comparable, and were evidence of stability of the residues in storage

Table 6.2.1.1-4 Chemical and Enzymatic Digestion of Seed Un-extracted Residues

	PRE-T Seed		POE-T Seed	
Fraction	Normalized Percent of TRR	Normalized mg/kg (ppm)*	Normalized Percent of TRR	Normalized mg/kg (ppm)*
Hexane	10.54	0.031	8.75	0.034
Acetonitrile	0.64	0.002	0.76	0.003
ACN/H_2O	49.20	0.143	52.89	0.206
Phosphate	0.25	0.001	1.42	0.006
Starch	1.29	0.004	1.27	0.005
Protein	10.06	0.029	8.18	0.032
Pectin	3.26	0.009	2.78	0.011
Cellulose	3.75	0.011	3.46	0.013
Hemicellulose	13.92	0.040	12.90	0.050
Total Extracted	**92.91**	**0.270**	**92.40**	**0.359**
Pellet (unextracted)	**7.09**	**0.021**	**7.60**	**0.030**

* Dicamba equivalents

4. Identification and Characterization of Radioactive Components of Soybean Forage, Hay and Seed

Radioactive components were isolated and purified by preparative HPLC using the same HPLC method that was used for quantitation. Further purification was conducted by a preparative HPLC method using methanol as the organic solvent which provided different selectivity than the acetonitrile mobile phase used in the primary method. Table 6.2.1.1-5 provides a summary of the quantitation and characterization of the metabolites in soybean forage, hay and seed of dicamba-tolerant soybean following dicamba pre-emergence applications. Table 6.2.1.1-6 provides a summary of the quantitation and characterization of the metabolites in soybean forage, hay and seed of dicamba-tolerant soybean following dicamba post emergence applications at the R1 growth stage.

Table 6.2.1.1-5 Metabolites detected in Soybean Forage, Hay and Seed following Pre-emergence treatment with Dicamba

			PRE-T Pre-Forage		PRE-T Forage		PRE-T Hay		PRE-T Seed	
Peak Number	Retention Time* (min)	Identification	Percent of Matrix TRR	Peak mg/kg (ppm)**	Percent of Matrix TRR	Peak mg/kg (ppm)**	Percent of Matrix TRR	Peak mg/kg (ppm)**	Percent of Matrix TRR	Peak mg/kg (ppm)**
1	4.2	Sugars	1.47	0.048	0.96	0.014	1.08	0.011	8.42	0.025
2	6.2	Unknown	0.26	0.008	0.40	0.006	nf	nf	1.22	0.004
3	7.0	DCGA Glucoside	2.77	0.09	1.14	0.016	3.45	0.036	1.60	0.005
6	12.7	Unknown	0.64	0.021	0.58	0.008	0.53	0.006	0.33	0.001
7	14.0	Unknown	0.2	0.007	nf	nf	0.50	0.005	0.72	0.002
8	14.6	DCGA Malonylglucoside	5.46	0.177	1.40	0.020	0.73	0.008	4.73	0.014
9	15.9	DCSA Glucoside	68.96	2.24	74.48	1.067	70.81	0.748	11.55	0.034
10	17.6	Unknown	nf	nf	nf	nf	nf	nf	1.26	0.004
11	18.2	DCSA HMGglucoside	7.62	0.247	5.21	0.075	6.67	0.070	8.73	0.025
12	19.4	Unknown	nf	nf	nf	nf	0.74	0.008	0.38	0.001
14	21.5	Unk DCSA/DCGA Conj.	0.29	0.009	1.26	0.018	1.64	0.017	0.75	0.002
15	22.2	Unknown	nf	nf	nf	nf	0.75	0.008	nf	nf
16	23.0	Unknown	0.62	0.02	0.41	0.006	1.07	0.011	0.18	0.001
18	24.0	Unk DCSA/DCGA Gluc.	nf	nf	0.55	0.008	0.51	0.005	nf	nf
22	26.3	DCSA	1.46	0.047	3.19	0.046	1.54	0.016	0.37	0.001
23	28.3	Dicamba	0.8	0.026	1.61	0.023	0.85	0.009	0.20	0.001
Triglycerides (from the hexane-extracted oil fraction)			na	na	na	na	na	na	13.87	0.040
Total - Quantitation of the Acetonitrile/Water Extractable Residues			**90.55**	**2.94**	**91.19**	**1.307**	**90.87**	**0.958**	**40.44**	**0.12**
Total Identified or Characterized Metabolites			**88.83**	**2.88**	**89.8**	**1.287**	**87.28**	**0.920**	**36.35**	**0.107**

* Retention times (RT) given are based on the RT in PRE-T forage as there were minor differences in RT between the matrices for those fractions. For the metabolites which were not found in forage, the RT given is the time found in seed.

**All mg/kg (ppm) values are expressed as dicamba equivalents.

Metabolite peaks 4, 20, 21 and 26 were observed in PRE-C seed only; metabolite peaks 5, 13, 17, 19, 24 and 25 were observed in POE-T matrices, but not PRE-T matrices.

Table 6.2.1.1-6 Metabolites detected in Soybean Forage, Hay and Seed following Post emergence treatment with Dicamba

			POE-T Forage		POE-T Hay		POE-T Seed	
Peak Number	Retention Time* (min)	Identification	Percent of Matrix TRR	Peak mg/kg (ppm)**	Percent of Matrix TRR	Peak mg/kg (ppm)**	Percent of Matrix TRR	Peak mg/kg (ppm)**
1	4.1	Sugars	nf	nf	0.49	0.190	9.15	0.036
2	5.3	Unknown	nf	nf	0.18	0.071	nf	nf
3	7.0	DCGA Glucoside	0.75	1.007	4.32	1.690	2.07	0.008
5	12.4	Unknown	0.36	0.478	nf	nf	0.31	0.001
6	13.1	Unknown	nf	nf	0.71	0.280	0.62	0.002
7	14.0	Unknown	nf	nf	nf	nf	0.57	0.002
8	14.4	DCGA Malonylglucoside	1.11	1.485	1.61	0.631	4.64	0.018
9	15.6	DCSA Glucoside	60.32	80.913	67.26	26.333	15.27	0.059
10	17.3	Unknown	0.14	0.189	nf	nf	0.84	0.003
11	18.0	DCSA HMGglucoside	1.14	1.535	2.48	0.970	9.61	0.037
12	19.6	Unknown	0.18	0.239	nf	nf	0.61	0.002
13	20.4	Unknown	0.18	0.239	nf	nf	0.48	0.002
14	21.6	Unk DCSA/DCGA Conj.	0.38	0.503	1.75	0.686	0.62	0.002
16	22.7	Unknown	0.40	0.541	0.95	0.373	0.36	0.001
17	23.5	Unknown	0.26	0.352	0.91	0.354	0.33	0.001
18	23.9	Unk DCSA/DCGA Gluc	0.12	0.164	nf	nf	nf	nf
19	24.5	Unknown	0.18	0.239	0.38	0.149	nf	nf
22	25.4	DCSA	4.08	5.473	1.93	0.757	0.46	0.002
23	27.9	Dicamba	24.21	32.473	12.33	4.828	0.64	0.003
24	29.9	Unknown	nf	nf	nf	nf	0.52	0.002
25	32.3	Unknown	nf	nf	nf	nf	0.11	0.0004
	Triglycerides (from the hexane-extracted oil fraction)		na	na	na	Na	10.76	0.042
Total – Quantitation of the Acetonitrile/Water Extractable Residues			**93.81**	**125.83**	**95.30**	**37.312**	**47.21**	**0.1814**
Total – Identified or Characterized Metabolites			**92.11**	**123.553**	**92.17**	**36.085**	**43.09**	**0.1674**

* Retention times (RT) given are based on the RT in PRE-T forage as there were minor differences in RT between the matrices for those fractions. For the metabolites which were not found in forage, the RT given is the time found in seed.

** All mg/kg (ppm) values are expressed as dicamba equivalents.

Metabolite peaks 4, 20, 21 and 26 were observed in PRE-C seed only; metabolite peak 15 was observed in PRE-T matrices, but not POE-T matrices.

5. Identification of metabolites in soybean matrices

HPLC analysis of the extractable residue showed that while there are at least 26 different ^{14}C zones in the HPLC chromatograms, DCSA glucoside (peak 9) was present in all soybean matrices and was the major metabolite in all pre- and post treatment matrices. Four other metabolites; DCGA glucoside (peak 3), DCGA malonylglucoside (peak 8), DCSA HMGglucoside (peak 11), DCSA (peak 22) and the parent dicamba (peak 23) were also identified in the dicamba tolerant soybean matrices (Tables 6.2.1.1-5, 6.2.1.1-6 and Figure 6.2.1.1-1). The identified and characterized components constituted 87-92% of the total TRR in forage and hay of the pre- and post emergence applications, while they constituted only 36-43% of the total TRR in the seeds of pre- and post emergence treatments. The remaining unidentified components of the extracted residue were characterized by hydrolysis to be glucosidic and other unknown conjugates of the metabolites DCSA and DCGA (peaks 18 and 14 respectively). These conjugates were present in PRE-T and POE-T forage, and PRE-T hay, constituting < 1.75% of peak 14 and < 1% of peak 18 of the TRR in each matrix.

The hexane extracts (oil fraction) of the PRE-T, POE-T, PRE-C and POE-C soybean seed contained 10.4%, 8.4%, 17.8% and 11.7% of the TRR, respectively, was composed of triglycerides (89.64% due to fatty acids and 5.05% due to glycerol).

The procedures used to identify the metabolites included:

- Comparison of the retention times and co-elution of the purified fractions (peaks) with dicamba, DCGA DCSA, 3-hydroxy-3-methylglutaric acid and glucose reference standards.
- Analysis using LC/ESI/MS in the negative ion mode for RT comparison and mass spectral identification
- ^{1}H-NMR experiment for the purified fraction of hydrolyzed DCSA HMGglucoside
- Mild acid and enzymatic hydrolysis for identification of sugar moieties in DCSA and DCGA conjugates
- Hydrolysis with 2 N HCl at 100 °C for 2 hours to determine the identity of DCSA and DCGA.

6. Proposed Metabolic Pathways

The proposed pathways for the metabolism of dicamba in dicamba-tolerant soybean are shown in Figure 6.2.1-1. The metabolism of dicamba in dicamba-tolerant soybean proceeds by initial demethylation of dicamba to form DCSA **22** through the action of the dicamba *O*-demethylase enzyme resulting from introduction of the dicamba mono-oxygenase (DMO) gene into dicamba-tolerant soybean. Only small amounts of free DCSA are observed in soybean matrices; rather, the DCSA exists largely as its 2-*O*-β-glucoside (DCSA glucoside **9**), some of which is further modified by esterification with 3-hydroxy-3-methylglutaric acid (HMGA) to form DCSA HMGglucoside **11.** As a minor pathway, DCSA is hydroxylated at the 5-position, presumably by a P-450 enzyme or other oxygenase, to form DCGA. DCGA is not observed as a free metabolite in soybean matrices but is present as the 5-*O*-β-glucoside (DCGA glucoside **3**). The DCGA glucose conjugate **3** is further converted by malonylation of the glucose moiety to DCGA malonylglucoside **8.**

III. CONCLUSIONS

On the basis of the data presented, residues found in dicamba-tolerant soybean foliage (pre-forage, collected 7 days after planting and the pre-mergence application, forage, collected after 7 days of the post-emergence application, and hay, collected after 27 days of the post emergence application following the application of dicamba at a slightly exaggerated rate of 2.5 lbs ae/acre (2.80 kg/ha) were as follows:

- DCSA glucoside **9** was the major metabolite in, constituting 60-75% of the TRR in pre-forage, forage and hay.
- DCSA HMGglucoside **11** constituted 5-8% of TRR in PRE-T pre-forage, forage and hay, and 1-2.5% of TRR in the POE-T forage and hay.
- DCSA **22** represented only 1.5-2% of TRR in hay and pre-forage, but somewhat larger amounts were observed in forage (3-4% of TRR).
- The analytical method involves acid hydrolysis to convert DCSA glycosides into DCSA. The sum of DCSA and its glycosides constitutes 66-83% of the TRR in pre-forage, forage and hay.
- DCGA glucoside **3** constituted 1-4% of TRR in forage and hay, with larger amounts present in the hay compared to the forage.
- DCGA malonylglucoside **8** represented 6% of the TRR in pre-forage, and less than 2% of TRR in forage and hay.
- The analytical method also converts DCGA glycosides into DCGA upon acid hydrolysis. The sum of DCGA and its glycosides constitutes 2-8% of the TRR in pre-forage, forage and hay.
- Unchanged dicamba was a significant component of the residue of treated plants only in the POE-T forage and hay (24% of TRR and 12% of TRR, respectively).
- Unknown conjugates of DCSA and DCGA were characterized in PRE-T and POE-T forage, and PRE-T hay, constituting in total < 2% of the TRR in each matrix.

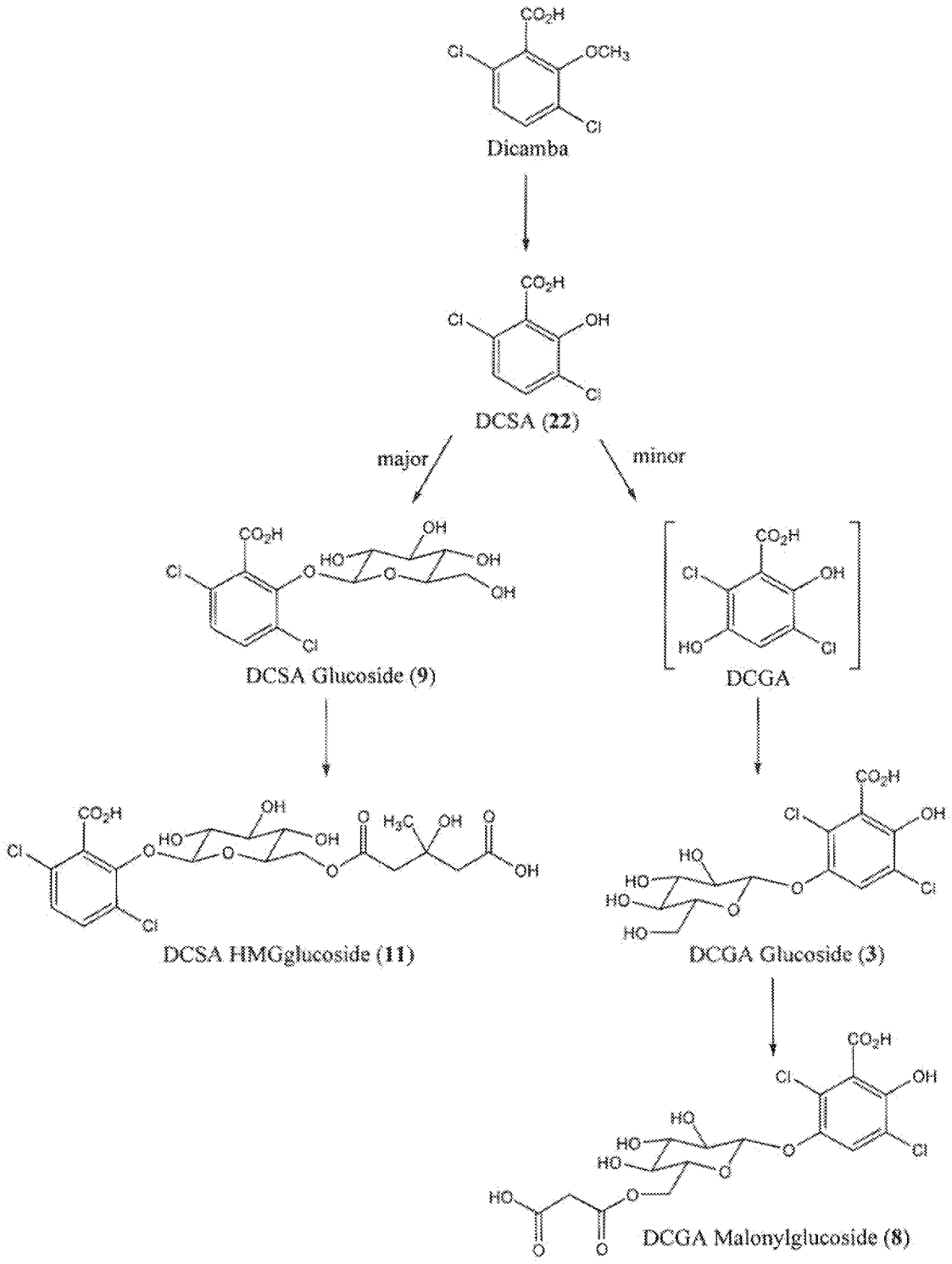
The identified and characterized extractable radioactivity in seeds of dicamba-tolerant soybean following the application of dicamba at a slightly exaggerated rate of 2.5 lbs ae/acre (2.80 kg/ha) comprised 50-53% of the TRR. An additional 29-32% of the seed TRR was characterized as radioactivity incorporated into natural plant constituents (biomolecules), primarily hemicellulose and protein. Residues found in seeds were as follows:

- DCSA glucoside **9** constituted 12% and 15% of TRR in the PRE-T and POE-T seed, respectively.
- DCSA HMGglucoside **11** represented 9 and 10% of TRR,
- DCSA **22** was a minor component of the seed residues (<1% of TRR each in PRE-T and POE-T seed).
- The sum of DCSA and its glycosides constitutes 21-25% of the TRR in the seed of pre- and post emergence applications.
- DCGA malonylglucoside **8** constituted *ca.* 5% of TRR in both the PRE-T and POE-T seed.
- DCGA glucoside **3** was also present in the seed constituting 1.6 and 2% of TRR in the PRE-T and POE-T seed, respectively.

- The sum of DCGA and its glycosides constitutes 6% of the TRR in the seed of pre- and post emergence applications.
- A polar material was characterized as sugars **1** representing 8 and 9% of TRR in PRE-T and POE-T seed, respectively.
- Dicamba **23** was detected at <1% of TRR each in PRE-T and POE-T seed.
- Other significant extractable seed residues, present in the oil fraction, were triglycerides (14% and 11% of TRR in PRE-T and POE-T seed, respectively).
- The seed residues in sugars, triglycerides and other biomolecules are presumed to result from uptake of $^{14}CO_2$ from metabolism of dicamba in the soil, and possibly by metabolism of dicamba to $^{14}CO_2$ and/or other small molecules in the plant, and incorporation into plant natural products through normal metabolic processes.

It was also concluded that while the amount of DCSA glucoside found in forage and hay was sharply reduced in seeds (from 60-75% in forage and hay to 12-15% in seed), the amount of DCGA, its glycosides and other conjugates increased in the seeds (6-7%) compared to forage and hay (2-8%) indicating transformation of DCSA into DCGA in the plant metabolic process. It is understood that DCGA and its conjugates are formed by the hydroxylation of DCSA as a result of the alternate metabolic route. The amount of DCGA , its glycosides and other conjugates may increase depending on the conditions favoring its formation.

Figure 6.2.1.1-1 Proposed Pathway for the Metabolism of Dicamba in Dicamba-Tolerant Soybean



4. Analytical Methods

4.1 Analytical standards and samples

Analytical standards

1. Dicamba

Common name	Dicamba
CAS #	1918-00-9
CAS name	Benzoic acid, 3,6-dichloro-2-methoxy-
IUPAC name	3,6-dichloro-*o*-anisic acid
Purity	>95%

2. 5-Hydroxydicamba

Common name	5-hydroxydicamba
CAS #	7600-50-2
CAS name	Benzoic acid, 2,5-dichloro-3-hydroxy-6-methoxy-
IUPAC name	2,5-dichloro-3-hydroxy-6-methoxybenzoic acid
Purity	>95%

3. DCSA

Common name	3,6-dichlorosalicylic acid
CAS #	3401-80-7
CAS name	Benzoic acid, 3,6-dichloro-2-hydroxy-
IUPAC name	3,6-dichloro-2-hydroxybenzoic acid
Purity	>95%

4. DCGA

Common name	3,6-dichlorogentisic acid
CAS #	18688-01-2
CAS name	Benzoic acid, 2,5-dichloro-3,6-dihydroxy-
IUPAC name	2,5-dichloro-3,6-dihydroxybenzoic acid
Purity	>95%

Internal standards

1.	$^{13}C_6$-Dicamba	3,6-dichloro-2-methoxybenzoic-1,2,3,4,5,6-$^{13}C_6$ acid, CAS # 1173023-06-7
2.	$^{13}C_6$-5-Hydroxydicamba	2,5-dichloro-3-hydroxy-6-methoxybenzoic-1,2,3,4,5,6-$^{13}C_6$ acid
3.	$^{13}C_6$-DCSA	3,6-dichloro-2-hydroxybenzoic-1,2,3,4,5,6-$^{13}C_6$ acid, CAS #1173019-34-5
4.	$^{13}C_6$-DCGA	2,5-dichloro-3,6-dihydroxybenzoic-1,2,3,4,5,6-$^{13}C_6$ acid

Analyzed samples

RAC: soybean forage, hay and seed

Processed: soybean hull, defatted flour, toasted defatted meal, protein isolate, protein concentrate, crude lecithin, degummed oil, refined bleached deodorized (RBD) oil, soymilk and tofu.

4.2 Methods for the determination of residues

The method "Analytical Method for the Determination of Dicamba and Its Major Metabolites in Soy Matrices by LC/MS/MS" (IIA 4.3/1) was designed for quantitative analysis of dicamba and its metabolites 5-hydroxydicamba and conjugates of 3,6-dichlorosalicylic acid (DCSA) and 3,6-dichlorogentisic acid (DCGA), (the latter two are analyzed as their respective acid hydrolysis products in dicamba tolerant soybean.

Table 4.2-1 Summary Parameters for the Analytical Method Used for the Quantitation of Dicamba and its metabolites in Soybean Matrices

Method ID	AG-ME-1321-01
Analytes	Dicamba, 5-OH dicamba, DCSA, DCGA
Extraction solvents/technique	ACN:H_2O (40:60) followed by acid hydrolysis
Cleanup strategies	LL partitioning
Instrument/Detector	ESI LC-MS/MS in the negative ion mode
Standardization method	External standardization with standards injected with each calibration set. Additionally, analyte-specific $^{13}C_6$-labeled internal standards were used to compensate for matrix effects.
Stability of standards	Stable in solvent for 201 days

4.3 Residues in and/or on plants, plant products, foodstuffs (of plant and animal origin), feedingstuffs

Reports:

IIA 4.3/1 MRID 47899501. PMRA No. 1894495. Foster, J.E., Mierkowski, M. and Miller, M.J. (2010). Analytical Method for the Determination of Dicamba and Its Major Metabolites in Soy Matrices by LC/MS/MS. Monsanto Report No. MSL0022661.

IIA 6.2.1.1/1 MRID 47899523. PMRA No. 1894536. Miller, M.J. and Mierkowski, M.J. (2010). Metabolism of Dicamba in Dicamba-Tolerant Soybeans. Monsanto Report No. MSL0022659.

IIA 6.3.1.2/1 MRID 47899524. PMRA No. 1894534. Moran, S.J. and Foster, J.E. (2010). Magnitude of Residues of Dicamba in Soybean Raw Agricultural and Processed Commodities after Application to MON 87708. Monsanto Report No. MSL0022660.

Guidelines: US EPA OCSPP 860.1340 Residue Analytical Method; OECD Series on Testing and Assessment No. 72, Series on Pesticides No. 39, Guidance Document on Pesticide Residue Analytical Methods, 2007 PMRA DACO 7.2.4

GLP: Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided.

Acceptability: Under the conditions and parameters used in the study, the analytical method is suitable for data collection of residues of dicamba and its metabolites 5-hydroxy dicamba and DCSA in dicamba-tolerant soybean. Determination of DCGA is however questionable and may not be suitable using the current method due to

poor recoveries obtained when spiked directly to the matrix as well as inconsistency with radio-validation at low levels.

Principle of the Method

The analytical method incorporates an acid hydrolysis step which converts conjugates of 3,6-dichlorosalicylic acid (DCSA) and 3,6-dichlorogentisic acid (DCGA),to their respective non-glycosidic hydrolysis products; DCSA and DCGA. The method converts the metabolites DCSA glucoside, DCSA HMGglucoside, and DCSA unidentified conjugates and reports the sum as DCSA in addition to any free DCSA present. Similarly, the metabolites DCGA glucoside, DCGA malonylglucoside, and unidentified DCGA conjugates are reported as DCGA. These analytes along with dicamba and 5-hydroxydicamba.are detected and quantitated by turbo ionspray ionization liquid chromatography-tandem mass spectrometry in the negative ion mode. Analyte-specific ^{13}C-labeled internal standards were utilized in the method to compensate for matrix effects in the LC/MS/MS analysis and method procedural losses. The residues are calculated as µg/g, ppm. Structures of the analytes and internal standards are shown in Figure 4.3.1-1 and 4.3.1-2.

Soybean matrices are extracted using 40:60 (volume/volume) acetonitrile:water. An aliquot of the extract is hydrolyzed in 1N HCl at 95 °C in a water bath. The hydrolysate is partitioned with 40:60 (volume/volume) ethyl acetate:isooctane and the organic phase is partially concentrated under vacuum. Water is then added to the organic phase, and the sample is further concentrated under vacuum until only the aqueous solution remains. The aqueous solution is filtered, acidified and analyzed by LC/MS/MS with turbo ion-spray ionization in the negative ion mode to quantitate dicamba, 5-hydroxydicamba, DCSA and DCGA. Minor preparation modifications were used for the processed tofu and lecithin such as specific dilution patterns. The radio-validation, which demonstrates the extraction efficiency and recovery of the method, was conducted using soybean hay and seed samples from the study, "Metabolism of Dicamba in Dicamba-Tolerant Soybeans" (IIA 6.2.1.1/1) in which [^{14}C]-dicamba was used as the test substance.

Recovery Findings

Results obtained for dicamba, 5-hydroxy dicamba, and DCSA in soybean forage, hay, seed and processed fractions (soybean hulls, defatted flour, toasted defatted meal, protein isolate, protein concentrate, crude lecithin, degummed oil, refined bleached deodorized (RBD) oil, soymilk and tofu) were generally within the acceptable range of 70-120% at fortification levels ranging from 0.010 to 2 µg/g. Recoveries of DCGA and the internal standard $^{13}C_6$-DCGA were found to be poor when these analytes were spiked directly onto hay and forage as well as when the analytes were spiked into a container containing matrix and extraction solution. The registrant proposed spiking DCGA and $^{13}C_6$-DCGA into the 10 mL extract after separation from the matrix to overcome this issue. The procedure's approach to overcome the poor recovery of DCGA and its labeled internal standard does not guarantee the extraction of incurred residues of DCGA and is not in accordance with OCSPP Residue Chemistry Guidelines 860.1340 or with PMRA Regulatory Directive Dir98-02 (Section 3.3.2). Recoveries of 5-hydroxydicamba were 64 and 67% at 0.005 and 0.01 ppm fortification levels, respectively in soybean seed. In defatted flour, recovery of 5-hydroxydicamba was also below acceptable limits and was 31% and 64% at fortification levels of 0.01 and 0.02 ppm respectively. Recovery of DCGA, when spiked to the extract and not to the matrix, was 68% at 0.005 ppm fortification level in soybean hay and 64% at 0.01 ppm fortification level in RBD oil. Recovery results are presented in Table 4.3-1.

Linearity

Calibration standard curves for each analyte were generated from reference standard solutions over a concentration range of 0.0005-0.50 µg/mL by plotting the ratio of the peak areas of the analyte and internal standard against the analyte concentration. A weighted (1/analyte concentration) quadratic curve was used to fit the calibration data. The correlation coefficient (r) for calibration curves generated during the validation phase was acceptable (≤0.9992) for all analytes in all sets.

Specificity

The use of mass spectrometry (MS) with multiple reaction monitoring (MRM) provide a specific method for the determination of the residues of dicamba as well as the metabolites 5-hydroxydicamba and the conjugates of DCSA and DCGA (determined as their respective acid hydrolysis products). The extraction of DCGA by acetonitrile:water (40:60) from forage and hay matrices was not specific. Stable labeled isotope ISs were used to correct for matrix effects. There are no interferences from the pesticides 2,4-dichlorophenoxyacetic acid (2,4-D) or 2,4-dichlorophenylacetic acid, which have the same precursor ion as dicamba and DCSA, respectively.

Limit of Quantitation

The limit of quantification (LOQ) is defined as the lowest concentration at which an acceptable recovery is obtained. The lowest level of method validation (LLMV) was 0.005 ppm for each analyte in soybean forage, hay and seed and was 0.01 ppm in soybean processed samples. LOQs were also determined statistically by the study authors for all four analytes in soybean matrices.

Repeatability

The relative standard deviations for recovery (accuracy) results at the 0.01 µg/g fortification level (near the LOQ for each analyte in each matrix) ranged from 2.9% to 9.5% for forage, hay and seed. At the levels tested (0.005, 0.010, 0.020 and 0.10 µg/g), RSD values were generally less than 20%. Limited repeatability data were obtained for processed fractions which could not be calculated due to insufficient number of replicates at several fortification levels. The precision data obtained during validation demonstrate that the method has satisfactory repeatability in soybean forage, hay and seed.

Reproducibility

Based on the performance of the method, reproducibility is expected to be good for all analytes except for DCGA.

Confirmation of the identity of residues

The results of endogenous method validation (radio-validation) (reference IIA 6.2.1.1/1) showed that the method accounts for dicamba residues and its metabolites 5-hydroxydicamba and DCSA in hay and seed of dicamba tolerant soybean. It is expected that the method similarly accounts for the dicamba residues and its metabolites in soybean forage. The results showed that the average overall recoveries of radioactivity through the method, including the extraction, hydrolysis, partitioning, evaporation and final filtration steps, were 62.9%, 64.3% and 25.5% for PRE-T hay, POE-T hay and POE-T seed, respectively. The largest losses of radioactivity in the method were in the partitioning step. Average recoveries from the partitioning step were 74% for the hay samples, and 59% for the seed sample.

Average extractabilities for the analytical method were comparable to the extractabilities obtained in the metabolism study, except for DCGA at levels of 0.025 ppm in POE-T seed and 0.032 ppm in PRE-T hay (Tables 4.3-2 and 4.3-3).

Confirmation of the identity of residues was achieved by comparison of the retention times of the radioactive peaks to those of corresponding reference standards, which demonstrated that DCSA, DCGA and dicamba were the only significant radioactive components of the final analyte solutions and that the metabolite 5-hydroxydicamba has not been detected. DCSA was the major component in all three samples, constituting 89.71%, 78.50% and 69.59% of the radioactivity in the final analyte solutions for PRE-T hay, POE-T hay and POE-T seed, respectively. Together, DCSA, DCGA and dicamba constituted 96.18%, 96.54% and 89.62% of the PRE-T hay, POE-T hay and POE-T seed HPLC profiles, respectively. The results obtained are summarized in the Table 4.3-3.

Conclusion

The analytical method described converts, by acid hydrolysis, DCSA and DCGA conjugates present in commodities of dicamba-treated soybean to DCSA or DCGA, respectively. These analytes along with dicamba and 5-hydroxydicamba are quantitated by LC/MS/MS in the method. The analytical method met validation acceptance criteria (70-120% recovery "accuracy" and RSD≤ 20%) at the majority of tested fortification levels ranging from 0.005 to 2 µg/g. The LOQ, defined as the lowest concentration at which an acceptable recovery is obtained, or LLMV (lowest level of method validation) for the four analytes in soybean forage, hay and seed was 0.005 ppm and was 0.01 ppm for soybean processed samples.

The results of endogenous method validation (radio-validation) showed that the average overall recoveries of radioactivity through the method, including the extraction, hydrolysis, partitioning, evaporation and final filtration steps, were 62.9%, 64.3% and 25.5% for PRE-T hay, POE-T hay and POE-T seed, respectively. The largest losses of radioactivity in the method were in the partitioning step. There was generally good agreement between the LC/MS/MS quantitation results and actual residue levels except for the case of low-level DCGA seed residues.
The analytical method is suitable for use as an accurate means of quantifying dicamba and its metabolites, 5-hydroxydicamba and 3,6-dichlorosalicylic acid (DCSA) in soybean raw agricultural and processed commodities. There were evidence of poor recovery and discrepancies between the recoveries of the method and radio-validation for 3,6-dichlorogentisic acid (DCGA), which suggests that the method may not be suitable for the determination of DCGA.

Table 4.3-1: Recovery (Accuracy) Results from Method Validation of Dicamba and Metabolites in Soybean Matrices

			Mean Percent Accuracy (% RSD)			
Matrix	**Fortification Level (µg/g)**	**Number of Tests**	**5-Hydroxy-dicamba**	**DCGA**	**DCSA**	**Dicamba**
Seed	0 (Control)*	7	ND**	<0.001	ND	ND
	0.005	7	63.7 (11.1)	88.0 (5.54)	107 (5.18)	103 (7.35)
	0.01	7	66.9 (9.19)	95.9 (2.88)	106 (3.55)	99.8 (5.85)
	0.02	7	71.3 (6.53)	97.4 (3.52)	105 (2.39)	97.9 (4.81)
	0.1	7	102 (2.25)	101 (2.76)	104 (2.12)	97.1 (2.97)
	2	2	104	87.7	97.3	87.8
Hay	0 (Control)	7	ND	<0.001	ND	ND
	0.005	7	77.4 (8.73)	68.4 (2.69)	97.4 (4.55)	ND
	0.01	7	80.0 (6.67)	82.9 (3.00)	105 (2.94)	86.3 (6.56)
	0.02	7	84.0 (9.13)	90.5 (1.82)	106 (2.01)	95.9 (10.5)
	0.1	7	102 (3.94)	100 (3.88)	107 (2.78)	105 (4.05)
	2	2	89.8	90.0	88.0	72.5
Forage	0 (Control)	7	ND	0.0014	ND	ND
	0.005	7	97.9 (7.49)	72.6 (5.57)	102 (5.22)	105 (9.30)
	0.01	7	99.3 (4.60)	80.9 (3.10)	110 (7.75)	101 (9.45)
	0.02	7	99.8 (3.52)	88.0 (2.77)	108 (2.42)	100 (5.83)
	0.1	7	105 (2.13)	90.0 (2.51)	106 (3.36)	105 (5.11)
	2	2	95.8	97.4	88.5	92.8
Hulls	0 (Control)	3	<0.005	<0.005	<0.005	ND
	0.01	2	74.4	78.4	115	92.5
	0.02	4	74.9 (15.3)	86.2 (8.39)	109 (1.42)	92.8 (6.80)
	0.05	3	74.8 (29.3)	88.3 (13.0)	104 (8.09)	96.1 (3.81)
	0.2	1	100	86.8	99.5	96.5
	0.4	2	91.6	90.9	98.3	101
	1.5	1	101	97.9	97.9	106
Defatted Flour	0 (Control)	4	0.007	<0.005	<0.005	<0.005
	0.01	2	31.0	83.0	102	79.3
	0.02	2	64.4	99.9	98.2	78.2
	0.05	1	84.3	84.8	103	96.3
	0.2	1	91.5	91.0	94.0	94.1
	0.4	3	99.4 (2.57)	96.0 (6.10)	104 (7.11)	103 (5.82)
	2	2	93.5	97.7	89.2	81.9
	3	1	98.5	96.3	92.3	97.6
Toasted Defatted Meal	0 (Control)	3	<0.005	<0.005	<0.005	ND
	0.01	2	70.7	81.8	92.9	88.7
	0.02	4	86.6 (10.8)	92.8 (7.42)	105 (11.8)	97.5 (5.42)
	0.05	3	86.5 (9.74)	92.0 7.85)	107 (7.70)	92.8 (10.8)
	0.2	1	101	83.2	101	94.0
	0.4	2	85.7	92.9	102	96.7
	1.5	1	95.3	104	93.8	88.0

			Mean Percent Accuracy (% RSD)			
Matrix	**Fortification Level (µg/g)**	**Number of Tests**	**5-Hydroxy-dicamba**	**DCGA**	**DCSA**	**Dicamba**
Soybean Protein Isolate	0 (Control)	4	ND	<0.005	<0.005	ND
	0.01	2	86.8	79.3	89.4	80.5
	0.02	2	94.1	82.9	94.7	87.1
	0.4	2	97.7	89.2	92.7	81.7
	0.8	2	98.0	93.6	100	87.1
	1.5	1	101	86.7	92.6	88.1
Soybean Protein Concentrate	0 (Control)	3	ND	<0.005	<0.005	ND
	0.01	2	80.7	82.7	96.3	81.0
	0.02	1	84.8	89.2	103	90.1
	0.2	1	106	97.1	105	90.1
	0.4	2	100	95.9	97.3	114
	1.5	1	105	89.3	90.6	94.3
Soybean Crude Lecithin	0 (Control)	8	<0.005	ND	<0.005	<0.005
	0.01	3	84.1 (7.93)	71.3 (7.54)	93.6 (4.90)	83.5 (18.1)
	0.2	3	98.9 (4.05)	78.8 (2.04)	96.7 (8.15)	86.1 (10.7)
	0.4	2	107	90.8	107	107
	2	2	103	101	108	107
Soybean Degummed Oil	0 (Control)	4	<0.005	<0.005	<0.005	ND
	0.01	3	84.9 (2.65)	70.8 (14.5)	96.0 (3.19)	98.5 (3.61)
	0.02	3	87.7 (2.20)	77.9 (19.6)	99.1 (2.42)	93.5 (5.16)
	0.05	2	93.7	82.2	101	86.8
	0.4	3	99.3 (8.38)	92.6 (7.20)	104 (3.49)	96.5 (12.1)
	2	2	101	105	99.4	103
Soybean Refined Bleached Deodorized (RBD) Oil	0 (Control)	4	<0.005	<0.005	<0.005	<0.005
	0.01	3	80.4 (9.65)	63.6 (5.17)	95.1 (1.03)	88.9 (4.31)
	0.02	3	86.7 (7.48)	73.2 (7.96)	98.2 (5.38)	87.6 (18.3)
	0.05	2	100	79.3	96.2	91.1
	0.2	1	90.0	94.5	99.7	105
	0.4	3	102 (8.76)	99.4 (5.29)	103 (4.94)	104 (8.32)
	2	2	100	103	107	102
Soymilk	0 (Control)	4	<0.005	<0.005	ND	ND
	0.01	3	83.5 (1.52)	72.6 (4.58)	101 (5.10)	95.1 (6.26)
	0.02	3	88.9 (7.30)	83.3 (9.56)	103 (8.58)	96.2 (11.5)
	0.05	2	94.5	86.5	104	83.9
	0.2	1	87.5	107	105	64.0
	0.4	3	92.9 (2.38)	87.9 (7.92)	94.1 (5.58)	90.7 (11.2)
	2	2	92.4	95.2	97.8	77.7
Tofu	0 (Control)	2	<0.005	<0.005	ND	ND
	0.01	2	92.7	77.8	109	77.7
	0.02	1	96.4	78.2	101	80.5
	0.05	1	100	92.6	103	80.9
	0.2	1	94.7	85.6	91.6	97.6
	0.4	3	84.5 (11.7)	85.7 (11.8)	82.5 (13.4)	81.2 (16.3)
	1.5	1	83.3	93.3	80.5	91.1

*Values reported for unspiked control samples are µg/g
**ND = not detected in any sample

Table 4.3-2: Comparison of the results (% recovery) obtained with respect to recoveries obtained in endogenous method validation (radio-validation) and in the metabolism study

Matrix	Results from metabolism study (% TRR)*	Recovery corrected method accountability
PRE-T hay	90.9	94.7
POE-T hay	95.3	94.2
POE-T seed	55.3	48.1

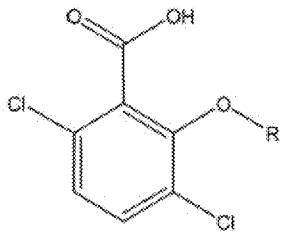
*Values were obtained from Table 6.2.1-3 in section 6.2.1.1 Metabolism, distribution and expression of residues in soybean.

Table 4.3-3: Comparison of the results (µg/g, ppm) obtained with respect to recoveries obtained in endogenous method validation (radio-validation) and in the metabolism study

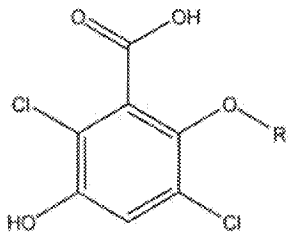
Sample	Metabolite/Analyte	Metabolite Quantitation (µg/g, ppm)*	Radio-validation Analysis (µg/g, ppm)**
PRE-T Hay	DCGA	0.032	0.068 (0.068, 0.067)
	DCSA	0.862	0.863 (0.878, 0.849)
	Dicamba	0.012	0.016 (0.015, 0.016)
POE-T Hay	DCGA	2.28	2.44 (2.59, 2.29)
	DCSA	28.0	27.7 (25.1, 30.3)
	Dicamba	4.88	3.74 (4.30, 3.18)
POE-T Seed	DCGA	0.025	0.005 (0.007, 0.004)
	DCSA	0.099	0.088 (0.090, 0.085)
	Dicamba	0.003	Not Detected

* DCGA values are mainly the sum of DCGA glucoside and DCGA malonylglucoside; DCSA values are mainly the sum of DCSA glucoside, DCSA 3-hydroxy-3-methylglutarylglucoside and free DCSA as determined in the soybean metabolism study final storage stability profiles.
** Average of duplicate sample analyses (values in parentheses are the results for the individual replicates).
PRE-T = pre-emergence treated; POE-T = post emergence treated.
All values are dicamba equivalents.

Figure 4.3-1: Analyte Structures

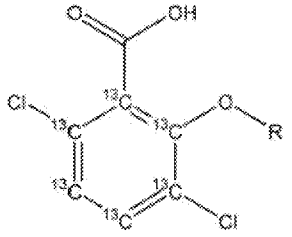


Dicamba, R = CH_3
3,6-Dichlorosalicylic Acid (DCSA), R = H

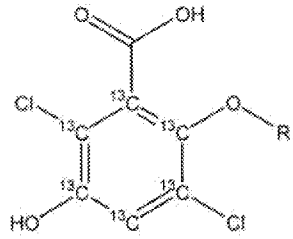


5-Hydroxydicamba, R = CH_3
3,6-Dichlorogentisic Acid (DCGA), R = H

Figure 4.3-2: Internal Standard Structures



$^{13}C_6$-Dicamba, R = CH_3
$^{13}C_6$-3,6-Dichlorosalicylic Acid (DCSA), R = H



$^{13}C_6$-5-Hydroxydicamba, R = CH_3
$^{13}C_6$-3,6-Dichlorogentisic Acid (DCGA), R = H