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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

DATE: 03-APR-01

SUBJECT: PP# 8F04954. **Mesotrione: Issues to be Presented to the Health Effects Division (HED) Metabolism Assessment Review Committee (MARC) 10-APR-01.** DP Barcode D273597. Chemical 122990. Case No. 289589. Submission No. S541377.

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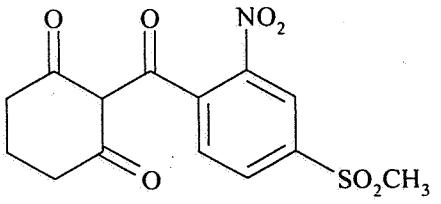
TO: Yan Donovan, Chemist, RAB2/HED (7509C)
Executive Secretary, MARC

Syngenta (formerly Zeneca Ag Products) has submitted a petition for permanent registration and the establishment of tolerances and for use of a new insecticide, mesotrione [2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione] (designated by the company code ZA1296), in/on field corn. Section F of the petition proposes the establishment of tolerances for residues of mesotrione *per se* in/on the following raw agricultural commodities (RACs):

Field corn	0.01 ppm
Field corn, fodder	0.01 ppm
Field corn, forage	0.01 ppm

Mesotrione is a triketone herbicide which inhibits the enzyme p-hydroxyphenylpyruvate dioxygenase (HPPD), disrupting the pigment biosynthesis in susceptible plants. Mesotrione is intended for preemergence and postemergence use for selective control of annual broadleaf weeds.

The structure of mesotrione is as follows:



1. RESIDUE CHEMISTRY SECTION

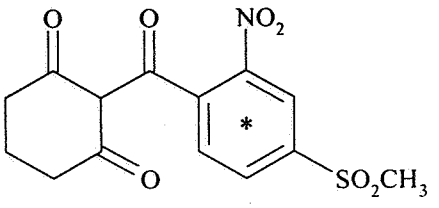
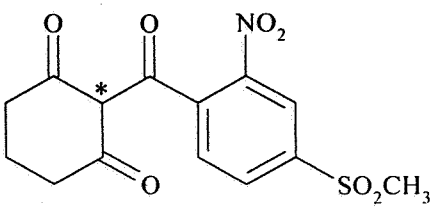
a. Use Information/Identification of Chemical

The petitioner provided a proposed label for the 4 lbs. a.i./gal suspension concentrate (SC) formulation (EPA File Symbol No. 100-RRGR; Product Name = Callisto™ Herbicide) proposed for use on all varieties of field corn, including production seed corn, and silage corn; use is prohibited on sweet corn, and popcorn. A maximum of two applications per season and a maximum seasonal application rate of 0.43 lbs ai/A/season are proposed. Mesotrione may be applied by either ground sprayers or by aerial application. For preemergence application, Callisto™ is proposed for use at 0.188-0.24 lbs ai/A. For postemergence application, mesotrione is proposed for a maximum rate of 0.188 lbs ai/A. In a single postemergence application, 0.094 lbs ai/A should not be exceeded.

The proposed label specifies that corn may be replanted immediately, following any crop failure. Soybeans, small grains, alfalfa, and clover may be planted 120 days after application. All other rotational crops may be planted the spring following application of mesotrione.

b. Summary of Plant and Livestock Metabolism Studies

The test substances for the plant and livestock metabolism studies and the confined rotational crop studies were uniformly ring-labeled [phenyl-^{14}C]mesotrione and [cyclohexane-2-^{14}C]mesotrione. The positions of the radiolabels are depicted below:

Phenyl label (PH):	Cyclohexane label (CY):
	

Plants

The qualitative nature of the residue in *field corn only* is adequately understood based on acceptable studies conducted on field corn.

Pending submission of additional storage stability data and information for the cyclohexane-label study, the qualitative nature of the residue in corn is adequately understood for purposes of this petition. Total radioactive residues (TRR, expressed as mesotrione equivalents) following a single preemergence application of uniformly ring-labeled [phenyl-^{14}C]mesotrione or [cyclohexane-^{14}C]mesotrione at 0.250-0.273 lb ai/A were respectively, 0.013 and 0.001 ppm in corn grain and 0.795 and 0.015 ppm in corn stover harvested at maturity (153 days after treatment, DAT). TRR were 0.356 and 0.067 ppm in immature corn forage harvested 27-28 days after the preemergence application of [phenyl-^{14}C]mesotrione or [cyclohexane-^{14}C]mesotrione, respectively. In a second plot of corn which received a single postemergence foliar application of [phenyl-^{14}C]mesotrione or [cyclohexane-^{14}C]mesotrione at 0.144-0.146 lb ai/A, TRR were 0.014 and 0.011 ppm in grain, 1.066 and 0.330 ppm in stover, and 0.244 and 0.098 ppm in immature forage, respectively.

Approximately 69-101% TRR were characterized/identified in immature and mature corn matrices from the metabolism studies. Mesotrione was identified at low levels in corn forage (0.4-3.0% TRR, 0.001-0.008 ppm) indicating that residues were extensively metabolized. Metabolite 4-OH ZA1296 was identified in forage and stover (0.7-3.8% TRR, 0.007-0.014 ppm) from the phenyl-label (PH) studies, and in forage from the cyclohexane-label (CY) studies at low levels (6.1-10.4% TRR, 0.006-0.007 ppm). Metabolites 4-OGlu ZA1296, MNBA, and AMBA were only identified in corn matrices from the PH studies; AMBA (free, acid-labile, base-labile, and hexose esters) metabolite levels were greater following postemergence treatment than preemergence treatment. Metabolite 4-OGlu ZA1296 was identified in forage from the PH studies at low levels (3.6-3.8% TRR, 0.009-0.013 ppm). AMBA was the major metabolite identified in forage (12.2-13.2% TRR, 0.032-0.043 ppm) and stover (13.6-28.2% TRR, 0.108-0.301 ppm) from the PH studies. MNBA was also identified in PH forage (3.4-19.7% TRR, 0.008-0.070 ppm) and stover (1.0-1.9% TRR, 0.008-0.019 ppm). A large amount of residues were characterized as minor compounds with diverse polarity. In the PH studies, extensive attempts (acid, base, and enzyme hydrolysis, acidic and basic TLC) were made to further characterize the unknown polar components. Based on mobility and responses to hydrolysis, the unknowns were considered to be similar to 4-OGlu ZA1296 and are likely polar conjugates, with endocons of neutral or amphoteric character. In the CY studies, the incorporation of radioactivity into carbohydrates, such as glucose, fructose, and malic acid, was demonstrated. The incorporation of radioactivity into lignin and cellulose was also characterized in postemergence stover.

The metabolite profiles differ significantly in the PH and CY studies. Little of the residue characterized from the PH studies resulted from the fixation of $^{14}CO_2$, while the identification of carbohydrates in the CY studies indicate that $^{14}CO_2$ is the major source of radioactive residues.

In an additional study reflecting the maximum proposed use pattern for mesotrione on corn, TRR following a single preemergence application at 0.27 lb ai/A and subsequent postemergence application at 0.16 lb ai/A of [phenyl-^{14}C]mesotrione were 0.03 ppm in corn grain and 0.57 ppm in corn stover harvested at maturity (91 DAT), and 0.27 ppm in immature forage harvested 48 DAT.

Residues of the parent, mesotrione, were not detected in this metabolism study. Metabolite 4-OH ZA1296 was identified only in corn forage at low levels (5.4% TRR, 0.01 ppm). AMBA, MNBA, and their conjugates were identified in both forage (2.2-4.6% TRR, 0.01 ppm) and stover (1.0-2.3% TRR, 0.01 ppm). As was observed in the separate preemergence and postemergence studies, minor compounds represented the largest amount of the radioactivity; however, each of these components was individually present at <0.01 ppm.

The HED Metabolism Committee previously met on 4/22/97 and determined (Memo, J. Stokes, 6/20/97) from a preliminary briefing on the metabolism and field trial studies (Memo, J. Stokes, 3/20/97), that the registrant should continue to analyze all field trial samples for mesotrione and MNBA, the major soil metabolite, in target and rotational crops. The Committee concluded that MNBA, a precursor of the AMBA, could be used as a potential marker for residues of AMBA for risk assessments if necessary because of difficulties associated with analysis for AMBA. AMBA was determined by the Metabolism Committee to have a projected toxicity less than the parent.

Table 1. TRR in samples of corn commodities treated with phenyl- or cyclohexane-labeled [^{14}C]mesotrione as a preemergence application at 0.250-0.273 lb ai/A (pre-E) or a postemergence application at 0.144-0.146 lb ai/A (post-E).

Commodity	TRR, ppm [^{14}C]mesotrione equivalents	
	Phenyl Label (PH)	Cyclohexane Label (CY)
Pre-E		
Forage	0.356	0.067
Stover [a]	0.795	0.015
Leaves	0.728	0.025
Stalks	0.068	0.004
Grain	0.013	0.001
Cobs	0.020	0.001
Post-E		
Forage [a]	0.244	0.098
Leaves	0.228	0.190
Stalks	0.015	0.014
Stover [a]	1.066	0.330
Leaves	0.969	0.649
Stalks	0.098	0.045
Grain	0.014	0.011
Cobs	0.027	0.019

[a] Calculated by the petitioner by summing TRR values for the leaves and stalks by weight ratio.

Table 2a. Summary of radioactive residues characterized/identified in corn commodities treated with [phenyl-^{14}C]mesotrione as a preemergence application at 0.250 lb ai/A (pre-E) or a postemergence application at 0.146 lb ai/A (post-E).

Fraction	Corn, forage Pre-E (TRR = 0.356 ppm)		Corn, stover Pre-E (TRR = 0.795 ppm)		Corn, forage Post-E (TRR = 0.244 ppm)		Corn, stover Post-E (TRR = 1.066 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]								
Mesotrione	2.2	0.008	<0.4	<0.003	0.4	0.001	<0.3	<0.003
4-OH ZA1296	3.8	0.014	0.9	0.007	3.0	0.007	0.7	0.007
4-OGlu ZA1296	3.8	0.013	<1.2	<0.010	3.6	0.009	<1.0	<0.010
MNBA	19.7	0.070	1.0	0.008	3.4	0.008	1.9 [b]	0.019
AMBA, total	12.2	0.043	13.6	0.108	13.2	0.032	28.2	0.301
AMBA, free	3.3	0.012	0.7	0.006	1.6	0.006	0.6	0.006
AMBA, acid-labile	3.5	0.012	3.4	0.027	3.5	0.009	7.2	0.077
AMBA, hexose esters	5.4	0.019	9.4	0.075	8.0	0.020	19.7	0.211
AMBA, base-labile	--	--	0.2	0.002	--	--	0.7	0.007
Total identified	**41.7**	**0.148**	**15.5**	**0.123**	**23.6**	**0.057**	**30.8**	**0.327**
Characterized								
Minor unidentified metabolites [c]	59.6	0.213	67.8	0.539	66.0	0.161	69.6	0.743
Total identified/characterized	**101.4**	**0.361**	**83.2**	**0.662**	**89.5**	**0.218**	**99.1**	**1.058**
Nonextractable	7.9	0.028	4.2	0.033	7.0	0.017	5.9	0.063

[a] See Attachment III for chemical names and structures of identified metabolites.

[b] Includes MNBA (1.2% TRR, 0.012 ppm) solubilized by potassium permanganate oxidation.

[c] Minor metabolites consist of compounds with diverse polarity and a large fraction which did not chromatograph as discrete entities.

Table 2b. Summary of radioactive residues characterized/identified in corn commodities treated with [cyclohexane-^{14}C]mesotrione as a preemergence application at 0.273 lb ai/A (pre-E) or a postemergence application at 0.144 lb ai/A (post-E).

Fraction	Corn, forage Pre-E (TRR = 0.067 ppm)		Corn, forage [a] Post-E (TRR = 0.098 ppm)		Corn, stover [a] Post-E (TRR = 0.330 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [b]						
Mesotrione	3.0	0.002	1.0	0.001	--	--
4-OH ZA1296	10.4	0.007	6.1	0.006	--	--
Total identified	**13.4**	**0.009**	**7.1**	**0.007**	--	--
Characterized						
Minor unidentified metabolites including carbohydrates [c]	56.7	0.038	68.4	0.067	34.2	0.113
Carbohydrates	23.9	0.016	--	--	--	--
Acid hydrolysate (minor unidentified metabolites including carbohydrates)	--	--	--	--	13.6	0.045
Solids from neutralization of acid hydrolysate	--	--	--	--	4.8	0.016
Lignin (DMSO extractable)	--	--	--	--	13.3	0.044
Cellulose (Schweizer's reagent extractable)	--	--	--	--	3.3	0.011
Total identified/characterized	**70.1**	**0.047**	**75.5**	**0.074**	**69.2**	**0.294**
Stalk fraction	--	--	7.1	0.007	7.3	0.024
Nonextractable	20.9	0.014	18.4	0.018	9.4	0.031

[a] Post-E forage and stover were divided into leaves and woody stalks with the leaves containing >90% of the residue. Characterization of the residues was performed on leaves only; however, the % TRR and ppm values reported above are corrected for the total (leaves + stalks) weight.

[b] See Figure 1 Attachment III for chemical names and structures of identified metabolites. The petitioner provided the summary values.

[c] Minor metabolites consist of compounds with diverse polarity, unretained fraction, and carbohydrates.

Table 3. TRR in samples of corn commodities treated with phenyl-labeled [^{14}C]mesotrione as a preemergence soil application at 0.27 lb ai/A and a subsequent postemergence application at 0.16 lb ai/A.

Commodity	TRR, ppm [^{14}C]mesotrione equivalents
Forage	0.27
Stover	0.57
Grain	0.03

7

Table 4. Summary of radioactive residues characterized/identified in corn commodities treated with a preemergence application of [phenyl-^{14}C]mesotrione at 0.27 lb ai/A and a subsequent postemergence application at 0.16 lb ai/A.

Fraction	Corn, forage (TRR = 0.27 ppm)		Corn, stover (TRR = 0.57 ppm)		Corn, grain (TRR = 0.03 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]						
4-OH ZA1296	5.4	0.01	--	--	--	--
AMBA	2.4	0.01	1.7	0.01	--	--
AMBA conjugates	4.6	0.01	2.3	0.01	--	--
MNBA	3.3	0.01	2.2	0.01	--	--
MNBA conjugates	2.2	<0.01	1.0	0.01	--	--
Total identified	**17.9**	**<0.05**	**7.3**	**0.04**	--	--
Characterized						
Minor unknown metabolites	45.7	0.14	47.7	0.27	46.9	0.01
2M acid hydrolysate	9.8	0.03	4.7	0.03	--	--
Total identified/characterized	**73.4**	**<0.22**	**59.7**	**0.34**	**46.9**	**0.01**
Nonextractable	22.6	0.06	23.5	0.13	46.9	0.01

[a] See Attachment III for chemical names and structures of identified metabolites. The petitioner provided the summary values.

8

Livestock

The qualitative nature of the residue in livestock is adequately understood based on lactating cow and laying hen metabolism studies.

Ruminants

Pending submission of additional storage stability data for the cyclohexane-label study, the dairy cow metabolism studies are acceptable. Following oral administration of [phenyl-^{14}C]mesotrione to a dairy cow for seven consecutive days at a feeding level of 11.91 ppm (700x and 650x the maximum theoretical dietary burdens (MTDBs) for beef and dairy cattle, respectively), the TRR (expressed as mesotrione equivalents) were 0.01-0.03 ppm in milk, 0.077 ppm in liver, 0.067 ppm in kidney, 0.002 ppm in muscle, ≤0.004-0.007 ppm in fat. Residues in milk plateaued on Day 2, and residues in tissues were highest in liver.

Following oral administration of [cyclohexanedione-2-^{14}C]mesotrione to a dairy cow for seven consecutive days at a feeding level of 9.9 ppm (590x and 540x the MTDBs for beef and dairy cattle, respectively), the TRR (expressed as mesotrione equivalents) were 0.006-0.079 ppm in milk, 0.110 ppm in liver, 0.110 ppm in kidney, 0.007 ppm in muscle, and 0.005-0.013 ppm in fat. Residues in milk plateaued on Day 4-5, and residues in tissues were highest in liver and kidney.

Approximately 80-88% TRR were characterized/identified in milk and tissues. The parent, mesotrione, was identified as the major metabolite in PH kidney (18.0% TRR, 0.012 ppm) and liver (10.3% TRR, 0.009 ppm), and CY kidney (14.4% TRR, 0.015 ppm) and liver (12.5% TRR, 0.013 ppm). AMBA was only identified in PH kidney (15.0% TRR, 0.010 ppm). [^{14}C]Lactose was identified as the major metabolite in CY milk (35.1% TRR, 0.025 ppm). Unknowns, none of which were present at >0.010 ppm, were characterized as aqueous or organosoluble. Additional characterization of the tissue extracts determined that a significant amount of radioactivity was associated with proteinaceous material in the liver.

In a separate study, following oral administration of [propyl-^{14}C]AMBA to a dairy cow for seven consecutive days at a feeding level of ~10 ppm, the TRR (expressed as AMBA equivalents) were 0.0030-0.0090 ppm in milk, 0.005 ppm in liver, 0.053 ppm in kidney, 0.000 ppm in muscle, and 0.000-0.018 ppm in fat. Residues in milk plateaued on Day 3, and residues in tissues were highest in kidney.

Approximately 86-88% TRR were characterized/identified in kidney and perirenal fat. The acid metabolite, AMBA, was identified in kidney (79.0% TRR, 0.038 ppm) and perirenal fat (61.6% TRR, 0.013 ppm). The petitioner stated that since AMBA was the major residue identified in kidney and perirenal fat, and also in urine (95.4% TRR), residues in kidney and perirenal fat are most likely a result of residual urine remaining in these tissues.

Table 5. TRR in samples of milk and edible tissues from two dairy cows following administration of [^{14}C]mesotrione at a feeding level of ~10 ppm (650x (PH) or 540x (CY)) for 7 consecutive days.

Matrix	TRR (ppm) mesotrione equivalents [a]	
	Phenyl-label	Cyclohexanedione-label
Milk		
Day 1	0.01 (**0.021** PM)	0.006
Day 2	0.03	0.052
Day 3	0.03	0.065
Day 4	0.03	0.067
Day 5	0.04	0.074
Day 6	0.03 (**0.036** AM)	0.078
Day 7	0.03	0.079
Day 8	0.03	0.072 (**0.070** AM)
Liver	0.077 (**0.085**)	0.110 (**0.101**)
Kidney	0.067 (**0.065**)	0.110 (**0.107**)
Muscle, forequarter	0.002	0.007
Muscle, hindquarter	0.002	0.007
Fat, subcutaneous	≤0.004	0.013
Fat, peritoneal	≤0.004	0.008
Fat, perirenal	0.007	0.005

[a] The mean of replicate combustion/LSC analyses is presented; except as noted, mil values represent combined AM and PM TRR. **Bolded** values were calculated by summing extractable and nonextractable radioactivity and were used by the petitioner for further determinations.

Table 6a. Summary of radioactive residues characterized/identified in milk and tissues of a dairy cow orally dosed with [phenyl-^{14}C]mesotrione at a feeding level of ~10 ppm (650x) for 7 consecutive days.

Fraction	Milk Day 6 AM (TRR = 0.036 ppm)		Liver (TRR = 0.085 ppm)		Kidney (TRR = 0.065 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]						
Mesotrione	–	–	10.3	0.009	18.0	0.012
AMBA	–	–	--	–	15.0	0.010
Total identified	**--**	**--**	**10.3**	**0.009**	**33.0**	**0.022**
Characterized						
Proteinaceous material	4.6	0.002	18.6	0.015	4.6	0.003
Butterfat fraction	0.6	<0.001	--	--	--	--
Aqueous	1.8	0.001	3.7	0.003	2.5	0.002
Aqueous acetone	3.9	0.001	--	--	--	--
DCM	3.6	0.001	--	--	--	--
EtOAc	44.1	0.016	--	--	--	--
Acetone	--	--	3.0	0.002	4.5	0.003
MeOH	--	--	8.3	0.007	4.0	0.003
Acidic MeOH	--	--	13.2	0.011	--	--
Unknowns	25.1	0.009	24.1	0.020	35.8	0.023
Total characterized/identified	**83.7**	**0.031**	**81.2**	**0.067**	**84.4**	**0.056**
Nonextractable	--	--	1.7	0.001	4.1	0.003

[a] Mesotrione and AMBA were identified and confirmed by different TLC systems. See Attachment III for chemical names and structures of identified metabolites.

Table 6b. Summary of radioactive residues characterized/identified in milk and tissues of a dairy cow orally dosed with [cyclohexanedione-^{14}C]mesotrione at a feeding level of ~10 ppm (540x) for 7 consecutive days.

Fraction	Milk, Day 8 AM (TRR = 0.070 ppm)		Liver (TRR = 0.101 ppm)		Kidney (TRR = 0.107 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]						
Mesotrione	–	--	12.5	0.013	14.4	0.015
Lactose	35.1	0.025	--	--	--	--
Total identified	**35.1**	**0.025**	**12.5**	**0.013**	**14.4**	**0.015**
Characterized						
Aqueous soluble unknowns	24.4	0.017	1.8	0.002	--	--
Organosoluble unknowns	10.5	0.007	4.2	0.004	9.2	0.010
Proteinaceous material	7.6	0.005	29.3	0.030	7.1	0.008
Butterfat	10.2	0.007	--	--	--	--
Aqueous soluble	--	--	12.1	0.012	11.9	0.013
Organic soluble	--	--	3.4	0.003	8.2	0.009
Acid soluble	--	--	10.0	0.010	--	--
Base soluble	--	--	7.5	0.008	--	--
Aqueous (anion exchange)	--	--	–	–	19.0	0.020
Adhered to ion exchange resin	--	--	--	--	10.1	0.011
Total characterized/identified	**87.8**	**0.061**	**80.8**	**0.082**	**79.9**	**0.086**
Nonextractable	--	--	5.4	0.005	--	--

[a] Mesotrione and lactose were identified by TLC and confirmed by HPLC.

Table 7. TRR in samples of milk and edible tissues from a dairy cow following administration of [^{14}C]AMBA at a feeding level of ~10 ppm for 7 consecutive days.

Matrix	TRR (ppm) AMBA equivalents [a]
Milk	
Day 1	0.0050
Day 2	0.0065
Day 3	0.0070
Day 4	0.0065
Day 5	0.0085
Day 6	0.0090
Day 7	0.0075
Day 8 (am only)	0.0030
Liver	0.005
Kidney	0.053 (**0.048**)

12

Matrix	TRR (ppm) AMBA equivalents [a]
Muscle, forequarter	0.000 [b]
Muscle, hindquarter	0.000 [b]
Fat, subcutaneous	0.003
Fat, omental	0.000 [b]
Fat, perirenal	0.018 (**0.021**)

[a] **Bolded** values were calculated by summing extractable and nonextractable radioactivity and were used by the petitioner for further determinations.

[b] As reported by the petitioner; no limits of detection were provided for these tissues.

Table 8. Summary of radioactive residues characterized/identified in tissues of a dairy cow orally dosed with [phenyl-^{14}C]AMBA at a feeding level of ~10 ppm for 7 consecutive days.

Fraction	Kidney (TRR = 0.048 ppm)		Perirenal Fat (TRR = 0.021 ppm)	
	% TRR	ppm	% TRR	ppm
Identified [a]				
AMBA	79.0	0.038	61.6	0.013
Total identified	**79.0**	**0.038**	**61.6**	**0.013**
Characterized				
Unknowns	0.5	<0.001	3.2	0.001
Aqueous soluble	5.9	0.003	21.3	0.004
Organic soluble	2.5	0.001	--	--
Total characterized/identified	**87.9**	**0.042**	**86.1**	**0.018**
Nonextractable	3.1	0.001	9.7	0.002

[a] AMBA was identified and confirmed by different TLC systems.

Poultry

The submitted poultry metabolism studies are acceptable. Following oral administration of [phenyl-^{14}C]mesotrione to laying hens for 10 consecutive days at a feeding level of ~10 ppm (1100x the MTDB for poultry), the TRR (expressed as mesotrione equivalents) were <0.003 ppm in egg whites, <0.003-0.024 ppm in egg yolks, 1.121 ppm in liver, ≤0.004 ppm in muscle, <0.003 ppm in peritoneal fat, and 0.042 ppm in subcutaneous fat with skin. Residues in egg yolks plateaued on the sixth day of dosing. Residues in tissues were highest in liver.

Following oral administration of [cyclohexanedione-^{14}C]mesotrione to laying hens for 10 consecutive days at a feeding level of ~10 ppm (1100x the MTDB for poultry), the TRR (expressed as mesotrione equivalents) were 0.012-0.025 ppm in egg whites, 0.002-0.094 ppm in egg yolks, 1.245 ppm in liver, 0.011-0.012 ppm in muscle, 0.010 ppm in peritoneal fat, and 0.048 ppm in subcutaneous fat with skin. Residues in egg whites and yolks plateaued on the fifth and eighth days of dosing, respectively. Residues in tissues were highest in liver.

Approximately 66-102% TRR were characterized/identified in eggs and tissues. The parent, mesotrione was the only identified compound; it was found in egg yolks, subcutaneous fat, and liver at 24-91% TRR (0.017-1.097 ppm). Incorporation of radioactivity into naturally occurring fatty acids such as palmitic, oleic, and stearic acid was tentatively observed in CY egg yolk. Additional unknowns were observed in both PH and CY egg and tissue matrices; each unknown fraction accounted for <0.01 ppm.

Table 9. TRR in samples of eggs and edible tissues from laying hens following administration of [^{14}C]mesotrione at a feeding level of ~10 ppm (1100x) for 10 consecutive days.

Matrix	TRR (ppm) mesotrione equivalents	
	Phenyl-label [a]	Cyclohexanedione-label [b]
Egg whites		
Day 1	<0.003	0.012
Day 2	<0.003	0.018
Day 3	<0.003	0.019
Day 4	<0.003	0.025
Day 5	<0.003	0.022
Day 6	<0.003	0.022
Day 7	<0.003	0.021
Day 8	<0.003	0.016
Day 9	<0.003	0.022
Day 10	<0.003	0.019
Composite [c]	--	**0.019**
Egg yolks		
Day 1	<0.003	0.002
Day 2	<0.004	0.007
Day 3	0.007	0.020

Matrix	TRR (ppm) mesotrione equivalents	
	Phenyl-label [a]	Cyclohexanedione-label [b]
Day 4	0.012	0.037
Day 5	0.016	0.055
Day 6	0.020	0.070
Day 7	0.019	0.085
Day 8	0.021	0.093
Day 9	0.022	0.094
Day 10	0.024	0.094
Composite [c]	**0.021**	**0.099, 0.097, 0.079**
Liver	1.121 (**1.234**)	1.245 (**1.209**)
Fat, subcutaneous with skin	0.042 (**0.037**)	0.048 (**0.047**)
Fat, peritoneal	<0.003	0.010 (**0.011**)
Muscle, breast	0.004	0.012 (**0.014**)
Muscle, thigh	<0.004	0.011 (**0.013**)

[a] TRR values are the average results for three hens.

[b] TRR values are the average results for two hens.

[c] Composite samples consisted of Day 6-7 CY egg whites from one hen and Day 9-10 CY egg whites from another hen, Day 6-10 PH egg yolks from 3 hens, and Day 9-10 CY egg yolks from 2 hens.

Table 10a. Summary of radioactive residues characterized/identified in eggs and tissues of laying hens orally dosed with [phenyl-^{14}C]mesotrione at a feeding level of ~10 ppm (1100x) for 10 consecutive days.

Fraction	Egg yolk (TRR = 0.021 ppm)		Subcutaneous fat (TRR = 0.037 ppm)		Liver (TRR = 1.234 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]						
Mesotrione	80.9	0.017	85.3	0.032	85.2	1.051
Total identified	**80.9**	**0.017**	**85.3**	**0.032**	**85.2**	**1.051**
Characterized						
Unknowns	4.0	0.001	4.6	0.002	2.2	0.027
1:1 ACN:water	--	-	1.4	0.001	--	--
Water with ACN rinsate	--	--	2.7	0.001	--	--
Aqueous	--	--	--	--	4.3	0.053
SDS precipitate	--	--	--	--	1.1	0.014
Total characterized/identified	**84.9**	**0.018**	**94.0**	**0.035**	**92.8**	**1.145**
Nonextractable	3.5	0.001	3.4	0.001	--	--

[a] Mesotrione was identified by TLC and confirmed by HPLC. See Attachment III for chemical name and structure.

Table 10b. Summary of radioactive residues characterized/identified in eggs and tissues of laying hens orally dosed with [cyclohexanedione-^{14}C]mesotrione at a feeding level of ~10 ppm (1100x) for 10 consecutive days.

Fraction	Egg white (TRR = 0.019 ppm)		Egg yolk (TRR = 0.079 ppm)		Subcutaneous fat (TRR = 0.047 ppm)		Liver (TRR = 1.209 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified [a]								
Mesotrione	–	–	24.0	0.019	71.2	0.033	90.7	1.097
Palmitic/oleic acid	--	--	29.5	0.023	--	--	--	--
Stearic acid	--	--	3.6	0.003	--	--	--	--
Total identified	--	--	**57.1**	**0.045**	**71.2**	**0.033**	**90.7**	**1.097**
Characterized								
Unknowns	53.7	0.010	10.9	0.009	1.2	0.001	--	--
Baseline/origin	6.7	0.001	4.2	0.003	0.3	<0.001	1.6	0.018
Aqueous	5.5	0.001	--	--	--	--	--	--
Hexane	--	--	--	--	3.7	0.002	--	--
ACN:water	--	–	–	–	9.2	0.004	--	--
Ethanol	--	--	13.5	0.010	--	--	--	--
pH 9 EtOAc	--	–	6.7	0.005	--	--	--	--
pH 2 EtOAc	--	–	4.9	0.004	--	--	--	--
Aqueous pH 1	--	--	4.5	0.004	--	--	--	--
SDS precipitate	--	--	--	--	--	--	4.8	0.058
Total characterized/identified	**65.9**	**0.013**	**101.8**	**0.082**	**85.6**	**0.040**	**97.1**	**1.174**
Nonextractable	23.7	0.005	--	--	10.6	0.005	1.8	0.022

[a] Mesotrione was identified by TLC and confirmed by HPLC; palmitic/oleic and stearic acids were tentatively identified by TLC. See Attachment III for chemical names and structures of identified metabolites.

Confined and Field Accumulation in Rotational Crop Studies

Confined Rotational Crop Study

The submitted confined rotational crop study is adequate. TRR accumulated at levels ≥0.01 ppm in the following rotational crop commodities of soybeans and wheat planted in sandy loam soil 30 days after treatment (DAT) with uniformly ring-labeled phenyl (PH) or cyclohexane-labeled (CY) [^{14}C]mesotrione at 0.274 lb ai/A (1.1x the maximum proposed preemergence rate for corn): soybean forage, hay, and soybeans, and wheat forage, hay, straw, and grain. TRR in PH samples ranged from 0.038 ppm in wheat grain to 2.58 ppm in wheat straw; in CY samples TRR were lower, ranging from 0.010 ppm in wheat grain to 0.059 ppm in wheat straw.

In rotational crop commodities of endive, radish, and wheat planted 120 days and 300 days after treatment of the soil with PH or CY [^{14}C]mesotrione at 0.411 lb ai/A (1x the maximum proposed seasonal rate for corn), TRR accumulated at levels ≥0.01 ppm in 120-DAT PH endive, radish tops and roots, and wheat forage, hay, 120-DAT CY wheat forage, hay, and straw, and 300-DAT PH endive, radish tops, and wheat forage, hay, straw, and grain. TRR in 120-DAT PH samples ranged from 0.014 ppm in wheat grain to 0.303 ppm in wheat forage; in 120-DAT CY samples, TRR ranged from 0.13 ppm in wheat hay to 0.043 ppm in wheat straw. TRR in 300-DAT PH samples ranged from 0.015 ppm in wheat grain to 0.197 ppm in wheat straw.

Approximately 51-97% TRR were identified/characterized in rotational crop commodities. Mesotrione, was identified at <0.01 ppm in rotational crop commodities, accounting for 1% and 2% TRR in 30-DAT PH wheat forage and 300-DAT PH wheat hay, and for 6-11% TRR in 30-DAT CY soybean and wheat commodities and 5% and 3% TRR in 120-DAT CY wheat forage and straw. MNBA and AMBA (free and conjugated) were the major identified metabolites in PH samples, but were not identified in CY samples. MNBA was identified at 8-62% TRR (0.003-0.625 ppm) in all samples analyzed from all rotational intervals. Free AMBA accounted for 9-17% TRR (0.024-0.330 ppm) in 30-DAT samples except wheat grain, 5-16% TRR (0.012-0.020 ppm) in120-DAT wheat forage, hay, and straw, and 6-7% TRR (0.001-0.014 ppm) in all 300-DAT samples analyzed. AMBA sulfate conjugate was a major metabolite in 30-DAT (9-17% TRR, 0.088-0.317 ppm), 120-DAT (8-12% TRR, 0.015-0.028 ppm), and 300-DAT (11-21% TRR, 0.009-0.026 ppm) wheat forage, hay, and straw, and was identified in 120-DAT radish roots at 8% TRR (0.003 ppm). Another AMBA conjugate was identified at 1-7% TRR (0.001-0.021 ppm) in wheat forage, hay, and straw from all rotations. In PH samples of 30-DAT soybeans and wheat grain, glucose accounted for 3% and 34% TRR (0.004 ppm and 0.013 ppm). Residues of the following metabolites were identified in rotational crops at 1-15% TRR, (<0.001-0.014 ppm): 4-OH ZA1296, 5-OH ZA1296 (CY only), 4-O-glucose ZA1296 (CY only), and xanthenone ZA1296 (CY only).

As in the corn metabolism study, the majority of residues characterized/identified in CY rotational crop commodities resulted from incorporation of $^{14}CO_2$ into natural products. Radioactivity was mostly unretained in the extractable fractions, and was characterized as carbohydrates such as glucose by HPLC analysis.

Based on the components identified, the results suggest that mesotrione is metabolized in rotational crops via a route similar to that demonstrated in primary crops.

Table 11. TRR in samples of rotational crop commodities grown in soil treated with [^{14}C]mesotrione at application rates of 0.274-0.275 lb ai/A (1.1x the proposed maximum preemergence rate) for 30-DAT crops or 0.411-0.412 lb ai/A (1x the proposed maximum seasonal rate) for 120- and 300-DAT crops.

Commodity	TRR (ppm) [^{14}C]mesotrione equivalents [a]		
	30-DAT [b]	120-DAT	300-DAT
Phenyl-label (PH) [c]			
Endive	--	0.046 (**0.053**)	0.018 (**0.019**)
Radish tops	--	0.033 (**0.048**)	0.011 (**0.009**)
Radish roots	--	0.036 (**0.037**)	0.005
Soybean forage	0.634 (**0.645**)	--	--
Soybean hay	0.417 (**0.462**)	--	--
Soybeans	0.139 (**0.145**)	--	--
Wheat forage	0.940 (**1.011**)	0.324 (**0.303**)	0.097 (**0.100**)
Wheat hay	0.738 (**0.756**)	0.111 (**0.127**)	0.043 (**0.044**)
Wheat straw	2.35 (**2.58**)	0.215 (**0.233**)	0.182 (**0.197**)
Wheat grain	0.035 (**0.038**)	0.012 (**0.014**)	0.015 (**0.015**)
Cyclohexane-label (CY)			
Endive	--	0.003	--
Radish tops	--	0.004	--
Radish roots	--	0.002	--
Soybean forage	0.026	--	--
Soybean hay	0.021	--	--
Soybeans	0.017	--	--
Wheat forage	0.056	0.017	0.002
Wheat hay	0.048	0.013	0.002
Wheat straw	0.059	0.043	0.006
Wheat grain	0.010	0.008	0.001

[a] -- = Not planted.

[b] DAT = Days after treatment.

[c] The mean of replicate combustion/LSC analyses is presented; **bolded** values were calculated by summing extractable and nonextractable radioactivity and were used by the petitioner for further determinations.

Table 12a. Summary of the characterization/identification of radioactive residues in rotational crop commodities grown in soil treated with [phenyl-^{14}C]mesotrione at application rates of 0.274 lb ai/A (1.1x the maximum proposed pre-emergence rate) for 30-DAT crops and 0.411 lb ai/A (1x the maximum proposed seasonal rate) for 120- and 300-DAT crops.

Metabolite [a]	30-DAT Soybean forage (TRR = 0.645 ppm)		30-DAT Soybean, hay (TRR = 0.462 ppm)		30-DAT Soybeans (TRR = 0.145 ppm)		30-DAT Wheat, forage (TRR = 1.011 ppm)		30-DAT Wheat, hay (TRR = 0.756 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified										
Mesotrione	--	--	--	--	--	--	1	0.012	--	--
MNBA	48	0.312	35	0.163	10	0.014	62	0.625	23	0.172
AMBA	11	0.069	7	0.034	17	0.024	9	0.086	11	0.083
AMBA sulfate conjugate	--	--	--	--	--	--	9	0.088	17	0.131
AMBA conjugate	--	--	--	--	--	--	4	0.036	3	0.021
4-OH ZA1296	--	--	--	--	--	--	1	0.014	--	--
Glucose	--	--	--	--	3	0.004	--	--	--	--
Total identified	**59**	**0.381**	**43**	**0.197**	**29**	**0.042**	**85**	**0.861**	**54**	**0.407**
Characterized										
Metabolite M1	1	0.004	1	0.003	7	0.010	--	--	--	--
Metabolite M2	2	0.011	3	0.013	--	--	--	--	2	0.012
Metabolite M5	--	--	--	--	--	--	--	--	--	--
Metabolite M12	--	--	--	--	--	--	--	--	1	0.011
Metabolite M13	--	--	--	--	--	--	--	--	1	0.009
Metabolite M14	--	--	1	0.004	--	--	--	--	--	--
Metabolite M15	--	--	2	0.008	--	--	--	--	--	--
Metabolite M18	1	0.009	2	0.009	4	0.005	--	--	2	0.018
Metabolite M19	--	--	--	--	--	--	--	--	2	0.017
Metabolite M20	1	0.005	--	--	--	--	--	--	--	--
Metabolite M22	--	--	--	--	--	--	--	--	--	--
Other (each <0.010) [b]	17	0.112	21	0.096	23	0.033	12	0.118	24	0.184
Base hydrolysate	--	--	--	--	3	0.005	--	--	--	--
Hexane	--	--	--	--	3	0.004	--	--	--	--
Total characterized/identified	**81**	**0.522**	**72**	**0.330**	**68**	**0.099**	**97**	**0.979**	**87**	**0.658**
Nonextractable	16	0.104	27	0.124	18	0.026	5	0.051	10	0.073

Table 12a (*PH study, continued).*

Metabolite [a]	30-DAT Wheat, straw (TRR = 2.580 ppm)		30-DAT Wheat, grain (TRR = 0.038 ppm)		120-DAT Endive (TRR = 0.053 ppm)		120-DAT Radish, roots (TRR = 0.037 ppm)		120-DAT Radish, tops (TRR = 0.048 ppm)		120-DAT Wheat, forage (TRR = 0.303 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified												
Mesotrione	–	–	--	–	–	--	–	–	--	–	–	–
MNBA	18	0.458	8	0.003	38	0.020	36	0.013	53	0.025	54	0.165
AMBA	13	0.330	--	--	--	--	--	--	--	--	6	0.019
AMBA sulfate conjugate	12	0.317	--	--	--	--	8	0.003	--	--	9	0.028
AMBA conjugate	1	0.021	--	--	--	--	--	--	--	--	7	0.021
4-OH ZA1296	--	--	--	--	--	--	--	--	--	--	--	--
Glucose	--	--	34	0.013	--	--	--	--	--	--	--	--
Total identified	**44**	**1.126**	**42**	**0.016**	**38**	**0.020**	**44**	**0.016**	**53**	**0.025**	**76**	**0.232**
Characterized												
Metabolite M1	--	--	--	--	3	0.001	--	--	--	--	--	--
Metabolite M2	1	0.022	--	--	--	--	--	--	--	--	--	--
Metabolite M5	--	--	--	--	3	0.001	--	--	--	--	--	--
Metabolite M12	--	--	--	--	--	--	--	--	--	--	--	--
Metabolite M13	1	0.017	--	--	--	--	--	--	--	--	--	--
Metabolite M14	--	--	--	--	--	--	--	--	--	--	--	--
Metabolite M15	--	--	--	--	--	--	--	--	--	--	--	--
Metabolite M18	--	--	--	--	--	--	--	--	--	--	--	--
Metabolite M19	--	--	--	--	--	--	--	--	--	--	--	--
Metabolite M20	--	--	--	--	--	--	--	--	--	--	--	--
Metabolite M22	1	0.015	--	--	--	--	--	--	--	--	--	--
Unknowns [b]	39	1.010	24	0.009	9	0.005	10	0.004	20	0.010	12	0.035
Base hydrolysate	--	--	--	--	--	--	--	--	--	--	--	--
Hexane	--	--	--	--	--	--	--	--	--	--	--	--
Total characterized/identified	**85**	**2.189**	**66**	**0.025**	**53**	**0.028**	**55**	**0.020**	**73**	**0.035**	**88**	**0.267**
Nonextractable	14	0.351	29	0.011	43	0.023	45	0.017	25	0.012	5	0.014

Table 12a (*PH study, continued*).

Metabolite [a]	120-DAT Wheat, hay (TRR = 0.127 ppm)		120-DAT Wheat, straw (TRR = 0.233 ppm)		300-DAT Endive (TRR = 0.019 ppm)		300-DAT Wheat, forage (TRR = 0.100 ppm)		300-DAT Wheat, hay (TRR = 0.044 ppm)		300-DAT Wheat, straw (TRR = 0.197 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified												
Mesotrione	–	--	–	–	--	–	–	–	2	0.001	--	--
MNBA	18	0.023	8	0.018	35	0.007	43	0.043	17	0.007	8	0.015
AMBA	16	0.020	5	0.012	6	0.001	7	0.007	7	0.003	7	0.014
AMBA sulfate conjugate	12	0.015	8	0.018	--	--	11	0.011	21	0.009	13	0.026
AMBA conjugate	1	0.001	1	0.002	--	--	5	0.005	4	0.002	1	0.003
4-OH ZA1296	--	--	--	--	--	--	--	--	--	--	--	--
Glucose	--	--	--	--	--	--	--	--	--	--	--	--
Total identified	**46**	**0.059**	**22**	**0.050**	**41**	**0.008**	**67**	**0.067**	**50**	**0.022**	**29**	**0.058**
Characterized												
Metabolite M1	--	--	--	–	13	0.002	--	--	--	–	--	--
Metabolite M2	--	--	--	--	--	--	--	--	--	--	--	--
Metabolite M5	--	--	--	--	--	--	--	--	4	0.002	3	0.005
Metabolite M12	1	0.002	--	--	--	--	--	--	--	--	2	0.003
Metabolite M13	--	--	--	--	--	--	--	--	--	--	--	--
Metabolite M14	--	--	--	--	--	--	--	--	--	--	--	--
Metabolite M15	--	--	--	--	--	--	--	--	--	--	--	--
Metabolite M18	--	--	--	--	--	--	--	--	--	--	--	--
Metabolite M19	--	--	--	--	--	--	--	--	--	--	--	--
Metabolite M20	--	--	--	--	--	--	--	--	--	--	--	--
Metabolite M22	--	--	--	--	--	--	--	--	--	--	--	--
Unknowns [b]	25	0.032	51	0.119	25	0.005	16	0.016	24	0.011	37	0.072
Base hydrolysate	--	--	--	--	--	--	--	--	--	--	--	--
Hexane	--	--	--	--	--	--	--	--	--	--	--	--
Total characterized/identified	**72**	**0.092**	**73**	**0.169**	**79**	**0.015**	**83**	**0.083**	**78**	**0.034**	**71**	**0.139**
Nonextractable	9	0.012	15	0.034	37	0.007	15	0.015	30	0.013	15	0.029

[a] Mesotrione and its metabolites were identified by HPLC; refer to Attachment III for chemical names and structures of identified metabolites

[b] "Other" radioactivity includes the blue zone, which was characterized as containing many AMBA conjugates, for 30-DAT soybean forage and hay, 30-DAT wheat forage and straw, and 120-DAT wheat straw

Table 12b. Summary of the characterization/identification of radioactive residues in rotational crop commodities grown in soil treated with [cyclohexane-^{14}C]mesotrione at application rates of 0.275 lb ai/A (1.1x the maximum proposed pre-emergence rate) for 30-DAT crops and 0.412 lb ai/A (1x the maximum proposed seasonal rate) for 120- and 300-DAT crops.

	30-DAT Soybean, forage (TRR = 0.026 ppm)		30-DAT Soybean, hay (TRR = 0.021 ppm)		30-DAT Wheat, forage (TRR = 0.056 ppm)		30-DAT Wheat, hay (TRR = 0.048 ppm)		30-DAT Wheat, straw (TRR = 0.059 ppm)	
Metabolite [a]	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Identified										
Mesotrione	11	0.003	10	0.002	17	0.009	7	0.003	6	0.003
XanH4	3	0.001	4	0.001	2	0.001	2	0.001	4	0.002
4-OGlu ZA1296	–	–	5	0.001	4	0.002	6	0.003	3	0.002
5-OH ZA1296	6	0.001	5	0.001	1	0.001	4	0.002	4	0.003
4-OH ZA1296	4	0.001	--	--	15	0.008	8	0.004	3.6	0.002
Total identified	**24**	**0.006**	**24**	**0.005**	**39**	**0.022**	**27**	**0.013**	**21**	**0.012**
Characterized										
Unknowns [b]	--	--	8	<0.002	8	0.005	13	0.006	12.9	0.008
Unretained [c]	20	0.005	22	0.005	12	0.007	20	0.009	24.9	0.015
Organic (ether or ACN)	2	<0.001	4	<0.001	10	0.006	1	<0.001	--	--
Acetone	5	0.001	2	<0.001	6	0.003	7	0.003	1	<0.001
Total characterized/identified	**51**	**<0.013**	**60**	**<0.013**	**75**	**0.042**	**68**	**<0.033**	**59**	**<0.035**
Nonextractable	29	0.008	27	0.006	15	0.008	22	0.011	28	0.017

Table 12b (*CY study, continued).*

Metabolite [a]	120-DAT Wheat, forage (TRR = 0.017 ppm)		120-DAT Wheat, straw (TRR = 0.043 ppm)	
	% TRR	ppm	% TRR	ppm
Identified				
Mesotrione	5	0.001	3	0.001
XanH4	3	<0.001	4	0.002
4-OGlu ZA1296	6	0.001	4	0.002
5-OH ZA1296	2	<0.001	2	0.001
4-OH ZA1296	12	0.002	6	0.003
Total identified	**28**	**<0.005**	**19**	**0.008**
Characterized				
Unknowns [b]	10	<0.002	11	<0.005
Unretained [c]	10	0.002	23	0.010
Organic (ether or ACN)	20	0.003	--	--
Acetone	0	<0.001	2	<0.001
Total characterized/identified	**68**	**<0.012**	**55**	**<0.024**
Nonextractable	24	0.004	32	0.014

[a] Mesotrione and its metabolites were identified by HPLC; refer to Attachment III for chemical names and structures of identified metabolites.

[b] Each unknown metabolite is present at ≤0.003 ppm.

[c] Carbohydrate HPLC analysis demonstrated that unretained residues included glucose and/or fructose in 30-DAT wheat straw and grain and 120-DAT wheat straw .

Field Accumulation in Rotational Crops

The limited field rotational crop study is acceptable. Residues of mesotrione and its metabolite MNBA were each less than the method LOQ (<0.01 ppm) in/on all rotational crop matrices (radish, roots and tops; soybean forage, hay, and seed; millet forage, hay, straw, and grain; and sorghum forage) from the 29- to 30-day plantback interval (PBI) following single preplant incorporated application made to the primary crop, field corn, of the 4 lb/gal SC formulation at 0.30 lb ai/A/application (~0.7x maximum proposed seasonal rate). Residues of mesotrione and its metabolite MNBA were each less than the method LOQ (<0.01 ppm) in/on all rotational crop matrices (radish, roots and tops; endive leaves; and wheat forage, hay, straw, and grain) from the 74- to 100-day PBI following two applications (preplant incorporated and postemergence) made to the primary crop, field corn, of the 4 lb/gal SC formulation at a total rate of 0.50 lb ai/A (~1.2x maximum proposed seasonal rate).

Provided the residues of concern are mesotrione and/or metabolite MNBA, these data support the PBIs proposed on the specimen label of 30 days for soybeans and sorghum, and 120 days for small grains, alfalfa, and clover.

d. Residue Analytical Methods - Plants, Livestock

Plants

The petitioner proposes HPLC method TMR0643B with fluorescence detection for the enforcement of tolerances for field corn forage, stover, and grain. Method validation recoveries indicate that this method adequately recovers residues of mesotrione and its metabolite MNBA from field corn forage, stover, and grain. Adequate radiovalidation and ILV data have been submitted for this method. This method was forwarded to ACL for a PMV (Memo, S. Levy, 11/09/99, D260569).

Livestock

Tolerances for livestock commodities are not required to support the proposed use of mesotrione on corn. Therefore, no residue analytical methods for livestock commodities are required.

e. Multiresidue Methods

A report on MRM testing of mesotrione (MRID# 44505224) has been received and was forwarded to FDA (Memo, S. Levy, 11/16/99, D260571) for updating PAM-I, Appendix I. Acceptable recoveries of mesotrione were obtained in corn forage using Protocol C and D. If the MARC determines that metabolites of mesotrione are of toxicological concern for purposes of tolerance expression and/or risk assessment, then an additional MRM study will be recommended by HED.

f. Crop Field Trials and Livestock Feeding Studies

Crop Field Trials

The petitioner has provided adequate residue data reflecting the maximum proposed use pattern of mesotrione on field corn forage, stover, and grain. The results of the field corn field trials indicate that residues of mesotrione *per se* will not exceed the proposed tolerance level of 0.01 ppm in/on field corn forage harvested 46-92 days and field corn stover and grain harvested 68-114 days following two applications of the 4 lb/gal SC formulation at 0.30 lb ai/A/application (preplant, at planting, preemergence, or preplant incorporated) and 0.20 lb ai/A/application (postemergence) for a total application rate of 0.50 lb ai/A (~1.2x maximum proposed seasonal application rate).

Residues of metabolite MNBA were also <0.01 ppm (nondetectable) in/on field corn forage, stover, and grain treated with the 4 lb/gal SC formulation according to the maximum proposed use pattern.

Livestock Feeding Studies

Because the residues observed from the ruminant and poultry metabolism studies are negligible, no data are required pertaining to this requirement. HED concludes that there is no reasonable expectation of finding quantifiable mesotrione residues of concern in eggs, milk, and the meat, fat, or meat byproducts of poultry or ruminants as a result of the proposed uses on field corn [Section 180.6(a)(3)].

g. International Considerations

There are no Codex, Canadian, or Mexican maximum residue limits (MRLs) established for mesotrione on the commodities included in this request. Thus, harmonization is not an issue.

2. TOXICOLOGY DATA

Mesotrione's primary mode of action is inhibition of the HPPD, an enzyme involved in the process of photosynthesis. It also inhibits mammalian HPPD that is involved in the catabolism of tyrosine in animals and humans. The resulting tyrosinemia causes toxic effects in the eye, liver, and kidney. Developmental effects involve delayed ossification of several bone structures. Tyrosinemia in young children has been linked to mental retardation and abnormal neurological symptoms. Mesotrione is not a mutagen or a carcinogen.

A series of rat metabolism studies with [^{14}C-phenyl]mesotrione indicated that mesotrione was readily absorbed and distributed in the body. Tissue distribution was about the same in both sexes, although one study showed higher residues in the kidneys in females, with the highest residues of the test compound in the liver and kidney. Higher doses resulted in higher residues in the liver and kidney, while repeated doses resulted in reduced accumulation of residues in all

tissues. Levels of radioactivity in tissues of iv-dosed animals were essentially the same as in orally-dosed animals. Over 50% of the administered dose was excreted in the urine in both sexes and around 25% was excreted in the feces. Females exhibited slightly higher total urinary excretion than males, and males had slightly increased total fecal excretion when compared to females. Increasing the dose or repeated doses had little effect on the pattern of excretion in both sexes. The overall pattern of excretion was similar between orally-dosed and intravenous-dosed rats. The metabolite profile was similar between the sexes in each group and between the single-dosed and repeated-dosed animals. The parent compound was the major component identified in the urine accounting for 47-64% of the dose. In addition, the following minor metabolites were identified: MNBA (1-4% of the dose), AMBA (3-12%), 5-hydroxymesotrione ($\leq$ 2%), and 4-hydroxymesotrione (3-6%). In bile cannulated rats administered [^{14}C-phenyl]mesotrione or [^{14}C-dione]mesotrione, the major component in fecal excreta and bile was the parent compound. Analysis of the bile identified mesotrione and 4-hydroxymesotrione as two minor components. Another minor component in the feces was 5-hydroxymesotrione.

AMBA and MNBA have low acute toxicity by the oral route (both are category IV) and MNBA has low acute dermal toxicity (category III). In bacterial reverse gene mutation tests, AMBA and MNBA were negative for mutagenicity in all tester strains under all conditions, except for an equivocal response with tester strain TA100 using the pre-incubation method for MNBA. Both metabolites are very weak inhibitors of HPPD as compared with the parent, mesotrione, and NTBC, a potent inhibitor of HPPD used in drug therapy. In a nonguideline 28-day oral toxicity study by gavage in rats, MNBA caused an increase in motor activity in females at all doses tested (15, 150, or 1,000 mg/kg/day). No other treatment-related effects were noted.

The doses and toxicological endpoints selected for various exposure scenarios are summarized in Table 13.

Table 13. Summary of Exposure Scenarios, Doses, Endpoints, and Studies Selected for Mesotrione.

EXPOSURE SCENARIO	DOSE [a] (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary	No appropriate endpoint was available to determine the Acute RfD for the general population or for the females 13-50 subpopulation.		
Chronic Dietary	LOAEL = 2.1 mg/kg/day UF = 300	Tyrosinemia in F_1 adults and F_{2a} pups and ocular discharge in F_1 pups	Multi-generation Reproduction Study Mouse
		Chronic RfD = 0.007 mg/kg/day	
Incidental Oral, Short-Term	NOAEL = 100 mg/kg/day	Decreased body weight gains during treatment and food consumption	Developmental Toxicity - Rat
Incidental Oral, Intermediate-Term	LOAEL = 2.1 mg/kg/day MOE = 300	Tyrosinemia in F_1 adults and F_{2a} pups and ocular discharge in F_1 pups	Multi-generation Reproduction Study Mouse
Dermal, Short-Term	LOAEL = 100 mg/kg/day MOE = 300	Delays in skeletal ossification and changes in *manus/pes* ossification assessments	Developmental Toxicity - Rat
Dermal, Intermediate-/ Long-Term	LOAEL = 2.1 mg/kg/day MOE = 300	Tyrosinemia in F_1 adults and F_{2a} pups and ocular discharge in F_1 pups	Multi-generation Reproduction Study Mouse
Inhalation, Short-Term	LOAEL = 100 mg/kg/day MOE = 300	Delays in skeletal ossification and changes in *manus/pes* ossification assessments	Developmental Toxicity - Rat
Inhalation, Intermediate-/ Long-Term	LOAEL = 2.1 mg/kg/day MOE = 300	Tyrosinemia in F_1 adults and F_{2a} pups and ocular discharge in F_1 pups	Multi-generation Reproduction Study Mouse

[a] NOAEL=no observed adverse effects level; LOAEL=lowest observed adverse effects level; MOE=margin of exposure.

28

3. ENVIRONMENTAL FATE DATA

The following is an excerpt from the DRAFT EFED memorandum dated 3/9/01 (electronic correspondence, A. Clem, 3/15/01):

The herbicide mesotrione and its major metabolites MNBA and AMBA are distinctly acidic compounds. This acidic/ionic property has a major influence on their behavior in environmental media at different pHs. As a suite, these compounds had low to virtually no sorption to tested soils/sediments at the more neutral pHs typical of agriculture, indicating high potential for leaching and runoff. Tending to offset the opportunity for leaching and runoff, parent was relatively short-lived. Generally, MNBA and AMBA were also relatively short-lived under aerobic conditions. However, as indicated further below, there is greater uncertainty about the persistence of MNBA and AMBA under suboxic conditions, such as would be found in subsoil and ground water. These metabolites would be more likely candidates for leaching and runoff than parent. Based on physicochemical properties, bioconcentration of mesotrione, MNBA and AMBA is not expected. Likewise, volatilization of mesotrione and its transformation products (except for carbon dioxide) is not indicated.

Based on laboratory studies, the primary routes of environmental transformation for parent mesotrione are aerobic and suboxic metabolism in soil and water. Numerous laboratory "half-lives" (more than 17) ranged from around four days to one month, depending on ambient conditions, especially pH (see main text). In relative, practical terms, photolysis is a minor degradative route. Mesotrione was stable against simple hydrolysis.

Sorption of mesotrione to soil organic matter and its aerobic soil metabolism half-lives (paired values for sorption and half-life for 17 soils) each correlate inversely with pH–the higher the pH, the lower the apparent sorption to soil organic matter and the shorter the half-life (see main text). Lower sorption to soil acts to increase available concentrations, while shorter half-lives act to decrease them. For mesotrione, the overall quantitative effect tended to normalize estimated environmental concentrations to a central value, regardless of pH.

Only three compounds, MNBA, AMBA, and carbon dioxide, were identified specifically as by-products in laboratory studies. Depending on conditions and time after application of parent, MNBA and AMBA can comprise up to approximately 60% of applied parent equivalent. Aerobic conditions favor MNBA, suboxic conditions favor AMBA. Half-lives for MNBA can only be crudely estimated from the available data and are highly uncertain. In at least two aerobic soils, MNBA half-lives appeared to be measured in one to several months, but seemed to be much shorter in others. Although, as for parent, metabolism rates for MNBA may show correlation with pH, this has not been pursued because of lack of sufficient, amenable data.

In a separate aerobic soil metabolism study with AMBA as the test substance in three soils, AMBA half-lives averaged 21 $\pm$ 5 days with an upper 90% confidence interval on the mean of 31 days. Data are insufficient to determine the range of variation of half-life with pH. Under suboxic conditions, half-lives for AMBA cannot be reliably established because of study deficiencies; a crude, reviewer-estimated first-order half-life for AMBA under the existing study conditions is 110 days, but may be longer with greater restriction of oxygen.

Under aerobic conditions, carbon dioxide was a ubiquitous product which issued from key positions in both rings of the mesotrione molecule. The cyclohexanedione ring was much more reactive in yielding carbon dioxide than the benzene ring. In some cases, carbon dioxide comprised up to about 80% of the radioactive dose after about six months. Increasingly difficult to extract soil residues tended to increase with time up to roughly15 to 50% of the dose, and then tended to decrease in roughly complementary fashion with increasing levels of carbon dioxide. This pattern clearly indicates progression to ultimate degradation/mineralization when there is sufficient aeration. However, when aeration was limited (suboxic conditions), carbon dioxide was only sparingly evolved from either ring. Under such conditions, as stated above, AMBA was a prominent metabolite which may be persistent when oxygen is in short supply.

Three terrestrial field dissipation studies on bare ground did not adequately account for the dissipation of

mesotrione. They did, however, provide supplemental aspects which are consistent with the laboratory findings of a relatively short residency time for mesotrione in soil. The registrant failed to identify any degradates in the field, or to clearly determine leaching potential.

4. DIFFERENCES IN THE METABOLISM AMONG THE RAT, PLANT AND LIVESTOCK

There is only one minor difference between the metabolic pathways of mesotrione (ZA1296) in the rat and in plants. In both plants and rats, mesotrione is metabolized to 4-hydroxyZA1296, MNBA, or AMBA. However, in plants, 4-hydroxyZA1296 can be further metabolized to MNBA; in rats, it appears that it does not metabolize to MNBA but is excreted. In ruminants, it appears that mesotrione is metabolized *only* to AMBA.

5. QUESTIONS TO THE MARC

1. Is there any scientific objection to establishing the tolerance and conducting risk assessment in terms of mesotrione *per se*? What are the residues of concern in drinking water?

2. Are additional mesotrione metabolites at the levels reported of special toxicological concern? If so, which one(s)? Do they warrant inclusion in the tolerance regulation? Separate regulation? Inclusion in the dietary risk assessment? Additional metabolism studies? Toxicological studies?

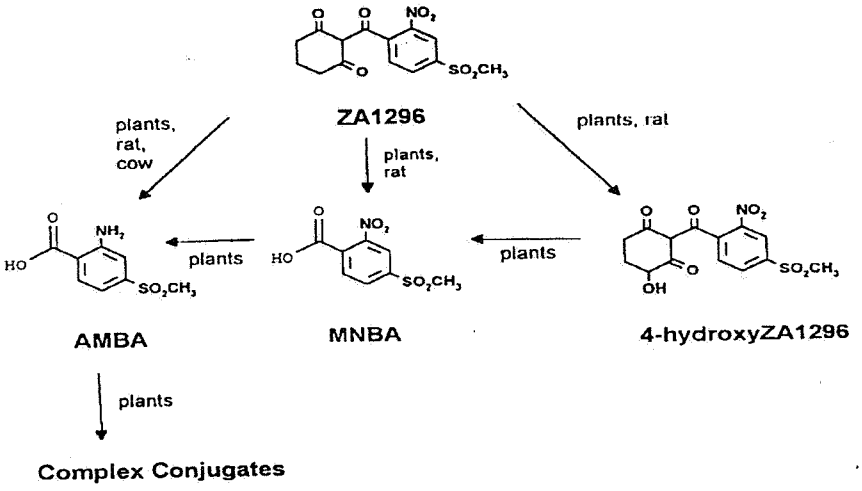
ATTACHMENTS

ATTACHMENT I - Proposed Metabolic Pathway for Mesotrione in Ruminants, Plants, and Rat.
ATTACHMENT II - Proposed Metabolic Pathway for Mesotrione in the Rat (from D259369).
ATTACHMENT III - Chemical Names and Structures of Mesotrione and its Metabolites Identified Metabolism Studies.

cc: S. Levy (RAB1/HED), D. Nixon (RAB1/HED), HED MARC File (C. Olinger, RRB1/HED), J. Stone (RD)
RDI: RAB1 Chemists (4/3/01), G.F. Kramer (3/28/01), G.J. Herndon (4/3/01)
S. Levy:806T:CM#2:(703)305-0783:7509C:RAB1

ATTACHMENT I - Proposed Metabolic Pathway for Mesotrione in Plants, Ruminants, and Rat.

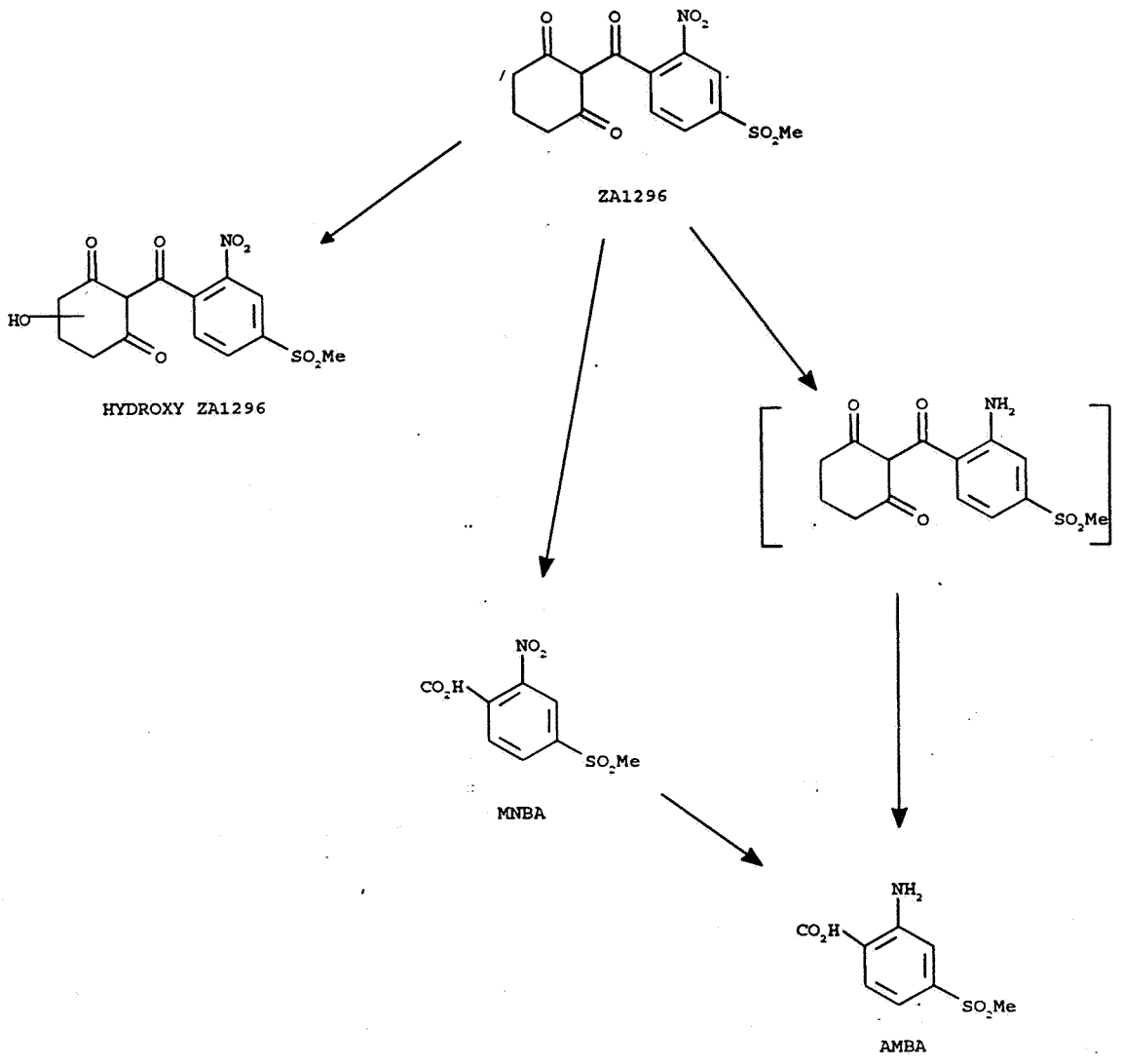
FIGURE I
METABOLIC PATHWAY FOR ZA1296



31

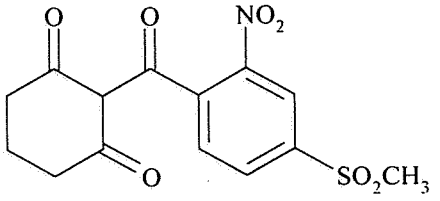
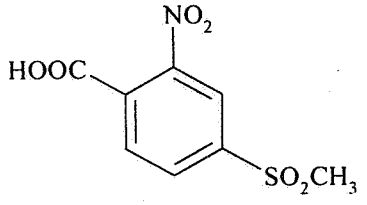
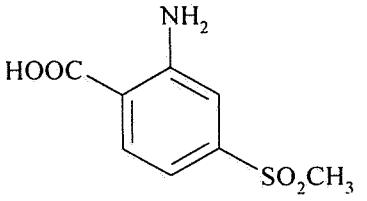
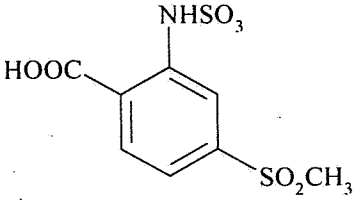
ATTACHMENT II - Proposed Metabolic Pathway for Mesotrione in the Rat (from D259369).

FIGURE 7 - PROPOSED BIOTRANSFORMATION PATHWAY FOR ZA1296 IN THE MOUSE



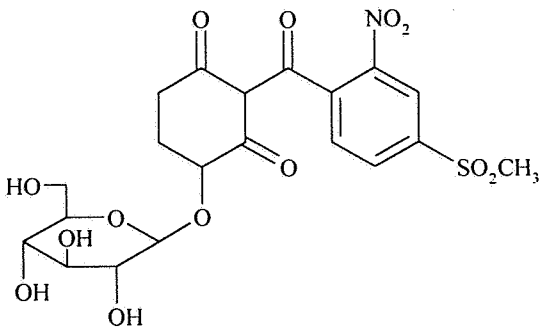
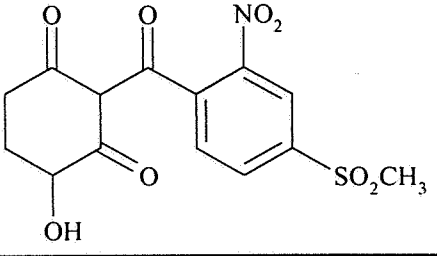
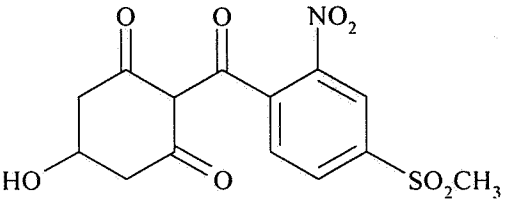
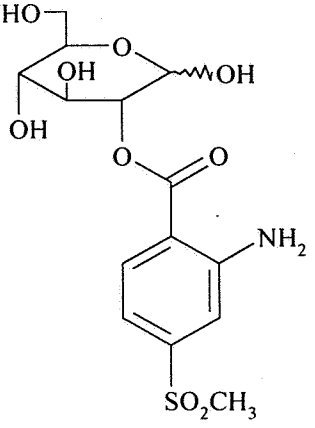
32

ATTACHMENT III - Chemical Names and Structures of Mesotrione and its Metabolites Identified in Metabolism Studies.

Common Name/Code Chemical Name	Chemical Structure	Matrices
Mesotrione **ZA1296** 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione		Field corn forage Cow liver and kidney Hen egg yolk, subcutaneous fat, liver Rotated soybean forage and hay, and wheat forage, hay, and straw
MNBA 4-(methylsulfonyl)-2-nitrobenzoic acid		Field corn forage and stover Rotated endive, radish roots and tops, soybean forage, hay, and soybeans, and wheat forage, hay, straw, and grain
AMBA [a] 2-amino-4-(methylsulfonyl)benzoic acid		Field corn grain, forage, and stover Cow kidney and perirenal fat [b] Rotated endive, soybean forage, hay, and soybeans, and wheat forage, hay, and straw,
AMBA sulfate conjugate		Rotated wheat forage, hay, and straw, radish root

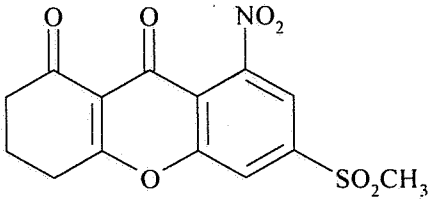
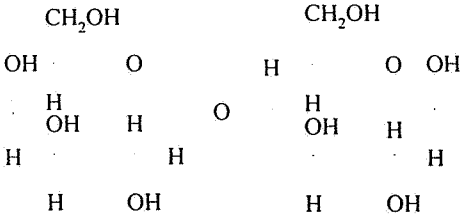
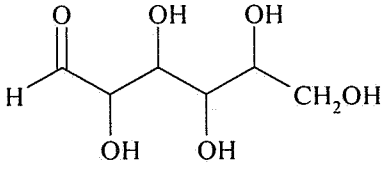
(continued; footnotes follow)

(continued).

Common Name/Code Chemical Name	**Chemical Structure**	**Matrices**
4-OGlu ZA1296 4-(β-D-glucosyloxy)-2-[4-(methylsulfonyl)-2-nitrobenzyl]-1,3-cyclohexanedione		Field corn forage Rotated soybean hay, and wheat forage, hay, and straw
4-OH ZA1296 4-hydroxy-2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione		Field corn forage and stover Rotated soybean forage, and wheat forage, hay, and straw
5-OH ZA1296		Rotated soybean forage and hay, and wheat forage, hay, and straw
EH1/EH2 hexose ester of AMBA (representative structure; other isomers possible)		Field corn forage and stover

(continued; footnotes follow)

(continued).

Common Name/Code Chemical Name	**Chemical Structure**	**Matrices**
ZA1296 tetrahydro xanthenone (XANH4) 3,4-dinitro-6-methylsulfonyl-1H-xanthene-1-9(2H)-dione		Rotated soybean forage and hay, and wheat forage, hay, and straw
Lactose		Cow milk
Palmitic/oleic acid [c]	$CH_3(CH_2)_{14}CO_2H$ or $CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2H$	Hen egg yolk
Stearic acid [c]	$CH_3(CH_2)_{16}CO2H$	Hen egg yolk
Glucose [c]		Rotated soybeans and wheat grain

[a] Includes conjugates.

[b] Identification in kidney from [^{14}C]mesotrione and [^{14}C]AMBA metabolism studies; identification in perirenal fat from [^{14}C]AMBA metabolism study only.

[c] Tentative identification.

35