DATA EVALUATION RECORD

MESOTRIONE 7/18/2002

Study Type: §85-1; Metabolism of [^{14}C-aromatic]Mesotrione in Mouse

Work Assignment No. 2-01-52LL (MRID 44537101)

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Mouse
OPPTS Number: 870.7485 OPP Guideline Number: §85-1[b]

DP BARCODE: D259369 SUBMISSION CODE: S541375
P.C. CODE: 122990 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Mesotrione (97% radiochemical purity)

SYNONYMS: ZA1296; 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione; 2-(4-mesyl-2-nitrobenzoyl)-cyclohexane-1,3-dione

CITATIONS: Gledhill, A.J. (1997) ZA1296: Excretion, tissue distribution and metabolism of a single oral dose (1 mg/kg and 100 mg/kg) in the mouse. Central Toxicology Laboratory, Cheshire, UK. Laboratory Report/Study No. CTL/R/1331/UM0573, November 13, 1997. MRID 44537101. Unpublished.

SPONSOR: Zeneca Ag Products, Wilmington, DE 19850-5458

EXECUTIVE SUMMARY: In this mouse metabolism study (MRID 44537101), [^{14}C-aromatic]mesotrione (97% radiochemical purity) was administered to CD-1:Crl(ICR)BR mice (4/sex/dose) as a single oral (gavage) dose at 1.00 or 100 mg/kg.

Absorption of [^{14}C-aromatic]mesotrione from the G.I. tract of mice was evident in low- and high-dose animals based upon the high level of urinary excretion. In both dose groups, overall renal and fecal excretion accounted for 67.27-90.94% of the dose within 24 hours of dosing, equivalent to 86-96% of the total excretion. The pattern of excretion was different for the low-dose sexes, but was similar between the high-dose sexes.

At the low-dose level, females exhibited higher total urinary excretion (1.4x) when compared to the males and males had increased total fecal excretion (1.8x) compared to the females. No further differences were noted in the low-dose sexes in levels of radioactivity recovered in the terminal cage wash, GI contents, or tissues/carcass. At the high-dose, male and female mice excreted 62.90-69.82% of the dose in the urine and 24.46-27.27% of the dose in the feces within 72 hours of administration. Radioactivity remaining in the carcass/tissues of both sexes from the high-dose group accounted for 0.28-0.41% of the dose. When compared to the low-dose mice,

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greater elimination occurred in the urine of the high-dose animals (1.2-1.5x). Additionally, radioactivity recovered in the tissues/carcass of high-dose animals was much lower (0.019-0.029x) in comparison to the low-dose animals and may indicate that bioaccumulation in the tissues may have been saturated at the high-dose level.

At 72 hours post-dose, ^{14}C-residues were highest in liver and kidney and lowest in heart, lung, muscle, bone, brain, and plasma. In low- and high-dose females, the higher concentration of radioactivity in the kidneys corresponded to the higher level of urinary excretion.

Except for the kidneys, levels of radioactivity in tissues and organs were similar between sexes 72 hours following a low-dose at 1.00 mg/kg. ^{14}C-residues levels in the kidneys were 4.3x higher in the females when compared to the males; this difference was the single sex-related variation. In the high-dose group, females had higher levels of radioactivity in the liver (1.7x), kidneys (5.7x), and fat (5.1x) when compared to males. Increasing the dose level from 1.00 to 100 mg/kg increased the concentration of radioactivity in tissues by 25.8x on average for both sexes, with the greatest increases occurring in fat (5.7-65x), carcass (11-46x), and whole blood (22-104x).

For metabolite characterization, 51-81% of the dose was identified in urine and fecal extracts. In mice from both the low- and high-dose group, free mesotrione was the major component identified in urine and feces, accounting for 49-65% of the dose in the low-dose group and 70-78% of the dose in the high-dose group. In low- and high-dose mice, minor components detected in fecal extracts included 4-(methylsulphonyl)-2-aminobenzoic acid (AMBA, 1-4% dose) and 2-nitro-4-(methylsulphonyl)-benzoic acid (MNBA, ≤2% dose). No urinary or fecal unknowns were detected which accounted for >5% of the administered dose.

When the high-dose mice were compared to the low-dose group, the metabolite profile was qualitatively similar. The only difference observed was in the low-dose group, in which the females excreted higher (1.5x) levels of parent compound in the urine than males.

In low-dose female mice, increased renal excretion of parent compound suggested a more efficient excretion route for the females. No apparent sex-related differences were noted in the rat metabolism studies submitted with the current study (MRIDs 44505101 through 44505106) regarding patterns of elimination or the metabolic profile.

This study is classified **acceptable (§85-1)** and <u>does</u> satisfy the requirement for a metabolism study.

<u>COMPLIANCE</u>: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

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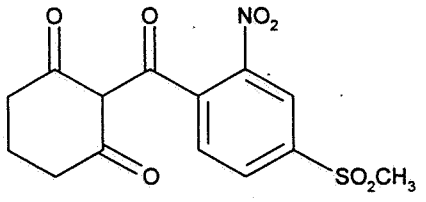
I. MATERIALS AND METHODS

A. Materials

1. Test compounds:
   [^{14}C-aromatic]Mesotrione
   Radiochemical purity: 97% (method unspecified)
   Specific activity: 1.42 GBq/mmol
   Code: Y06684/218

   Mesotrione (unlabeled)
   Purity: 99.3% a.i. (w/w)
   Code: Y06684/008
   Description: Cream solid
   Contaminants: Not specified
   Storage: Ambient temperature, in the dark
   CAS #: 104206-82-8
   Structure:



2. Vehicle: 1% sodium bicarbonate solution

3. Test animals: Species: Mouse
   Strain: CD-1:Crl(ICR)BR
   Age: Not specified
   Weight at study initiation: 18-25 g
   Source: Charles River
   Housing: During acclimation, mice were housed in groups of the same sex in stock mouse cages. During the in-life phase, animals were housed in pairs in glass metabolism cages.
   Acclimation period: At least 4 days and 1 day in metabolism cages
   Diet: Pelleted RM1 diet (Special Diet Services, Ltd, Stepfield, Wiltham, Essex, UK), ad libitum, except for 12 hours prior to dosing and approximately 2 hours following dosing
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 22 ± 3 °C
   Humidity: 30-70%
   Air Changes: At least 15/hour
   Photoperiod: 12-hr photoperiod

4. Observations: Observations were not reported.

5. Preparation of dosing solutions: For the low-dose groups, undiluted [^{14}C-aromatic] mesotrione was dissolved in sodium bicarbonate solution. The composition of the final dosing solution was 0.26 mg mesotrione/g; the specific activity of the test substance in the dosing solution was 4.19 MBq/mg of mesotrione. For the high-dose groups, [^{14}C-aromatic]mesotrione was dissolved in sodium bicarbonate solution and isotopically diluted with non-labeled mesotrione. The composition of the final dosing solution was 25.4 mg mesotrione/g and 2.1 MBq/g of dosing solution; the specific activity of the test substance in the dosing solution was 81 kBq/mg of mesotrione. The radiochemical purity of each ^{14}C-dosing solution was determined by HPLC analysis prior to and after dosing; however, the results of these analyses were not reported.

B. Study Design - These studies were designed to determine the absorption, metabolism, distribution, and excretion of [^{14}C-aromatic]mesotrione in mice as a function of a single oral dose. The study consisted of two groups of CD-1 mice (4/sex/dose group) that were dosed once with [^{14}C-aromatic]mesotrione orally (gavage) at target doses of 1.00 or 100 mg/kg/day. Animals were randomly assigned to dose groups. Actual doses for animals in each test group were within 90-110% nominal of the 1.00 mg/kg dose and 100.5-105.8% nominal of the 100 mg/kg dose; actual average doses for each test group are presented in Table 1.

Dose levels were selected based on previous metabolism studies in rats administered this compound. In these rat studies (MRIDs 44505101 through 44505106), the low-dose level (1.00 mg/kg) was stated to represent a NOAEL while the high-dose level (100 mg/kg) was stated to exceed the NOAEL by 100 fold.

The in-life portions of these studies were conducted from June 19, 1997 to November 10, 1997.

Table 1. Dose groups for [^{14}C-aromatic]mesotrione mouse metabolism study.

Dose Group	Nominal dose (mg/kg)	Actual average ^{14}C-dose (mg/kg) [a]	# animals per group	Comments
Single oral high dose	100.0	Male: 102.5 Female: 101.3	4/sex	Mass Balance Study: Urine and feces samples were collected at 6 (urine only), 12, 24, 36, 48, and 72 hours post-dose. Organ and tissue samples were collected at sacrifice (72 hours post-dose). Pooled samples of urine and feces from each dose group were used for metabolite identification and characterization.
Single oral low dose	1.00	Male: 1.03 Female: 1.00	4/sex	

a Data were obtained from Appendix B, page 50 of the study report. Calculated by reviewers.

1. Dosing - The test animals were dosed orally by gavage at a target dose of 1.00 or 100 mg/kg body weight and a target volume of 4 mL/kg body weight. Animals were fasted for 12 hours prior to dosing with [^{14}C-aromatic]mesotrione and for approximately 2 hours post-dosing. Animals were weighed prior to ^{14}C-dosing to determine dose per animal and the actual dose administered was determined by weighing the syringe and catheter assembly prior to and immediately after dosing.

2. Sampling - Urine samples were collected over solid carbon dioxide from each animal at 6, 12, 24, 36, 48, and 72 hours post-dose, and feces were collected separately at 12, 24, 36, 48, and 72 hours post-dose. At each collection, cages were rinsed with approximately 5 mL of sodium bicarbonate solution and the rinsate was added to the respective urine sample from that interval. At 72-hours post-dose, animals were sacrificed by cardiac puncture under halothane Ph Eur anesthesia and the following tissue/organ samples were collected: blood, bone (femur), brain, digestive tract and contents, fat, heart, kidneys, liver, lung, muscle, ovaries, residual carcass, spleen, and testes. An aliquot of blood was centrifuged to obtain plasma. The cages were thoroughly washed with sodium bicarbonate solution followed by 1M HCl and the rinsate was retained.

   Samples of tissues, blood, cage wash, and excreta were stored at -20°C until analysis.

3. Radioassay - Samples of urine, plasma, and cage wash were analyzed for total radioactivity directly by liquid scintillation counting (LSC). Feces were homogenized and radioassayed by LSC following combustion. Samples of tissues, organs, digestive tract, and carcass were digested in tissue solubilizer prior to radioassay by LSC. Liver and abdominal fat were homogenized prior to solubilization. Bone was cut into pieces and analyzed by combustion. Whole blood was analyzed by combustion. All samples were radioassayed in duplicate. The reported limit of detection for tissue samples at the low-dose level was <0.0005µg equivalents of mesotrione/g of tissue and at the high-dose level was <0.0257µg equivalents of mesotrione/g of tissue.

4. Metabolite characterization in excreta - For identification, characterization, and quantitation of metabolites in excreta, aliquots of urine samples collected 0-48 hours post-dose and fecal samples collected from 0-12 hours post-dose were pooled by dose group/sex. Urine samples were analyzed directly by HPLC utilizing several solvent systems. Feces samples were extracted sequentially with 1% acetic acid (4 hours) and ethanol. Sample extracts were then dried, reconstituted in a solvent suitable for injection onto an HPLC column, and then eluted with several solvent systems; radioactivity levels in the 12-48 hour samples were below the limit of quantitation of the HPLC. Extraction efficiencies ranged from 65-77%. Metabolite identities were confirmed using MS and HPLC/MS.

5. Statistics - For urine, fecal, and tissue/blood samples, radioactivity in terms of concentration (μg equivalents/g) and the % of administered dose was reported for individual samples and as the mean (with ± S.D.) of four animals/sex/dose group.

II. RESULTS

A. Absorption, excretion, and distribution - Absorption of [^{14}C-aromatic]mesotrione from the G.I. tract of mice was evident in both low- and high-dose animals based upon the high level of urinary excretion. In both dose groups, overall renal and fecal excretion accounted for 67.27-90.94% of the dose within 24 hours of dosing, equivalent to 86-96% of the total excretion.

Following oral dosing of [^{14}C-aromatic]mesotrione at 1.0 mg/kg, differences in elimination between the sexes were observed as follows: during the first 6 hours post-dose, females excreted higher levels of radioactivity in the urine (1.6x) when compared to the males; at 12 hours post-dose, males excreted higher levels of radioactivity in the feces (1.5x) when compared to the females; after 72 hours post-dose, total urinary excretion by the females was higher (1.4x) when compared to the males, while total fecal excretion by the males was higher (1.8x) when compared to the females. The pattern of excretion was different for the low-dose sexes and similar between the high-dose sexes.

a) [^{14}C-aromatic] single oral low dose: Following oral dosing with [^{14}C-aromatic] mesotrione at 1.0 mg/kg, excretion in the urine was rapid with 31.35% and 51.08% of the dose being excreted in the urine of males and females, respectively, within 6 hours of dosing (Table 2), equivalent to 77% and 87% of the total urinary excretion in males and females, respectively. Differences in elimination between the sexes were observed as follows: during the first 6 hours post-dose, females excreted higher levels of radioactivity in the urine (1.6x) when compared to the males; at 12 hours post-dose, males excreted higher levels of radioactivity in the feces (1.5x) when compared to the females; after 72 hours post-dose, total urinary excretion by the females was higher (1.4x) when compared to the males, while total fecal excretion by the males was higher (1.8x) when compared to the females. Overall excretion, in urine and feces, was essentially complete within 24 hours and accounted for 67.27% and 76.46% of the dose in males and females, respectively, equivalent to 86% and 96% of the total excretion in males and females.

Table 2. Recovery over time of radioactivity in excreta of mice following a single oral dose of [^{14}C-aromatic]mesotrione at 1.00 mg/kg.[a]

Sample	Percent of radioactive dose administered						
	Males						
	6 hr	12 hr	24 hr	36 hr	48 hr	72 hr	Total
Urine	31.352	3.073	2.554	2.231	0.859	0.564	40.634
Feces	ns	24.885	5.410	2.605	0.629	4.131	37.660
Total	31.352	27.958	7.964	4.836	1.488	4.695	78.294
Sample	Females						
	6 hr	12 hr	24 hr	36 hr	48 hr	72 hr	Total
Urine	51.083	4.399	1.737	0.673	0.435	0.278	58.605
Feces	ns	16.626	2.611	0.841	0.217	0.582	20.877
Total	51.083	21.025	4.348	1.514	0.652	0.860	79.482

a Data are the mean of four animals/sex at each sampling interval and were obtained from Tables 1 and 2, pages 37 and 38 of the study report.

ns = not sampled

b) [^{14}C-aromatic] single oral high dose: The pattern of excretion was similar for males and females. Following oral dosing of [^{14}C-aromatic]mesotrione at 100 mg/kg, excretion in the urine was rapid with 42.78-44.54% of the dose being excreted in the urine within 6 hours of dosing (Table 3), equivalent to 61-71% of the total urinary excretion. Overall excretion, in urine and feces, was essentially complete within 24 hours and accounted for 82.83-90.94% of the dose, equivalent to 92-96% of the total excretion.

Table 3. Recovery over time of radioactivity in excreta of mice following a single oral dose of [^{14}C-aromatic]mesotrione at 100 mg/kg.[a]

Sample	Percent of radioactive dose administered						
	Males						
	6 hr	12 hr	24 hr	36 hr	48 hr	72 hr	Total
Urine	44.538	13.380	1.089	2.158	1.596	0.142	62.903
Feces	ns	22.014	1.809	0.570	2.484	0.395	27.272
Total	44.538	35.394	2.898	2.728	4.080	0.537	90.175
Sample	Females						
	6 hr	12 hr	24 hr	36 hr	48 hr	72 hr	Total
Urine	42.780	22.203	3.370	0.990	0.393	0.085	69.821
Feces	ns	18.057	4.527	0.978	0.615	0.280	24.457
Total	42.780	40.260	7.897	1.968	1.008	0.365	94.278

a Data are the mean of four animals/sex at each sampling interval and were obtained from Tables 3 and 4, pages 39 and 40 of the study report.

ns = not sampled

2. Overall excretion and recovery of the administered dose - The overall recovery of dosed radioactivity in excreta, tissues, and cage washes was 90.78-95.08% from mice in both dose groups.

Excretion patterns of male and female mice after 72 hours of administering a single oral dose of [^{14}C-aromatic]mesotrione indicated minor differences between the sexes at the low-dose level. Low-dose females exhibited higher total urinary excretion (1.4x) when compared to the males and males had increased total fecal excretion (1.8x) when compared to the females (Table 4). No differences were noted in the low-dose sexes in levels of radioactivity recovered in the terminal cage wash, GI contents, or tissues/carcass. No sex-related differences in the overall pattern of excretion were noted when the dose was increased to 100 mg/kg. At the high-dose, male and female mice excreted 62.90-69.82% of the dose in the urine and 24.46-27.27% of the dose in the feces within 72 hours of administration. Radioactivity remaining in the carcass/tissues of both sexes from the high-dose group accounted for 0.28-0.41% of the dose. When compared to the low-dose mice, greater elimination occurred in the urine of the high-dose animals (1.2-1.5x). Additionally, the % of the dose recovered in the tissues/carcass of high-dose animals was much lower (0.019-0.029x) in comparison to the low-dose animals and may indicate that bioaccumulation in the tissues may have been saturated at the high-dose level.

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Table 4. Recovery of radioactivity in excreta, and tissues/carcass of mice dosed with [^{14}C-aromatic]mesotrione at 1.0 or 100 mg/kg.[a]

Dose group	Percent of radioactive dose administered			
	Single low oral dose		Single high oral dose	
Sample	Male	Female	Male	Female
Urine	40.634	58.605	62.902	69.821
Feces	37.660	20.879	27.272	24.457
Cage Wash	0.622	0.898	0.310	0.374
GI Tract Contents at Termination	0.096	0.052	0.018	0.020
Tissues/Carcass [b]	14.785	14.138	0.277	0.410
Total	93.797	94.572	90.779	95.082

a Data are the mean of four animals/sex at each sampling interval and were obtained from Tables 1 through 4 pages 37 through 40 and Tables 5 through 8, pages 41 through 44.
b Includes radioactivity associated with the digestive tract, excised organs and tissue, and the carcass.

3. Tissue distribution - The concentration of radioactivity in tissues and organs of mice in the two dose groups at 72 hours following ^{14}C-dosing are presented in Table 5. ^{14}C-Residues were highest in liver and kidney and lowest in heart, lung, muscle, bone, brain, and plasma. In the low- and high-dose females, an increased concentration of radioactivity was observed in the kidneys and corresponded to the higher level of urinary excretion.

Except for the kidneys, levels of radioactivity in tissues and organs were similar between sexes 72 hours following a low-dose at 1.00 mg/kg. ^{14}C-residues levels in the kidneys were 4.3x higher in the females when compared to the males; this difference was the single sex-related variation.

In the high-dose group, females had higher levels of radioactivity in the liver (1.7x), kidneys (5.7x), and fat (5.1x) when compared to the males.

Increasing the dose level from 1.00 to 100 mg/kg increased the concentration of radioactivity in tissues by 25.8x on average for both sexes, with the greatest increases occurring in fat (5.7-64.5x), carcass (10.5-45.8x), and whole blood (22-104x).

a) [^{14}C-aromatic] single oral low-dose: Except for the kidneys, levels of radioactivity in tissues and organs were similar between sexes 72 hours following a low-dose at 1.00 mg/kg. For both sexes, ^{14}C-residues were highest in liver (male, 2.84 µg/g; female, 2.61 µg/g) and kidneys (male, 0.19 µg/g; female, 0.80 µg/g). Levels in the kidneys were 4.3x higher in the females when compared to the males; this difference was the

single sex-related variation. Tissues with the lowest ^{14}C-residues (≤0.002 µg/g) were muscle, bone, brain, and plasma.

b) [^{14}C-aromatic] single oral high-dose: Compared to males, the females had higher levels of radioactivity in the liver (1.7x), kidneys (5.7x), and fat (5.1x). For both sexes, ^{14}C-residues were highest in liver (male, 2.86 µg/g; female, 4.97 µg/g) and kidneys (male, 0.21 µg/g; female, 1.21 µg/g). Tissues with the lowest ^{14}C-residues (≤0.042 µg/g) were heart, lung, muscle, brain, gonads, and plasma.

Table 5. Distribution of radioactivity in blood, tissues, and organs of mice sacrificed at various intervals following a single oral dose of [^{14}C]mesotrione at 1 or 100 mg/kg. [a]

Dose group	Concentration of radioactivity (µg equivalents/g)			
	[^{14}C] mesotrione			
	Single oral low dose [b]		Single oral high dose [c]	
Tissue/organ	Male	Female	Male	Female
Heart	0.002	0.006	<0.040	<LOD
Lung	0.005	0.003	0.031	<0.042
Liver	2.843	2.614	2.862	4.974
Spleen	0.005	<0.008	<LOD	<LOD
Kidney	0.187	0.798	0.210	1.205
Muscle	0.002	0.001	0.029	<0.026
Bone	0.002	<0.002	<0.086	<LOD
Brain	<0.001	<0.001	<LOD	<LOD
Fat	0.009	0.004	0.051	0.258
Digestive tract	0.005	0.005	0.192	0.202
Testicles	0.006	na	<0.027	na
Ovaries/Uterus	na	<0.018	na	<LOD
Carcass	0.013	0.004	0.136	0.183
Whole blood	0.021	0.006	0.463	0.624
Plasma	<0.002	<0.001	<0.032	0.041

a Data were obtained from Tables 5 through 8, pages 41 through 44. Data are the average of 4 animals/ sex/dose group.
b LOD <0.0005µg equiv/g.
c LOD <0.0257µg equiv/g.
na not applicable

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B. Metabolite Characterization

1. Metabolites in excreta - Quantitative HPLC/MS analyses isolated up to 4 distinct components in urine and 3 distinct components in fecal extracts. Results from the analyses of urine and fecal extracts from the low- and high-dose groups treated with [^{14}C-aromatic]mesotrione are summarized in Table 6. The proposed pathway for biotransformation of mesotrione in mice (as obtained from page 36 of the MRID) is presented in Appendix 1.

   A total of 55-81% was identified in urine and fecal extracts. Although there were minor differences in levels of metabolites between males and females and between dose groups, the metabolite profile was qualitatively similar between the sexes in each group and between the low-dose and high-dose groups. In mice from each of these groups, free mesotrione was the major metabolite identified in urine and feces, accounting for 49-65% of the dose in low-dose group and 70-78% of the dose in the high-dose. No urinary or fecal unknowns were detected which accounted for >5% of the administered dose.

   In female mice, renal excretion of parent compound was increased and indicated a more efficient excretion route for the females. No apparent sex-related differences were noted in the rat metabolism studies submitted with the current study (MRIDs 44505101 through 44505106) regarding patterns of elimination or the metabolic profile.

Table 6. Metabolite profile in excreta of mice following dosing with [^{14}C-aromatic]mesotrione at 1.00 or 100 mg/kg.

Dose Group	Percent of administered dose			
	Single oral low dose		Single oral high dose	
Metabolite/fraction	Male	Female	Male	Female
Identified Urinary Metabolites [a]				
Mesotrione	39	58	61	70
AMBA	T	--	1	--
Hydroxy mesotrione	--	--	1	T
MNBA	T	--	T	--
Identified Fecal Metabolites [a]				
Mesotrione	10	7	9	8
AMBA	4	2	1	2
MNBA	2	T	1	1
Total identified Metabolites	**55**	**67**	**74**	**81**
Urinary Unknowns [a]				
Unknown I	1	--	1	1
Unknown II	T	--	T	--
Unknown III	T	--	--	--
Fecal Unknowns [a]				
Unknown I	1	T	1	1
Unknown II	1	--	--	--
Unknown III	--	T	T	T
Unknown IV	T	T	T	--
Unknown V	T	T	T	--
Unknown VI	T	1	--	T
Unknown VII	T	T	2	2
Unknown VIII	--	T	--	--
Unknown IX	--	T	--	--
Total Isolated Unknowns	**3**	**1**	**4**	**4**
Unanalyzed Fractions				
Urine (48-72 hr) [b]	0.564	0.278	0.142	0.085
Residual fecal solids (0-12 hr) [c]	6.221	4.655	7.704	4.153
Feces (12-72 hr) [b]	12.775	4.253	5.258	6.400
Cage wash [b]	0.622	0.898	0.310	0.374
Tissues/carcass [b]	14.881	14.190	0.295	0.230
Total accounted for [d]	**93.063**	**92.274**	**91.709**	**96.242**

a Data are from separate HPLC/MS analyses of pooled urine (0-48 hour) and fecal (0-12 hour) extracts from 4 mice/sex/dose group and were obtained from Tables 9-12, pages 45-48 of study report.
b Data were obtained from Tables 2 through 4 of this review, including GI tract contents.
c Data represent the unextracted radioactivity from pooled 0-12 hour fecal samples and were calculated by reviewers using data obtained from Tables 2 and 3 of this review and pages 26 and 27 of study report.
d Total accounted for = (Total identified) + (Total isolated unknowns) + (Total unanalyzed).
T Noted by sponsor as trace amount accounting for <0.5% of administered dose
-- Not detected

a) [^{14}C-aromatic] single oral low dose: A total of 3 components were isolated from urine of males and females. For both sexes, the largest single fraction identified in urine was the parent compound that accounted for 39-58% dose. Other trace (defined by sponsor as accounting for <0.5% dose) components detected in urine of males only included MNBA and AMBA.

Parent compound was also the major components in feces, with free mesotrione accounting for 7-10% of the dose. Both MNBA (≤2% dose) and AMBA (2-4% dose) were also detected in feces.

A total of 55-67% of the dose was identified in excreta of low-dose mice, with the major component being parent compound. The metabolite profile was qualitatively the same between the sexes; females excreted higher (1.5x) levels of parent compound in the urine. Fecal and urinary unknowns in both sexes accounted for 1-3% of the dose.

b) [^{14}C-aromatic] single oral high dose: A total of 4 components were isolated from urine of males and females. For both sexes, the major component identified in urine was parent mesotrione, accounting for 61-70% of the dose.

Parent compound was also the major component in feces and accounted for 8-9% of the dose. Minor components detected in fecal extracts included AMBA (1-2% dose) and MNBA (1% dose).

A total of 74-81% of the dose was identified in excreta of high-dose mice, with the major component being parent compound. The metabolite profile was quantitatively and qualitatively the same between the sexes. Fecal and urinary unknowns in both sexes accounted for 4% of the dose.

Compared to the low-dose group, the metabolite profile was qualitatively the same in high-dose mice. The only difference was observed in the low-dose group, in which the females excreted higher (1.5x) levels of parent compound in the urine than males. The excretion patterns of the low-dose females and the high-dose males and females were similar.

III. DISCUSSION

A. Investigator's Conclusions - The study author concluded that following administration of a single oral dose of 1.00 or 100 mg/kg of [^{14}C-aromatic]mesotrione, absorption was similar at both dose levels and in both sexes. Excretion was rapid and predominantly in the urine. For the low-dose animals, there was an apparent sex-related difference, with males excreting less (40.6%) of the dose in urine than females (58.6%) and excreting more in feces (males, 37.7%; females 20.9%); however, fecal excretion values for males had a large standard

deviation and may be indicative of an anomalous result for a single animal. For the high-dose mice, the sex difference was much less pronounced.

The highest tissue concentrations were observed in the liver and kidneys. For the low-dose, a sex-related difference was observed in the levels of radioactivity in the kidneys (males, 0.19 µg equiv/g; females, 0.80 µg equiv/g). For the high-dose mice, liver and kidney concentrations were only slightly higher than the low-dose tissues and a similar sex-related difference was observed in the radioactivity levels in the kidney (males, 0.2 µg equiv/g; females, 1.2 µg equiv/g).

The molecule was not extensively metabolized by the mouse, since most of the absorbed dose was excreted as parent compound in the urine and feces. Urine was the primary route of elimination for both dose groups with the parent compound accounting for approximately ≥94% of urinary radioactivity and equivalent to 30-70% of the administered dose.

B. Reviewer's Discussion - Dosed radioactivity was quantitatively recovered from each dose group, with 90.78-95.08% of the dosed radioactivity being recovered in urine, feces, cage washes, and tissues within 72 hours of dosing.

Absorption of [^{14}C-aromatic]mesotrione from the G.I: tract of mice was evident in both low- and high-dose animals based upon the high level of urinary excretion. In mice dosed orally with [^{14}C-aromatic]mesotrione at either 1.00 or 100 mg/kg, the pattern of excretion was different for the low-dose sexes and similar between the high-dose sexes. In both dose groups, overall renal and fecal excretion accounted for 67.27-90.94% of the dose within 24 hours of dosing, equivalent to 86-96% of the total excretion.

Following oral dosing of [^{14}C-aromatic]mesotrione at 1.0 mg/kg, differences in elimination between the sexes were observed as follows: during the first 6 hours post-dose, females excreted higher levels of radioactivity in the urine (1.6x) when compared to the males; at 12 hours post-dose, males excreted higher levels of radioactivity in the feces (1.5x) when compared to the females and after 72 hours post-dose total urinary excretion by the females was higher (1.4x) when compared to the males, while total fecal excretion by the males was higher (1.8x) when compared to the females.

No differences were noted in the low-dose sexes in levels of radioactivity recovered in the terminal cage wash, GI contents, or tissues/carcass. No sex-related differences in the overall pattern of excretion were noted when the dose was increased to 100 mg/kg. At the high-dose, male and female mice excreted 62.90-69.82% of the dose in the urine and 24.46-27.27% of the dose in the feces within 72 hours of administration. Radioactivity remaining in the carcass/tissues of both sexes from the high-dose group accounted for 0.28-0.41% of the dose. When compared to the low-dose mice, greater elimination occurred in the urine of the high-dose animals (1.2-1.5x). Additionally, the % of the dose recovered in the tissues/carcass of high-dose animals was much lower (0.019-0.029x) in comparison to the low-dose animals and may indicate that bioaccumulation in the tissues may have been saturated at the high-dose level.

Regarding tissue distribution, ^{14}C-residues were highest in liver and kidney and lowest in heart, lung, muscle, bone, brain, and plasma. In the low- and high-dose females, an increased concentration of radioactivity was observed in the kidneys and corresponded to the higher level of urinary excretion.

Except for the kidneys, levels of radioactivity in tissues and organs were similar between sexes 72 hours following a low-dose at 1.00 mg/kg. ^{14}C-residues levels in the kidneys were 4.3x higher in the females when compared to the males; this difference was the single sex-related variation. Following a high-dose at 100 mg/kg, higher levels of radioactivity were noted in the liver (1.7x), kidneys (5.7x), and fat (5.1x) in females when compared to males. Increasing the dose level from 1.00 to 100 mg/kg increased the concentration of radioactivity in tissues by 25.8x on average for both sexes, with the greatest increases occurring in fat (5.7-65x), carcass (11-46x), and whole blood (22-104x).

For metabolite characterization, up to 55-81% of the dose was identified in urine and fecal extracts. In mice from both the low- and high-dose group, free mesotrione was the major metabolite identified in urine and feces, accounting for 49-65% of the dose in the low-dose group and 70-78% of the dose in the high-dose group. In low- and high-dose mice, minor components detected in fecal extracts included AMBA (1-4% dose) and MNBA (≤2% dose). No urinary or fecal unknowns were detected which accounted for >5% of the administered dose.

When the high-dose mice were compared to the low-dose group, the metabolite profile was qualitatively similar. The only difference observed was in the low-dose group, in which the females excreted higher (1.5x) levels of parent compound in the urine than males.

In low-dose female mice, increased renal excretion of parent compound indicated a more efficient excretion route for the females. No apparent sex-related differences were noted in the rat metabolism studies submitted with the current study (MRIDs 44505101 through 44505106) regarding patterns of elimination or the metabolic profile.

This is study is classified **acceptable (§85-1)** and <u>does</u> satisfy the guideline requirements for a metabolism study.

C. Study deficiencies - No deficiencies were noted.

Appendix 1

FIGURE 7 - PROPOSED BIOTRANSFORMATION PATHWAY FOR ZA1296 IN THE MOUSE