DATA EVALUATION RECORD

Mesotrione (ZA1296) 8/19/2000

Study Type: Non-Guideline, Supplemental to 230 Series, Systemic Exposure Following a Single Dermal Application to Human Subjects

Work Assignment No. 2-01-52OO (MRID 44920803)

Prepared for
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U.S. Environmental Protection Agency
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Disclaimer

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DATA EVALUATION RECORD

STUDY TYPE: Non-guideline
OPPTS Number: N/A

OPP Guideline Number: N/A

DP BARCODE: D259369
P.C. CODE: 122990

SUBMISSION CODE: S541375
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Mesotrione (ZA 1296) (9.1-39.8% a.i.)

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

CITATION: Hall, M.G. (1998) ZA1296: Investigation of Systemic Exposure Following a Single Dermal Application of Spray Formulations to Healthy Male Volunteers. Central Toxicology Laboratory, Cheshire, UK. Laboratory Project Identification Number QH0020. July 24, 1998. MRID 44920803. Unpublished.

SPONSOR: Zeneca Ag Products, Wilmington, Delaware

EXECUTIVE SUMMARY: In this special study (MRID 44920803), ZA 1296 (mesotrione (ZA1296; Lot/Batch # WF2515, 9.1% a.i. and WF2381, 39.8% a.i.) was administered as a single dermal application to 18 volunteer human males. The volunteers were assigned to one of 3 study phases (6 volunteers/phase) and received nominal doses of 4mg (5ug/cm^2 - WF2515), 4mg (5ug/cm^2 - WF2381), or 25.6mg (32ug/cm^2 - WF2381) mesotrione. The application sites wree washed after 10 hours. The volunteers were monitored for 5 days post-dosing, and then participated in post-study medical examinations 4-7 days after discharge. The objective of this study was to determine the urine and plasma concentrations of mesotrione following dermal application, and to define the dose-response relationship for increased plasma tyrosine. The study was conducted in accordance with the Declaration of Helsinki (1964) including all amendments up to and including the South Africa revision (1996).

No treatment-related changes in vital signs, ophthalmoscopic observations, or hematology, clinical chemistry, or urinalysis parameters were observed. No quantifiable concentrations of mesotrione were detected in the plasma or in urine samples from phases 1 and 2. Detectable levels occurred in urine at 2-5/13 timepoints in phase 3 and ranged from 5-9 ng/ml vs. a detection limit of 5-6 ng/ml. Comparison of tyrosine post-dose area under the curve (AUC) and Cp_{max}

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values with pre-test values indicated that dermal application of mesotrione had no discernable effect on plasma tyrosine concentration.

The number of volunteers reporting mild, transient itching was increased in phase 3 (4 volunteers at 3 timepoints) compared to phases 1 and 2 (1 volunteer at 1 timepoint. each). Other symptoms included reports of a burning sensation 30 minutes after dose application (1 volunteer in phase 1) and a stinging sensation 9 hours after dose application (1 volunteer in phase 2): these symptoms were also mild and transient in nature. All volunteers in phase 1 (14 timepoints). 1 volunteer in phase 2 (2 timepoints), and 6 volunteers in phase 3 (10 timepoints) experienced erythema.

Results of the tape strip analyses indicated that the percentage of the applied dose remaining at the application site decreased between the 10-hour (35-54.7% remaining) and 24-hour (10.3-20.0% remaining) timepoints in all phases of the study. Analysis of the swabs from each phase of the study indicated that a large proportion of the applied dose was removed from the application site during the washing procedure (36.8-49.6%). T-shirt analysis demonstrated that additional test-substance was transferred from the application site to the t-shirts between 10 and 24 hours post-dosing for each phase (8.65-29.7%). The tape strip results could not be analized as the majority were below the limit of detection.

Mesotrione appeared to remain in the stratum corneum and was easily removed by washing or contact with clothing.

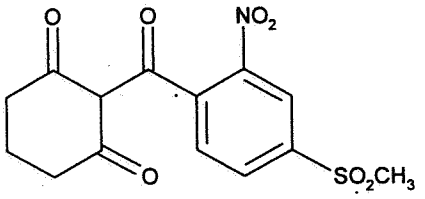
The submitted study is classified as **acceptable/non-guideline**.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test materials: Mesotrione (ZA1296)
   Description: Tan liquid, light beige opaque liquid
   Lot/Batch #: WF2515, WF2381
   Purity (w/w): WF2515: 9.1% a.i.; WF2381: 39.8% a.i.
   Stability of compound: Stable up to at least 10 days at 4°C
   CAS #: 104206-82-8
   Structure:



First formulation

Name	ZA 1290 100g/l SC formulation (WF2515)
Source	Zeneca Ag Products
Color	Tan
Physical state	Liquid
Batch reference number	16214-25-4
CTL test substance reference number	Y06684/221
ZA1296 content	9.1%w/w
Storage conditions	In darkness at less than 40°C
Expiry Date	26th October 1999

Second formulation

Name	ZA1296 480g/l SC Formulation (WF2381)
Source	Zeneca Ag Products
Color	Light beige opaque
Physical state	Liquid
Batch reference number	16565-9-2
CTL test substance reference number	Y06684/220
ZA1296 content	39.8% w/w
Storage conditions	In darkness at less than 25°C
Expiry Date	26th October 1999

Wetting agent

Name	Agri-Dex
Source	Zeneca Ag Products
Color	Amber
Physical state	Liquid
Batch reference number	16565-12-1
CTL test substance reference number	Y09893/001
Purity	Assumed 100% (not certified)
Storage conditions	In darkness at room temperature
Expiry Date	30th April 2000

Certificates of analysis (reference numbers 16491-15 and 16374-34) are retained in the CTL archives

2. Vehicle: Agri-Dex (wetting agent); added to dose preparations for phases 2 and 3.

3. Test subjects: Human male volunteers

Age and weight at the start of dosing: Between 18-55 years (inclusive): 60 to 90 kg (inclusive)
Source: Inveresk Clinical Research (ICR) Volunteer Panel
Testing Facility: ICR, Edinburgh, Scotland
Diet: Identical standard meals at comparable time points in order to minimize variability in plasma tyrosine concentrations. All beverages were decaffeinated from midnight before each dosing day until 24 hours post-dosing.
Acclimation period: 2 days

B. STUDY DESIGN

1. Purpose - The purpose of this study was to determine the urine and plasma concentrations of mesotrione following dermal application, and to define the dose-response relationship for increased plasma tyrosine.

2. In life dates - start: 11/21/97 end: 12/10/97

3. Subject assignment - The volunteers were assigned to the test groups shown in Table 1.

Table 1. Study design [a]

Phase	Test substance[b]	Nominal Dose (mg)[c]	Dose per unit area (µg/cm²)	Males Assigned
1	WF2515	4	5	6
2	WF2381	4	5	6
3	WF2381	25.6	32	6

a Data obtained from the study report, page 28.
b The test substance for phase 1 contained a wetting agent however, the test substance for phase 2 and 3 required the addition of a wetting agent (Agri-Dex).
c It was stated that achieved concentrations were approximately 86% of the nominal values.

4. Dose selection rationale - Dose levels were selected based on field strength dilutions of the test substance, the maximum practical dermal application area (800 cm²), the

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maximum practical application volume (4 ml), and a previous clinical oral study (Hall 1998).

5. Dosing preparation and analysis - The test substances were applied as aqueous dilutions of concentrated spray formulations. Dose preparations were prepared on the day of application. The stability of the test substances was established prior to study initiation by analyzing a 1:100 dilution of WF2515 and a 1:75 dilution of WF2381 after storage at 4°C for up to 10 and 20 days, respectively.

   Results - Stability analysis (range as % of day 0): 97.0-100%

6. Dose administration - The dosing preparation was mixed immediately before application and spread evenly over the test area (a 800 cm^2 grid on the back of each volunteer). The volunteers remained lying face down for approximately 5 minutes following application, and then remained shirtless while engaging in sedentary activities. Ten hours after dosing, the test area was swabbed with distilled water and allowed to dry for approximately 20 minutes. A 6 cm^2 area of the application site was then tape-stripped using low tack tape in order to remove layers of the stratum corneum. The volunteers wore cotton t-shirts continuously until 24 hours post-dosing. The t-shirts were assayed for test -substance residues. Tape-stripping was repeated 24 hours after dosing and again after showering (25 hours post-dosing).

7. Statistics - Data were summarized and presented in tabular format. Area under the curve (AUC) values for tyrosine were subjected to a paired Student's t-test and/or analysis of variance.

C. METHODS

1. Observations - All volunteers were subjected to pre- and post-study medical examinations, which included measurement of heart rate, blood pressure, resting ECG, and ophthalmoscopy. Oral temperature, pulse, and blood pressure were recorded pre-dose and at 2, 6, 12, 23, 96, and 120 hours post-dosing. The dose area was examined for signs of local reaction at 0.5, 1, 2, 6, 9, 23, and 48 hours post-dosing. Symptoms of local tolerability at the application site were determined by questioning at the time of examination.

2. Blood - Blood was collected from all volunteers at the pre-study and post-study medical examinations. The checked (X) hematology and clinical blood chemistry parameters were examined. Additionally, blood was collected at 7 pre-dosing time points and 17 post-dosing time points for plasma tyrosine and mesotrione determination.

a. Hematology

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count		Reticulocyte count
	Blood clotting measurements (Thromboplastin time)		
X	(Activated partial thromboplastin time) (Clotting time)		
X	(Prothrombin time)		

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorus	X	Total cholesterol
X	Potassium		Globulin
X	Sodium	X	Glucose
			Direct bilirubin
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum protein (TP)
	Cholinesterase (ChE)	X	Triglycerides
X	Creatine phosphokinase	X	Uric acid
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (ALT)		
X	Serum aspartate aminotransferase (AST)		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

7. Urinalysis - Urine was collected during the pre-study and post-study medical examinations. The checked (X) parameters were examined. In addition, urine was collected for creatinine and mesotrione analysis at 12-hour intervals for 48 hours prior to dosing and then at 2-hour intervals for the first 4 hours post-dosing, at 4-hour intervals for 4-12 hours post-dosing, and 12-hour intervals for the remainder of the study.

	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen

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II. RESULTS

A. Observations

1. Symptomology - The number of volunteers reporting mild, transient itching was increased in phase 3 (4 volunteers at 3 timepoints) compared to phases 1 and 2 (1 volunteer at 1 timepoint, each). Other symptoms included reports of a burning sensation 30 minutes after dose application (1 volunteer in phase 1) and a stinging sensation 9 hours after dose application (1 volunteer in phase 2); these symptoms were also mild and transient in nature. All volunteers in phase 1 (14 timepoints), 1 volunteer in phase 2 (2 timepoints), and 6 volunteers in phase 3 (10 timepoints) experienced erythema. No edema or induration was noted during the study.

2. Vital signs - No treatment-related changes in vital signs, physical examinations, pulmonary function tests, or electrocardiography were observed during the study.

3. Ophthalmoscopy - No treatment-related changes were observed between pre- and post-study ophthalmoscopic examinations.

B. Blood analyses

1. Hematology - No treatment-related differences in hematology parameters were observed between the pre- and post-study measurements.

2. Clinical chemistry - No treatment-related differences in clinical chemistry parameters were observed between the pre- and post-study measurements.

3. Plasma tyrosine concentration - Post-dose tyrosine area under the curve (AUC) values were slightly increased relative to pre-dose values in phase 1 volunteers (24 hours: ↑10%, $p \leq 0.01$; 48 hours: ↑7%, not statistically significant). When comparing post-dose tyrosine AUC values among treated groups, phase 2 volunteers had lower values (↓13-20%, $p \leq 0.05$) than phase 1 or phase 3 volunteers over 24 hours, and lower values (↓9%, $p \leq 0.05$) than phase 1 values over 48 hours. Tyrosine $C_{p_{max}}$ appeared to be unaffected by the test substance. Plasma tyrosine data are presented as an attachment (study report Tables 6-8, pages 60-62)

4. Plasma mesotrione concentration - No quantifiable concentrations of mesotrione were detected.

C. Urinalysis - No treatment-related differences in urinalysis parameters were observed between the pre- and post-study measurements.

1. Creatinine excretion - Comparison of daily creatinine excretion for each volunteer indicated consistent total urinary output. Incomplete urine collection was evidenced in only 1 volunteer.

2. Mesotrione excretion - No quantifiable concentrations of mesotrione were detected in urine samples from phases 1 and 2. Detectable levels occurred at 2-5/13 timepoints in phase 3 and ranged from 5-9 ng/ml vs. a detection limit of 5-6 ng/ml. Mesotrione excretion data are presented as an attachment (study report Table 11, page 71).

D. Other analyses

1. Tape strips - The tape strip results could not be analyzed as the majority were below the limit of detection. Results of the tape strip analyses indicated that the percentage of the applied dose remaining at the application site decreased between the 10-hour (35-54.7% remaining) and 24-hour (10.3-20.0% remaining) timepoints in all phases of the study. It was stated that these decreases were statistically significant; however, the level of statistical significance was not indicated in the data. Tape strip analysis data are presented as an attachment (study report Table 19, page 94).

2. Swabs and T-shirts - Analysis of the swabs from each phase of the study indicated that a large proportion of the applied dose was removed from the application site during the washing procedure (36.8-49.6%). T-shirt analysis demonstrated that additional test-substance was transferred from the application site to the t-shirts between 10 and 24 hours post-dosing for each phase (8.65-29.7%). Swab and t-shirt analysis data are presented as an attachment (study report Table 20, page 95).

III. DISCUSSION

A. Investigator's conclusions - No adverse effects were observed in human males receiving field-strength dermal applications of mesotrione. Mesotrione appeared to have no effect on plasma tyrosine concentration. There were no quantifiable concentrations of mesotrione in the urine samples from phase 1 and 2 volunteers; only low concentrations of mesotrione were detected in some phase 3 samples. There were no quantifiable concentrations of mesotrione detected in the plasma during any phase of the study. Analysis of the tape strips, swabs, and t-shirts indicated that the test substance was easily removed by washing or contact with clothing, and that mesotrione residues were minimal after 25 hours post-dosing.

B. Reviewer's discussion - In this special study, mesotrione was administered as a single dermal application to 18 volunteer human males. The volunteers were assigned to one of 3 study phases (6 volunteers/phase) and received nominal doses of 4 (WF2515), 4 (WF2381), or 25.6 (WF2381) mg mesotrione. The volunteers were monitored for 5 days post-dosing, and then participated in post-study medical examinations 4-7 days after discharge. The objective of this study was to determine the urine and plasma concentrations of mesotrione following

dermal application, and to define the dose-response relationship for increased plasma tyrosine.

No treatment-related changes in vital signs, ophthalmoscopic observations, or hematology, clinical chemistry, or urinalysis parameters were observed. No quantifiable concentrations of mesotrione were detected in the plasma or in urine samples from phases 1 and 2. Detectable levels occurred in urine at 2-5/13 timepoints in phase 3 and ranged from 5-9 ng/ml vs. a detection limit of 5-6 ng/ml.

The number of volunteers reporting mild, transient itching was increased in phase 3 (4 volunteers at 3 timepoints) compared to phases 1 and 2 (1 volunteer at 1 timepoint, each). Other symptoms included reports of a burning sensation 30 minutes after dose application (1 volunteer in phase 1) and a stinging sensation 9 hours after dose application (1 volunteer in phase 2); these symptoms were also mild and transient in nature. All volunteers in phase 1 (14 timepoints), 1 volunteer in phase 2 (2 timepoints), and 6 volunteers in phase 3 (10 timepoints) experienced erythema.

Post-dose tyrosine area under the curve (AUC) values were slightly increased relative to pre-dose values in phase 1 volunteers (24 hours: ↑10%, $p \leq 0.01$; 48 hours: ↑7%, not statistically significant). When comparing post-dose tyrosine AUC values among treated groups, phase 2 volunteers had lower values (↓13-20%, $p \leq 0.05$) than phase 1 or phase 3 volunteers over 24 hours, and lower values (↓9%, $p \leq 0.05$) than phase 1 values over 48 hours. Tyrosine Cp_{max} appeared to be unaffected by the test substance.

Analysis of the swabs from each phase of the study indicated that a large proportion of the applied dose was removed from the application site during the washing procedure (36.8-49.6%). T-shirt analysis demonstrated that additional test-substance was transferred from the application site to the t-shirts between 10 and 24 hours post-dosing for each phase (8.65-29.7%). The tape strip results could not be analyzed as the majority were below the limit of detection and the limit was unacceptably high.

Mesotrione appeared to remain in the stratum corneum and was easily removed by washing or contact with clothing. The data presented demonstrated that dermally applied mesotrione was not absorbed.

The submitted study is classified as **acceptable/non-guideline**.

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