DATA EVALUATION RECORD

5/9/2000

MESOTRIONE (ZA 1296)

Study Type: §84-2; Micronucleus Assay in Mice

Work Assignment No. 2-1-52 JJ (MRID 44373527)

Prepared for

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DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in mice
OPPTS NUMBER: 870.5395 OPP Guideline Number: §84-2

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

TEST MATERIAL (PURITY): ZA 1296 (mesotrione, 98.1% a.i.)

SYNONYMS: 2-[4-(Methylsulphonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione

CITATION: Griffiths, K. and Mackay, J.M. (1994). ZA 1296: An Evaluation in the Mouse Micronucleus Test. Zeneca Central Toxicology Laboratory (CTL), Alderley Park Macclesfield, Cheshire, UK. Laboratory Project ID. CTL/P/4249. February 11, 1994. MRID 44373527. Unpublished.

SPONSOR: Zeneca Ag Products, Zeneca Inc., Wilmington, DE

EXECUTIVE SUMMARY:

In a bone marrow micronucleus assay (MRID 44373527), groups of 5 CD-1 mice/sex were treated by gavage with ZA 1296 (mesotrione, 98.1% a.i.) in deionized water at a single dose of 500 mg/kg. Five mice/sex received the vehicle alone. Mice were observed for mortality and clinical signs of toxicity at 24 and 48 hours post-dosing. Five mice/sex each from the vehicle and ZA 1296-treated groups were sacrificed at 24 or 48 hours after treatment; bone marrow cells were harvested at each sacrifice time and scored for micronucleated polychromatic erythrocytes (MPCEs). Five mice per sex received a single gavage dose of cyclophosphamide (65 mg/kg) as the positive control and bone marrow was harvested at 24 hours post-treatment.

The selected dose, 500 mg/kg, was based on a preliminary toxicity test at 320-2000 mg/kg that showed deaths occurring at ≥800 mg/kg. In the micronucleus test, one ZA 1296-treated male was found dead 4 hours after treatment. ZA 1296 was not toxic to the bone marrow (PCE:NCE ratio), and **ZA 1296 gave a negative response for the induction of micronucleated polychromatic erythrocytes in bone marrow at both sampling times.** The sensitivity of this test to detect a genotoxic response was demonstrated by the significant ($p<0.01$) increase in MPCEs induced by the positive control (cyclophosphamide).

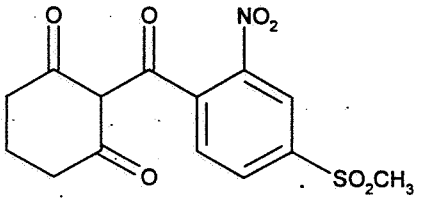
This study is classified as **acceptable (§84-2)** and does satisfy the FIFRA Test Guideline requirements for an *in vivo* cytogenetic mutagenicity test.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: ZA 1296 (mesotrione)
   Description: Light beige solid
   Lot/Batch #: P6
   Purity: 98.1% a.i.
   Stability of compound: Not specified. It was stated that the test material was used within the stated expiration date, based on information from the Sponsor. Stored at room temperature in the dark.
   CAS #: 104206-82-8
   Structure (Not present in study report; presumably copied from independent sources).



   Vehicle used: Sterile double deionized water
   Other comments: ZA 1296 formed a suspension in the vehicle.

2. Control Materials:
   Vehicle/Final volume/Route of administration: Sterilized double deionized water/20 mL per kg body weight/gavage

   Positive/Final dose(s)/Route of administration: Cyclophosphamide in physiological saline/65 mg per kg/gavage

3. Test compound administration:
   Preliminary toxicity test:
   Volume of test substance administered: 20 mL/kg body weight
   Route of administration: Gavage

   Micronucleus test:
   Volume of test substance administered: 20 mL/kg body weight
   Route of administration: Gavage

   Dose levels used:
   Preliminary study: 320, 500, 800, and 2000 mg/kg
   Micronucleus test: 500 mg/kg

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4. Test animals:
   a. Species: Mouse Strain: CD-1 Age: 4-12 weeks old (4-11 weeks, preliminary toxicity test; 5-12 weeks, micronucleus test)
   Weight: Males 30.0-38.8 g Females 22.1-28.5 g (micronucleus test)
   Source: Charles River Breeding Laboratories, Margate, UK

   b. No. animals used per dose:
   Preliminary toxicity test: 2 males 0 females at 320 and 2000 mg/kg; 5 males 5 females at 500 and 800 mg/kg
   Micronucleus test: 5 males 5 females
   Negative control: 5 males 5 females
   Positive control: 5 males 5 females

   c. Properly maintained? **Yes**

B. TEST PERFORMANCE

1. Treatment and Sampling Times:
   a. Test compound
   Dosing: x once ___ twice (24 hr apart) ___ other (describe)

   Sampling (after last dose): ___ 6 hr ___ 12 hr x 24 hr x 48 hr ___ 72 hr (mark all that are appropriate), ___ other (describe):

   b. Negative and/or vehicle control
   Dosing: x once ___ twice (24 hr apart) ___ other (describe)

   Sampling (after last dose): ___ 6 hr ___ 12 hr x 24 hr x 48 hr ___ 72 hr (mark all that are appropriate), ___ other (describe):

   c. Positive control
   Dosing: x once ___ twice (24 hr apart) ___ other (describe)

   Sampling (after last dose): ___ 6 hr ___ 12 hr x 24 hr ___ 48 hr ___ 72 hr (mark all that are appropriate), ___ other (describe):

2. Tissues and Cells Examined:

   x bone marrow ___ other (list):

   No. of polychromatic erythrocytes (PCE) examined per animal:

~~1120~~ 5

1,000 ; an additional 2,000 PCEs were examined in each treated and control female sacrificed at 48 hours
No. of normochromatic erythrocytes (NCEs; more mature RBCs) examined per animal: 1,000 total erythrocytes were counted, and the PCE:NCE ratio was calculated.
Other (if other cell types examined, describe):

3. Details of slide preparation:
At 24 and 48 hours after dosing, animals (5/sex) from the negative control and ZA 1296-treated groups were euthanized by asphyxiation in halothane or in a rising CO_2 concentration followed by cervical dislocation. All positive control animals (5/sex) were sacrificed 24 hours after dosing. Marrow was removed from the femurs and bone marrow smears were prepared. The slides were air-dried then the smears were stained with polychrome methylene blue-eosin. The slides were coded prior to scoring.

4. Statistical methods
The incidence of MPCEs and the percentage of PCEs were determined for each animal and treatment group. Data were summarized by sex and dose groups for different time points and were compared to the vehicle control values using analysis of variance. Extended counts from treated and control females sacrificed at 48 hours were analyzed as an independent data set and combined with the respective original counts using analysis of variance. Data for MPCEs were transformed prior to analysis. Dose group values were analyzed for statistical significance using a one-sided Student's *t*-test. Statistical significance was determined at $p<0.05$ and $p<0.01$.

5. Evaluation Criteria
The criteria for a valid test were that the cyclophosphamide should induce a significant increase in MPCEs compared to the vehicle control values, and that the test material should be tested at a level that causes a decrease in the ratio of PCEs to NCEs or at the maximum tolerated dose level.

A positive response was a reproducible, statistically and biologically significant increase in the frequency of MPCEs or percentage of PCEs for any dose level relative to the vehicle control at a given sampling time.

II. REPORTED RESULTS

A. Solubility/Analytical Determinations
The dose formulations were not analyzed for stability, homogeneity, or actual concentrations.

B. Preliminary Toxicity Assay
Two CD-1 male mice received a single dose of ZA 1296 by gavage at dose levels of 320 or 2000 mg/kg. In addition, five CD-1 mice/sex received a single dose of ZA 1296 by gavage

at 500 or 800 mg/kg. The mice were observed for mortality and clinical signs of toxicity at 5 days (320 mg/kg group) or 4 days (all other groups) post-dosing.

Deaths occurred in the 800 mg/kg (3/5 males, 5/5 females) and 2000 mg/kg (1/2 males) groups. Based on these results, 500 mg/kg was selected as the dose level in the micronucleus assay. The results of the preliminary toxicity tests were presented in Study Report Appendix B, page 28.

C. Micronucleus Assay:

Five CD-1 mice/sex/dose received a single dose of ZA 1296 by gavage at a dose level of 500 mg/kg. Two additional mice/sex received 500 mg/kg ZA 1296 to serve as replacements in the event of mortality. Five mice/sex received the vehicle (sterile double deionized water) alone, and another five mice/sex received the positive control, cyclophosphamide. Mice were observed for mortality and clinical signs of toxicity until sacrifice. Five mice/sex from the vehicle and ZA 1296-treated groups were sacrificed at 24 or 48 hours post-dosing. All positive control mice (5/sex) were sacrificed at 24 hours post-dosing.

One ZA 1296-treated male was found dead at 4 hours post-dosing. No other deaths or significant clinical signs were observed in the treated mice. Bone marrow slides were prepared from all 5 mice/sex/group at each sampling time and scored for MPCEs and the PCE:NCE ratio. A statistically significant ($p<0.05$) increase in mean MPCE incidence per 1000 PCEs (2.2 vs. 0.6 in vehicle controls) was observed in bone marrow cells collected from female mice 48 hours after dosing. The increase was due to the high number of MPCEs in bone marrow cells from one treated female compared to MPCEs in bone marrow from the other four treated females (7 vs. 0-2). Extended analysis of an additional 2,000 PCEs from the ZA 1296-treated and vehicle control females 48 hours after dosing showed no statistical or biological differences in mean MPCE incidence (0.9 for both groups). There was also no significant increase in MPCEs compared to vehicle controls when the original and extended 48-hour samples were combined (1.3 treated vs 0.8 controls). ZA 1296 did not cause a significant increase in MPCEs compared to vehicle controls in bone marrow cells collected from male mice 24 or 48 hours after dosing or from female mice 24 hours after dosing. The group mean number of MPCEs per 1,000 PCEs varied between 0.0 and 0.4 in bone marrow cells collected from treated and control males at 24 and 48 hours after dosing. The group mean number of MPCEs per 1,000 PCEs was 0.6 in bone marrow cells collected from treated female at 24 hours after dosing compared to 1.0 in corresponding vehicle control females. The positive control, cyclophosphamide, induced statistically significant ($p<0.01$) increases in mean number of MPCEs per 1,000 PCEs in bone marrow cells collected from males (25.6) and females (24.8) at 24 hours after dosing. The PCE:NCE ratios for the treated groups were not statistically different compared to the controls at 24 or 48 hours post-dosing. The results of the mutagenicity assays were presented as mean values in six tables and as individual data in two appendices in the study report (Study Report Tables 1-6, pages 21-26 and Study Report Appendices F and G, pages 33-36). The results of the micronucleus assay are compiled and summarized in Table 1 below.

Table 1. Summary of Mutagenicity Assays Results.[a]

Dose (mg/kg)	24 hours			48 hours						
	Total no. PCEs examined[b]	Mean Percent PCE	Mean incidence MPCE[c]	Total no. PCEs examined	Mean Percent PCE	Mean incidence MPCE	Total no. PCEs examined[d]	Mean incidence MPCE	Total no. PCEs examined[e]	Mean incidence MPCE
Males										
Vehicle control	1,000	53.0 ± 8.9	0.4 ± 0.9	1,000	51.0 ± 4.8	0.0 ± 0.0	NS	NS	NS	NS
500	1,000	58.4 ± 12.6	0.4 ± 0.6	1,000	63.9 ± 5.3	0.0 ± 0.0	NS	NS	NS	NS
Cyclophosphamide	1,000	47.6 ± 6.8	25.6 ± 9.7**	NS	NS	NS	NS	NS	NS	NS
Females										
Vehicle control	1,000	48.7 ± 9.0	1.0 ± 0.7	1,000	58.2 ± 7.4	0.6 ± 0.9	2,000	0.9 ± 0.2	3,000	0.8 ± 0.4
500	1,000	53.5 ± 8.3	0.6 ± 0.6	1,000	50.0 ± 9.6	2.2 ± 2.8*	2,000	0.9 ± 0.8	3,000	1.3 ± 1.3
Cyclophosphamide	1,000	56.7 ± 3.1	24.8 ± 7.1**	NS	NS	NS	NS	NS	NS	NS

a Data extracted from study report Tables 1-6, pages 21-26.
b Total number of PCEs examined/animal.
c Mean incidence of micronucleated polychromatic erythrocytes per 1000 PCEs.
d Extended analysis of an additional 2,000 PCEs per animal.
e Combined original (1,000 PCEs) and counts (2,000 PCEs) counts per animal.
NS Not sampled.
* Significantly different from the negative control, $p<0.05$.
** Significantly different from the negative control, $p<0.01$.

III. DISCUSSION/CONCLUSIONS

A. Investigator's Conclusions

Since no statistically or biologically significant increases in the incidence of MPCEs were observed in extended counts or when the original and extended counts were combined, the small increase in incidence of MPCEs in treated females at 48 hours for the original sample was not reproducible and of no biological significance. Therefore, ZA 1296 is considered negative for clastogenic activity in this test system.

B. Reviewer's Discussion

ZA 1296 was considered toxic at 500 mg/kg, based on the death of one treated male observed 4 hours after dosing. ZA 1296 was not toxic to the bone marrow and was negative in this in vivo micronucleus assay when tested at a dose level of 500 mg/kg. Although a statistically significant ($p<0.05$) increase in mean MPCE incidence was observed in bone marrow cells collected from female mice 48 hours after dosing, extended analysis of additional PCEs from these females showed no statistical or biological significance compared to the controls. The sensitivity of this test to detect a genotoxic response was demonstrated by the significant ($p<0.01$) increase in MPCEs induced by the positive control (cyclophosphamide). ZA 1296 was adequately tested and found to be non-genotoxic in this *in vivo* micronucleus assay.

C. Study Deficiencies

No deficiencies that would alter the conclusions of this study were noted. Although the dose formulations were not analyzed for actual concentrations, clinical signs of toxicity (death of one male) were observed. Several minor deficiencies noted in the study that are not considered to affect the validity of the study results are:

- The criteria for a valid test were not listed.
- Historical negative-control data were not provided.