Lisa Austin, PhD: Signature: _____
Registration Action Branch 1, Health Effects Division (7509C) Date_____
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DATA EVALUATION RECORD

STUDY TYPE: *In vivo* Mammalian Spermatogonial Chromosome Aberration Test-mouse
OPPTS 870.5380 [§84-2]; OECD 483

PC CODE: 080805 **DP BARCODE:** D310325

TEST MATERIAL (PURITY): Propazine (96.9%)

SYNONYMS: Not provided

CITATION: Erexson, G. L. (2003) Chromosomal Aberrations *In Vivo* in Mouse Spermatogonial Cells with Propazine. Covance Laboratories Inc., 9200 Leesburg Pike, Vienna, VA 22182-1699. Study Number: 23988-0-474OECD, February 28, 2003. MRID 46171701. Unpublished.

SPONSOR: Griffin, LLC, Valdosta, Georgia

EXECUTIVE SUMMARY:
In a spermatogonial chromosome aberration test (MRID 46171701), 6 male Crl:CD-1®(ICR)BR mice/dose were treated via gavage with a single dose of propazine, (96.9 % a.i., lot # SG90502205) at doses of 0, 500, 1000, or 2000 mg/kg. Spermatogonial cells were harvested 24 hours post-treatment. The vehicle was 0.5% aqueous carboxymethyl cellulose (CMC).

Propazine was tested up to the limit dose, 2000 mg/kg. There were no clinical signs of toxicity or mortality in any of the treated animals. Cytotoxicity was not observed in spermatogonia cells at any dose level. The mitotic index was slightly decreased in the high dose group and slightly increased in the low and mid dose groups, relative to controls. There was no increase in chromosomal aberrations in any treated group when compared to controls. The vehicle and positive control values were appropriate. Historical control data were not provided for chromosome aberrations in mouse spermatogonial cells, but were provided for mouse bone marrow cells. **There is no evidence for a biologically or statistically significant increase over the vehicle control values in the number of cells with chromosome aberrations following treatment with propazine**.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for an *In vivo* Mammalian Spermatogonial Chromosome Aberration test (OPPTS 870.5380; OECD

483).

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:

Propazine

Description: white powder
Lot/Batch #: SG90502205
Purity: 96.9 % a.i.
CAS # of TGAI: Not provided

Solvent Used: 0.5% aqueous carboxymethyl cellulose (CMC)

2. Control Materials:

Negative control (if not vehicle) :	None	**Final Volume:**	**Route:**
Solvent:	0.5% aqueous carboxymethyl cellulose	**Final Volume:**10 mL/kg	**Route:** gavage
Positive control :	Mitomycin C in water	**Final Dose(s):** 0.2 mg/mL	**Route:** Intraperitoneal injection

3. Test animals:

Species: Mouse
Strain: Crl:CD-1®(ICR)BR
Age/weight at study initiation: 8 weeks/30.7-38.4g
Source: Charles River Laboratories, Raleigh, NC
No. animals used per dose 6 males
Properly Maintained? Yes

4. Test compound administration:

	Dose Levels (mg/kg)	Final Volume	Route
Preliminary:	0, 250, 500, 1000, 2000	10 mL/kg	gavage
Main Study:	0, 500, 1000, 2000	10 mL/kg	gavage

B. TEST PERFORMANCE

1. Treatment and Sampling Times:

a. Vehicle control:

Dosing:	x	once		twice (24 hrs apart)				Other		
Sampling (after last dose):		6 hr		12 hr	x	24 hr		48 hr		72 hr
Other:										

b. Positive control:

Dosing:	x	once		twice (24 hrs apart)				Other		
Sampling (after last dose):		6 hr	x	18 hr		24 hr		48 hr		72 hr
Other:										

2. <u>Cytogenetic Assay</u>:

a. Test compound:

Dosing:	x	once		twice (24 hrs apart)				Other		
Sampling (after last dose):		6 hr		12 hr	x	24 hr		48 hr		72 hr
Other:										

b. Spindle inhibition

Inhibition used/concentration: colchicine/4mg/kg
Administration time: 5 hours (before cell harvest)

<u>c. Cytotoxicity Assessment</u>: Cytotoxicity was determined by the mitotic index (MI) from 1000 cells per animal from five animals per group.

d. Details of slide preparation: Approximately five hours prior to sacrifice (method not reported), the mice were injected intraperitoneally with approximately 4 mg/kg of colchicine. Both testes from each animal were harvested and placed in Hank's Balanced Salt Solution. Seminiferous tubules were teased out into a 1% sodium citrate solution. Spermatogonia preparations were based on procedures by Brewen and Preston (1978, see references). Slides from five of the six animals in each treatment and control group were analyzed. Slides were coded prior to scoring.

e. Metaphase analysis

No. of cells examined per dose: 100

Scored for structural?	x	Yes		No
Scored for numerical?		Yes	x	No
Coded prior to analysis?	x	Yes		No

f. Evaluation criteria: Cells were evaluated for chromosome and chromatid breaks, gaps, fragments and deletions, exchanges, multiple aberrations, and multiple aberrations plus exchanges. The MI was determined from 1000 cells per animal. The criterion for a positive response was a statistically significant, dose-related increase in the number of structural aberrations for at least one dose level. Gaps were not counted as significant aberrations. Open breaks were considered as indicators of genetic damage as were configurations resulting from the repair of breaks. The latter included translocations, multiradial, rings, multicentrics, etc. Cells with more than one aberration were considered to indicate more genetic damage than those containing evidence of single events. The vehicle control group was acceptable if the mean value was between zero and the highest value in the historical control range. The positive control was acceptable if the mean value was significantly higher than the vehicle control.

g. Statistical analysis: All the data were analyzed by ranked analysis (nonparametric) techniques for heterogeneity (Hollander and Wolfe, 1973) and trend (Thakur, 1984), where applicable. The alpha level (p-value) to determine statistical significance was 0.05. The statistics are considered appropriate.

II. REPORTED RESULTS

Propazine was prepared fresh prior to use. The preweighed quantity of propazine was mixed with 0.5% CMC to form a homogenous suspension. The dose preparations were held at ambient temperature prior to dosing and stirred during the dosing procedure. For the chromosome aberrations assay, duplicate 2-mL samples were taken from the top, middle, and bottom portions of the low- and high- dose levels and from the middle portion of the intermediate-dose level and stored at ambient temperature. Propazine concentrations with nominal values of 50, 100, and 200 mg/mL were analyzed and found to be 94, 97, and 99% of the theoretical values, respectively. The recommended storage condition for propazine was in ambient temperature. The concentration analysis is acceptable.

A. PRELIMINARY CYTOTOXICITY ASSAY: Twelve male mice, approximately 8 weeks old, weighing 30.3-34.4g and dosed with propazine ranging from 250-2000 mg/kg were used in the rangefinding assay. All animals were examined for signs of toxicity and/or mortality immediately after dosing, 1 hour after dosing and on days 1 and 2 after dosing. All animals appeared normal during all of the aforementioned examinations and at the end of the observation period. Based on these results, 500, 1000, and 2000 mg/kg were selected for the final chromosomal aberration assay.

B. CYTOGENETIC ASSAY: Propazine dose levels of 0, 500, 1000 and 2000 mg/kg were tested in the chromosomal aberrations assay. All animals were examined for signs of toxicity and/or mortality immediately after dosing, 1 hour after dosing and on days 1 and 2 after dosing. There were no clinical signs of toxicity or mortality in any of the treated animals. Spermatogonial cells were harvested at 24 hours post-treatment in all treatment groups and the vehicle control; and 18 hours post-treatment in the positive control group. Cytotoxicity was not observed in spermatogonial cells at any dose level. The mitotic index was slightly decreased in the high dose group and slightly increased in the low and mid dose groups, relative to controls. There was no increase in chromosomal aberrations in any treated group when compared to controls. The vehicle and positive control values were appropriate. Historical control data were provided for chromosome aberrations in mouse bone marrow cells, not in spermatogonial cells which were used in this study. Results of the cytogenetic assays are summarized in Table 1.

TABLE 1. Cytogenetic assay with Propazine, 24-hour post-treatment harvest (500 cells/treatment)[a]							
Treatment	Gaps	Chromosome Aberrations			Aberrant Metaphases (%)[c]	Other[d]	% Mitotic Index
		simple breaks[b]	chromatid exchange	chromosome exchange			
CMC (10 mL/kg)[e]	11	0	0	0	0.0 ± 0.00	0	0.94 ± 0.17
Propazine (mg/kg)							
500	15	3	0	1	0.8 ± 0.37	0	1.16 ± 0.21
1000	9	0	0	1	0.2 ± 0.20	0	1.18 ± 0.18
2000	8	1	0	0	0.4 ± 0.40	0	0.86 ± 0.16
Mitomycin C (2.0 mg/kg)	9	6	28	21	10.6 ± 1.29*	7	0.94 ± 0.18

[a] Data summarized from Tables 1, 2 and 3, pages 17-19 in Study Report, MRID 46171701.
[b] Simple breaks include chromatid and chromosomes breaks and acentric fragments.
[c] Percentage of metaphases with structural aberrations excluding gaps
[d] Other = sum of metaphases with more than 1 aberration.
[e] CMC controls (0.5% carboxymethyl cellulose, 476 cells/treatment)
*Statistically significant $p < 0.01$

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The investigator concluded that propazine was negative for the induction of structural damage under the conditions of exposure in this assay up to the limit dose (2000 mg/kg).

B. REVIEWER COMMENTS: The reviewer agrees with the investigator's conclusion. Propazine was tested up to the limit dose, 2000 mg/kg. There were no clinical signs of toxicity or mortality in any of the treated animals. Cytotoxicity was not observed at any dose level. The mitotic index was slightly decreased in the high dose group and slightly increased in the low and mid dose groups, relative to controls. There was no increase in chromosomal aberrations in any treated group when compared to controls. The vehicle and positive control values were appropriate. Historical control data were not provided for chromosome aberrations in mouse spermatogonial cells, but were provided for mouse bone marrow cells. There is no evidence for a biologically or statistically significant increase over the vehicle control values in the number of cells with chromosome aberrations following treatment with propazine. This is an **Acceptable/Guideline** study.

C. STUDY DEFICIENCIES: The following deficiencies were noted, but would not alter the conclusions of this DER:
- There was only one sampling time at the highest dose.
- The ratio of spermatogonial mitotic cells to the first and second meiotic metaphases were not reported.

-Historical control data were not provided for chromosome aberrations in mouse spermatogonial cells.

REFERENCES:

Brewen, G. and Preston, R. J. Analysis of chromosome aberrations in mammalian germ cells, Chemical Mutagens: Principles and Methods for Their Detection, Vol. 5, (eds.) Hollaender, A. and deSerres, F.J., pp. 127-150, Plenum Press: New York (1978).