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DATA EVALUATION RECORD

STUDY TYPE: *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus assay in Mice; OPPTS 870.5395 [§84-2]; OECD 474.

DP BARCODE: DP317750
PC CODE: 072506

Decision No: 353040
REGISTRATION No: Section 3

TEST MATERIAL (PURITY): Silver chloride (99.5% a.i.)

SYNONYMS: EK2004-0092

CITATION: Erexson, G.L. (2004). *In Vivo* Mouse Micronucleus Assay. Covance Laboratories Inc. (Vienna, Virginia). Study Number 6132-202, January 5, 2005, MRID 464533-02. Unpublished.

SPONSOR: Eastman Kodak Company, Building 320, Kodak Park, Rochester, New York 14652-6272

EXECUTIVE SUMMARY:

In a preliminary dose range-finding, three mice/sex/dose Crl:CD-1® (ICR) BR were dosed at 125, 250, 500, 1000, and 2000 mg/kg of silver chloride. Animals were observed for clinical toxicity signs directly after dosing of test article, 5 - 20 minutes, 5 hours, 1 day, and 2 days post dosing. Based on clinical observations the maximum tolerated dose was found to be 125 mg/kg. No differences were observed between sexes and only males were used in the micronucleus assay.

In the micronucleus assay (MRID 464533-02), Silver Chloride (>99.5% a.i.) was formed into a homogenous suspension in corn oil (Lot/Batch No. 12-424) and administered at the target dose level were 31.25, 62.4, and 125 mg/kg by i.p injection. Bone marrow was harvested at 24 hours, 6 animals/dose level. At 48 hours, 12 additional animals were sacrificed - 6 animals for vehicle control and 6 for the 125 mg/kg dose level. Three additional animals were also dosed at 125 mg/kg bw to ensure the survival of 6 animals for bone marrow extraction. Corn Oil (20 mL/kg) was administered by i.p. injections. Positive control article, cyclophosphamide (Sigma, Lot No. 113K1406, 99.8% a.i.) was dissolved in sterile deionized water, administered once by oral gavage at 80 mg/kg, and the bone marrow of the animals was harvested after 24 hours.

The test article induced signs of clinical toxicity, rough hair coat and/or hunched posture, at 62.5

and 125 mg/kg. The test article did not induce statistically significant increases in micronucleated PCEs when compared to vehicle control responses. There was statistically significant decrease in PCE:NCE (immature/mature erythrocytes) ratio for the 125 mg/kg dose group, indicating that the test article was cytotoxic to the bone marrow.

This study is classified as **Acceptable-Guideline** and satisfies the **Guideline** requirement (OPPTS 870.5395; OECD 474) for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided. (The quality assurance statement noted that no audits were conducted on the report).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Silver Chloride

Description:	White powder with chunks
Batch/Sample #:	Not reported; Lot. No. JAB-128V3
Purity:	Not reported
CAS # of TGAI:	Not reported
Structure:	Not reported
Solvent Used:	Corn Oil (Lot/Batch No. 12-424)

2. Control Materials:

Vehicle:	Corn Oil	**Final Volume:** 20 mL/kg	**Route:** Intraperitoneal Injection
Positive control :	Cyclophosphamide (monohydrate)	**Final Dose(s):** 80 mg/kg	**Route:** Oral Gavage

3. Test animals:

Species:	Mouse
Strain:	Crl:CD-1® (ICR) BR strain
Age/weight at study initiation:	For the dose rangefinding assay, 8 to 10 week old adult male and female Crl:CD-1®(ICR) BR mice weighed 30.4-38.4 g (males) and 24.3-29.6 g (females). 8-week old male Crl:CD-1®(ICR) BR mice weighing 29.9-36.3 g were used for the micronucleus assay.
Source:	Charles River Laboratories, Raleigh, NC
No. animals used per dose	Dose range finding study (3 males; 3 females); Micronucleus assay (6 males; 12 males for the vehicle control, and 15 for the highest dose of 125 mg/kg)
Properly Maintained?	Yes

4. Test compound administration:

	Dose Levels	Final Volume	Route
Preliminary:	125, 250, 500, 1000, and 2000 mg/kg	20 mL/kg	Intraperitoneal Injection
Main Study:	31.25, 62.5, 125 mg/kg	20 mL/kg	Intraperitoneal Injection

B. TEST PERFORMANCE

1. Treatment and Sampling Times:

Test compound, vehicle, and positive controls:

Dosing:		once								
Sampling (after last dose):		24 hr		48 hr						
Other:										

2. Tissues and Cells Examined:

Bone marrow:	hind limb bones (tibia)
No. of polychromatic erythrocytes (PCE) examined per animal:	2000/animal
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal:	not reported
Other (if other cell types examined, describe): erythrocytes (to determine ratio of PCE:NCE) per animal	at least 500/animal

3. Details of Slide Preparation: The hind limb bones (tibia) were removed from animals and marrow was flushed from the first five surviving animals per group. The bone marrow was transferred to individual centrifuge tubes and combined with 3-5 mL of fetal bovine serum. The cells were centrifuged creating a marrow pellet, a portion of the pellet is spread on slides, and air-dried. Slides were coded prior to analysis, fixed in methanol (30 minutes), stained with a combination of May-Gruenwald and Giemsa stain and coverslips were permanently mounted.

4. Evaluation Criteria: A positive result was defined as a statistically significant increase in the number of micronucleated PCEs for at least one of the dosing levels and a statistically significant dose-related response. A negative result was defined as any deviation from the above criterion. In addition to statistical analysis, the final evaluation included biological relevance consideration.

5. Statistical Methods: Assay data analysis was performed using analysis of variance (Winer, 1971) on untransformed proportions of cells with micronuclei per animal and on untransformed PCE:NCE ratios when the variances were homogeneous. Ranked proportions were used for heterogenous variances. If the analysis of variance was statistically significant ($p \leq 0.05$), Dunnett's t-test was used to determine which dose group.

Winer, B.J., Statistical Principles in Experimental Design, Second Ed., McGraw-Hill: New York (1971).

II. REPORTED RESULTS

A. ANALYTICAL ANALYSIS: The micronuclei frequency was determined by analyzing the number of Micronucleated PCEs from at least 2000 PCEs/animal. The PCE:NCE ratio was determined by scoring the number of PCEs and NCEs observed while scoring at least 500 erythrocytes per animal. The staining procedure permitted the differentiation, by color, of PCEs and NCEs.

B. PRELIMINARY TOXICITY ASSAY: Three mice/sex were exposed once to the test material via Intraperitoneal injection at concentrations of 125, 250, 500, 1000, and 2000 mg/kg bw. Animals were observed daily for clinical signs of toxicity including mortality. In the 125 mg/kg group one male mouse died. There were no surviving male or female mice in any of the higher concentrations. One male (1000 mg/kg) and one female (500 mg/kg) were sacrificed due to signs of excessive toxicity.

TABLE 1. Clinical Observations- Dose Rangefinding Assay

Target Dose Level (mg/gk)	Sex	Animal ID	Time After Dosing					
			IPD	5-20 min. PD	1 hr. PD	5 hr. PD	1 day	2 day
125	M	2031	0	NP	NP	NP	3,4	5
		2032	0	NP	NP	NP	0	0
		2035	0	NP	NP	NP	3,4	0
	F	2042	0	NP	NP	NP	0	0
		2047	2	NP	NP	NP	0	0
		2049	0	NP	NP	NP	0	0
250	M	2432	0	NP	NP	NP	5	-
		2433	0	NP	NP	NP	5	-
		2434	0	NP	NP	NP	5	-
	F	2435	0	NP	NP	NP	5	-
		2436	0	NP	NP	NP	5	-
		2437	0	NP	NP	NP	5	-
500	M	2030	0	1,2	1,2,3,4	5	-	-
		2033	0	1,2	1,2,3,4	5	-	-
		2037	0	1,2	1,2,3,4	5	-	-
	F	2040	0	1,2	0	0 to 6	-	-
		2043	0	1,2	0	5	-	-
		2048	0	1,2	0	5	-	-
1000	M	2028	0	1,2	1,2,3,4	5	-	-
		2029	0	1,2	1,2,3,4	0 to 6	-	-
		2034	0	1,2	1,2,3,4	5	-	-
	F	2038	0	1,2	1,2,3,4	5	-	-
		2039	0	1,2	1,2,3,4	5	-	-
		2046	0	1,2	1,2,3,4	5	-	-

2000	M	2026	0	1,2	1,2,3,4	5	-	-
		2027	0	1,2	1,2,3,4	5	-	-
		2036	0	1,2	1,2,3,4	5	-	-
	F	2041	0	1,2	1,2,3,4	5	-	-
		2044	0	1,2	1,2,3,4	5	-	-
		2045	0	1,2	1,2,3,4	5	-	-

[a] Data obtained from page 20 in the study report.

Key: 0=Normal, 1=hunched posture, 2=irregular respiration, 3=slightly hypoactive, 4=squinted eyes, 5=found dead, 6=animal sacrificed due to excessive toxicity; IPD=Immediately post dosing, PD=Post dosing, NP=Not performed, to=followed by

No significant differences in toxicity were observed between male and female mice, therefore, only male mice were selected for the micronucleus assay. The lowest dose of 125 mg/kg was selected to be the highest dose for the micronucleus assay.

C. **MICRONUCLEUS ASSAY:** Male mice in groups of 6 per dose or controls were harvested 24 hours after dosing (exceptions: 6 additional animals for 125 mg/kg and vehicle control dose level for harvest after 48 hours as well, and 3 additional animals for 125 mg/kg to ensure that 6 animals survived to harvest). Signs of toxicity included rough haircoat and/or hunched posture in all mice at 62.5 mg/kg and 125 mg/kg dose groups one hour post dose. At the 125 mg/kg dose group at the 48 hour time plot there was a statistically significant decrease in PCE:NCE ratio compared to vehicle control, indicating that the test article or its metabolites is cytotoxic to the target tissue (bone marrow). The vehicle control, corn oil, induced appropriate percent micronucleated PCEs when compared to historical control data. The positive control, cylophosphamide, induced a statistically significant increase in micronucleated PCEs as compared to the vehicle control (mean and standard error =1.23 ± 0.24%). The test article tested negative under study condition in the micronucleus assay.

TABLE 1. MICRONUCLEUS SUMMARY DATA[a]

TREATMENT	DOSE	HARVEST TIME	% MICRONUCLEATED PCEs MEAN OF 2000/ANIMAL ± S.E.	RATIO PCE:NCE MEAN ± S.E.
CONTROLS				
Vehicle	Corn Oil (20 mL/kg)	24 hr 48 hr	0.03 ± 0.02 0.05 ± 0.02	0.53 ± 0.07[b] 0.51 ± 0.04
Positive	CP 80 mg/kg	24 hr	1.23 ± 0.24*	0.60 ± 0.06
TEST ARTICLE	31.25 mg/kg	24 hr	0.07 ± 0.03	0.48 ± 0.06
	62.5 mg/kg	24 hr	0.01 ± 0.04	0.49 ± 0.03
	125 mg/kg	24 hr	0.07 ± 0.02	0.44 ± 0.05
		48 hr	0.05 ± 0.03	0.30 ± 0.04 [b,**]

[a] Data obtained from page 23 in the study report.
CP= Cyclophosphamide
PCE= Polychromatic erythrocyte
NCE= Normochromatic erythrocyte
* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
**Significantly greater than the corresponding vehicle control, $p \leq 0.05$.
[b] The registrant presented values of 0.54 ± 0.07 and 0.31 ± 0.04 compared to reviewers' values presented above when verifying the date. Variations of a hundredth can be attributed to rounding. These variations in the numerical values do not adversely affect the conclusions presented in the study.

Tables 2 (24 hr harvest time point) and 3 (48 hr harvest time point) show the number of micronucleated PCEs/2000 PCEs per animal and treatment doses and the ratios of PCE:NCE for the male mouse micronucleus assay.

TABLE 2. MICRONUCLEUS TEST-24 HOUR HARVEST INDIVIDUAL MALE DATA[a]

TREATMENT	DOSE	ANIMAL NUMBER	# MN PCEs/ 2000 PCEs	RATIO PCE:NCE
24 HOUR HARVEST	MALE			
VEHICLE CONTROL	Corn Oil	2602	1	0.47
		2606	0	0.78
		2624	0	0.38
		2628	0	0.48
		2632	2	0.56
POSITIVE CONTROL	CP 80 mg/kg	2600	21	0.63
		2601	39	0.49
		2603	32	0.54
		2610	14	0.81
		2613	17	0.53
TEST ARTICLE	31.25 mg/kg	2595	1	0.42
		2596	0	0.43
		2609	1	0.33
		2620	1	0.62
		2633	4	0.61
	62.5 mg/kg	2593	1	0.47
		2597	0	0.58
		2598	4	0.52
		2608	3	0.42
		2615	2	0.47
	125 mg/kg	2614	2	0.62
		2616	1	0.42
		2618	0	0.36
		2619	2	0.36
		2626	2	0.45

[a] Data obtained from page 24 in the study report.
CP= Cyclophosphamide
PCE= Polychromatic erythrocyte
# MN PCEs= Micronucleated PCEs
NCE= Normochromatic erythrocyte

TABLE 3. MICRONUCLEUS TEST-48 HOUR HARVEST INDIVIDUAL MALE DATA[a]

TREATMENT		ANIMAL NUMBER	# MN PCE'S/2000 PCES	RATIO PCE:NCE
48 HOUR HARVEST				
VEHICLE CONTROL	Corn Oil	2594 2604 2607 2622 2625	0 1 2 0 2	0.49 0.66 0.48 0.41 0.51
TEST ARTICLE	125 mg/kg	2599 2611 2612 2617 2631	0 3 0 1 1	0.4 0.26 0.38 0.32 0.16

[a] Data obtained from page 25 in the study report.
PCE= Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte

III. DISCUSSION and CONCLUSIONS

A. CONCLUSIONS: In the range finding study, a maximum tolerated dose of 125 mg/kg was established based on clinical observations. No differences were observed between sexes and only males were used in the micronucleus assay. The test article induced signs of clinical toxicity, rough hair coat and/or hunched posture, at 62.5 and 125 mg/kg of silver chloride. There was statistically significant decrease in the PCE:NCE ratio for the 125 mg/kg dose group, indicating that the test article was cytotoxic to the target issue - bone marrow. The test article did not induce statistically significant increases in micronucleated PCEs at any dose level when compared to vehicle control responses.

Note 1: More animals were used in the study than could be accounted for in the various tables. Samples were based on at least 2000 PCEs/animal. The PCE:NCE ratio was determined by scoring the number of PCEs and NCEs observed while scoring at least 500 erythrocytes per animal.

Note 2: The study report indicates that the study sponsor, Eastman Kodak Company, was responsible for determination and documentation of identity, strength, purity, stability and uniformity of the test article and the determination of stability, homogeneity, and concentration of the dosing preparations. If correct documentation with test substance purity, storage stability, and dose preparations were performed by the sponsor, then the study is considered to be Acceptable-Guideline

D. STUDY CLASSIFICATION: This study is classified as **Acceptable-Guideline** and satisfies the guideline requirement (OPPTS 870.5395; OECD 474) for *in vivo* cytogenetic mutagenicity data, Mouse Micronucleus Study.