DATA EVALUATION RECORD

BASELINE GASOLINE VAPOR CONDENSATE

STUDY TYPE: *In vivo* MAMMALIAN CYTOGENETICS - MICRONUCLEUS ASSAY; [OPPTS 870.5395(§84-2)]

Prepared for

Office of Transportation and Air Quality
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue
Washington, D.C. 20460

Prepared by

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Office of Transportation and Air Quality **Date**_____

DATA EVALUATION RECORD

STUDY TYPE: *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus assay in rat bone marrow [OPPTS 870.5395 (§84-2)] OECD 474.

TEST MATERIAL (PURITY): Baseline gasoline vapor condensate

SYNONYMS: None provided

CITATION: Mason, C. (2001*) Satellite procedure baseline gasoline vapor condensate rat micronucleus test. Huntingdon Life Sciences Ltd., Eye Research Centre, Eye, Suffolk, England. Laboratory Study ID: Huntingdon Life Sciences Report No. APT 004/012980. End of evaluation phase of study was April 10, 2001*. Unpublished *[The image file did not contain a final study date; it is assumed that a signed and dated copy is available].

SPONSOR: Huntingdon Life Sciences, Princeton Research Centre, Mettlers Road, East Millstone, NJ 08875-2360

EXECUTIVE SUMMARY: In a Crl: CD® IGS BR albino rat bone marrow micronucleus assay (C. Mason, 2001), five rats/sex/dose were treated by inhalation of a vapor in the breathing air via whole-body exposures for 4 weeks (for 6 hours per day for 5 days per week) with baseline gasoline vapor condensate at target exposure concentrations of 0, 2000, 10,000, or 20,000 mg/m^3. Dosimetry indicated that actual exposure concentrations slightly exceeded target concentrations. Bone marrow cells were harvested at 24 hours after the final (20^{th}) exposure. The vehicle was air.
No sign of toxicity of the test substance was observed during the study. Baseline gasoline vapor condensate is assumed to have been tested at an adequate concentration based on effects seen in some other part of the study. The vehicle and positive controls induced the appropriate responses. **There was not a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow at any exposure concentration.**

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

COMPLIANCE: Signed and dated GLP and Quality Assurance statements were provided*. No Data Confidentiality statement was provided. *[The image file did not contain signatures with corresponding dates; it is assumed that a signed and dated copy is available].

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Baseline gasoline vapor condensate

Description: Not provided
Lot/Batch #: Not provided
Purity: Not provided
CAS # of TGAI: Not provided
Structure Not provided
Solvent Used: Air

2. Control materials:

Negative control (if not vehicle) :		**Final Volume:**	**Route:**
Vehicle:	Air	**Final Volume:**	**Route:** Breathing
Positive control :	Cyclophosphamide	**Final Dose(s):** 40 mg/kg	**Route:** Intraperitoneal injection

3. Test animals:

Species: Rat
Strain: Crl: Sprague Dawley-derived CD® IGS BR
Age/weight at study initiation: Approximately 6 weeks/ males 177-219 g, females 142-177 g
Source: Charles River Laboratories, Kingston, NY
No. animals used per dose 5 Males; 5 females
Properly Maintained? Yes

4. Test compound administration:

	Target air concentration levels for 6-hours per day on 5 days per week for 4 weeks	**Final Volume**	**Route**
Preliminary:	None	Not applicable	None
Main Study:	2000, 10,000, or 20,000 mg/m^3 of breathing air	Not applicable	Inhalation

B. TEST PERFORMANCE:

1. Treatment and sampling times:

a. Test compound:

Dosing:		Once		Twice (24 hrs apart)			x	Other		
Sampling (after last dose):	x	24 hr		12 hr		24 hr		48 hr		72 hr
Other: by inhalation for 6 hours per day on 5 days per week for 4 weeks										

b. Negative and/or vehicle control:

Dosing:		Once		Twice (24 hrs apart)			x	Other		
Sampling (after last dose):	x	24 hr		12 hr		24 hr		48 hr		72 hr
Other: by inhalation for 6 hours per day on 5 days per week for 4 weeks										

c. Positive control:

Dosing:	x	Once		Twice (24 hrs apart)				Other		
Sampling (after last dose):	x	24 hr		12 hr		24 hr		48 hr		72 hr
Other:										

2. Tissues and cells examined:

Bone marrow	
No. of polychromatic erythrocytes (PCE) examined per animal:	2000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal:	# In at least 1000 erythrocytes
Other	

3. Details of slide preparation: Mice were killed by CO_2 asphyxiation. The bone marrow was harvested in fetal calf serum (FCS) from both femurs and centrifuged at about 150 × g for about 5 minutes. After the supernatant was drawn off, the cell pellet was resuspended by aspiration with a Pasteur pipette, and a small drop of cells was spread onto a clean glass slide. Four slides were prepared per animal. Following air drying and fixing in methanol, smears were stained using a modified Feulgen staining method that specifically stains DNA-containing bodies deep purple while leaving mast cell granules unstained. Those mast cell granules, which are found in rat bone marrow cells, can be confused with micronuclei using some other staining methods. In the modified Feulgen method the actual stains are Mayer's Haemalum and acridine orange. Slides were mounted and coded prior to analysis.

4. Evaluation criteria: Micronuclei from smears examined by light microscopy were identified by meeting the following criteria: (a) being large enough to discern morphological characteristics, (b) having a generally rounded shape with a clearly defined outline, (c) being deeply stained, but not black, and similar in color to the nuclei of other cells, (d) lying in the same focal plane as the cell, (e) lacking internal structure, and (f) lacking micronuclear-like debris in the area surrounding the cell. For each mouse, the incidence of micronucleated polychromatic erythrocytes (PCEs) was determined among 2000 PCEs, and the proportion of PCEs was assessed by examination of at least 1000 total erythrocytes. For each treatment and control group, the mean number of micronucleated PCEs was reported as well as the percentage of total erythrocytes that were PCEs. Criteria for a positive result were a statistically significant dose-related increase in the incidence of micronucleated PCEs in a treatment group in comparison with the vehicle control group and individual and/or group mean values that exceed the laboratory historical control range. Results were considered negative if the individual and group mean incidences of micronucleated PCEs in the treatment group were not statistically significantly higher than the incidences for the vehicle control group and when these values fell within the historical solvent/vehicle control range.

5. Statistical methods: Differences in the incidences of micronucleated PCEs in treated and control groups were analyzed for statistical significance using a nonparametric statistical test (StatXact, CYTEL Software Corporation, Cambridge, Massachussetts) in which one-sided p-values were calculate by permutation. Comparisons of percentages of PCEs among total erythrocytes made use of exact versions of Wilcoxon's sum of ranks test and Jonckheere's Test. The level of significance used when deciding if there were statistically significant effects was an α of 0.01. These methods are appropriate, although less stringent tests with the

commonly used α of 0.05 might have been preferable. The reviewer rechecked the statistical comparisons regarding numbers of PCEs with micronuclei by using a one-sided Fisher Exact Test for 2 x 2 tables, and the smallest p value for comparisons of the individual experimental groups with the vehicle control was 0.23. Thus, the choice of α made by the investigators was of no importance regarding induction of micronuclei in the current report. The reviewer also rechecked the statistical comparisons regarding the percentages of total erythrocytes that were PCEs using the Kruskal-Wallis test. Excluding comparisons involving the positive control, the smallest p value obtained was 0.15 in a comparison of all experimental groups and the vehicle control group, with the sexes combined. Thus, again the choice of α made by the investigators was of no importance regarding this endpoint.

II. **REPORTED RESULTS**: Dosimetry using an IR analytical procedure was carried out to determine how well the target concentrations of the test substance in air were achieved. By analytical methods the concentration in air was determined four times on each of the 20 exposure dates for both males and females. The four values for each sex on each date were averaged, with the result that there were 40 mean concentrations reported (2 per day) for each of the three target concentrations. The means ± SDs of these 40 analytical values for each of the target concentrations of 2000, 10,000, and 20,000 mg/m^3 were 2061 ± 67, 10,136 ± 380, and 20,476 ± 765 mg/m^3, respectively. It is accordingly concluded that, on average, all three of the target concentrations were exceeded to a small extent.

A. **PRELIMINARY TOXICITY ASSAY:** No preliminary toxicity assay was reported, and no rationale was presented for the dose selection in the micronucleus assay.

B. **MICRONUCLEUS ASSAY:** Five rats/sex/dose were treated by inhalation of a vapor in the breathing air via whole-body exposures for 4 weeks (for 6 hours per day for 5 days per week) with baseline gasoline vapor condensate at target exposure concentrations of 0, 2000, 10,000, or 20,000 mg/m^3. Bone marrow cells were harvested 24 hours after the final (20th) exposure The vehicle was air. No toxicity was seen at any exposure concentration. Results of the micronucleus assay are summarized in Table 1. No statistically significant increase in the percentage of micronucleated PCEs over vehicle control values was seen at any test material concentration in either sex or in the pooled data from both sexes. All experimental values were well within the historical solvent/vehicle control range, which was presented graphically in the report. The group means regarding the incidence of micronuclei in experimental groups (sexes combined) ranged from 0.6-0.8 micronuclei per 2000 cells, and according to the graph the corresponding range in the historical solvent/vehicle control was 0-2.3 micronuclei per 2000 PCEs. The vehicle and positive control values were appropriate. There was no difference between the sexes in the induction of micronuclei by the test substance. The route of exposure was appropriate in view of the most likely means of human exposure.

TABLE 1. Summary of micronucleus assay		
Treatment	**Mean ± SD of percentage of PCEs among total erythrocytes at 24 hours**	**Mean % Micronucleated PCEs at 24 hours**
Vehicle[1]		
Male	46.8 ± 4.3	0.020
Female	55.6 ± 8.2	0.040
Pooled	51.2 ± 7.7	0.030
2000 mg/m^3		
Male	37.0 ± 11.1	0.030
Female	48.2 ± 3.8	0.040
Pooled	42.6 ± 9.8	0.035
10,000 mg/m^3		
Male	41.6 ± 5.7	0.050
Female	48.0 ± 3.4	0.030
Pooled	44.8 ± 5.6	0.040
20,000 mg/m^3		
Male	42.4 ± 6.8	0.030
Female	45.6 ± 9.6	0.030
Pooled	44.0 ± 8.0	0.030
Positive control[2]		
Male	30.2 ± 5.8 *****	0.91 *******
Female	23.2 ± 4.8 *****	0.49 *******
Pooled	26.7 ± 6.2 *******	0.70 *******

Data summarized from Table 2 (C. Mason, 2001), pp.18-19.
1 Air
2 Cyclophosphamide (40 mg/kg)
***** Statistically significant, $p < 0.01$ (as calculated by reviewer using Mann-Whitney U test)
******* Statistically significant, $p < 0.001$

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The investigators concluded that baseline gasoline vapor condensate did not increase the incidence of micronucleated PCEs in rat bone marrow in the study. They also concluded that the test substance did not cause a statistically significant decrease in the proportion of PCEs among the total erythrocyte.

B. REVIEWER COMMENTS: The reviewer agrees with the investigators' conclusions. Baseline gasoline vapor condensate did not increase the incidence of micronucleated PCEs over vehicle control values at any test concentration in either sex, thus indicating no clastogenic or aneugenic activity. The test material was tested to a concentration of 20,000 mg/m^3 in the breathing air for 6 hours per day on 5 days per week for 4 weeks. Proper experimental protocol was followed, and the vehicle and positive control values were appropriate. This is an **Acceptable/Guideline** study.

C. STUDY DEFICIENCIES: No study deficiencies were identified.